Plant metabolism

Studies on the metabolism of fludioxonil when applied as a seed treatment and as foliar treatment were reported to the Meeting but no information was provided on post-harvest degradation. Pyrrole and phenyl-labelled compounds were used with radiochemical purity >97–>99%.

Foliar application

Grapes (Nicollier, 1991, Report 3/91; 1993, Report 89GN14PR2). In a field study in Sisseln, Switzerland three grapevines (Riesling/Sylvaner) were sprayed three times at 3-week intervals with [pyrrole-4-^{14}C]fludioxonil formulated as WP 50 at an approximate rate of 500 g ai/ha (50 g ai/100 l), twice the typical maximum field use rate of 250 g ai/ha. The application to two of the vines was directed at the fruit, and to the third included the leaves.

Grapes and leaves were sampled 0-day (Interval 1) and 1 month (Interval 2) after the first application, and 0-day (Interval 3) and 14 days (Interval 4) and at maturity (Interval 5, 35 days) after the third.

The grapes and leaves were washed with methanol/water (1:1), and the grapes pressed. At harvest, a portion of the *unwashed* grapes was frozen, crushed and pressed. Unwashed grapes were fermented to produce wine. Grapes, presscake, seeds and leaves were homogenised with liquid nitrogen and the TRR was measured by combustion and liquid scintillation counting (LSC) of sub-samples. Methanol/water (80:10) followed by hot methanol (Soxhlet) were used to extract samples, and extracts and washes analysed by 1- or 2-dimensional TLC. Some samples were cleaned up first using silica gel column chromatography. Unextracted radioactivity was determined by combustion and LSC of the extracted residue. Enzyme cleavage was used to determine sugar-conjugated metabolites.

Ancillary cell culture and grape leaf incubation experiments were conducted to aid the identification of radiolabelled residues. Liquid chromatography employing silica gel or XAD-4 was used in the clean-up stages. Metabolites were identified by TLC and HPLC, enzyme cleavage, and acetylation and methylation, and finally either by co-chromatography or mass spectrometry and NMR.

At harvest (Interval 5) fruit and leaves contained 56% and 43% of the radioactivity respectively immediately after the final application when 57% remained on the surface of the fruit and 32%, 2.6% and 8.2% was in the presscake, seeds, and juice respectively. The surface radioactivity on the grapes decreased from 87% shortly after the third application to 57% of the TRR at harvest. Total residues *after washing* and pressing the harvested grapes were 6.0 mg/kg as fludioxonil in the presscake (fludioxonil 2.0 mg/kg), 1.7 mg/kg in the seeds (fludioxonil 0.63 mg/kg), and 0.30 mg/kg in the juice (fludioxonil 0.14 mg/kg). Results are shown in Table 12.

Table 12. Distribution of radioactivity and residual fludioxonil (CGA 173506) after three applications of [pyrrole-4-^{14}C]fludioxonil (Nicollier, 1991, Report 3/91; 1993, Report 89GN14PR2).

Interval	Sample	Total residues (mg/kg) [1]	(%) [2]	Balance (%) [3]	Parent (mg/kg)	Surface (%) [4]	Extract (%) [4]	Soxhlet (%) [4]	Unext. (%) [4]	Total (%) [4]
0.5 h after 1st application Interval 1 (07/13/1989)	Leaves	4.9	100		4.1	87.9	11.8	0.3	0.0	100.0
	Grapes	21.	100		19.	98.5	1.4	0.1	0.0	100.0
26 days after 1st application, 1 day before 2nd Interval 2	Leaves	3.0	79		1.9	84.0	9.4	2.6	4.4	100
	Grapes	2.1	52		1.7	85.4	11.5	0.4	2.2	100

Interval	Sample	Total residues (mg/kg) [1]	(%) [2]	Balance (%) [3]	Parent (mg/kg)	Surface (%) [4]	Extract (%) [4]	Soxhlet (%) [4]	Unext. (%) [4]	Total (%) [4]
0.5 h after 3rd application	Leaves	12	100		10	92.8	4.8	1.5	1.8	101
	Grapes									
	wash	-		87.0		87.0				
Interval 3	cake	2.1		9.0	1.0		83.1	3.5	12.0	99
(08/30/1989)	juice	0.28		4.0	0.12		100.0			100
	Total Grapes	5.0	100	100.0	4.5	87.0	11.5	0.3	1.1	100
	Leaves	13	120		11.	91	7.5	0.8	0.9	100
14 days after	Grapes									
3rd application	wash			74		74				
	cake	2.5		19	0.59		61.	5.1	42	110
Interval 4	juice	0.37		7.5	0.17		100			100
	Total Grapes	3.4	64	100.	2.4	74	19.	1.0	8.0	102
	Leaves	5.2	42.		3.6	52.	42.	1.8	3.8	100.
35 days after 3rd	Grapes									
application	wash	-		57.		57.				
	cake	6.0		32.	2.02		67.	4.4	23.	94.
Interval 5	seeds	1.7		2.6	0.63		74.	4.0	16.	94.
(10/04/1989)	juice	0.30		8.2	0.14		100.			100.
	Total Grapes	2.8	58	100	2.0	57	31.	1.5	7.6	98

n.a.: not analysed.
[1] Fludioxonil equivalents.
[2] Leaf and grape radioactivity at interval 2 as % of that recovered at interval 1, and at intervals 4 and 5 as % of that recovered at interval 3
[3] 100%: total ^{14}C found in the grapes
[4] % relative to combustion value except for interval 1 where it was determined by sum of extracted + unextracted radioactivity

Unextracted total grape radioactivity increased from 1.1% shortly after the third application to 7.6% of the TRR at harvest, and from 1.8% to 3.8% of the TRR in the leaves at harvest.

Unwashed harvested grapes used for juice contained total radioactive residues of 2.5 mg/kg, including 1.7 mg/kg fludioxonil. Pressing these grapes demonstrated that 77% of the radioactivity remained in the presscake, 23% in the juice, and combined residues were 10 mg/kg (fludioxonil-6.8 mg/kg) and 0.77 mg/kg (fludioxonil 0.59mg/kg) respectively. Wine and sediment residues were 0.43 mg/kg (fludioxonil 0.34 mg/kg) and 53 mg/kg (fludioxonil 39. mg/kg) respectively. Results are shown in Tables 13 and 14.

Table 13: Distribution of radioactivity in unwashed grapes at harvest after three applications of [pyrrole-4-^{14}C]fludioxonil, PHI 35 days (Nicollier, 1991, Report 3/91; 1993, Report 89GN14PR2).

Sample	Total residues (mg/kg) [1]	Balance (%) [2]	Parent (mg/kg)
Grapes			
Cake	10	77	6.8
Juice	0.77	23	0.59
Total Grapes	2.5	100	1.7

[1] Fludioxonil equivalents.
[2] % of total radioactivity found in grapes

Table 14. Distribution of radioactivity in wine from grapes at harvest after three applications of [pyrrole-4-^{14}C]fludioxonil, PHI 35 days (Nicollier, 1991, Report 3/91; 1993, Report 89GN14PR2).

Sample	Total Residues (mg/kg)[1]	Parent (mg/kg)[1]	Balance (%)[2]	Extracted radioactivity (%)[3]	Soxhlet (%)[3]	Unextracted (%)[3]	Total (%)[3]
Juice	0.77						
Wine	0.43	0.34	60	100	-	-	100
Sediment	53	39	40	91	1.2	6.1	98
Juice total	0.72	0.55	100				

[1] Fludioxonil equivalents
[2] % relative to total radioactivity found in juice.
[3] % of radioactivity in individual processed fractions (wine and sediment)

90-100% of the radioactive residues in the grapes could be extracted with organic solvents (Table 14). Methanol/water extracted 61% to 83% of the TRR in the presscake and 74% of the TRR in the harvested seeds, and Soxhlet extraction an additional 3.5% to 5.1% from the former and 4% from the latter.

The partitioning of extracted residues is shown in Table 15.

Table 15. Partitioning of extracted radioactivity in vines and processed grapes after three applications of [pyrrole-4-^{14}C]fludioxonil (Nicollier, 1991, Report 3/91; 1993, Report 89GN14PR2).

Sample	Extracted radioactivity (%)		
	Organic phase	Water phase	Total
Leaves	81	13	94
Juice	58	51	110
Cake	78	16	95
Seeds	78	18	96
Wine	63	34	97
Sediment	94	3.2	97

TLC analysis of the extracted radioactive residues from the juice, seeds and presscake yielded numerous components (Table 16). Fludioxonil constituted 70% of the radioactivity in the whole fruit with 16 further components, none of which exceeded >2.4% of the TRR. The metabolites SYN 518579 (II_2), SYN 518581 (I_{3b}), SYN 518580: (II_{3a}), CGA 344623: (I_4), CGA 308103: (I_{11}) and the glucose conjugate of CGA 308103: (I_6) were identified. Fewer metabolites were found in the leaves than in the fruit, with fludioxonil representing 69% of the TRR (Table 16).

TLC quantification showed that fludioxonil represented most of the radioactivity in wine and sediment (79% and 74% respectively**,** Table 17)**.** The metabolites were the same as those in the juice. None were >3.1% of the radioactivity in the processed fraction.

Table 16. Quantification of metabolites in grapes at harvest and separated fractions after three applications of [pyrrole-4-^{14}C]fludioxonil (Nicollier, 1991, Report 3/91; Nicollier, 1993, Report 89GN14PR2).

Sample	Metabolite fractions (%) [1,2,3]																		Par-ent II_4	Un-re-solved	Sub-total	Soxh-let (%) [2]	Unext. (%) [2]	Total (%) [2]
	I_1	I_2	I_3 * I_{3b}	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}	I_{13}	I_{14}	I_{15}	II_1	II_2	II_3						
Grapes																								
Washings a)			0.3									0.5					1.7		96	1.8	100.	-	-	100.
Washings b)			0.2									0.3					1.0		55	1.1	58.	-	-	58.
Juice a)	9.7	2.6	8.6	2.0	1.8	2.9	2.3	1.1	0.9	1.0	1.4	2.0	1.0	1.7		0.8	3.2	1.5	45.	19.	109.	-	-	109.
Juice b)	0.8	0.2	0.7	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1		0.1	0.3	0.1	3.7	1.6	9.0	-	-	9.0
Cake a)	4.0		1.6	0.4		0.2	0.8	-	0.2	0.2	0.2	0.2	-	0.3		3.1	1.0	2.0	34.	15.	64.	4.4	23.	91.
Cake b)	1.3		0.5	0.1		0.1	0.3	-	0.1	0.1	0.1	0.1	-	0.1		1.0	0.3	0.4	11.	4.9	20.	1.4	7.2	29.
Seeds a)	4.0	0.5	2.6	0.5		0.3	1.7	0.8	0.9	0.5	-	-	0.5	0.6	0.5	-	3.7	1.6	36.	17.	72.	4.0	16.	92.
Seeds b)	0.1	-	0.1	-	-		0.1	-	-	-	-	-	-	-	-	-	0.1	0.1	0.9	0.4	1.8	0.1	0.4	2.3
Whole grape	2.4		1.5	0.2	0.2	0.3	0.6	0.1	0.2	0.2	0.2	0.6	0.1	0.2		1.1	1.7	0.6	70.	8.0	88.	1.5	7.6	98.
Leaves																								
Washings	0.2		0.2	-															50.	1.5	52.	-		520
Leaves	1.6	0.8	2.4	-		0.9	0.1	0.2	-	-	-	-	-	0.3	-	-	1.8	5.6	19.	6.6	39.	1.8	3.8	45.
Total	1.8	0.8	2.6	-		0.9	0.1	0.2	-	-	-	-	-	0.3	-	-	1.8	5.6	69.	8.1	91.	1.8	3.8	97.

n.d.: not detected or <LOQ (<0.003 mg/kg)
a) % of radioactivity in individual fruit parts
b) % of radioactivity in whole grape
[1] Quantification by 2-dimensional TLC in two solvent systems.
[2] % of sum of surface and penetrated radioactivity
[3] Metabolite fractions numbered according to position in systems SS I and SS II
* In identification phase, zone I_3 was renamed I_{3b} to differentiate it from an I_3 zone in wheat
Metabolite I_{3b}: SYN 518581
Metabolite I_4: CGA 344623
Metabolite I_6:glucose conjugate of CGA 308103
Metabolite I_{11}: CGA 308103.
Metabolite II_2: SYN 518579
Metabolite II_3 contains trace amounts of II_{3b} identified as CGA 265378.
Metabolite II_{3a}: SYN 518580

Table 17. Quantification of metabolite fractions in wine and grape sediment from grapes at harvest after three applications of [pyrrole-4-^{14}C]fludioxonil (Nicollier, 1991, Report 3/91; 1993, Report 89GN14PR2).

Sample	Metabolite Fractions (%) [1,2,3]																		Par-ent II_4	Un-re-solved	Sub-total	Soxh-let (%) [2]	Unext. (%) [2]	Total (%) [2]
	I_1	I_2	I_3 * I_{3b}	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}	I_{13}	I_{14}	I_{15}	II_1	II_2	II_3						
Wine	1.7		0.6	3.1	0.6	1.3		0.3			2.1					1.9	2.1		79	4.4	97.	-	-	97.
Sediment	0.3	0.4	0.6	0.2						0.1	0.5					2.1	1.5	2.3	74.	6.6	88	1.2	6.1	96.

[1] Quantification by 2-dimensional TLC using systems SS I and SS II
[2] % of total radioactivity found in individual parts
[3] Metabolite fractions according to position in two solvent systems.
* In identification phase, zone I_3 was renamed I_{3b} to differentiate it from an I_3 zone identified in wheat
Metabolite I_{3b}: SYN 518581
Metabolite I_4: CGA 344623
Metabolite I_6:glucose conjugate of CGA 308103
Metabolite I_{11}: CGA 308103.
Metabolite II_2: SYN 518579
Metabolite II_3 contains trace amounts of II_{3b}, CGA 265378.
Metabolite II_{3a}: SYN 518580

Tomatoes. In a trial on greenhouse-grown plants (Krauss, 1992, Report 1/92, addenda 1, 04/07/1995 and 2, 12/11/1966) eight 2- to 3-week old Capello plants were sprayed three times at two-week intervals with ^{14}C-fludioxonil formulated as WP 50 at a rate of 50g ai/ 100 l/application (1500 l/ha), equivalent to 750 g ai/ha/application. This is a grossly exaggerated rate for field uses.

Samples of tomatoes and leaves were collected at the first (Interval 1) and last (Interval 2) application. At maturity (Interval 3, harvest, 40 days after the last application) all tomatoes and leaves were sampled.

Tomatoes were washed with methanol/water (1:1) to determine surface residues, frozen in liquid nitrogen and homogenised for analysis. The sum of the radioactivity found in the washings and in the fruit (by combustion) was considered as the total ^{14}C-residue. Homogenised plant material was extracted with methanol/water (8:2) by blending and shaking, followed by Soxhlet extraction with methanol. The remainder was combusted to determine the unextracted residue. The combined methanol/water extracts were concentrated and partitioned with dichloromethane. The resultant organic and aqueous phases were analysed by two-dimensional thin-layer chromatography (2-D TLC). Liquid chromatography using Amberlite XAD-4 resin, HPLC, and enzyme cleavage were employed for clean-up and isolation. The metabolites were also compared by 2-D TLC with those obtained in the grape study. The grape metabolites were identified by spectroscopic means.

The distribution of TRR in the tomatoes is summarised in Table 18.

Penetration of radioactivity from the treated fruit surface to the interior of the fruit increased over time. Surface radioactivity represented 84%, 72% and 41% of the fruit TRR after the first application, after the last application, and at harvest (40-day PHI) respectively. The residues in the tomatoes decreased from 0.36 mg/kg shortly after the final application to 0.28 mg/kg at harvest, and in the leaves from 11 mg/kg to 7.0 mg/kg.

Extraction of radioactivity from fruit at all three sampling intervals was almost quantitative (93% to 102% of the TRR).

Table 18. Total radioactivity and residual ^{14}C-fludioxonil in greenhouse-grown tomato plants after three foliar applications of [pyrrole-4-^{14}C]fludioxonil at approximately 50g ai/100 l/application (1500 l/ha/application) (Krauss, 1992, Report 1/92, and addenda 1, 04/07/1995 and 2, 12/11/1966).

Interval	Sample	Total residue (mg/kg) [1]	Fludioxonil (mg/kg)	Surface (%)	Non-surface			Total (%)
					Cold ext. (%)	Soxhlet (%)	Unextracted. (%)	
0 days after application 1 Interval 1	Tomatoes	0.20	0.19	84.	16.	0.2	0.1	100.
	Leaves	5.8	5.4		97.	0.6	0.2	98
28 days after application 1 0 days after application 3 Interval 2	Tomatoes	0.36	0.31	72.	29.	0.6	0.9	102
	Leaves	11.	9.5		95.	0.2	0.6	96
68 days after application 1 40 days after application 3 Interval 3	Tomatoes	0.28	0.20	41.	50.	2.0	5.8	98
	Leaves	7.0	4.8		92.	1.2	4.2	98.

LOQ for combustion: 0.001 mg/kg, LOQ for TLC analysis of plant material: 0.001 mg/kg
[1] fludioxonil equivalents: Tomatoes: sum of surface rinse and extracts. Leaves: direct combustion (no rinse).

Most of the radioactivity from the harvested tomatoes was organosoluble with 88% of the radioactivity of the surface wash and 79% of the fruit extract partitioning into the organic phase.

Water-soluble radioactivity was 3.7% and 12% respectively. Leaf extracts showed 90% organosoluble and 8.5% water-soluble radioactivity.

The metabolite pattern in tomatoes was complex. Fludioxonil represented 73% of the radioactivity in the harvested fruit. TLC analysis produced 11 metabolite zones in addition to fludioxonil (Table 19). In total the metabolite fractions represented 6.6% of the total radioactivity, with no single fraction above 1.6% of the TRR. Metabolites SYN 518579 (III_2), SYN 518581 (I_{3b}), SYN 518580: (III_3), CGA 344623: (I_4) and the glucose conjugate of CGA 308103: (I_6) were identified by 2-D TLC comparison with the metabolites found in grapes together with metabolite CGA 192155 (I_5), identified by co-chromatography with its authentic reference standard. The identified metabolites represented a total of 3.6% of the TRR in tomato fruit. Trace amounts of sugar conjugates (approximately 0.2% of the TRR) could be detected in water-soluble material of the tomato extracts.

A similar metabolic pattern was found in the leaves. Fludioxonil represented 69% of the harvested leaf radioactivity. In total the metabolite fractions constituted 7.9% of the TRR in the leaves with 4.6% identified as SYN 518579 (III_2), SYN 518581 (I_{3b}), SYN 518580 (III_3), CGA 344623 (I_4), the glucose conjugate of CGA 308103 (I_6) and CGA 192155 (I_5).

At harvest (Interval 3) unextracted radioactivity in the fruit and leaves was 5.8% and 4.2% of the TRR respectively (Table 19).

Table 19. Quantification (TLC) of metabolite fractions in tomatoes at maturity after three foliar treatments with [pyrrole-4-^{14}C]fludioxonil (Krauss, 1992, Report 1/92 plus addenda 1, 04.07.1995 and 2, 12.11.1966).

Sample	Radioactivity in metabolite fractions (% of TRR)													Soxhlet (%)	Un-extracted (%)	Total (%)
	I_1	I_2	I_{3a}	I_{3b}	I_4	CGA 192155 I_5	I_6	I_{11}	III_1	III_2	III_3	Fludioxonil III_4	Un-resolved			
Tomatoes																
Surface (0.11 mg/kg)	1.0	0.2	--	1.0	0.2	0.2	-	-	-	1.0	0.7	85	3.2	-	-	93
Fruit (after wash) (0.16 mg/kg)	2.1	0.7	-	1.4	0.5	-	<LOQ [2]	0.2	1.4	2.1	-	67 [3]	10.	2.6 [4]	10.	98
Total fruit [1]	1.6	0.5	-	1.2	0.4	0.1	<LOQ [2]	0.1	0.8	1.6	0.3	73 [3]	7.3	1.5 [4]	5.8	94
Leaves (7.0 mg/kg)	0.6	0.1	0.2	0.7	0.2	0.1	0.1	-	2.4	1.8	1.7	69. [3]	12.	1.0 [4]	4.2	94

[1] % of total residues in surface and fruit.
[2] LOQ: 0.001 mg/kg.
[3] Includes parent content of Soxhlet and all other fractions.
[4] Parent content subtracted from Soxhlet fraction.

Metabolite I_{3b}: SYN 518581
Metabolite I_4: CGA 344623
Metabolite I_5: CGA 192155
Metabolite I_6: the glucose conjugate of CGA 308103
Metabolite III_2: SYN 518579
Metabolite III_3: SYN 518580

Peaches. US studies were conducted during the 1996 and 1997 growing seasons at the Northeast Research Station, Hudson, NY and Western Research Station, Sanger, CA. The test substance, uniformly labelled on the phenyl ring, was formulated as 50% WP and diluted in water/acetone (9:1) for application.

For each of the three tests an isolated branch on a mature peach tree grown outdoors that would yield ~2 kg of fruit and could be covered by a predetermined volume of formulated test substance to achieve complete coverage with minimal run-off was selected. In the NY tests three foliar applications were made at 280 or 2800 g ai/ha/application beginning at petal fall and repeated at intervals of 30 and 33 days for a total of 840 or 8400 g ai/ha/season. Samples of leaves were collected 0 and 28 days and mature fruit harvested 28 days after treatment. In the CA test [^{14}C]fludioxonil was applied at petal fall at 2100g ai/ha and again 35 days later at 6300 g ai/ha (total 8400g ai/ha/season, tenfold rate. Samples of leaves were collected 0 and 114 days after treatment and of immature and mature fruit 30 and 114 days after the second application.

Samples stored frozen at the test site were later shipped frozen, combusted for TRR determination within 14-65 days of harvest, and radioassayed by LSC. The LOQs for the radioassays were 0.005-0.006 mg/kg for the 1x NY test and 10x CA test, and 0.022 mg/kg for the 10x NY test. ^{14}C-Residues were <LOQ in all fruit and leaf control samples. ^{14}C residues in the fruit and leaves harvested 28 days after the last application at the two rates were 3.5 and 46. mg/kg in leaves, and 0.083 and 0.98 mg/kg in fruit, reflecting approximately the 10-fold difference in application rates. ^{14}C residues in fruit from the 10x CA test (0.26 mg/kg; 114-day PHI) were four times lower than those detected in the fruit from the 10x NY test (0.98 mg/kg; 28-day PHI); residues in the immature fruit from the 10x CA test 30 days after treatment were 0.83 mg/kg (Table 20).

Table 20. TRR in or on fruit and leaves of peaches harvested after 2-3 foliar applications of [phenyl^{14}C]fludioxonil, 0.84 or 8.4 kg ai/ha, 1 or 10x foliar rate (Peffer, Report 156-96, 1999).

Location	Sample	PHI (days)	Total radioactive residues (mg/kg)[1]	
			1x	10x
NY	Leaves	0	13	140
		28	3.5	46
	Mature fruit	28	0.083	0.98
CA	Leaves	0	Not determined	360
		114		38
	Immature fruit	30		0.83
	Mature fruit	114		0.26

[1] Mean of triplicate analyses as fludioxonil equivalents.

Radioactive residues in homogenized fruit and leaves were extracted 3-4 times with ACN/water/acetic acid (80:20:1), and the extracts combined, concentrated, and analysed by TLC and HPLC. Cold extraction released 88-101% of the TRR from fruit and >94% from mature leaves. A small amount remained in the post-extraction solids of 1x-treated fruit (9.5% of the TRR, 0.008 mg/kg), which was not further analysed.

The unextractable residues in 10x treated fruit (7-10% of the TRR, 0.026-0.069 mg/kg) were twice subjected to sequential microwave-assisted extraction, twice with 2-propanol/water (8:2) and once with 3.0 N HCl, each for a total of 37 minutes at sequential temperatures of 100°, 120° and 150°C. The released radioactivity in the alcohol and acid fractions was partitioned with methyl *tert*-butyl ether (MtBE), and the organosoluble ^{14}C-residues (1-7% of the TRR) were analysed by TLC and/or HPLC; the aqueous fractions (0.8-1.5% of the TRR) were not further analysed.

Extracts from 1x treated leaves were purified before analysis on a C_{18}-SPE column to remove plant pigments and other non-polar interferences. The column was eluted successively with ACN/water (80:20) and solvents such as ACN, MeOH, and $CHCl_3$. The ACN eluates and MeOH/$CHCl_3$ fractions were each combined, concentrated, and analysed by TLC and HPLC; components of the combined ACN fraction (71% of the TRR, 2.5 mg/kg) were resolved by TLC and HPLC. Radioactivity in the combined MeOH/$CHCl_3$ fraction (12.3% of the TRR, 0.43 mg/kg) was not resolved by TLC owing to extensive sample interference, and HPLC analysis produced peaks eluting after those from the standards and mature fruit extracts. The study author suggested that the late-eluting radioactivity may have been associated with sample components or perhaps incorporated in leaf pigments. As these peaks were not observed in fruit samples or in 10x treated leaves, they were not examined further. Unextractable radioactivity accounted for 9.5-13% of the TRR (0.45-4.4 mg/kg) in leaves and was not further analysed.

Extractable radioactive residues were analysed 2-D TLC on silica gel plates developed with ethyl acetate/1-propanol/water (62/24/12) and chloroform/methanol/formic acid/water (75:20:4:2) for definitive sample analyses and seven other solvent systems were also used for selected analyses. Radioactive residues in mature fruit extracts were detected using an AMBIS Radioanalytic Imageing System or Fuji BAS 1000 Phosphor Imager; visualized ^{14}C-residues were scraped from the plates and quantified by LSC. For TLC of leaf extracts radioactivity was quantified by direct integration using the AMBIS system. Identification was by comparison with fludioxonil, CGA-339833, CGA-344623, CGA-308103, CGA-192155, CGA-340351, CGA-265378, CGA-260766, CGA-257777, CGA-227731, CGA-308565, CGA-335892, CGA-336293, and CGA-339836 standards visualized with UV light. A C_{18} HPLC column equipped with an in-line radioactivity monitor and UV detection (271 nm) and using a mobile phase gradient of ACN to 0.05 M ammonium formate with 5% ACN was also used with additional HPLC systems for chromatography of selected isolated metabolites.

After an additional bulk extractions of 10x-treated fruit for further characterisation, selected aliquots of the XAD-4 organic eluants from aqueous extracts were incubated overnight in 0.1M NaOAc buffer (pH 4.6) at 37°C with either cellulase to cleave sugar-conjugated metabolites, or glucosidase to cleave specifically glucose-conjugated metabolites.

The following compounds were isolated from 10x treated fruit and leaves and identities determined using MS and/or NMR analysis: the parent compound, CGA-339833, the 2-keto-5-hydroxy and 2-hydroxy-5-keto analogues of fludioxonil, glucose conjugates of oxidized fludioxonil (metabolites P10a, P10b, P8b), the hexose conjugate of oxidized fludioxonil (metabolite P8), and CGA-339833.

The distribution and identification of ^{14}C-compounds in the fruit and leaves treated with [^{14}C]fludioxonil are shown in Tables 21 and 22.

Table 21. ^{14}C-residues in peaches harvested after 2-3 foliar applications of [phenyl-U-^{14}C]fludioxonil (Peffer, Report 156-96, 1999).

Compound or fraction	Fruit 1x (28-day PHI) (TRR 0.083 mg/kg)		Fruit 10x (28-day PHI) (TRR 0.98 mg/kg)		Fruit 10x (114-day PHI) (TRR 0.27 mg/kg)	
	% of TRR [1]	mg/kg [2]	% of TRR	mg/kg	% of TRR	mg/kg
Fludioxonil [3]	22	0.018	62	0.60	36	0.091
CGA-344623	3.7	0.003	0.8	0.008	2.8	0.007
CGA-339833	5.6	0.005	2.3	0.022	4.1	0.010
P8 (oxidized fludioxonil sugar conjugates)	1.3	0.001	0.6	0.006	0.7	0.002
P8b (oxidixed fludioxonil glucose conjugate)	0.4	<0.001	ND	--	ND	--

Compound or fraction	Fruit 1x (28-day PHI) (TRR 0.083 mg/kg)		Fruit 10x (28-day PHI) (TRR 0.98 mg/kg)		Fruit 10x (114-day PHI) (TRR 0.27 mg/kg)	
	% of TRR [1]	mg/kg [2]	% of TRR	mg/kg	% of TRR	mg/kg
P10a, P10b (oxidized fludioxonil glucose conjugates)	11	0.009	3.7	0.036	7.1	0.018
CGA-308103	3.7	0.003	1.4	0.014	1.4	0.004
2-keto-5-hydroxy-fludioxonil SYN518579			0.8	0.007	0.3	0.001
2-hydroxy-5-keto-fludioxonil SYN518579	1.6	0.001	1.4	0.014	0.7	0.002
CGA-192155	1.7	0.001	1.5	0.015	1.0	0.002
Total identified	**51**	**0.042**	**74**	**0.72**	**54**	**0.14**
CGA-308565[4]	2.0	0.002	2.1	0.021	0.9	0.002
Unknown TLC Regions [5]	21.5	0.018	9.5	0.093	18.9	0.048
Total characterised/identified	**74**	**0.062**	**86**	**0.84**	**74**	**0.19**
Unresolved [6]	21	0.018	14	0.14	15	0.038
Post-extraction Solids (PES)	9.5	0.008	7.1	0.069	10	0.026

[1] Uncorrected for recovery.
[2] [^{14}C]fludioxonil equivalents.
[3] CGA-265378 co-eluted with parent and was characterised by HPLC as possible minor metabolite in fruit accounting for 0.9-1.7% of the TRR.
[4] Tentative identification.
[5] 6-9 regions each consisting of ≤8.3 % of the TRR, except for the origin of 114-day PHI fruit (10.2% of the TRR; 0.026 mg/kg) which was shown, after enzyme treatment and A25 chromatography, to be a multicomponent mixture (each ≤0.013 mg/kg) including entrapped parent and neutral and acid metabolites.
[6] TLC quadrants 1-4 scraped into eight vials (two each quadrant) after scraping of major spots.

Table 22. ^{14}C-residues in peach leaves harvested 28 days after three foliar applications of [phenyl-U-^{14}C]fludioxonil (Peffer, Report 156-96, 1999).

Compound or fraction	Leaves (1x rate) (TRR 3.5 mg/kg)		Leaves (10x rate) (TRR 46. mg/kg)	
	% of TRR [1]	mg/kg [2]	% of TRR	mg/kg
Fludioxonil	3.6	0.13	67	30.
CGA-344623	4.0	0.14	0.9	0.41
CGA-339833	5.5	0.19	2.5	1.1
P8 (oxidized fludioxonil-sugar conjugate)	1.1	0.040	0.3	0.16
P8b (oxidized fludioxonil-glucose conjugate)	1.8	0.062	0.6	0.26
P10a, P10b (oxidized fludioxonil-glucose conjugates)	4.0	0.14	1.8	0.82
CGA-308103	2.6	0.092	1.4	0.62
2-keto-5-hydroxy-fludioxonil	1.4	0.050	1.2	0.57
2-hydroxy-5-keto-fludioxonil	5.4	0.19	3.2	1.4
CGA-192155	1.7	0.060	1.6	0.72
Total identified	**31**	**0.90**	**80**	**37.**
CGA-308565[3]	4.5	0.16	3.9	1.8
CGA-265378	4.8	0.17	5.7	2.6
CGA-339833 [4]	4.0	0.14	3.0	1.4

Compound or fraction	Leaves (1x rate) (TRR 3.5 mg/kg)		Leaves (10x rate) (TRR 46. mg/kg)	
	% of TRR [1]	mg/kg [2]	% of TRR	mg/kg
CGA-344623 [4]	1.3	0.045	0.6	0.26
Unknown TLC Regions [5]	27	0.95	13	6.0
Organosoluble	12	0.43	NA	--
Total characterised/identified	**85**	**2.8**	**106**	**49**
Unresolved [6]	2.6	0.092	12.	5.6
Post-extraction Solids (PES)	13	0.45	9.5	4.4

[1] Not corrected for recovery.
[2] [^{14}C]fludioxonil equivalents.
[3] Tentatively identified by HPLC.
[4] ^{14}C-activity co-migrating with leaf components characterised as CGA-344623 or CGA-339833.
[5] 7 or 8 regions each accounting for4.3% of the TRR, except for the origin of 1x leaves which accounted for 10.9% of the TRR (0.384 mg/kg).
[6] TLC quadrants 1-4 scraped into eight vials (two each quadrant) after scraping of major spots.

Samples of fruit were extracted and profiled within 4 months of harvest, and final characterisation of residues was within 14-32 months. HPLC profiles from the original extracts and those at the end of the study period were similar, indicating that fludioxonil residues are stable in peaches.

Onions. The metabolism of [phenyl-U-^{14}C]fludioxonil in green onions was studied at a California field location in the USA (Kennedy, Report 153-97, 1999). A 50 WP formulation was applied as a foliar spray twice with a 14-day interval, at intended rates equivalent to seasonal use rates of 1116 g ai/ha and 5580 g ai/ha. Actual treatment rates were 557 g ai/ha and 683 g ai/ha (total 1240 g ai/ha) and 2793g ai/ha and 3376 g ai/ha (total 6169 g ai/ha)

Green onions (whole plants) were sampled immediately after each application, 7 days after the last application (early harvest), 14 days after the last application (mature harvest), and 28 days after the last application (delayed harvest).

Homogenized samples were combusted and the TRR determined by LSC. Uncombusted samples were extracted with acetonitrile/water (80:20) or acetonitrile/water/acetic acid (80:20:1) using a Polytron homogeniser, concentrated and profiled by TLC and HPLC. The extracts from preparative extractions were chromatographed on a C-18 column, which was washed with acetonitrile and eluted with chloroform. The fractions from the loading and washing steps were combined. Selected solutions were evaporated to leave an aqueous fraction, which was partitioned with heptane and then methyl *tert*-butyl ether (MTBE) to separate the organosoluble radioactivity. The water-soluble radioactivity from the partition was subjected to C-18 solid-phase extraction (SPE). Additional clean-up was by A-25 anion exchange chromatography. Organic and aqueous fractions were analysed by TLC and HPLC. Selected extracts were hydrolysed with β-glucosidase. Microwave-assisted extractions of selected unextracted residues (post-extraction solids, PES) were with 2-propanol followed by acid and base hydrolysis. MS and NMR were used to identify metabolites.

The distribution of the TRR in the onion plants is shown in Table 23. Control samples did not contain any detectable residues.

The residues in the whole plants decreased from 2.4 mg/kg shortly after the second application to 0.98 mg/kg 28 days later at the lower treatment rate, and from 13. mg/kg to 4.7 mg/kg at the higher treatment rate. Extractable radioactivity for the lower rate decreased from 95% after the first application to 62% after 28 days, and for the higher treatment from 96% to 79%. Unextracted radioactivity increased from 1.2% and 4.4% after the two lower treatments to 25% in the 14-day PHI samples, with similar results at the higher rate.

Table 23. Distribution and extraction characteristics of radioactivity in extracts of field-grown green onions after foliar treatment with [phenyl-U-^{14}C]fludioxonil (Kennedy, Report 153-97, 1999).

Treatment /Location	Sample	TRR (mg/kg)	Extracted [1]		Unextracted		Recovery[2]
			% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
Low rate/California							
	Whole plant post 1st application	1.5	95	1.4	1.2	0.018	96
	Whole plant post 2nd application	2.4	95	2.2	4.4	0.10	99
	Whole plant – 7-day PHI	1.8	76[3]	1.4[3]	21[3]	0.38[3]	98
	Whole plant-14-day PHI	1.6	73	1.2	25	0.39	98
	Whole plant-28-day PHI	0.98	62.	0.60	22.	0.21	84.
High rate/California							
	Whole plant post 1st application	14	96	13	0.8	0.11	96
	Whole plant post 2nd application	13	98	13	4.3	0.58	102
	Whole plant-7-day PHI	10	79	7.9	18	1.8	97
	Whole plant-14-day PHI	10	78	7.8	28	2.8	106
	Whole plant-28-day PHI	4.7	79	3.7	28	1.3	107

[1] Toal extracted before C-18 acetonitrile flash chromatography.
[2] Extracted % of TRR + unextracted (PES) % of the TRR.
[3] Average: extraction carried out in two parts

The distribution of extracted residues between organic and aqueous phases is shown in Table 24. For the 1X treatment 44% of the radioactivity in the 7-day PHI samples was organosoluble, and for the 5X treatment the proportion decreased from 67% in 7-day PHI samples to 48% in 28-day PHI samples. The corresponding water-soluble radioactivity increased from 17% to 26% over the same period.

Table 24. Characterisation of extracted radioactivity from field-grown green onions treated with foliar applications of [phenyl-U-^{14}C]fludioxonil (Kennedy, Report 153-97, 1999).

Sample	TRR	Heptane soluble	MTBE soluble	Total organosoluble [1]		C-18 ACN eluant	C-18 MeOH eluant	C-18 aqueous L+W[2]	Total water-soluble [3]	
	mg/kg	% of TRR	% of TRR	% of TRR	mg/kg	% of TRR	% of TRR	% of TRR	% of TRR	mg/kg
1X, 7-day PHI	1.8	15	29	44	0.79	12	6.5	7.7	26	0.47
5X, 7-day PHI	10	40	27	67	6.7	8.9	3.8	4.6	17	1.7
5X, 14-day PHI	10	24	27	51	5.1	5.0	3.0	13.	21	2.08
5X, 28-day PHI	4.7	25	24	49	2.3	4.3	3.9	17	26	1.2

[1] Heptane soluble fraction + MTBE soluble fraction
[2] Load to wash fractions
[3] C-18 ACN eluant + C-18 MeOH eluant + C-18 aq L+W fractions.

Quantification of the metabolites is shown in Table 25. Fludioxonil was the main residue with 38% to 54% of the TRR in 7-day PHI samples, 36% to 49% of the TRR in 14-day PHI samples and 12% to 31% of the TRR in 28-day PHI samples. Metabolites CGA 265378, CGA 308103, SYN 518579, CGA 192155, CGA 344623, and CGA 339833 were also found, with none above 7% of the TRR. The residue percentages characterised ranged from 64% to 82% at all intervals and both treatment rates, and percentage identified from 29% to 67%.

Microwave-assisted extraction of the 7-day PHI samples, followed by acid hydrolysis for the 14- and 28-day PHI samples, released an additional 11% to 18% of the TRR, most of which was organosoluble. Small amounts (<2.0%) of fludioxonil, CGA-265378, CGA-308103, and CGA-192155 were found after the microwave extraction. The residues remaining (PES) varied from <0.1% to as much as 7.6% of the TRR.

Table 25. Quantification of metabolites in green onions treated with foliar applications of [phenyl-U-^{14}C]fludioxonil (Kennedy, Report 153-97, 1999).

Source	Components	1X rate		5X rate	
		% of TRR	mg/kg	% of TRR	mg/kg
7-day PHI					
	Total mg/kg	100	1.8	100	10.
Aqueous acetonitrile extracts		76		79	
	% of TRR profiled	77		79	
A [1]	Fludioxonil	38	0.69	54	5.4
B [1]	CGA-265378	1.2	0.022	4.1	0.41
E	CGA-308103	4.4	0.079	2.4	0.24
F	CGA-192155	2.6	0.047	1.2	0.12
P15	SYN 518579	1.2	0.022	0.9	0.090
O16	MS/partial structure	1.5	0.027	4.0	0.40
H	CGA-344623	2.6	0.047	0.9	0.090
I	CGA-339833	0.8	0.014	1.4	0.14
Unknowns	Multiple zones	17.	0.30	2.9	0.29
Unresolved	Low level spots			1.3	0.13
O	Origin	7.7	0.14	5.0	0.50
Total		77	1.4	79	7.8
Initial unextracted[2]		23	0.41	18	1.8
Microwave-extracts		16	0.29	11	1.1
Microwave-aqueous[3]	(not analysed)	2.9	0.052	3.1	0.31
Microwave-MTBE [4]		11	0.20	8.2	0.82
	% of TRR profiled [4]	8.6	0.16	8.2	0.82
A	Fludioxonil	0.3	0.005	0.3	0.030
B	CGA-265378	0.5	0.009	0.5	0.050
E	CGA-308103	0.3	0.005	0.2	0.020
F	CGA-192155	0.3	0.005	0.2	0.020
P15	SYN 518579	0.5	0.009	0.2	0.020
O16	MS/partial structure	1.0	0.018	0.8	0.080
Unknowns	Multiple zones	2.0	0.036	2.2	0.22
Unresolved		3.7	0.067	3.6	0.36
O	Origin	0.1	0.002	0.1	0.010
Total		12	0.21	11	1.1
Final unextracted[2]	Post-microwave	7.6	0.14	4.8	0.48
Overall total		96	1.7	95	9.4
% Characterised [6]		82	1.5	82	8.2
% Identified [7]		53	0.95	67	6.7
14-day PHI					
	Total mg/kg	100	1.6	100	10.

Source	Components	1X rate		5X rate	
		% of TRR	mg/kg	% of TRR	mg/kg
Aqueous acetonitrile extracts		73		78	
	% of TRR profiled	67		78	
A [1]	Fludioxonil	36	0.56	49	4.9
E	CGA-308103	4.5	0.071	2.0	0.20
F	CGA-192155	1.7	0.027	2.2	0.22
P15	SYN 518579	0.9	0.014	0.5	0.050
O16	MS/partial structure			1.7	0.17
H	CGA-344623	1.3	0.020	1.3	0.13
I	CGA-339833	2.7	0.042	1.4	0.14
Unknowns	Multiple zones	5.6	0.088	4.2	0.42
Unresolved				7.9	0.79
O	Origin	15	0.24	7.3	0.73
Total		67	1.1	78	7.8
Initial unextracted[2]		25	0.39	28	2.8
Microwave-extracts	Microwave	15	0.24	11	1.1
Acid/base-extracts [3]	Acid/base hydrolysis			4.5	0.45
Microwave-aqueous	(not analysed)	4.1	0.064	2.7	0.27
Microwave-MTBE[4]		10	0.16	9.0	0.90
	% of TRR profiled [4]	10	0.16	8.3	0.83
A	Fludioxonil	1.1	0.017	0.4	0.04
B	CGA-265378			0.5	0.05
E	CGA-308103	0.4	0.006	0.3	0.03
F	CGA-192155	0.2	0.003	0.3	0.03
P15	SYN 518579	0.9	0.014	0.3	0.03
O16	MS/partial structure	1.4	0.022	0.9	0.09
Unknowns	Multiple zones	3.2	0.050	2.0	0.20
Unresolved		3.0	0.047	3.5	0.35
O	Origin	0.3	0.005	0.2	0.02
Total		15	0.229	15	1.6
Final unextracted[2]	Post microwave	7.5	0.12		
Final unextracted [5]	Post acid/base hydrolysis			<0.1	<0.010
Overall Total		90	1.4	93	9.4
% Characterised [6]		75	1.2	75	7.5
% Identified [7]		47	0.74	58	5.8
28-day PHI					
	Total mg/kg	100	0.98	100	4.7
Raw extracts		62		79	
	% of TRR profiled	56		75	
A [1]	Fludioxonil	12	0.112	31	1.4
B [1]	CGA-265378	2.6	0.025	6.8	0.32
E	CGA-308103	5.5	0.054	3.0	0.14
F	CGA-192155	2.5	0.024	2.2	0.10
P15	SYN 518579	1.4	0.014	1.6	0.075
O16	MS/partial structure			4.0	0.19
H	CGA-344623	1.7	0.017	2.0	0.093
I	CGA-339833	2.8	0.027	2.2	0.10
Unknowns	Multiple zones			7.0	0.33
Unresolved				5.4	0.25
O	Origin	28	0.275	9.4	0.44
Total		56	0.549	75	3.5
Initial unextracted[2]		22	0.213	28	1.3
Microwave-Extracts	Microwave	13	0.123	18	0.86
Acid -Extracts [3]	Acid hydrolysis			1.8	0.084
Microwave-Aqueous[4]	(not analysed)	2.5	0.024	3.8	0.18
Microwave-MTBE[4]		9.5	0.093	15	0.71
	% of TRR profiled [4]	7.9	0.077	15	0.69
A	Fludioxonil CGA-173506	0.5	0.005	0.9	0.042

Source	Components	1X rate		5X rate	
		% of TRR	mg/kg	% of TRR	mg/kg
B	CGA-265378	0.8	0.008	1.1	0.051
E	CGA-308103	0.4	0.004	0.5	0.023
F	CGA-192155	0.4	0.004	0.6	0.028
P15	SYN 518579	0.5	0.005	0.7	0.033
O16	MS/partial structure	1.3	0.013	1.8	0.084
Unknowns	Multiple zones	3.9	0.038	4.0	0.19
Unresolved		0.2	0.002	4.6	0.22
O	Origin			0.4	0.019
Total		10	0.102	20	0.94
Final unextracted[2]	Post microwave	6.7	0.065		
Final unextracted [5]	Post acid/base hydrolysis			6.2	0.29
Overall total		73	0.716	101	4.7
% Characterised [6]		64	0.625	79	3.7
% Identified [7]		29	0.280	53	2.4

[1] CGA 265378 co-eluted with fludioxonil in zone A/B. Quantitative values based on ratio of metabolites by HPLC.
[2] Initial unextracted (post-extraction solids): residues remaining after aqueous acetonitrile extraction, further extracted using microwave techniques or acid/base hydrolysis. Final unextracted (post-extraction solids): residues remaining after microwave extraction.
[3] Extract from acid hydrolysis not fractionated or further analysed.
[4.] Metabolites in MTBE-soluble fraction of microwave extracts identified by co-chromatography (TLC) with available standards and/or characterised by comparison of chromatographic behaviour (TLC and HPLC) with known metabolites. Water-soluble fraction of extracts not analysed.
[5] For the 5X sample unextracted residue combusted after acid hydrolysis.
[6] Sum of values for all quantified chromatographic peaks.
[7] Sum of values for regions A, B, E, F, P15, H, and I.

Lettuce. Iceberg Floreal lettuce plants were treated three times at 10-day intervals with a WP 50 formulation of [pyrrole-4-^{14}C]fludioxonil in St Aubin, Switzerland (Stingelin, Report 98JS29, 2000, amendments 01/10/2000 and 10/02/2003) at a rate corresponding to 200 g ai/ha/application and sampled 1 h, and 6 and 13 days after the last application.

In an auxiliary experiment a threefold treatment rate (600 g ai/ha/application) was used for characterisation purposes. Plants and soil were sampled as above.

Plant samples cut just above the soil surface and free of soil were chopped, homogenized with liquid nitrogen and combusted and the TRR determined by LSC. An aliquot of the homogenised plant material was extracted with methanol/water (80:20) five times or until any additional extraction yielded <5% of the first extract using a mechanical shaker and centrifugation. For metabolite isolation a single extraction with methanol and then was used. The unextracted residues (PES) were determined by combustion. The methanol/water extracts were analysed by TLC. The crude extracts were also concentrated to the aqueous phase and extracted with dichloromethane. The organosoluble material was analysed by TLC and the water-soluble remainder cleaned up by C-18 and Serdolit® PAD I solid-phase extraction. Samples were further cleaned up and analysed using HPLC. Selected aqueous phases were hydrolysed using cellulase and β-glucosidase. Metabolites were identified by mass spectrometry and NMR.

The TRR in lettuce heads were 5.3 mg/kg (0-day PHI), 1.3 mg/kg (6-day PHI), and 0.64 mg/kg (13-day PHI) for the 1X rate, and for the 3X rate correspondingly higher. Fludioxonil residues from the 1X treatment decreased from 4.0 mg/kg to 0.29 mg/kg and those from the 3X rate from 11. mg/kg to 0.58 mg/kg during the 13 days after treatment. Most of the radioactivity in the heads was extracted, decreasing from 109% to 94% of the TRR in the 1X samples and from 104% to 91% in the 3X samples over the 13-day period, while the unextracted increased from 1.9% to 9.0% of the TRR in the 1X lettuce and from 1.7% to 6.4% in the 3X lettuce over the same period (Table 26).

Table 26. Distribution of radioactivity in heads of field-grown lettuce treated with foliar applications of [pyrrole-4-^{14}C]fludioxonil in Switzerland (Stingelin, Report 98JS29, 2000).

Treatment/Days after 3rd treatment	TRR, mg/kg [1]	Parent, mg/kg	% of TRR [2]		
			Extracted	Unextracted	Total
1X rate					
0	5.3	4.0	109	1.9	110
6	1.3	0.76	97	5.7	103
13	0.64	0.29	94	9.0	103
3X rate					
0	19	11	104	1.7	105
6	5.8	2.7	104	3.6	108
13	2.0	0.58	91	6.4	97

n.a. :not analysed
LOQ: lettuce heads. 0.002 mg/kg
[1] Fludioxonil equivalents
[2] TRR determined by combustion

The quantification of the parent and metabolite fractions in the lettuce extracts after the 1X and 3X treatments is summarised in Tables 27 and 28.

54% to 74% of the TRR from the 1X rate was the parent compound and the results for the 3X samples were similar, 62% to 87%. Twenty metabolite fractions were detected and no metabolite or zone constituted more than 4% of the TRR at either rate.

The metabolites identified by TLC co-chromatography with reference compounds or by MS and NMR were CGA 308103, CGA 339833, CGA 344623, CGA 192155, CGA 265378, the lactic acid conjugate of fludioxonil, the N-or O-glucose conjugate of CGA 308103, and the glucose conjugate of CGA 344623.

Table 27. Quantification of metabolite fractions in heads of lettuce treated with foliar applications of [pyrrole-4-^{14}C]fludioxonil (1X rate) (Stingelin, Report 98JS29, 2000).

pHI (days)[1]	0		6		13	
TRR (mg/kg)	5.3		1.3		0.64	
Extract	E1		E1		E1 + E2	
Metabolite fractions	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
I_1 (start)	2.3	0.12	2.3	0.030	3.5	0.022
I_2 CGA 344623 glucose conjugate	1.9	0.10	1.1	0.015	2.4	0.015
I_{2a}	<LOQ	<LOQ	0.8	0.011	1.5	0.010
I_3[2]	1.5	0.082	1.7	0.022	1.8	0.011
I_4 CGA 344623	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
I_{4a}	<LOQ	<LOQ	0.3	0.004	0.4	0.002
I_{4b} fluxioxonil lactic acid conjugate	<LOQ	<LOQ	<LOQ	<LOQ	0.4	0.002
I_{4c} fludioxonil lactic acid conjugate	0.2	0.012	1.0	0.013	1.7	0.011
I_{4d}	0.2	0.012	0.4	0.005	0.6	0.004
I_{4e}	0.4	0.022	0.8	0.011	1.5	0.010
I_5 CGA 308103 N- or O- glucose conjugate	<LOQ	<LOQ	0.5	0.007	0.9	0.006
I_6	<LOQ	<LOQ	0.7	0.009	1.2	0.007
I_{10} CGA 192155	2.2	0.116	0.5	0.006	0.6	0.004
I_{10a}	<LOQ	<LOQ	0.3	0.004	0.3	0.002
I_{11} CGA 308103	<LOQ	<LOQ	0.2	0.003	0.2	0.001
I_{12}	0.5	0.025	1.1	0.014	0.8	0.005

pHI (days)[1]	0		6		13	
TRR (mg/kg)	5.3		1.3		0.64	
Extract	E1		E1		E1 + E2	
Metabolite fractions	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
I_{12a}	<LOQ	<LOQ	0.7	0.009	0.4	0.003
I_{12b}	<LOQ	<LOQ	1.2	0.015	0.8	0.005
I_{12c}	<LOQ	<LOQ	0.3	0.004	0.3	0.002
I_{12d}	<LOQ	<LOQ	0.3	0.004	0.2	0.001
I_{13}	1.3	0.067	1.8	0.023	1.6	0.010
I_{14} CGA 265378	1.1	0.057	2.2	0.029	1.8	0.011
I_{15} fludioxonil (CGA 173506)	74	3.9[3]	68	0.90[3]	54	0.34[3]
I_{16}	0.9	0.049	1.6	0.021	1.2	0.007
I_{17}	<LOQ	<LOQ	0.4	0.005	0.5	0.003
Unresolved radioactivity	23.	1.2	8.8	0.12	16.	0.103
Sub total	109.0	5.815	97.4	1.275	94.	0.60
Extract E2	n.a.		n.a.		1.1	
Unextracted	1.9		5.7		9.0	
Total	110		103		104	
Identification	81		76		64	

LOQ: c. 0.2-1.2% of the TRR depending on the size of the radioactive zone. 0 days - c. 0.047 mg/kg, 6 days - c. 0.007 mg/kg, 13 days - c. 0.003 mg/kg.
n.a.: not analysed
[1] after 3rd application
[2] I_3: two fractions I_{3a} and I_{3b} : 1st is (CGA 339833)
[3] fludioxonil determined by TLC system 1 generally gave higher values than those by TLC system II

Table 28. Quantification of metabolite fractions in heads of lettuce treated with foliar applications of [pyrrole-4-^{14}C]fludioxonil (3X rate) (Stingelin, Report 98JS29, 2000).

PHI (days)[1]	0		6		13	
TRR (mg/kg)	19.		5.8		2.0	
Extract	E1		E1		E1 + E2	
Metabolite fractions	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
I_1 (start)	0.9	0.17	1.7	0.098	2.6	0.052
I_2 CGA 344623 glucose conjugate	0.9	0.174	1.8	0.104	2.2	0.044
I_{2a}	<LOQ	<LOQ	0.5	0.029	0.7	0.014
I_3[2]	1.3	0.252	1.5	0.086	1.9	0.038
I_4 CGA 344623	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
I_{4a}	<LOQ	<LOQ	0.4	0.023	0.3	0.006
I_{4b} fudioxonil lactic acid conjugate	0.2	0.039	<LOQ	<LOQ	0.2	0.004
I_{4c} fludioxonil lactic acid conjugate	0.2	0.039	0.4	0.023	1.1	0.022
I_{4d}	<LOQ	<LOQ	0.2	0.012	0.4	0.008
I_{4e}	0.1	0.019	0.6	0.035	1.2	0.024
I_5 CGA 308103 N- or O- glucose conjugate	<LOQ	<LOQ	0.5	0.029	0.6	0.012
I_6	<LOQ	<LOQ	<LOQ	<LOQ	0.7	0.014
I_{10} CGA 192155	0.2	0.039	0.5	0.029	0.5	0.010
I_{10a}	<LOQ	<LOQ	<LOQ	<LOQ	0.2	0.004
I_{11} CGA 308103	<LOQ	<LOQ	0.3	0.017	0.2	0.004
I_{12}	0.4	0.078	0.3	0.017	0.5	0.010
I_{12a}	<LOQ	<LOQ	<LOQ	<LOQ	0.3	0.006
I_{12b}	<LOQ	<LOQ	<LOQ	<LOQ	0.5	0.010
I_{12c}	<LOQ	<LOQ	<LOQ	<LOQ	0.2	0.004
I_{12d}	<LOQ	<LOQ	<LOQ	<LOQ	0.4	0.008
I_{13}	1.2	0.233	1.4	0.081	1.1	0.022
I_{14} CGA 265378	1.2	0.233	1.1	0.063	1.7	0.034
I_{15} fludioxonil (CGA 173506)	87	17[3]	80	4.6[3]	62	1.2[3]
I_{16}	1.2	0.23	1.1	0.063	0.9	0.018
I_{17}	<LOQ	<LOQ	<LOQ	<LOQ	0.4	0.008
Unresolved radioactivity	8.5	1.6	11.	0.63	9.9	0.20
Sub total	104.	20.	103.	6.0	91.	1.8

PHI (days)[1]	0		6		13	
TRR (mg/kg)	19.		5.8		2.0	
Extract	E1		E1		E1 + E2	
Metabolite fractions	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Extract E2	n.a.		n.a.		1.1	
Unextracted	1.7		3.6		6.4	
Total	105		107		98	
Identification	91		86		70	

LOQ: c. 0.1-1.7% of TRR depending on size of radioactive zone. 0 days - c. 0.095 mg/kg, 6 days - c. 0.030 mg/kg, 13 days - c. 0.007 mg/kg.
n.a.: not anaysed
[1] after 3rd application
[2] I_3: two fractions, I_{3a} and I_{3b} . 1st is CGA 339833
[3] Fludioxonil determined by TLC system 1 generally gave higher values than those by TLC system II .

Seed treatment

Potatoes. In a field trial in Klus, Switzerland, Bintje seed potatoes were treated with [pyrrole-4-^{14}C]fludioxonil, formulated as FS 100, at a rate corresponding to 2.5 g ai/100 kg seed (Kruass, Report 13/93, 1993). After being dried, the potatoes were wrapped and stored until planting the next day. Plants were sampled at 0, 40 days 71 and at harvest 95 days after treatment (Intervals 1, 2, 3, and 4). Tubers were present on the plants after 71 days, and at harvest were separated into peel and flesh.

Samples were frozen with liquid nitrogen and homogenized. The TRR were determined by combustion and LSC of representative sub-samples. Homogenized plant material was extracted with methanol/water (8:2) by blending and shaking, followed by Soxhlet extraction with methanol. Unextracted residues were determined by combustion after the Soxhlet extraction. The extracts were analysed by 2-D TLC. In some cases, the methanol/water was concentrated and extracted with dichloromethane to produce an organosoluble and water-soluble fraction, which were also analysed by 2-D TLC. Selected samples were subjected to liquid chromatography clean-up using XAD-4 resin before TLC. Some water-phase material was subjected to enzyme cleavage to release sugar conjugates.

The distribution of radioactivity in the plants and in various samples at intervals is shown in Table 29. Total residues in the tubers shortly after treatment were 8.6 mg/kg with a fludioxonil content of 8.4 mg/kg. Translocation of radioactivity from treated tubers to leaves or into new growth was limited. In the new tubers, the TRR was only 0.006 mg/kg at 71 days after treatment and at harvest. The radioactivity at harvest was 48/52% peel/flesh: approximately half was in the peel (equivalent to a residue of 0.031 mg/kg) and half in the flesh (equivalent to 0.004 mg/kg). In leaves, the TRR was 0.022 mg/kg (mg/kg), 0.019 mg/kg and 0.024 mg/kg, 40 days and 71 days after treatment and at harvest respectively.

The characterisation, identification, and quantification of the extracted radioactive residues are shown in Table 30.

Only the peel at harvest had sufficient radioactivity for chromatographic characterisation. Five zones, I_1, I_{1a}, I_2, I_{4a}, and fludioxonil (II_4), were defined and quantified by TLC. The major component was fludioxonil (21% of the TRR in the whole new tuber at harvest with the highest remaining component being 1.6% of the TRR.

Four zones were defined and quantified by TLC in the leaves at harvest. These were I_1, I_2, CGA 192155 (I_{10}) and fludioxonil (II_4). CGA 192155 and fludioxonil constituted 1.9% and 0.8% of the TRR respectively in leaves at harvest.

Unextracted radioactivity represented 35% of the TRR in new peels at harvest and was too low in the flesh to characterise, and in leaves increased over time from 21% 40 days after treatment to 46% at harvest.

Table 29: Distribution and penetration of radioactivity and residual fludioxonil in potato substrates after treatment of the seed potatoes with [pyrrole-4-^{14}C]fludioxonil at 2.5 g ai/100 kg (Kruass, Report 13/93, 1993).

Days after treatment	Sample	Total residues (mg/kg)[1]	(%)[2]	Parent (mg/kg)	Extracted radioactivity cold ext. (%)[3]	Soxhlet ext. (%)[3]	Unextracted (%)[3]	Total (%)[3]
0 days Interval 1	Treated tubers	8.6	100	8.4	99	n.a.	1.3	100
40 days Interval 2	Leaves	0.022	100	<0.001	77.	5.3	21.	103
	Treated peels	3.4	96	3.2	101	1.8	5.5	109
	Treated flesh	0.024	4.3	0.009	105.	1.0	5.3	111
	Whole treated tuber	0.49	100	0.44				
71 days Interval 3	Leaves	0.019	100	<0.001	75	3.2	28.	106
	New tubers	0.006	100	n.a.	n.a.	n.a.	n.a.	n.a.
95 days Interval 4	Leaves	0.024	100	<0.001	49	2.2	46	97
	New tuber peels	0.031	48	0.014	67	6.7	35	109
	New tuber flesh	0.004	52	n.a.	n.a.	n.a.	n.a.	n.a.
	Whole new tuber	0.006	100	0.003				

n.a.: not analysed
LOQ: combustion: 0.001 mg/kg, LOQ: TLC analyses of plant material: 0.001 mg/kg
[1] fludioxonil equivalents
[2] % of radioactivity in sample.
[3] % of radioactivity in sample.

Table 30. Quantification of metabolite fractions in potatoes at maturity after seed treatment with [pyrrole-4-^{14}C]fludioxonil at 2.5 g ai/100 kg (Kruass, Report 13/93, 1993).

Sample	Metabolite Fractions (% of TRR)							Soxhlet (% of TRR)	Unextracted (% of TRR)	Total (%) [1]
	I_1 [2]	I_{1a} [3]	I_2 [3]	I_{4a}	CGA 192155 I_{10}	Fludioxonil II_4 [4]	Un-resolved			
New tuber peels	3.4	2.5	1.4	1.2	-	44 [5]	6.3	5.6 [6]	35	100
New tuber flesh	n.a.									
Whole new tuber	1.6	1.2	~0.7	~0.6	-	21[5]	n.a.			
Leaves	18	-	1.3	-	1.9	0.8	27	2.2	46	97

[1] Sum of fractions: % by combustion of total residues.
[2] Radioactivity remaining at the origin in TLC analysis in analytical system I.
[3] Diffuse spot in TLC analysis and/or partial overlap
[4] I_{12} = II_4 = fludioxonil.
[5] Includes parent content of Soxhlet and all other fractions.

Rice. Plant uptake, distribution, and metabolism of [pyrrole-4-^{14}C]fludioxonil were studied in a greenhouse trial (Fleishmann, Report ABR-90099, 1991, amendment 10/12/1993). Rice seeds (variety Labonnet) were soaked in a 267 mg/kg solution of ^{14}C-fludioxonil formulated as a 5% powder, a treatment rate equivalent to 6.5 g ai/100 kg seed, and plants grown from the seed.

Whole plants were sampled at 25% maturity (38 days) and 50% maturity (76 days), and stalk, hulls, and grain at maturity (152 days).

The seed coating solution was analysed by TLC to establish that degradation was insignificant. Treated seeds were combusted to determine the initial seed dressing. Whole plants and mature fractions were homogenized before combustion. Samples were combusted and the TRR determined by LSC.

The distribution of the TRR in the plants is shown in Table 31. Treated seeds contained an initial coating equivalent to 65. mg/kg of fludioxonil.

Translocation of radioactivity from treated seeds to aerial plant parts and/or uptake of radioactivity by roots were minimal. The maximum total radioactive residue of 0.004 mg/kg was in whole plants sampled at 25% maturity and the TRR 38 and 76 days after treatment was 0.004 mg/kg and <0.002 mg/kg respectively. At harvest the mature stalks, hulls, and grain contained total residues of <0.002 mg/kg, 0.002 mg/kg and <0.002 mg/kg respectively.

Both extracted and unextracted radioactivity was too low in all the plant samples to pursue characterisation and/or identification.

Table 31. Residues found in greenhouse-grown rice plants after seed treatment with [pyrrole-4-^{14}C]fludioxonil (Fleishmann, Report ABR-90099, 1991).

Sampling	Interval (days)	TRR in ^{14}C-fludioxonil equivalents (mg/kg)				
		Treated seed	Whole plant	Stalk	Hulls	Grain
Treated seeds	0	65	-	-	-	-
25% maturity	38	-	0.004	-	-	-
50% maturity	76	-	<0.002	-	-	-
100% maturity	152	-	-	<0.002	0.002	<0.002

Wheat. The metabolism of [pyrrole-4-^{14}C]fludioxonil was studied in greenhouse- and field-grown spring wheat plants by Gentile (Report 15/91, 1991, addendum 11/28/1996). In the greenhouse seeds were individually syringed with a mixture of blank formulation (A-8207) and [pyrrole-4-^{14}C]fludioxonil, corresponding to approximately 13 g ai/ha and covered with soil in beakers. Plants were sampled 11, 18, 25, 32, 39, 46, and 53 days after application and separated into leaves and roots.

For the field experiment seeds were treated at a rate of 7.4 g ai/100 kg seed with ^{14}C-fludioxonil as an FS 037 formulation containing A-8207, corresponding to approximately 15 g ai/ha. Plants were sampled 48 (stalks), 83 (ears and stalks) and 106 days (straw, husks and grain) after treatment, corresponding to ear emergence (Interval 1), milky stage (Interval 2) and maturity (Interval 3).

Plant fractions were homogenized with liquid nitrogen, extracted with acetonitrile/water (8:2) and analysed by TLC. The extracts were cleaned up by XAD-4 resin and analysed by HPLC. Enzyme cleavage was used to determine sugar conjugates. Mass spectrometry was attempted on selected fractions. Reference metabolites isolated from grapes treated with ^{14}C-fludioxonil were also used to aid in the identification process.

To obtain higher residues for characterisation in an auxiliary experiment, one-month old spring wheat plants were injected with a DMSO solution of radiolabelled fludioxonil (Gentile, Report 27/92, 1993) and harvested 40 and 69 days later.

In a second short-term stem injection experiment using an acetone solution of ^{14}C-fludioxonil for the injections immature plants were after harvested three weeks and the work-up of green plant samples was as described above.

The distribution of the TRR in the greenhouse-grown wheat plants is shown in Table 32. Translocation of radioactivity from treated seeds to aerial plant parts and/or uptake of radioactivity by roots was low ranging from 0.9% and 22.6% of the applied radioactivity 11 days after treatment to 3.1% and 13.0% after 53 days in the leaves and roots respectively.

Table 32. Distribution of fludioxonil in wheat after treatment of the seeds with [pyrrole-4-^{14}C]fludioxonil (greenhouse experiment) (Gentile, Report 15/91, 1991).

Interval	Sample	Total residues		Parent (mg/kg)[1]	Extracted radioactivity (%) [3]	Unextracted (%)[3]	Total (%)[3]
		mg/kg [1] ± SD (%)	(%) [2]				
11 days	Leaves	0.32 ± 55.	0.9	0.005	96	3.6	100
	Roots	8.6 ± 30	23	2.8	86	14	100
	Plant total		24				
	Soil total	0.015 ± 3.8	78	0.013	97	3.3	100
	Total		102				
18 days	Leaves	0.36 ± 10.	3.9	0.015	86	14	100
	Roots	5.5 ± 42.	20	1.4	79	21	100
	Plant total		24				
	Soil total	0.015 ± 3.9	75	0.012	94	6.2	100
	Total		99				
25 days	Leaves	0.16 ± 28.	3.1	<0.001	86	14	100
	Roots	2.9 ± 11.	14	0.81	68	32	100
	Plant total		17				
	Soil total	0.015 ± 3.8	78	0.012	91	8.6	100
	Total		95				
32 days	Leaves	0.14 ± 12.	3.8	0.004	76	24	100
	Roots	3.3± 7.5	18	0.89	58	42	100
	Plant total		22				
	Soil total	0.015 ± 0.0	77	0.012	89	11	100
	Total		99				
39 days	Leaves	0.081 ± 15.	3.1	<0.001	88	12	100
	Roots	2.2 ± 8.2	13	0.46	48	52	100
	Plant total		16				
	Soil total	0.016 ± 3.7	80	0.012	89	11	100
	Total		96				
46 days	Leaves	0.067 ± 3.9	3.3	<0.001	87	13	100
	Roots	1.3 ± 20.	10	0.15	38	62	100
	Plant total		13.5				
	Soil total	0.015 ± 10	75	0.010	85	15	100

Interval	Sample	Total residues		Parent (mg/kg)[1]	Extracted radioactivity (%) [3]	Unextracted (%)[3]	Total (%)[3]
		mg/kg [1] ± SD (%)	(%) [2]				
	Total		89				
53 days	Leaves	0.056 ± 30.	3.1	<0.001	78	22	100
	Roots	1.9 ± 22.	13	0.20	32	68	100
	Plant total		16				
	Soil total	0.016 ± 6.2	83	0.010	83	17	100
	Total		99				

[1] Fludioxonil equivalents.
[2] % of radioactivity applied.
[3] % of total radioactivity found in sample, determined by sum of extracted and unextracted radioactivity.
n.a.: not analysed

Results from the field experiment are summarized in Table 33. Translocation of radioactivity from treated seeds to aerial plant parts (stalks, husks, and grain) was low. Radioactive residues at harvest were 0.015 mg/kg, 0.005 mg/kg and 0.003 mg/kg in stalks, husks, and grain respectively.

Table 33: Distribution of radioactivity and residual fludioxonil in spring wheat after seed treatment with [pyrrole-4-^{14}C]fludioxonil (long-term experiment) (Gentile, Report 15/91, 1991).

Interval	Sample	Total residues		Parent (mg/kg)[1]	Extracted radioactivity (%)		Unextracted (%)[3]	Total (%)[3]
		mg/kg [1]	(%) [2]		Cold [3]	Soxhlet [3]		
48 days	Stalks	0.005	100	n.a.	80	n.a.	36	120
Ear emergence	Soil							
	0-5 cm	0.035	91	0.017	70	7.4	29	106
Interval 1	5-10	0.002	4.5	n.a.	n.a.	n.a.	n.a.	
	10-20	<0.001	3.3	n.a.	n.a.	n.a.	n.a.	
	20-30	<0.001	1.3	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.007	100					
83 days	Stalks	0.004	100	n.a.	n.a.	n.a.	n.a.	
Milk stage	Ears	0.002	100	n.a.	n.a.	n.a.	n.a.	
Interval 2	Soil							
	0-5 cm	0.013	91	0.004	53	6.2	53	112
	5-10	<0.001	5.2	n.a.	n.a.	n.a.	n.a.	
	10-20	<0.001	3.2	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	<0.001	0.9	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.003	100					
106 days	Stalks	0.015	100	n.a.	41	14.	63	120
Maturity	Husks	0.005	100	n.a.	n.a.	n.a.	n.a.	
Interval 3	Grain	0.003	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.048	90	0.017	53	6.1	43	102
	05-10	0.003	5.5	n.a.	n.a.	n.a.	n.a.	

Interval	Sample	Total residues		Parent (mg/kg)[1]	Extracted radioactivity (%)		Unextracted (%)[3]	Total (%)[3]
		mg/kg [1]	(%) [2]		Cold [3]	Soxhlet [3]		
	10-20	<0.001	2.7	n.a.	n.a.	n.a.	n.a.	
	20-30	<0.001	2.2	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.008	100					

[1] fludioxonil equivalents.
[2] % of radioactivity in sample; % of radioactivity in soil layers.
[3] % of radioactivity in sample, determined by combustion.
n.a. not analysed

In the injection experiment 78% of the radioactivity remained at the injection point. At harvest, mature stalks, husks and grain contained 20%, 0.9% and 0.2% respectively of the applied radioactivity (Table 35).

Table 34. Distribution of radioactivity in greenhouse-grown spring wheat after stem injection with [pyrrole-4-^{14}C]fludioxonil (Gentile, Report 27/92, 1993).

Interval	Sample	Total Residues		Parent (mg/kg)	Extracted radioactivity (%)		Unextracted (%)[2]	Total (%)[3]
		mg/kg [1]	(%) [2]		Cold [2]	Soxhlet [2]		
Harvest (69 days after injection)	Grain	0.46	0.2	0.19	79	0.7	20	102
	Husks	8.8	0.9	4.2	89	0.7	10	101
	Stalks	75.	20.	41.	84	1.5	15	99
	Injection point	-	78					
	Plant total	-	100					

[1] Fludioxonil equivalents.
[2] % of total extracted + unextracted activity.
[3] % of total radioactivity determined by combustion.

The quantification of the metabolite fractions from the stem injection experiment is shown in Table 35. Fifteen zones were quantified. Fludioxonil (II_4) represented the largest fraction at 36%, 49% and 49% of radioactivity in the grain, husks and stalks, and unresolved activity 8.7%, 13% and 13% of the TRR respectively. Cellulase treatment released only 1% of sugar conjugates.

Metabolites SYN 518579 (II_2), SYN 578580 (II_3), CGA 308103 (I_{13}), CGA 192155 (I_{10}), CGA 339833 (I_3) and CGA 344623 (I_4) were identified in the grain, husks and straw, but none above 2.6% of the TRR. The short-term stem injection experiment yielded an organosoluble fraction in green parts that co-chromatographed on 2-D TLC with CGA 265378 (II_{3b}).

Table 35. Quantification of metabolite fractions in mature spring wheat after stem injection with [pyrrole-4-^{14}C]fludioxonil (Gentile, Report 27/92, 1993).

Sample	Metabolite fractions (% of TRR) [1]																Soxhlet (%) [1]	Un-extracted (%) [1]	Total (%) [1]
	I_1	I_2	I_3	I_4 + I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}	I_{13} II_1	I_{14} II_2	II_3	II_4 *	Un-resolved			
Grain (0.37 mg/kg)	8.2	14	0.7	2.6	n.a.	0.3	n.a.	n.a.	1.0	n.a.	1.0	1.1	0.8	0.3	36	8.7	0.7	20	95

Sample	Metabolite fractions (% of TRR) [1]																Soxhlet (%) [1]	Un-extracted (%) [1]	Total (%) [1]
	I_1	I_2	I_3	I_4 + I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}	I_{13} II_1	I_{14} II_2	II_3	II_4 *	Un-resolved			
Husks (7.8 mg/kg)	7.7	6.2	2.1	1.3	0.9	0.2	n.a.	0.5	1.7	0.2	0.2	ca 2.5	ca 1.5	0.8	49	12.7	0.7	10.0	98
Straw (63 mg/kg)	3.1	2.6	2.5	1.1	0.3	0.3	0.1	0.2	1.2	0.1	0.6	1.7	1.6	0.6	49.	13.	1.5	14.7	95

* II_4 : parent fludioxonil
[1] % of total residues found in sample by combustion. Quantified by 2-D TLC two TLC systems.

Metabolite I_3: CGA 339833
Metabolite I_4: CGA 344623
Metabolite I_{10}: CGA 192155.
Metabolite I_{13}: CGA 308103.
Metabolite II_2: SYN 518579
Metabolite II_3: SYN 518580

Cotton. Plant-uptake, distribution and metabolism of [pyrrole-4-^{14}C]fludioxonil were studied in greenhouse-grown cotton plants (Close, 1998). Cotton seeds (Delta & Pineland 5415) were treated with an FS formulation at 2.5 g or 5.0 g fludioxonil per 100 kg seeds and grown in pots in a sandy loam soil.

Samples at maturity (186 days after planting) consisting of cotton seed, lint, and gin trash (leaves and stalks, forage, allowed to dry) were combusted and the TRR determined by LSC.

Samples with sufficient radioactivity were extracted and partitioned. The residues in the cotton seed were extracted with hexane and then, together with those in seed and gin trash, with acetonitrile/water (80:20). After evaporation of the acetonitrile, the aqueous fraction was partitioned with methylene chloride. Radioactivity was measured by combustion and LSC. This characterised radioactive residues as organosoluble, water-soluble, and unextracted.

The distribution of theTRR in the cotton plants is shown in Table 36. Translocation of radioactivity from treated seeds to aerial plant parts and/or uptake of radioactivity by roots were very low for both treatment rates. For the 2.5 g ai/100 kg seed treatment, mature cotton seed, lint, and gin trash contained radioactive residues equivalent to 0.003 mg/kg, for the 5 g ai/100 kg mature seed (undelinted) and lint 0.012 mg/kg, and for gin trash 0.011 mg/kg.

At 2.5 g ai/100 kg seed residues were too low to be characterised. Extracted radioactive residues in seed and gin trash from the 5 g ai/100 kg seed treatment were <0.005 mg/kg. In cotton seed, extracted residues constituted approximately 33% of the TRR, with organosoluble radioactivity representing 24% (0.003 mg/kg), and in gin trash approximately 19% of the TRR, with organosoluble 4.2% (<0.001 mg/kg). In cotton seed unextracted radioactivity constituted approximately 74% of the TRR, and in gin trash approximately 83%.

Table 36. Distribution of radioactivity in greenhouse-grown cotton plants after treatment of the seed with [pyrrole-4-^{14}C]fludioxonil at 2.5 g/100 kg and 5.0 g/100 kg seed (Close, 1998).

Treatment (g ai/100 kg seed)	Sample	Residue (mg/kg)	Fraction	% of TRR	Conc. (mg/kg)
2.5	Mature cotton seed	0.003			
	Lint	0.003			
	Cotton forage (gin trash)	0.003			

Treatment (g ai/100 kg seed)	Sample	Residue (mg/kg)	Fraction	% of TRR	Conc. (mg/kg)
5.0	Mature cotton seed	0.012		100	0.012
			Hexane	24	0.003
			Acetonitrile/water (80:20) [2]	88	0.011
			Dichloromethane	0.0	<0.001
			Aqueous	8.7	0.001
			Unextracted	74	0.009
			Total recovery [1]	106	0.013
	Lint	0.012			
	Cotton forage (gin trash)	0.011		100	0.011
			Acetonitrile/water (80:20) [2]	54	0.006
			Dichloromethane	4.2	<0.001
			Aqueous	15	0.002
			Unextracted	83	0.009
			Total recovery [3]	103	0.011

[1] calculated by adding hexane fraction, aqueous fraction, dichloromethane fraction, and unextracted residues.
[2] procedural problems led to over-estimation of results.
[3] calculated by adding aqueous fraction, dichloromethane fraction, and unextracted residues.

Soya beans. In a study by Close of the plant-uptake, distribution and metabolism of [pyrrole-4-^{14}C]fludioxonil in greenhouse-grown plants (Report ABR-97033, 1998b) seeds (Novartis Seeds 3474) were treated with a 4FS formulation at 5.0 g ai/100 kg seed.

Plants were sampled at the sixth node stage (above ground portion, soya bean forage, 28 days after planting), at mid full-bloom (above ground portion, soya bean hay, 38 days after planting) and at maturity (stalks, dry beans and dry hulls 133 days after planting).

Homogenized samples were combusted and the TRR determined by LSC, then extracted with acetonitrile/water (80:20), and the extract evaporated to leave an aqueous fraction which was partitioned with dichloromethane. Both the aqueous and organic layers were assayed by LSC. The water-soluble radioactivity was passed through a C-18 SPE cartridge. Non-retained fractions were collected and retained residues eluted with acetonitrile and methanol. All the cartridge fractions were analysed by LSC. The acetonitrile and methanol fractions were combined and analysed by HPLC and TLC. Selected samples of water-soluble fractions were incubated with β-glucosidase, and of unextracted residues treated with cellulase.

The uptake and distribution of the radioactivity into the soya bean plants is summarized in Table 37. Early forage taken 28 days after planting contained the highest radioactive residues at 0.096 mg/kg, the hay 38 days after planting contained a TRR of 0.041 mg/kg, and at harvest (133 days) stalks, beans, and hulls contained residues of 0.005 mg/kg, 0.015 mg/kg, and 0.012 mg/kg respectively.

Table 37. Distribution of radioactivity in greenhouse-grown soya bean plants after treatment of the seed with [pyrrole-4-^{14}C]fludioxonil at 5.0 g/100 kg seed (Close, Report ABR-97033, 1998b).

Sample	Residue (mg/kg)	Fraction	% of TRR	mg/kg
Soya bean forage 6th node	0.096		100	0.096
28 days		Acetonitrile/water (80:20)	1201	0.12
		Organosoluble	9.5	0.009
		Water-soluble	73	0.070
		Non-retained	3.5	0.003
		Acetonitrile	41	0.040
		Methanol	15	0.015
		Unextracted	22	0.021
		Recovery 2	104	0.10
Soya bean forage 6th node	0.096		100	0.096
38 days		Acetonitrile/water (80:20)	85	0.082
		Organosoluble	7.7	0.007
		Water-soluble	66	0.063
		β-glucosidase, dichloromethane	3.2	0.003
		β-glucosidase, aqueous	67	0.065
		Unextracted	19	0.018
		Recovery 2	92	0.088
Soya bean hay 50% flowering	0.041		100	0.041
		Acetonitrile/water (80:20)	2801	0.12
		Organosoluble	2.1	0.001
		Water-soluble	54	0.022
		Non-retained	6.1	0.002
		Acetonitrile	40.	0.016
		Methanol	2.9	0.001
		Unextracted	32	0.013
		Recovery 2	88	0.036
Soya bean stalks	0.005			
Dry beans, harvest	0.015		100	0.015
133 days		Acetonitrile/water (80:20)	2.0	0.000
		Organosoluble	4.4	0.001
		Water-soluble	10	0.002
		Non-retained	8.7	0.001
		Acetonitrile	4.2	0.001
		Methanol	1.1	0.000
		Unextracted	93	0.014
		Cellulase extracted		
		Organosoluble	0.0	0.000
		Water-soluble	16	0.002
		Recovery[2]	107	0.017
Dry hulls	0.012			

[1] high owing to poor replication/counting.
[2] calculated by adding aqueous and organic fractions, and unextracted residues.

The organosoluble residue from forage, hay, and dry beans was analysed using HPLC. The major component in forage matched the retention time of CGA 227731 (1.9% of the TRR and 0.002 mg/kg). Components matching the retention times of CGA 260766, CGA 340351, and CGA 192155 were also present each at levels of <1.4% of the TRR and ≤ 0.001 mg/kg. CGA 227731 was tentatively identified as the major component in soya bean hay (1.5% of the TRR and <0.001 mg/kg) with a small amount of CGA 260766 (0.5% of the TRR and <0.001 mg/kg). One peak was present in dry beans (1.5% of the TRR and <0.001 mg/kg) that did not match any standard. Residues were not confirmed using a second chromatographic system owing to the low level of radioactivity in these fractions (<10% of the TRR, <0.01 mg/kg).

The water-soluble radioactive fraction from forage, hay, and dry beans was passed through C-18 cartridges. The retained fractions after elution with organic solvent contained 57% (0.055 mg/kg) of the forage TRR, 43% (0.017 mg/kg) of the hay and 5% (c. 0.001 mg/kg) of the dry bean, and the corresponding unretained fractions were <10% of the TRR or 0.003 mg/kg. HPLC indicated the presence of CGA 260766, CGA 340351, CGA 227731, CGA 308103, CGA 192155 and CGA 257777 in some of the C18-fractions but confirmatory chromatography was inconclusive. HPLC fractionation showed that no component could be >0.01 mg/kg. Treatment of the forage water-soluble residue with β-glucosidase released only 3.2% of the TRR. The radioactivity was too low for further analysis.

Unextracted radioactivity represented 19%-22%, 32%, and 93% of the TRR in forage, hay, and dry beans respectively, and the residue from dry beans was treated with cellulase which released 18% of the TRR. None of the released radioactivity was organosoluble and only 16% was water-soluble.

Metabolic pathways consistent with the foliar and seed treatment metabolism studies are shown in Figure 2.

Figure 2: Proposed metabolic pathways of fludioxonil in or on plants (foliar, seed treatment).

Environmental fate in soil

Numerous studies were reported on degradation, dissipation under field conditions, adsorption/desorption, mobility, resdiues in succeeding crops, and fate in water. However on the basis of decisions made by the 2003 JMPR (Report 2003, General Items) and confirmed by the 2004 CCPR only aerobic soil degradation and dissipation (seed treatment) and rotational crops are of concern for fludioxonil. Some information on the photolysis of fludioxonil on soil was also considered.

Aerobic degradation

Laboratory studies on fludioxonil under dark conditions in a range of soils showed slow breakdown. Mineralisation to CO_2 was the main breakdown route (4-45%), together with formation of bound residues (8–27%). Mineralisation of [phenyl-U-^{14}C]fludioxonil (Minet, Report 4/93, 1994; Reishmann, Report 7/95, 1994) occurred more rapidly than that of the [pyrrole-4-^{14}C]-labelled compound at 20°C (Kirkpatrick, Report HRC/CBG485/90818, 1991; Abildt, Report 1/91, 1991; Ellgehausen, Report 1/92, 1992; Ellgehausen, Report 91EH08, 1992; Minet, Report 92MU01-1, 1994; Minet, Report 15/93, 1994). Extractable radioactivity was <10% of that applied; individual degradation products could not be isolated or characterised owing to the low amounts formed.

The extent of mineralisation and formation of bound residues under aerobic conditions depended on the initial concentration of fludioxonil, label position, soil moisture content and temperature, and initial microbial activity of the soil. In laboratory studies carried out under sterile aerobic conditions degradation of both phenyl and pyrrole-labelled fludioxonil was negligible (Kirkpatrick, Report HRC/CBG485/90818, 1991; Minet, Report 4/93, 1994; Adam, Report 97DA01, 1998).

In contrast degradation of fludioxonil on soil exposed to light was rapid. [Pyrrole-4-^{14}C]fludioxonil at 5 kg ai/ha equivalent on 1 mm layers of sandy loam soil was exposed to a xenon arc light source for up to 44 days (natural sunlight at 30°N equivalent). There was extensive degradation of the ai over this period and a biphasic decline curve (Kirkpatrick, Report CBG569A, CBG 569B, 1994).

On the basis of the best fit to a first-order model, fludioxonil in the irradiated compartment (i.e. at or near the soil surface) initially represented about 40% of the applied radioactivity (AR) and degraded with a half life of <1 day. Fludioxonil in the unirradiated compartment (partially or completely shielded from irradiation) initially represented about 50-60% of the AR with a half-life of 50 days.

In a similar study with [phenyl-U-^{14}C]fludioxonil, photoproducts extracted from the soil were co-chromatographed with those extracted after irradiation of the [pyrrole-4-^{14}C]fludioxonil-treated soil (Kirkpatrick, Report CBG 49064, 1994; Report HRC/CBG516/901362, 1994). Many of the degradates contained both radiolabels, the most significant ones formed from breakdown of the pyrrole ring. Three metabolites were identified: CGA 265378 with a maximum of about 1% of the AR, CGA 339833 9% and CGA 192155 10%. No other photoproduct represented >2% of the AR.

Cumulative evolution of volatiles (mainly CO_2) over the equivalent to about 44 days of natural sunlight accounted for 8% and 9% of the AR for the phenyl and pyrrole-labelled fludioxonil respectively

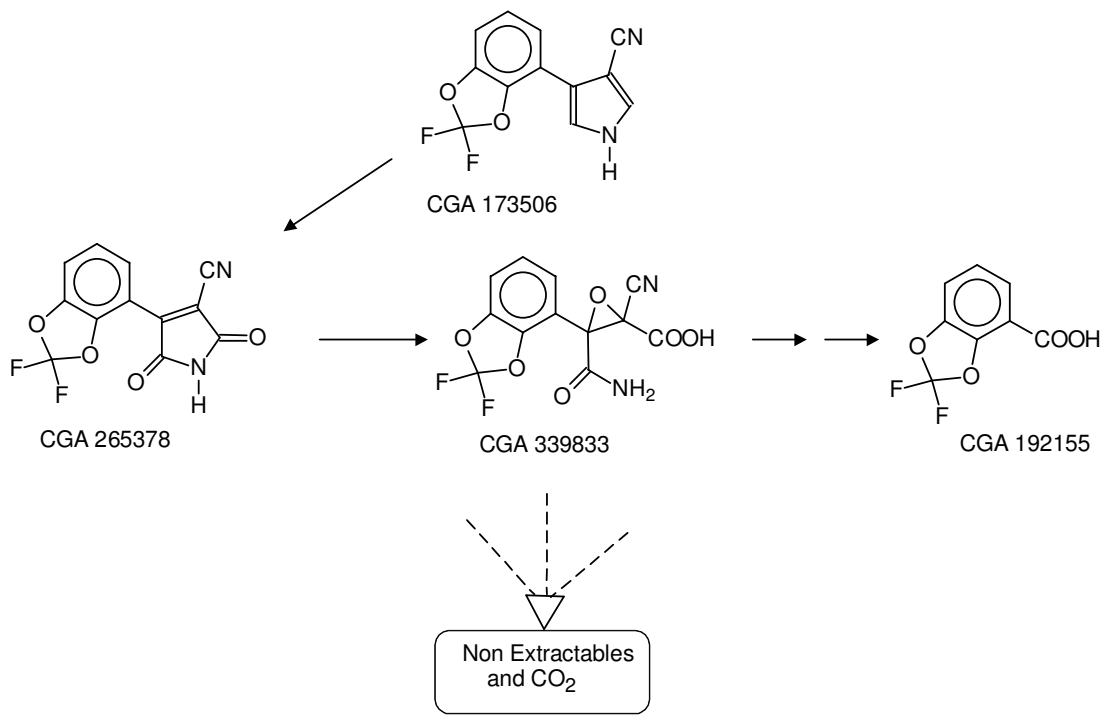
The importance of photodegradation of fludioxonil on the soil surface in field dissipation was demonstrated using [pyrrole-4-^{14}C]fludioxonil (Gentile, Report 89BG02PR3, 1993). A sandy loam plot was sprayed with the labelled compound as a WP 50 formulation at 550 g ai/ha, and divided into two areas, one of which was covered with 2 cm soil immediately after application (covered area) and the other was left uncovered. Over a period of 62 days both areas were protected with a tarpaulin at night and during rain but otherwise were unprotected. Over the initial 62 days the fludioxonil concentration in the 0-10 cm layer decreased from 0.43 mg/kg to 0.40 mg/kg in the covered and from 0.43 mg/kg to 0.34 mg/kg in the exposed area. Unextractable radioactivity increased to about 12% and 30% of the total soil residue in the covered and exposed areas respectively, indicating that the

formation of bound residues is the result of degradation and not of irreversible adsorption of the ai. The two main products in soil extracts from the light-exposed area were identified as CGA 192155 and CGA 339833 and amounted to 13% and 8% of the total residue in the light-exposed soil but only about 2% each in the covered area.

The biphasic decline curves of fludioxonil in the exposed and covered areas were analysed by a two-compartment model. In the former fludioxonil dissipated rapidly with half-lives of <0.1 days in the upper exposed layer and 60 days in the lower (40% and 60% of the applied radioactivity respectively), and in the covered soil 0.4 days in the upper and 255 days in the lower layer (19% and 81% of the AR respectively). Combined rates of both layers gave half-lives of 16 and 187 days for the light-exposed and covered plots respectively.

The proposed pathways of the degradation of fludioxonil in light-exposed soil are shown in Figure 3.

Figure 3: Proposed pathways for the degradation of fludioxonil in light-exposed soil.



Wheat. In two field studies in England *(*Walser, Report 211/98, 1999; Report 212/98, 1999*)* fludioxonil-treated winter wheat seeds were drilled into a clay and a sandy loam soil over a period of 3 years at 12.5 g ai/ha. No residues ≥0.02 mg/kg (LOQ) were found in the soil. In a third field study at two sites in Canada *(*Purdy, Report CER04110/97, 1998*)* maize seed was treated at 2.2 g ai/100 kg seed (corresponding to 0.59 g ai/ha). Dissipation in the soil was monitored using analytical techniques with an LOD of 0.001 mg/kg for fludioxonil, CGA 265378 and CGA 192155. Only fludioxonil was detected and only in the 0-10 cm soil layer. Residues were variable for the first 5-12 days and then steadily decreased from 0.011-0.013 mg/kg to about 0.003 mg/kg between days 79 and 121. By inclusion of a calculated initial (uniform) concentration of 0.028 and 0.024 mg/kg at the two sites, half-lives of 26 and 54 days were calculated assuming first order kinetics.

Rotational crops. Four studies were reported to the Meeting.

In an outdoor confined accumulation study (Gentile, Report 89BG03PR1, 1992) [pyrrole-4-^{14}C]fludioxonil was sprayed onto bare ground in a WP50 formulation in Switzerland at a rate of 750 g ai/ha. The uptake, distribution, and degradation in the following rotational crops were investigated:

Transplanted 90 days after application:	Lettuce (Soraya)
Sown 140 days after application:	Winter wheat (Zenta)
Sown 320 days after application:	Sugar beets (KWS)
Sown 345 days after application:	Maize (Blizzard)

Winter wheat, sugar beets, and corn were sown directly onto the plot and lettuce seedlings were transplanted after the specified ageing periods. Plant samples were collected at selected intervals and stored frozen. Soil cores (0-5 cm, 5-10 cm, 10-20 cm, and 20-30 cm layers) were taken at each planting and plant sampling.

Fresh and dry plant samples were homogenized and the TRR determined by combustion and LSC. The results are shown in Tables 38-41. Characterisation or identification of residues was not attempted owing to the very low residues observed in all four crops.

Table 38. Distribution of radioactivity and residual fludioxonil in rotation lettuce after a bare ground application of [pyrrole-4-^{14}C]fludioxonil at 750 g ai/ha (Gentile, Report 89BG03PR1, 1992).

Days after treatment/ growth stages	Sample	Total residues		Parent	Extracted radioactivity		Unextracted	Total
					Cold	Soxhlet		
		mg/kg [1]	% of TRR [2]	mg/kg	% of TRR [3]	% of TRR [3]	% of TRR [3]	% of TRR [3]
93 days	Soil							
	0-5 cm	0.43	84	0.12	49	3.6	48	101
	5-10 cm	0.052	9.2	0.007	46	3.1	52	100
	10-20 cm	0.010	4.7	n.a.	52	2.6	44	99
	20-30 cm	0.003	2.1	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.069	100					
120 days/ 50% maturity	Lettuce heads	0.006	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.154	70	0.006	38	4.8	58	101
	5-10 cm	0.036	16	0.003	32	3.5	62	97
	10-20 cm	0.011	12	<0.001	37	2.4	59	98
	20-30 cm	0.002	1.7	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.033	100					
152 days/ maturity	Lettuce heads	0.006	100	n.a.	n.a.	n.a.		
	Soil							
	0-5 cm	0.234	59	0.051	38	3.8	58	100
	5-10 cm	0.080	26	0.015	36	2.9	59	98
	10-20 cm	0.013	11	0.001	51.	1.9	46.	99
	20-30 cm	0.005	3.9	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.044	100					

n.a. : not analysed
LOD for combustion: 0.001 mg/kg; for the TLC of plant and soil material: 0.001 mg/kg
[1] fludioxonil equivalents
[2] % of summed radioactivity in soil layers
[3] % of radioactivity in sample determined by combustion

Table 39. Distribution of radioactivity and residual fludioxonil in rotational winter wheat after a bare ground application of [pyrrole-4-14C]fludioxonil at 750 g ai/ha (Gentile, Report 89BG03PR1, 1992).

Days after treatment/ growth stages	Sample	Total residues		Parent	Extracted radioactivity		Unextracted	Total
		mg/kg [1]	% of TRR [2]	mg/kg	Cold	Soxhlet	% of TRR [3]	% of TRR [3]
					% of TRR [3]	% of TRR [3]		
152 days before sowing of winter wheat	Soil							
	0-5 cm	0.23	59.	0.051	38	3.8	58	100
	5-10 cm	0.08	25.	0.015	36	2.9	59	98
	10-20 cm	0.013	11.	0.001	51	1.9	46	99
	20-30 cm	0.005	3.9	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.044	100					
196 days/ fall cutting	Whole tops	0.005	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.275	49	0.071	34	7.3	61	102
	5-10 cm	0.116	21	0.023	30	6.5	66	103
	10-20 cm	0.045	22	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.011	7.8	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.065	100					
321 days/ 25% maturity	Whole tops	0.002	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.19	58	0.038	31	4.2	61	96
	5-10 cm	0.055	17.1	0.008	26	4.1	68	98
	10-20 cm	0.030	16	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.009	8.7	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.047	100					
377 days/ 50% maturity	Whole tops	0.002	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.13	55	0.022	26	4.4	67	97
	5-10 cm	0.060	20	0.007	27	4.2	76	107
	10-20 cm	0.037	21	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.004	3.8	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.044	100.0					
429 days/ maturity	Stems	0.008	100	n.a.	n.a.	n.a.	n.a.	
	Husks	0.004	100	n.a.	n.a.	n.a.	n.a.	
	Grains	0.002	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.290	70	0.066	33	3.2	53	89
	5-10 cm	0.077	18	0.012	27	11.	58	97
	10-20 cm	0.021	9.2	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.005	2.9	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm		100					

n.a. - not analysed
LOD: for combustion: 0.001 mg/kg, for TLC of plant and soil material: 0.001 mg/kg
[1] fludioxonil equivalents
[2] % of summed radioactivity in soil layers
[3] % of radioactivity in sample determined by combustion

Table 40. Distribution of radioactivity and residual fludioxonil in rotational sugar beets after a bare ground application of [pyrrole-4-^{14}C]fludioxonil at 750 g ai/ha (Gentile, Report 89BG03PR1, 1992).

Days after treatment/ growth stages	Sample	Total residues		Parent	Extracted radioactivity		Unextracted	Total
		mg/kg [1]	% of TRR [2]	mg/kg	Cold	Soxhlet	% of TRR [3]	% of TRR [3]
					% of TRR [3]	% of TRR [3]		
321 days/ before sowing of sugar beets	Soil							
	0-5 cm	0.37	49	0.10	38	9.8	51	98
	5-10 cm	0.33	30	0.10	52	13.	36.	101
	10-20 cm	0.096	20	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.006	1.9	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.14	100					
377 days/ 25% maturity	Tops	0.002	100	n.a.	n.a.	n.a.	n.a.	
	Roots	0.002	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.190	71	0.036	30	9.7	57.	96

Days after treatment/ growth stages	Sample	Total residues		Parent	Extracted radioactivity		Unextracted	Total
					Cold	Soxhlet		
		mg/kg [1]	% of TRR [2]	mg/kg	% of TRR [3]	% of TRR [3]	% of TRR [3]	% of TRR [3]
	5-10 cm	0.058	16	0.007	24	15.	65.	104
	10-20 cm	0.018	11	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.003	2.2	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.051	100					
419 days/ 50% maturity	Tops	<0.001	100	n.a.	n.a.	n.a.	n.a.	
	Roots	0.002	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.201	48	0.038	27.9	6.2	62	96
	5-10 cm	0.122	27	0.022	27.8	17	62	107
	10-20 cm	0.030	19	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.009	6.3	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.056	100					
519 days/ maturity	Tops	<0.001	100	n.a.	n.a.	n.a.	n.a.	
	Roots	0.001	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-05 cm	0.147	44	0.019	25	6.4	66	97
	5-10 cm	0.135	29	0.019	24	5.4	68	97
	10-20 cm	0.048	24	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.004	3.0	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.058	100					

n.a. : not analysed. LOD for combustion: 0.001 mg/kg; for TLC of plant and soil material: 0.001 mg/kg
[1] fludioxonil equivalents
[2] % of summed radioactivity in soil layers
[3] % of radioactivity in sample determined by combustion

Table 41. Distribution of radioactivity and residual fludioxonil in rotational maize after a bare ground application of [pyrrole-4-^{14}C]fludioxonil at 750 g ai/ha (Gentile, Report 89BG03PR1, 1992).

Days after treatment/ growth stages	Sample	Total residues		Parent	Extracted radioactivity		Unextracted	Total
					Cold	Soxhlet		
		mg/kg [1]	% of TRR [2]	mg/kg	% of TRR [3]	% of TRR [3]	% of TRR [3]	% of TRR [3]
352 days before sowing of corn	Soil							
	0-5 cm	0.18	66	0.034	31	4.4	61	96
	5-10 cm	0.053	14	0.008	26	4.7	63	94
	10-20 cm	0.028	18	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.003	2.2	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.047	100					
377 days 25% maturity	Whole tops	0.003	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.330	56	0.070	34	8.4	57	99
	5-10 cm	0.18	23	0.035	31	5.4	63	100
	10-20 cm	0.051	16	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.011	5.2	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.093	100					
419 days 50% maturity	Whole tops	<0.001	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.19	50	0.030	27	5.8	66	99
	5-10 cm	0.10	24	0.011	22	2.9	69	93
	10-20 cm	0.033	19	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.011	8.3	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.055	100					
519 days maturity	Stalks	0.005	100	n.a.	n.a.	n.a.	n.a.	
	Cobs	<0.001	100	n.a.	n.a.	n.a.	n.a.	
	Grains	<0.001	100	n.a.	n.a.	n.a.	n.a.	
	Soil							
	0-5 cm	0.15	44	0.019	25	6.4	66	97
	5-10 cm	0.14	29	0.019	24	5.4	68	97
	10-20 cm	0.048	24	n.a.	n.a.	n.a.	n.a.	
	20-30 cm	0.004	3.0	n.a.	n.a.	n.a.	n.a.	
	Soil 0-30 cm	0.058	100					

n.a. - not analysed
LOD for combustion: 0.001 mg/kg
[1] fludioxonil equivalents
[2] % of summed radioactivity in soil layers
[3] % of radioactivity in sample determined by combustion

[Pyrrole-4-^{14}C]fludioxonil in methanol was sprayed onto bare ground in California, USA at a rate of 50 g ai/acre (124 g ai/ha). Uptake, distribution, and degradation were investigated in the following rotational crops (Thalacker, Report CHW 6117-329, 1996):

Sown 33 days and 90 days after application:	Spring wheat (Aldura), mustard (Florida Broadleaf), and turnips (Purple Top White Globe)

Spring wheat, mustard, and turnips were sown directly into the plot after the specified ageing periods. (The trial initially set up was discontinued owing to high background values of fludioxonil found in pre-treatment soil cores). Plant samples were collected at selected intervals as were soil cores but the latter will not be discussed further.

Plant samples were homogenized with solid CO_2 to keep them frozen. Subsamples were combusted to determine the TRR, and residues in each rotational crop sample were extracted with methanol/water (80:20) fractionated by evaporation of the methanol and partitioning of the resulting aqueous solution with dichloromethane. Additional fractionation of the water-soluble and the unextracted radioactivity was by enzymatic, acidic or basic procedures. Selected extract fractions were profiled using HPLC and/or 2D-TLC. Unextracted radioactivity was determined by combustion and LSC. The results are shown in Table 42.

Table 42. Distribution of radioactivity and residual fludioxonil in rotation crops after a bare ground application of [pyrrole-4-^{14}C]fludioxonil at 124 g ai/ha (Thalacker, Report CHW 6117-329, 1996).

Sample	Description	Harvest interval (days)	TRR mg/kg	Extracted						Unextracted		Recovery[1]	
				MeOH:H_2O (80:20)		Aqueous		Organic					
				% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
33 day plant back													
0-15 cm Soil	Pre-application	0	<0.001										
0-15 cm Soil	Post application	0	0.029										
0-15 cm Soil	Day of planting	33	0.041										
Root crop	Mature turnip tops	129	0.007	63	0.004	63	0.004	18	0.001	30	0.002	110	0.007
Root crop	Mature turnip roots	129	0.002										
Leafy vegetable	Mature mustard greens	129	0.006	95	0.006	70	0.004	32	0.002	29	0.002	130	0.008
Small Grain	25% mature wheat forage	109	0.058	80	0.047	57	0.033	26	0.015	32	0.019	115	0.067
				65	0.038	59	0.034	7.9	0.005	32	0.018	98	0.057
	Mature wheat straw	175	0.12	59	0.071	56	0.067	6.5	0.008	35	0.042	98	0.12
				63	0.075	56	0.067	7.0	0.008	34	0.041	98	0.12
	Mature wheat grain	175	0.006	39	0.002	33	0.002	14	0.001	49	0.003	97	0.006

[1] Sum of aqueous and organic fraction, and unextracted residues.

HPLC indicated that fludioxonil constituted 2.4% of the TRR (0.001 mg/kg) in the organosoluble fraction of 25% maturity wheat forage. Other radioactive peaks corresponded to CGA 265378, CGA 192155, and CGA 308103, but all were <1.5% of the TRR (<0.001 mg/kg). The residues were not verified by a second chromatographic system. At full maturity in harvest wheat straw fludioxonil had decreased to 0.3% of the TRR (<0.001 mg/kg). CGA 308103 (1.1% of the TRR, 0.001 mg/kg) and CGA 192155 (0.9% of the TRR, 0.001 mg/kg) were detected by co-chromatography with non-radiolabelled standards. The residues were not verified using a second chromatographic system.

HPLC analysis of the water-soluble fraction of 25% maturity wheat forage suggested the presence of CGA 227731 (10.7% of the TRR, 0.006 mg/kg), CGA 260766 (4.2% of the TRR, 0.002 mg/kg), and CGA 192155 (2.3% of the TRR, 0.001 mg/kg). Two unidentified polar peaks were 18.3% (0.011 mg/kg), and 12.7% (0.007 mg/kg) of the TRR. Co-chromatography using 2D-TLC confirmed the presence of CGA 227731.

HPLC of the water-soluble fraction from wheat straw harvested at full maturity suggested the presence of CGA 227731 (11.1% of the TRR, 0.013 mg/kg), CGA 260766 (4.8%, 0.006 mg/kg), CGA 340351 (1.2%, 0.001 mg/kg), and CGA 192155 (1.8%, 0.002 mg/kg). Two unidentified polar peaks were 13.1% (0.016 mg/kg), and 11.7% (0.014 mg/kg) of the TRR. Co-chromatography using 2D-TLC did not confirm HPLC identifications.

Enzyme treatment of the aqueous fractions from immature forage and mature straw with β-glucosidase released approximately 3% of the TRR (0.002-0.003 mg/kg). The HPLC profiles were similar to those of the untreated fractions.

Treatment of the wheat forage and straw aqueous fractions with three concentrations of HCl or NaOH released a maximum of 7% of the TRR into the organosoluble fraction. HPLC of the organosoluble fractions indicated the presence of CGA 340351, CGA 308103, CGA 260766, CGA 227731, CGA 265378 and CGA 192155 but these metabolites were not confirmed by a second system. None of these components was above 3.4% of the TRR (0.004 mg/kg). HPLC of the water-soluble radioactivity indicated the presence of CGA 308103, CGA 227731, CGA 192155 and CGA 340351. No component tentatively identified was above 5.1% of the TRR (0.003 mg/kg) except CGA 227731, which appeared to be highest in full maturity wheat straw at 15.9% of the TRR (0.019 mg/kg) after treatment with 1.0 N NaOH. This was not confirmed by a second chromatography system.

Treatment of the unextracted residues from wheat forage with 0.1N NaOH under reflux released 24.5% of the TRR (0.014 mg/kg) and of the full maturity straw 13.8% (0.017 mg/kg). HPLC of the forage extract produced five radioactive peaks that appeared to co-elute with CGA-308103, CGA 227731, CGA 260766, CGA 192155, and CGA 257777. None of these components was above 2.3% of the TRR (0.001 mg/kg) and none were confirmed by a second chromatography method. No defined peak areas were detected when wheat straw was subjected to the same treatment.

Treatment of the unextracted residues with cellulase and base reduced the unextracted radioactivity to 0.35% of the TRR (<0.001 mg/kg) and 2.04% (0.002 mg/kg), and with cellulase and acid to 11.4% of the TRR (0.007 mg/kg) and 22.1% (0.027 mg/kg) in the immature wheat forage and mature wheat straw respectively. The HPLC profiles of the compounds released by cellulase was similar to the aqueous profile. The components released by acid treatment were more polar than the standards by HPLC.

[Pyrrole-4-^{14}C]fludioxonil in methanol was sprayed onto bare ground in California, USA at a rate of 62 g ai/ha. Uptake, distribution, and degradation of ^{14}C-fludioxonil were investigated in the following rotational crops (Close, Report ABR-97005, 1997):

Sown 32 and 90 days after application:	Spring wheat (Yacaro rojo), mustard (Florida Broadleaf), and radishes (Cherry Belle)

Spring wheat, mustard, and radishes were sown directly into the plot after the specified ageing periods.

Plant samples were extracted, fractionated and analysed as above

The results are summarized in Table 43.

Table 43. Distribution of radioactivity and residual fludioxonil in rotation crops after a bare ground application of [pyrrole-4-14C]fludioxonil at 25 g ai/acre (Close, Report ABR-97005, 1997):

Sample	Description	PHI (days)	TRR (mg/kg)	Extracted						Unextracted		Recovery[1]	
				MeOH:H2O (80:20)		Aqueous		Organic					
				% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
32 day plant back													
0-15 cm Soil	Pre-application	0	<0.002										
0-15 cm Soil	Post application	0	0.021										
0-15 cm Soil	Day of planting	32	0.018										
0-15 cm Soil	Final harvest	206	0.010										
Root crop	Mature radish tops	117	0.002										
Root crop	Mature radish roots	117	0.002										
Leafy vegetable	Mature mustard greens	117	<0.002										
Small grain	25% mature wheat forage	117	0.011	58	0.0064	44	0.0048	15	0.0017	40	0.0044	99	0.0109
	50% mature wheat forage	135	0.008										
	Mature wheat straw	204	0.056	54	0.030	47	0.026	3.6	0.002	49	0.027	99	0.055
				60	0.034	52	0.029	2.5	0.001	47	0.026	102	0.056
	Mature wheat grain	204	0.015	34	0.005	29	0.004	8.9	0.001	69	0.010	107	0.015

[1] Sum of aqueous and organic fraction, and unextracted residues.

The radioactivity in the mature mustard and radishes was too low to characterise, as it was below or at the limit of quantification (0.002 mg/kg).

The organic fraction of wheat straw was analysed by HPLC and suggested one major component, CGA 308103 (1.1% of the TRR, <0.001 mg/kg). The residue was not confirmed owing to the low level of radioactivity in the fraction (4.6% of the TRR, 0.003 mg/kg). The organosoluble radioactivity in wheat grain (8.9% of the TRR, 0.001 mg/kg) was too low to analyse by HPLC.

The water-soluble fraction of the straw was also analysed by HPLC (C-18 retained and unretained fractions) and eluted in the vicinity of CGA 227731 (22% of the TRR, 0.012 mg/kg), CGA 260766 (6.5%, 0.004 mg/kg), CGA 192155 (1.4%, <0.001 mg/kg) and CGA 265378 (1.1%, <0.001 mg/kg). The HPLC assignments were not confirmed owing to the low level of radioactivity in the fraction (32% of the TRR, 0.018 mg/kg, C-18 retained fraction and 12% of the TRR, 0.007 mg/kg, C-18 unretained fraction).

The water-soluble fraction of wheat grain was analysed by HPLC (C-18 retained and non-retained fractions). Most of the radioactivity eluted near CGA 308103 (12.2% of the TRR, 0.001 mg/kg), some eluting near CGA 340351 (2.0%, <0.001 mg/kg). The HPLC assignments were not confirmed.

Treatment of the wheat straw aqueous fraction with enzyme released 2.1% of the TRR (0.001 mg/kg).

Treatment of the unextracted residue of wheat straw and grain with enzyme released 16% of the TRR (0.009 mg/kg) and 21% of the TRR (0.003 mg/kg) respectively. In straw 1.0% of the TRR (0.001 mg/kg) was organosoluble and 7.6% (0.004 mg/kg) was water-soluble, and in grain none was organosoluble and 11% (0.002 mg/kg) was water-soluble. The extracted residue was not analysed chromatographically.

Tentative identifications of CGA 265378, CGA 192155, CGA 308103, CGA 227731 and CGA-260766 in the wheat straw extracts and CGA 308103 and CGA 340351 in the grain were made using one chromatographic system. All were <0.01 mg/kg except CGA 227731 found at 0.012 mg/kg in the straw.

[Phenyl-U-^{14}C]fludioxonil in methanol was sprayed onto bare ground in California, USA at an exaggerated rate of 452 g ai/acre (1117 g ai/ha). Uptake, distribution, and degradation of ^{14}C-fludioxonil were investigated in the following rotational crops (Thalacker, Report 117-97, 1999):

Planted 30, 90 and 210 days after application:	Spring wheat (Yacaro rojo), Mustard (Florida Broadleaf), and Radishes (Cherry Belle)

Spring wheat, mustard, and radishes were sown directly into the plot after the specified ageing periods and plant and soil samples collected at intervals.

Plant samples were homogenized using a food grinder or a Wiley Mill with liquid nitrogen or solid CO_2 to keep the samples frozen. Sub-samples were combusted to determine the TRR. The radioactive residues in the crop samples were extracted with acetonitrile (ACN)/water (80:20) and initially cleaned up using C-18 chromatography. The acetonitrile was evaporated and the remaining aqueous solution was partitioned with methyl *tert*-butyl ether. Further fractionation of the water-soluble components was with cellulase, and of the unextracted radioactive residues by enzymatic, acidic and basic procedures. Selected organic and water-soluble fractions were profiled using HPLC and/or two-dimensional 2D-TLC. Unextracted radioactivity was determined by combustion and LSC.

The results are summarized in Tables 44 and 45.

Table 44. Distribution of radioactivity in rotation crops and soil after a bare ground application of [phenyl-U-14C]fludioxonil at 1120 g ai/ha (Thalacker, Report 117-97, 1999):

Plant-back interval, days	Sample	TRR mg/kg	ACN/H_2O extract		Organic (MtBE) fraction		Aqueous fraction		Unextracted		Recovery, % of TRR
			% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	
30	Mature Mustard Leaves	0.033	56	0.018	11.	0.004	34	0.011	52	0.017	108
	Mature Radish Leaves	0.11	62	0.066	18	0.019	41	0.043	42	0.045	104
	Mature Radish Tuber	0.14	49	0.066	22	0.029	21	0.029	54	0.073	103
	25% Mature Wheat Forage	0.080	65	0.052	14.	0.011	48	0.039	26	0.021	91.
	50% Mature Wheat Forage	0.067	70.	0.047	22.	0.015	51	0.034	29	0.020	100
	Mature Wheat Straw	0.36	44	0.16	16.	0.059	24	0.087	53	0.190	97.
	Mature Wheat Grain	0.058	16	0.009	8.2	0.005	13	0.008	81	0.047	97.
	Soil post application	0.43	101	0.44	NA	NA	NA	NA	1.8	0.008	103.
	Soil at planting	0.32	70.	0.23	NA	NA	NA	NA	45	0.089	94
	Soil at 50% mature wheat harvest	0.20	53	0.11	NA	NA	NA	NA	45	0.089	98
90	Mature Mustard Leaves	0.044	79	0.035	40	0.017	48	0.021	13	0.006	92
	Mature Radish Leaves	0.021	75	0.016	19.	0.004	31	0.006	37	0.008	112.
	Mature Radish Tuber	0.019	94	0.016	53	0.010	35	0.007	12	0.002	106
	25% Mature Wheat Forage	0.15	76	0.012	30	0.045	49	0.075	18	0.027	94
	50% Mature Wheat Forage	0.095	78	0.074	19	0.018	59	0.056	22	0.021	100
	Mature Wheat Straw	0.14	46	0.063	16.	0.022	27	0.036	59	0.080	105
	Mature Wheat Grain	0.021	10	0.002	4.3	0.001	8.3	0.002	87	0.018	98
	Soil post application	1.03	99	1.02	NA	NA	NA	NA	1.6	0.016	101
	Soil at planting	0.31	64	0.20	NA	NA	NA	NA	38	0.12	101
	Soil at 50% mature wheat harvest	0.15	26	0.039	NA	NA	NA	NA	68	0.105	93
210	Mature Mustard Leaves	0.050	84	0.042	40.	0.020	40	0.020	32	0.016	116
	Mature Radish Leaves	0.022	67	0.015	21.	0.005	41	0.009	40	0.009	106
	Mature Radish Tuber	0.019	63	0.012	23	0.004	29	0.005	40	0.008	103
	25% Mature Wheat Forage	0.11	73	0.080	38	0.042	52	0.057	19	0.021	92
	50% Mature Wheat Forage	0.089	66	0.058	30	0.027	35	0.031	31	0.027	96
	Mature Wheat Straw	0.11	40	0.043	17	0.018	22	0.023	57	0.061	97
	Mature Wheat Grain	0.019	13	0.003	3.9	0.001	7.0	0.001	85	0.016	98
	Soil at planting	0.28	45	0.13	NA	NA	NA	NA	48	0.14	93
	Soil at 50% mature wheat harvest	0.22	18	0.039	NA	NA	NA	NA	71	0.16	88

Table 45. Identification of metabolites in rotation crops after a bare ground application of [phenyl-U-14C]fludioxonil at 1120 g ai/ha (Thalacker, Report 117-97, 1999).

Sample	TRR mg/kg	% of TRR Organic	Fludioxonil		CGA-192155		CGA-308103		CGA-265378		CGA-308565		CGA-339833		CGA-344623		Total characterised	
			% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
30-DAT																		
Mature Mustard Leaves	0.033	11	Not profiled															
Mature Radish Leaves	0.11	18	2.0	0.002	8.0	0.008	1.6	0.002	1.2	0.001	1.6	0.002	0.7	<0.001	1.6	0.002	16.7	0.018
Mature Radish Tuber	0.14	22	11.8	0.016	4.8	0.007	0.3	<0.001	1.6	0.002	0.7	<0.001	0.4	<0.001	0.7	0.001	20.	0.027
25% Mature Wheat Forage	0.080	14	0.5	<0.001	7.2	0.006	1.1	<0.001	0.9	<0.001	0.9	<0.001	1.1	<0.01	1.1	<0.001	13.	
50% Mature Wheat Forage	0.067	22	3.5	0.002	7.5	0.005	2.1	0.001	1.4	<0.001	1.0	<0.001	1.7	0.001	1.3	<0.001	18.	0.012
Mature Wheat Straw	0.36	16	0.2	<0.001	4.4	0.015	0.2	0.001	0.1	0.001	ND	NA	0.1	<0.001	0.3	0.001	5.3	0.019
Mature Wheat Grain	0.058	8.2																
90-DAT																		
Mature Mustard Leaves	0.044	40	0.8	<0.001	28	0.012	1.1	<0.001	ND	NA	ND	NA	0.9	<0.001	ND	NA	30	0.013
Mature Radish Leaves	0.021	19.	Not profiled															
Mature Radish Tuber	0.019	53	4.3	<0.001	38	0.007	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA	43	0.008
25% Mature Wheat Forage	0.15	30	0.7	0.001	19	0.029	0.7	0.001	0.9	0.001	1.7	0.003	3.1	0.005	2.1	0.003	28	0.044
50% Mature Wheat Forage	0.095	19	0.5	<0.001	13	0.012	0.5	<0.001	0.5	<0.001	0.8	<0.001	1.4	0.001	1.6	0.002	18	0.017
Mature Wheat Straw	0.14	16	0.2	<0.001	7.0	0.010	0.6	<0.001	0.3	<0.001	0.3	<0.001	ND	NA	1.7	0.002	10	0.014
Mature Wheat Grain	0.021	4.3																
210-DAT																		
Mature Mustard Leaves	0.050	40	2.1	0.001	10.4	0.005	1.5	<0.001	ND	NA	ND	NA	ND	NA	ND	NA	14	0.007
Mature Radish Leaves	0.022	21	Not profiled															
Mature Radish Tuber	0.019	23	Not profiled															
25% Mature Wheat Forage	0.11	38	1.9	0.002	18.2	0.020	3.9	0.004	1.6	0.002	2.2	0.002	3.7	0.004	3.0	0.003	34	0.038
50% Mature Wheat Forage	0.089	30	1.3	0.001	1.0	0.009	1.8	0.002	ND	NA	ND	NA	1.6	0.001	1.1	<0.001	16	0.014
Mature Wheat Straw	0.11	17	0.2	<0.001	7.8	0.008	0.5	<0.001	0.2	<0.001	0.3	<0.001	4.6	0.005	0.9	<0.001	14	0.016
Mature Wheat Grain	0.019	3.9	Not profiled															

HPLC and 2 D-TLC analyses of the organosoluble fractions of various crop tissues from the 30-day plant back identified residues of fludioxonil, CGA 192155, CGA 308103 and CGA 344623. CGA 265378, CGA 308565 and CGA 339833 were indicated by HPLC. The same compounds were indicated in the organosoluble fractions of crop tissues from the 90 and 210-day plant back. Only fludioxonil and CGA 192155 were detected at or above 0.01 mg/kg. Fludioxonil constituted 0.2% to 11.8% of the TRR (<0.001 to 0.016 mg/kg), the main metabolite CGA 192155 4.8% to 38.3% of the TRR (0.005 to 0.029 mg/kg), and the minor metabolites CGA 308103, CGA 265378, CGA 308565, and CGA 344623 each <4% of the TRR (<0.005 mg/kg). CGA339833 reached 4.6% in mature wheat straw.

HPLC analysis of the water-soluble fractions from mature straw indicated the presence of fludioxonil and CGA 192155 below 0.02 mg/kg but these assignments were not confirmed by TLC. CGA 308103, CGA 265378, CGA 308565, CGA 344623 and CGA 339833 were also indicated by HPLC in some fractions but again unconfirmed.

Treatment of aqueous samples from 25% mature (90 and 210-day plant back) and 50% mature (90-day plant back) forage and mature wheat straw (30-day plant back) with cellulase released 3.1% to 10.0% of the TRR (0.009 to 0.014 mg/kg) as organosoluble residues. CGA 192155 was the major component at a maximum of 0.01 mg/kg. No other components exceeded 0.002 mg/kg.

Enzyme treatment of the unextracted residues from 30-day plant back radish tuber (containing 54.4% of the TRR) and mature wheat straw (containing 53.4% of the TRR) released 3.41% of the TRR (0.005 mg/kg) and 4.58% (0.016 mg/kg) respectively. HPLC analyses of the released material showed a void volume peak and diffuse base-level radioactive residues. Further sequential treatment of these unextracted fractions with 0.1M HCl, 1.0 M HCl, 0.1M NaOH and 1.0M NaOH released totals of 24.8% of the TRR (0.033 mg/kg) and 37.1% (0.132 mg/kg) of radioactive residues. After the base treatment, 8.5% (0.011 mg/kg) and 7.1% (0.025 mg/kg) remained unextracted. After each treatment more of the released radioactivity was water-soluble than organosoluble. Chromatographic profiling of the organosoluble residues indicated the presence of fludioxonil, CGA 192155, CGA 308103, CGA 265378, CGA 308565, CGA 339833 and CGA 344623. None of these were above 2.1% of the TRR (0.007 mg/kg). Most of the water-soluble residues were more polar than available reference standards by HPLC..

The highest radioactive residues were seen in cereal straw (0.36 mg/kg) and radish tuber (0.14 mg/kg) at the 30-day planting interval. At the 90 and 210-day planting intervals total radioactive residues were at or below 0.05 mg/kg in all food crops and <0.2 mg/kg. in animal feed items. Fludioxonil was present between 0.2% and 12% of the TRR (<0.001 to 0.016 mg/kg). CGA 192155, the major identified metabolite, was present between 4.4% and 38% of the TRR (0.005 to 0.046 mg/kg), and was no higher than 0.012 mg/kg in food crops. The minor metabolites CGA 308103, CGA 265378, CGA 308565, CGA 339833 and CGA 344623 were <4% of the TRR (0.005 mg/kg).

In further rotational crop trials fludioxonil (50WP formulation at a rate of 282 g ai/ha/application, total 1126 g ai/ha) was applied four times at 7-day intervals to bare soil in California, Florida, and New York (Joseph, Report 174-97, 1999). Cyprodinil was also applied as a 75 WG formulation at a rate of 561 g ai/ha. The California site was the location of previous confined rotational studies using ^{14}C-fludioxonil (see above).

Wheat, turnips, and leaf lettuce were sown or planted 30, 90, 150 and 210 days after the last application. Plant samples collected at selected intervals and kept frozen before analysis were homogenised and extracted with acetonitrile/water (90:10). An aliquot was evaporated to an aqueous solution, methanol and water added, and the sample loaded onto a phenyl solid-phase extraction cartridge. Fludioxonil was eluted with acetone/water and the eluate evaporated to the aqueous solution and partitioned with methyl *tert*-butyl ether. After solvent exchange to hexane, the residues were further cleaned up on a silica extraction cartridge. Residues were determined by normal phase HPLC with UV detection at 268 nm. These procedures are described in Analytical Method AG-597B (see below). The limit of quantification was 0.01 mg/kg for all samples.

Samples of wheat forage, hay, straw and grain, turnip tops and roots, and leaf lettuce from the 30-day planting were analysed for fludioxonil (and cyprodinil). No detectable residues of fludioxonil (<0.01 mg/kg, duplicate samples) were found in any of the samples at any location so the samples from the longer plant-back intervals were not analysed.

Results with pyrrole- and phenyl-labelled fludioxonil were similar.They showed that fludioxonil was not taken up into rotational crops. The metabolism of fludioxonil in rotational crops was the same as that seen in target crop studies and is characterised by oxidation and cleavage of the pyrrole ring. No metabolites indicating cleavage of the bond between the phenyl and the pyrrole rings were observed, showing that either ^{14}C label is suitable for metabolic studies.

RESIDUE ANALYSIS

Analytical methods

Analytical methods for the determination of residues of fludioxonil in crops and animal products (meat, fat, liver, kidney, milk and eggs) were reported.

Two HPLC methods, one with column switching (REM 133.04, AG-631A) and one without (AG- 597), and a multiresidue method (DFG S19) are described for determination of fludioxonil residues in plant materials. They determine fludioxonil only. Method REM 133.04 (Mair, Report REM-133-04, 1993) was developed from REM 133.01 (Hohl, Report REM-133-01, 1989*)* and REM 133.03 (Maffezzoni, Report RES 133.03, 1996). Homogenized plant samples are extracted with methanol. An aliquot of the filtered extract is diluted with water and passed through a phenyl solid-phase extraction cartridge. The analyte is eluted from the cartridge with acetone. The eluate is diluted with water, saturated with sodium chloride and partitioned against hexane/diethyl ether (8:2). The organic phase is evaporated to dryness and reconstituted with hexane/isopropanol (9:1). Determination is by normal-phase HPLC using a two-column switching system with UV detection at 268 nm. Typically, the column is switched from a C-18 column to a phenyl column immediately before elution of fludioxonil.

Wine is diluted with water and treated in the same way as the methanol extract of plant material.

Residues of fludioxonil can be confirmed by GC-MS using an ion trap and diagnostic ions m/z 248, 127, 154 and 182.

Validation was carried out as part of the method development and showed that the overall average recovery for the method from 30 samples was 91% (range 71-111%), Table 46. The lowest practical level of determination is stated to be 0.02 mg/kg for plant material and soil, and 0.005 mg/kg for wine. The demonstrated limit of quantification was 0.04 mg/kg, except 0.01 mg/kg for wine.

Table 46: Recoveries by analytical method REM 133-04 for determination of fludioxonil in crops (Mair, Report REM-133.04, 1993).

Sample	Fortification level	Recovery [%]		SD (rel)	No.
	[mg/kg]	Mean	Range	[%]	
Grapes	0.04	88	83, 93	-	2
	0.4	88	88, 88	-	2
Wine	0.01	90	90	-	1
	0.1	89	89	-	1
Tomatoes	0.04	91	81-97	7.1	4
	0.4	89	87-91	1.7	4
Strawberries	0.04	104	104	-	1
	0.4	87	87	-	1
Maize (on cob)	0.04	90	90	-	1
	0.4	87	87	-	1
Egg plant	0.04	87	79, 95	-	2

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
	0.4	80	79, 82	-	2

The range of crops and limit of quantification was extended by Tribolet (Report 210/01, 2001). Four crop samples and a wine sample were each fortified at 0.02 mg/kg and 0.2 mg/kg. Control samples were also analysed for each crop, and in each case the residues were quoted as <0.02 mg/kg. The data are shown in Table 47. The overall mean recoveries for each of the samples range from 83-89% with relative standard deviations in the range 7.4-14.7%. The LOQ was 0.02 mg/kg for the crops and 0.005 mg/kg for wine.

Table 47. Recoveries by analytical method REM 133-04 for determination of fludioxonil in crops (Tribolet, Report 210/01, 2001).

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
Strawberries	0.02	85	73-97	12	5
	0.2	81	72-87	7.7	5
Apples	0.02	95	84-112	12.	5
	0.2	83	76-88	6.4	5
Wheat grain	0.02	92	81-106	11	5
	0.2	81	70-89	10.	5
Grapes	0.02	88	71-119	21.	5
	0.2	84	79-89	4.7	5
Wine	0.005	84	76-95	9.8	5
	0.05	84	77-88	5.2	5

Radiovalidation of the method was provided by Mair (Report 10/96, 1996) using samples of tomato from the metabolism study. Three sub-samples of tomato previously treated with radiolabelled fludioxonil and harvested at maturity were analysed to determine the amount of fludioxonil. The total radioactive residue (TRR) in the tomato sample was 0.106 mg/kg fludioxonil equivalents and 73% was identified as fludioxonil. In the sub-samples taken, the expected residue of fludioxonil was therefore 0.077 mg/kg. The extraction method solubilized 89% (0.094 mg/kg) of the total radioactivity and the residue of fludioxonil in the samples was determined as 0.051 mg/kg, or 48% of the TRR. The method showed a good extraction of the residues and an overall recovery of fludioxonil of 66% (0.051/0.077 mg/kg)**.**

The second HPLC method (AG-597A, AG-597B) for the determination of fludioxonil in plant commodities (Campbell, Report CGA173506/0773, 1996; Williams, Report ABR-97080, 1998) is typically used in the USA for crop field trials. Crop samples are homogenized with acetonitrile/water (9:1) and filtered. An aliquot is evaporated to remove acetonitrile, diluted with saturated salt solution and partitioned with methyl *tert*-butyl ether (MTBE). For fodder only, the aliquot is partitioned with hexane before evaporation of acetonitrile, diluted with saturated salt solution and partitioned with hexane/MTBE (4:1). Toluene is added to the organic phase, the MTBE evaporated and hexane added to the sample. Grape, wine, grain and tuber samples are cleaned up on a silica SPE cartridge and the analyte eluted with dichloromethane/toluene (1:1). Forage, hay and fodder samples are cleaned up on a silica cartridge and the analyte eluted with dichloromethane/toluene (1:3).

Eluates from the silica cartridges are further cleaned up on a phenyl SPE cartridge, the analyte being eluted with acetone. The eluate is evaporated to dryness and reconstituted in mobile phase. Determination is by normal phase HPLC (amino column) with UV detection at 268 nm. No column switching is involved.

Maize oil samples are dissolved in hexane and partitioned with acetonitrile. The acetonitrile extract is evaporated to dryness and reconstituted in toluene. Clean up is on a Florisil SPE cartridge eluted with 2% acetone in toluene. The eluate is evaporated to dryness and reconstituted in mobile phase. Determination is by reverse-phase HPLC (C-18 column) with UV detection at 268 nm.

Validation results are shown in Table 48. The LOQ of the method is commodity-specific and in the 0.01–0.05 mg/kg range

The extraction efficiency of the method was determined using the rice grain and stalk samples from greenhouse-grown rice given a foliar treatment with ^{14}C fludioxonil. Mean extractabilities of the TRR were 53% (CV 5.7%, n = 3) for 1x treated grain (0.28 kg ai/ha, PHI 52 days), 90% (CV = 0.6%, N=3) for 1x treated stalks, 119% (CV 7.8%, n=3) for 5x treated grain (1.4 kg ai/ha, PHI 50 days) and 86% (CV 3.7%, n=3) for 5x treated rice stalks. HPLC analysis of residues was good from the 1x treated stalks and 5x treated stalks and grain (range 61-112%), but poor from the 1x treated grain (range 29-39%). No comparison could be made with the percentage of the TRR determined as fludioxonil in the metabolism study as the study was not reported to the Meeting.

Table 48. Recoveries by analytical method AG-597B for determination of fludioxonil in crops (Campbell, Report CGA173506/0773, 1996).

Sample	Fortification level [mg/kg]	Recovery [%]		No.
		Mean	Range	
Maize grain	0.01	109	105-114	3
	0.05	87	87	1
	0.1	95	95	1
Maize forage	0.01	83	79, 86	2
	0.05	86	86	1
	0.1	86	86	1
	0.2	83	83	1
Maize forage	0.02	81	72-90	1
	0.1	100	100	1
	0.5	72	72	1
Maize fodder	0.01	72	70, 73	2
	0.05	89	89	1
	0.10	82	82	1
	0.20	87	87	1
Sorghum grain	0.05	66	66	1
	0.10	102	102	1
Sorghum hay	0.01	96	95-96	2
	0.05	90	90	1
	0.10	76	76	1
	0.20	71	70, 72	2
Sorghum fodder	0.01	83	75, 90	2
	0.05	70	70	1
	0.10	86	86	1
	0.20	81	81	1
Potato tuber	0.01	85	75-101	3
	0.05	92	92	1
	0.10	79	79	1
Rice grain	0.01	83	72, 94	2
	0.05	113	109-117	2
Rice stalks	0.05	79	79	1
	0.10	94	94	1
	2.0	85	85	1
	8.0	94	94	1
White grapes	0.02	91	88, 93	2
	0.2	134	134	1
	0.5	73	73	1
Red grapes	0.02	90	86, 93	2

Sample	Fortification level [mg/kg]	Recovery [%]		No.
		Mean	Range	
	0.1	96	96	1
	1.0	93	93	1
White wine	0.02	96	92, 100	2
	0.05	73	73	1
	0.5	96	96	1
Red wine	0.02	114	114	1
	0.2	76	76	1
	1.0	79	79	1

In a further validation of the method (Williams, Report ABR-97080, 1998) the range of crops was extended and minor modifications made to the method for particular samples. Recoveries are shown in Table 49.

LC/MS conditions were developed for confirmatory detection and quantification of residues in lima beans and as a primary determination system for alfalfa hay, clover hay and black pepper. LC/MS was used in single ion monitoring mode on ion 247.

Table 49. Recoveries by analytical method AG-597B for determination of fludioxonil in crops (Williams, Report ABR-97080, 1998).

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
Radish tops	0.01	97	92, 101	-	2
	0.02	95	93, 97	-	2
	0.05	98	95, 100	-	2
Radish tops	0.01	98	85-120	12	10
	0.02	104	94-112	4.9	10
Dried peas	0.01	101	99, 102	-	2
	0.02	85	64, 105	-	2
	0.05	101	97, 104	-	2
Turnip tops	0.01	110	109, 111	-	2
	0.02	104	103, 104	-	2
	0.05	100	98, 101	-	2
Succulent peas with pod	0.01	102	72-120	15	10
	0.02	101	77-120	13	10
Wheat grain	0.01	90	88, 91	-	2
	0.02	79	61-89	20	3
	0.05	79	71-94	16	3
Head lettuce[1]	0.01	107	104, 110	-	2
	0.02	87	84, 90	-	2
	0.05	112	105, 118	-	2
Celery [1]	0.01	85	82, 87	-	2
	0.02	94	93, 95	-	2
	0.05	97	96, 97	-	2
Spinach[1]	0.01	81	71, 91	-	2
	0.02	88	86, 89	-	2
	0.05	94	90, 98	-	2
Cantaloupe fruit[1]	0.01	88	81, 94	-	2
	0.02	76	60, 92	-	2
	0.05	89	85, 93	-	2
Summer squash[1]	0.01	108	104, 112	-	2
	0.02	105	104, 105	-	2
	0.05	104	101, 106	-	2
Cucumber[1]	0.01	93	84, 101	-	2
	0.02	102	101, 103	-	2
	0.05	97	96, 97	-	2

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
Carrot root[1]	0.02	96	88-103	6.3	5
	0.05	108	102-112	4.2	4
Sugar beet top[1]	0.01	98	95, 100	-	2
	0.02	93	91, 95	-	2
	0.05	95	93-, 6	-	2
Sugar beet root[1]	0.02	109	108, 110	-	2
	0.05	108	107, 108	-	2
Bulb onion dry[1]	0.02	111	110, 112	-	2
Green onion[1]	0.01	106	101-113	5.1	4
	0.02	95	95	-	1
	0.05	97	97	-	1
Bean with pod[1]	0.01	110	109, 110	-	2
	0.02	98	96, 100	-	2
	0.05	96	95, 96	-	2
Bean without pod[1]	0.01	99	98, 100	-	2
	0.02	101	100, 101	-	2
	0.05	100	100	-	1
Pea without pod[1]	0.01	94	92, 96	-	2
	0.02	87	85, 89	-	2
	0.05	88	85, 91	-	2
Sorghum asp. grain fraction[1]	0.01	109	108, 109	-	2
	0.02	110	105, 114	-	2
	0.05	104	101, 107	-	2
Tomato fruit[1]	0.01	112	104, 120	-	2
	0.02	105	105	-	2
	0.05	105	103, 107	-	2
Pepper chilli[1]	0.01	101	99, 103	-	2
	0.02	94	93, 95	-	2
	0.05	92	91, 93	-	2
Pepper bell[1]	0.01	113	111, 115	-	2
	0.02	117	115, 118	-	2
	0.05	115	113, 116	-	2
Fresh basil[1]	0.01	102	99, 104	-	2
	0.02	101	99-103	-	2
	0.05	101	100, 101	-	2
Turnip root[1]	0.01	113	109, 116	-	2
	0.02	108	105, 111	-	2
	0.05	117	114, 119	-	2
Broccoli[1]	0.01	114	113, 115	-	2
	0.02	108	105, 111	-	2
	0.05	106	102, 110	-	2
Mustard greens[1]	0.01	107	106, 107	-	2
	0.02	104	101, 106	-	2
	0.05	99	94, 104	-	2
Chives (fresh)[1]	0.01	105	104, 106	-	2
	0.02	103	102, 103	-	2
	0.05	100	99, 101	-	2
Rape seed[1]	0.01	97	90, 103	-	2
	0.02	98	96, 99	-	2
	0.05	93	92, 93	-	2
Lettuce, leaf[1]	0.01	104	92-117	8.1	12
	0.02	99	90-110	7.9	6
	0.05	102	92-109	7.2	5
Cucumber[1]	0.01	98	88-109	8.8	11
	0.02	100	81-112	11	6
	0.05	93	75-105	14	6
Radish roots[1]	0.01	96	74-110	14	10
	0.02	108	95-123	11	8
	0.05	110	104, 116	-	2

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
Rice straw[2]	0.01	100	99, 100	-	2
	0.02	103	96, 109	-	2
	0.05	101	99, 102	-	2
Sweet corn forage[2]	0.01	107	105, 108	-	2
	0.02	96	95, 97	-	2
	0.05	101	100, 101	-	2
Peanut hay[2]	0.02	116	115, 116	-	2
	0.05	120	117, 122	-	2
Wheat forage[2]	0.01	99	84-115	16	3
	0.02	96	75- 111	20	3
	0.05	104	99-107	4.2	3
Wheat hay[2]	0.01	96	81, 110	-	2
	0.02	101	85-108	14	3
	0.05	99	93-104	5.7	3
Soya forage[2]	0.01	108	103, 113	-	2
	0.02	91	89, 93	-	2
	0.05	95	92, 97	-	2
Clover forage[2]	0.01	101	93, 109	-	2
	0.02	99	93, 104	-	2
	0.05	91	83, 100	-	2
Alfalfa forage[2]	0.01	83	83	-	2
	0.02	84	84	-	1
	0.05	80	77, 83	-	2
Celery seed[2]	0.01	102	100, 104	-	2
	0.02	95	94, 96	-	2
	0.05	96	91, 100	-	2
Cabbage[2]	0.01	72	71, 73	-	2
	0.02	92	89, 95	-	2
	0.05	88	84, 92	-	2
Peanut meal[2]	0.01	80	73, 86	-	2
	0.02	99	86, 111	-	2
	0.05	107	105, 109	-	2
Field corn flour[3]	0.01	105	102, 107	-	2
	0.02	109	107-110	-	2
	0.05	92	92	-	2
Soya beans[3]	0.01	105	104, 106	-	2
	0.02	114	113, 114	-	2
	0.05	106	105, 107	-	2
Soya asp. grain fraction[3]	0.01	73	66, 79	-	2
	0.02	106	104, 107	-	2
	0.05	91	80, 102	-	2
Soya hay[3]	0.01	101	99, 103	-	2
	0.02	109	107, 110	-	2
	0.05	96	95, 96	-	2
Sunflower seeds[3]	0.01	110	107, 112	-	2
	0.02	92	82, 102	-	2
	0.05	99	89, 109	-	2
Maize starch[3]	0.01	111	108, 114	-	2
	0.02	111	110, 111	-	2
	0.05	101	101	-	2
Maize grits[3]	0.01	108	10, -110	-	2
	0.02	112	111, 113	-	2
	0.05	100	99, 100	-	2
Maize asp. grain fraction[3]	0.01	106	104, 107	-	2
	0.02	107	107	-	2
	0.05	103	102, 104	-	2
Maize fodder[3]	0.01	90	86, 94	-	2
	0.02	91	89, 92	-	2
	0.05	92	92	-	2

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
Rice grain[3]	0.01	104	103, 105	-	2
	0.02	107	106, 108	-	2
	0.05	97	96, 97	-	2
Lima bean, dry[3]	0.01	95	79-109	17.	4
	0.02	98	86-110	13.	4
	0.05	99	92-106	6.3	4
Basil dried[3]	0.02	105	99, 110	-	2
	0.05	95	92, 98	-	2
Chives dried[3]	0.01	80	64, 96	-	2
	0.02	122	122	-	1
	0.05	88	87, 89	-	2
Peanut nutmeg[3]	0.01	81	79, 82	-	2
	0.02	72	64, 80	-	2
	0.05	84	80, 88	-	2
Cotton undelinted seed[3]	0.05	100	91-106	5.9	8
	0.5	103	103	-	1
Rape seed oil[4]	0.01	72	71, 73	-	2
	0.02	76	76	-	1
	0.05	127	96, 158	-	2
Peanut oil, refined[4]	0.01	94	90, 96	-	2
	0.02	85	80, 89	-	2
	0.05	89	88, 89	-	2
Cotton seed oil, refined[4]	0.05	77	75, 78	-	2
Wheat straw[3]	0.01	99	89-100	6.0	3
	0.02	94	90-97	3.7	3
	0.05	83	80-85	3.0	3
Alfalfa hay[5]	0.01	103	101, 105	-	2
	0.02	97	96, 98	-	2
	0.05	114	111, 116	-	2
Clover hay[5]	0.01	81	79, 83	-	2
	0.02	73	64, 82	-	2
	0.05	116	115, 117	-	2
Black pepper[5]	0.01	106	90, 122	-	2
	0.02	108	93, 123	-	2
	0.05	90	87, 93	-	2
Lima beans, dried[5]	0.01	106	105, 107	-	2
	0.02	95	93, 96	-	2
	0.05	86	85, 87	-	2

[1] using method AG-597B as written for grapes, wine, grain and potato tubers with alternative procedure for forage crops
[2]using method AG-597B as written for forage and hay samples
[3]using method AG-597B as written for fodder samples
[4]using method AG-597B as written for oil samples
[5]using method AG-597B as written for fodder samples using LC/MS detection

An LOQ of 0.01 mg/kg was achieved in radish roots and tops, turnip roots and tops, beet tops, green onion (whole plant), head and leaf lettuce, celery, spinach, broccoli, cabbage, mustard greens, succulent beans (with and without pod), beans (dry seed), succulent peas (with and without pod), peas (dry seed) soya (asp. grain fraction, dry beans, forage and hay), tomato, peppers (bell and non-bell), cantaloupe, cucumber, summer squash, maize (starch, grits, flour, asp. grain fraction), rice grain and straw, sorghum (asp. grain fraction), wheat (grain, straw, forage and hay), maize forage and fodder, alfalfa forage and hay, clover forage and hay, basil (fresh and dried), chives (fresh and dried), celery seed and black pepper, sunflower seeds, rape (oil and seed), cotton (undelinted seed, gin trash, hulls, meal and oil) and peanuts (nutmeat, meal, hay and oil). An LOQ of 0.02 mg/kg was achieved in carrot, sugar beet roots, dry bulb onions, peanut hay and dried basil.

Control values were quoted as less than the corresponding LOQ samples. Reagent blanks and control samples were free of interfering substances at the retention time of fludioxonil.

The original method (without modifications) was validated by the U.S. EPA (Willet, Report D217129, 1995). The method was as described in the manufacturer's document, with very minor modifications to use the available equipment. The limit of detection was estimated as 0.002 mg/kg. Findings are summarized in Table 50. Recovery from forage was unacceptable with the unmodified method.

Table 50. Recoveries by analytical method AG-597A for determination of fludioxonil in crops (Willet, Report D217129, 1995).

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
Maize grain	0.02	95	98.0, 92.5		2
	0.04	104	106, 101		2
Sorghum grain	0.05	93	93.8, 92.0		2
	0.1	96	99.2, 91.8		2
Corn forage	0.02	67	56.5-94.0	18	4
	0.04	41	31.3-55.3	12	3

Method AG-597B was also validated by the US EPA for stone fruits, strawberries, and bulb vegetables (Donovan, Report D272959, 2001). The limit of detection and LOQ for these commodities were 0.003 mg/kg and 0.008 mg/kg respectively.

A multi-residue method, based on DFG S19 (extended revision) was developed for routine monitoring of fludioxonil in samples of plant material (Pelz, Report SYN-0103V, 2001). The extractions of fludioxonil were according to extraction module E1 for orange and tomato, E2 for wheat grain and E7 for rape seed. Extracts were separated by gel permeation chromatography and analysed by capillary gas chromatography (30 m fused silica capillary DB-5, 60–180^{o}C) using mass selective detection (MSD) with the molecular ion (m/z 248) and 2 fragment ions (m/z 154 and 127).

In method DFG S19 (extended revision) has been validated for determination of residues of fludioxonil in fortified samples of tomato (as representative of commodities with high water content), orange (as representative of fruits with high acid content), wheat (as representative of cereals and other dry crops), and rape (as representative of commodities with high fat content). Recovery experiments were conducted with fortifications of 0.02 mg/kg and 0.2 mg/kg for each sample. Control samples were analysed in duplicate and fortified samples in quintuplet for each fortification level.

The demonstrated LOQ was 0.02 mg/kg. The stated limit of detection (LOD) was 0.004 mg/kg. Analysis of unfortified homogenized control samples yielded no residues of fludioxonil above the limit of detection, indicating that no background levels of fludioxonil or interferences were present in any of the samples before the beginning of the study. The linearity of the detector response covered a working range of 0.01-4.0 µg/ml fludioxonil.

Recoveries were within the required range 70-110% (except oranges where the mean value at 0.02 mg/kg was 114%). The data are presented in Table 51.

Table 51. Recoveries by analytical method DFG S19 (extended revision) for determination of fludioxonil in crops (Pelz, Report SYN-0103V, 2001).

Sample	Fortification level [mg/kg]	Recovery [%]		SD* (rel) [%]	No.
		Mean	Range		
Tomato	0.02	104	98-108	3.8	5
	0.2	107	97-114	6.9	5
Orange	0.02	114	107-117	4.2	5
	0.2	105	97-111	5.5	5
Wheat	0.02	97	86-106	7.7	5

Sample	Fortification level [mg/kg]	Recovery [%]		SD* (rel) [%]	No.
		Mean	Range		
	0.2	101	94-108	5.1	5
Rape	0.02	106	99-109	5.3	5
	0.2	92	84-96	5.1	5

The applicability of the multi-residue to wine was confirmed (Dieterle, Report 108-93, 1993). The LOQ was 0.01 mg/kg, defined by the lower fortification level tested. (Table 52).

Table 52. Recoveries by analytical method DFG S19 (extended revision) for determination of fludioxonil in wine (Dieterle, Report 108-93, 1993).

Sample	Fortification level [mg/kg]	Recovery [%]		No.
		Mean	Range	
Wine	0.01	102	95, 108	2
	0.1	99	92, 105	2

An independent validation of the multi-residue method was carried out on fortified samples of tomato and rape seed (Stenhauer, Report SYN-0104V, 2001). The data are presented in Table 53.

Table 53. Recoveries by analytical method DFG S19 (extended revision) for determination of fludioxonil in crops (Stenhauer, Report SYN-0104V, 2001).

Sample	Fortification level [mg/kg]	Recovery [%]		SD (rel) [%]	No.
		Mean	Range		
Tomato	0.02	104	97-113	6.5	5
	0.2	95	93-97	2.0	5
Rape	0.02	107	95-115	7.9	5
	0.2	93	80-110	14	5

Fludioxonil was tested according to the FDA Multiresidue Methods using sorghum grain, field corn grain and potatoes as the test samples according to protocols C, D and E (Willett, Report D206301, 1995). The compound was inadequately recovered from sorghum grain and field corn grain. Potatoes were tested as the representative non-fatty crop using protocol D. Recoveries ranged from 49 to 121% at the 0.05 mg/kg fortification level and from 78 to 87% at 0.5 mg/kg.

An HPLC method was reported for the determination of fludioxonil residues in livestock commodities (Vienneau, Report AG-616B, 1996). The method converts residues of fludioxonil and its metabolites to CGA 192155 (2,2-difluoro-1,3-benzodioxole-4-carboxylic acid), and the total residues are quantified in terms of CGA 192155 by external calibration. An independent laboratory has validated the in-house method.

Homogenized samples of animal tissues, milk and eggs are extracted by reflux with ammonium hydroxide/acetonitrile. The extract is filtered, acidified and partitioned with toluene. The organic phase of liver, eggs and chicken fat extracts is cleaned up by solid-phase extraction cartridge (silica for liver and eggs and C-18 for chicken fat). The cleaned up extract and the original extracts for other substrates are evaporated to dryness and heated with potassium permanganate and aqueous sodium hydroxide to oxidize fludioxonil and its oxidizable benzopyrrole metabolites to CGA-192145. The oxidation is quenched with sodium metabisulphite. The oxidized extract is filtered, acidified and partitioned with dichloromethane before clean-up by silica solid-phase extraction. Determination is by column switching reversed-phase HPLC (Supelcosil LC-C1 plus Supelcosil LC-8-DB analytical) with

UV (230 nm) detection. Residues are expressed as fludioxonil. A confirmatory HPLC system using an alternative column (Suplcosil LC-C1 plus Supelcosil LC-CN) is also described. Residue values are converted to mg/kg fludioxonil using a conversion factor of 1.23 (MW of fludioxonil ÷ MW of CGA-192155).

The method was validated. The overall recovery was found to be 83% with a standard deviation of 8.6%. Extraction and accuracy were shown to be acceptable by the analysis of samples from the goat and hen metabolism studies. Fortified samples were also analysed by the confirmatory HPLC method where the overall mean recovery was 80% with a relative standard deviation of 22%.

The limit of quantification was 0.01 mg/kg fludioxonil equivalents in meat and milk and 0.05 mg/kg in eggs, liver, kidney and fat. Recovery data are shown in Table 54.

Table 54. Recoveries by analytical method AG-616B for determination of fludioxonil in animal tissues, milk and eggs (Vienneau, Report AG-616B, 1996).

Analyte	Sample	Fortification level [mg/kg]	Recovery [%]		CV [%]	No.
			Mean	Range		
Fludioxonil	Cattle milk	0.01	83.5	83, 84	-	2
and metabolites		0.05	83	83	-	1
as	Goat milk	0.01	83.7	77-94	10.8	3
CGA-192155		0.60	82	82	-	1
	Beef round muscle	0.01	78.5	78, 79	-	2
		0.10	79	79	-	1
	Beef tenderloin	0.01	75.5	74, 77	-	2
		0.05	71	71	-	1
	Goat leg muscle	0.01	73	69, 77	-	2
		0.05	79	79	-	1
	Poultry lean meat	0.01	94	90, 98	-	2
		0.05	79	79	-	1
	Beef liver	0.05	77.5	70, 85	-	2
		0.10	69	69	-	1
	Goat liver	0.05	88	88	-	2
		6.0	94	94	-	1
	Poultry liver	0.05	89	89	-	2
		0.10	91	91	-	1
	Beef kidney	0.05	89	87, 91	-	2
		0.10	86	86	-	1
	Goat kidney	0.05	78.5	69, 88	-	2
		3.0	90	90	-	1
	Beef omental fat	0.05	89.5	80, 99	-	2
		0.10	83	83	-	1
	Goat perirenal fat	0.05	95.5	87, 104	-	2
		0.06	94	94	-	1
	Poultry perirenal fat	0.05	74.5	66, 83	-	2
		0.10	89	89	-	1
	Poultry eggs	0.05	83.5	82, 85	-	2
		0.50	75.7	68-81	-	3

The validation data for the confirmatory method (alternative HPLC column) for AG-616 is presented in Table 55.

Table 55. Recoveries by confirmatory method for AG-616B (Vienneau, Report AG-616B, 1996; Perez, Report ADPEN-901-95-1023, 1996).

Analyte	Sample	Fortification level [mg/kg]	Recovery [%]		No.
			Mean	Range	
Fludioxonil	Cattle milk	0.01	92	83, 101	2
and metabolites		0.05	76	76	1
as	Goat milk	0.01	86.5	83, 90	2
CGA-192155		0.60	81	81	1
	Beef tenderloin	0.01	76.5	71, 82	2
		0.05	65	65	1
	Goat leg muscle	0.01	63.5	37, 90	2
		0.05	78	78	1
	Poultry lean meat	0.01	133	95, 171	2
		0.05	92	92	1
	Beef liver	0.05	69.5	69, 70	2
		0.10	66	66	1
	Goat liver	0.05	101	88, 114	2
		6.0	89	89	1
	Poultry liver	0.05	86	85,	2
		0.10	95	95	1
	Beef kidney	0.05	88.5	88,	2
		0.10	88	88	1
	Goat kidney	0.05	77	69,	2
		3.0	88	88	1
	Beef omental fat	0.05	58	51, 65	2
		0.10	73	73	1
	Goat perirenal fat	0.05	64	63, 65	2
		0.06	60	60	1
	Poultry perirenal fat	0.05	45	37, 53	2
		0.10	80	80	1

An independent laboratory validation was carried out on the method (Perez, Report ADPEN-901-95-1023, 1996). Results are presented in Table 56. The validation was successful at a fortification of 0.05 mg/kg for eggs and liver and at 0.01 mg/kg for milk. A second independent laboratory validation was carried out to determine the ruggedness of the method (Tang and Baldi, 1996).

Table 56. Method validation and concurrent method recoveries of Method AG-616B from fortified untreated samples of milk and animal tissues (Perez, Report ADPEN-901-95-1023, 1996; Tang and Baldi, Study 102-96, 1996).

Commodity	Fortification level, mg/kg	% Recovery[1]
Independent Laboratory Validation (Perez, Report ADPEN-901-95-1023, 1996)		
Liver	0.05, 0.10	85-107 (n=4)
Milk	0.01, 0.10	83-109 (n=4)
Eggs	0.05, 0.10	63 (n=1); 73-99 (n=3)
Concurrent Method Recoveries		
Dairy cattle, milk	0.01-0.20	58-68 (n=6), 70-101 (n=35)
Dairy cattle, round muscle	0.01, 0.20	82, 85 (n=2)
Dairy cattle, tenderloin muscle	0.01, 0.10	72, 76 (n=2)
Dairy cattle, liver	0.05, 0.50	78, 80 (n=2)

Commodity	Fortification level, mg/kg	% Recovery[1]
Dairy cattle, kidney	0.05, 0.20	88, 100 (n=2)
Dairy cattle, perirenal fat	0.05, 0.20	80, 80 (n=2)
Dairy cattle, omental fat	0.05, 0.10	85, 88 (n=2)
Independent Laboatory Valiadation (Tang and Baldi, Reort 102-96, 1996)		
Liver	0.05, 0.25	80-85 (n=4)
Milk	0.01, 0.05	70-75 (n=4)
Eggs	0.05, 0.25	75-79 (n=4)

[1] No. of samples (n) in parentheses; recoveries outside the acceptable 70-120% range are listed separately.

Samples from the goat and poultry metabolism studies were used for radioavalidation of the method (Vienneau, Report AG-616B, 1996). Extraction of the total residue ranged from 50% to 85% for goat tissues and was 69% for eggs. In the metabolism studies the figures were 32%-76% and about 70% of the TRR respectively, so extraction by the method is acceptable. The overall recovery of fludioxonil and benzopyrrole metabolites ranged from 36% to 58%. The total percentage of the TRR identified in the metabolism studies ranged from 14 to 83% for the goat metabolism and 69% in eggs. The results are summarized in Table 57.

Table 57. Extraction and recoveries of [^{14}C]fludioxonil as determined by method AG-616B (Vienneau, Report AG-616B, 1996).

Sample	TRR [1] (mg/kg)	Extraction[1,2] (mg/kg)	Mean extraction of TRR (%)	LSC Total Residue from HPLC[1] (mg/kg)	Fludioxonil by HPLC (mg/kg)[3]	Recovery[5] (%)	Total % of TRR identified from metabolism studies[6]
Goat milk, day 2	0.59	0.50	85	0.28	0.34	56	78
Goat muscle	0.045	0.030	68	0.015	0.018	40	52
Goat liver	6.6	3.3	50	1.92	2.36	36	14
Goat kidney	2.8	1.84	65	0.95	1.16	41	48
Goat fat	0.063	0.041	65	0.026	0.031[4]	49	83
Hen eggs	0.52	0.36	69	0.24	0.30	58	69

[1] Average of three samples.
[2]Residues (fludioxonil plus benzopyrrole metabolites) determined by HPLC and converted with a factor of 1.23.
[3]Conversion factor 1.23
[4] Less than the LOQ for fat.
[5]Fludioxonil equivalents by HPLC divided by the TRR in the sample.
[6]See Tables 5 and 10 above.

Stability of pesticide residues in stored analytical samples

The stability of fludioxonil residues under deep freeze storage (<-18°C) was examined in cereal grain and straw samples (Bass, Report 621/7-1012, 1995), grapes (Mair, Report 131/93, 1996), maize, sorghum and potato substrates (Eudy, Report ABR-97108, 1997), tomatoes (Tribolet, Report 222/98, 2000a), apples (Tribolet, Report 221/98, 2000b), and peas and rape seed (Tribolet, Report 210/00, 2002).

Single period assessments were made of the storage stability of fludioxonil in fortified samples of peach (Thompson & Ediger, Report A6934, 1999a), plum (Thompson & Ediger, Report 06943, 1999b), cherry (Thompson and Ediger, Report 06933, 1999c), raspberry (Starner, Report

06838, 2001), blueberry (Thompson, Report 06724, 2001), cabbage (Arsenovic, Report 07121, 2002a), broccoli (Arsenovic, Report 07122, 2002b), carrots (Hong Chen, Report 07090, 2002), and fresh chives (Hong Chen, Report 07126, 2002),.

Samples were stored in polyethylene containers in deep freeze rooms under conditions corresponding to those used for storage of residue samples (≤ -20°C). The analytical methods were REM 133 (European samples) and AG 597 (US samples).

Fludioxonil residues were shown to be stable over the periods for which they were stored (depending on the sample) under these conditions. The results are summarized in Tables 58 and 59. Samples in Table 59 were not analysed at the time of fortification (day 0) and the results are therefore of limited value.

Table 58: Storage stability of fludioxonil residues in frozen crop substrates (multi-period studies).

Crop (fortification level, mg/kg) Reference	Storage (months)	Procedural recovery (%)	Mean procedural recovery (%)	Fludioxonil remaining in stored samples					
				Individual samples (mg/kg)			Mean (mg/kg)	Mean corrected (mg/kg)[1]	% of fortification not corrected
Cereal	0	86 82 71	80	0.43	0.41	0.36	0.40	0.50	80
grain	3	94 97	96	0.35	0.42	0.43	0.40	0.42	80
(fortified,	6	101 101	101	0.50	0.47	0.48	0.48	0.48	96
0.50 mg/kg)	12	85 91	88	0.42	0.39	0.44	0.42	0.47	84
Bass, Report 621/7-1012, 1995	24	102 98	100	0.44	0.48	0.44	0.45	0.45	90
Cereal	0	106 103 97	102	0.53	0.51	0.48	0.51	0.51	102
straw	3	72 81	77	0.38	0.38	0.34	0.37	0.48	74
(fortified,	6	72 79	76	0.46	0.42	0.37	0.42	0.55	84
0.50 mg/kg)	12	74 90	82	0.50	0.53	0.52	0.52	0.63	104
Bass, Report 621/7-1012, 1995	24	80 81	81	0.41	0.42	0.48	0.44	0.54	88
Apples	0	93 90	92	0.43	0.46	0.46 0.47	0.46	0.49	92
(fortified,	1	85 88	87	0.43		0.43	0.43	0.49	86
0.50 mg/kg)	3	87 86	87	0.45		0.40	0.43	0.49	86
Tribolet,	6	87 89	88	0.42		0.43	0.43	0.48	86
Report 221/98,	12	86 88	87	0.42		0.42	0.42	0.48	84
2000b	24	89 80	85	0.41		0.41	0.41	0.48	82
Tomatoes	0	88 89	89	0.44	0.45	0.42 0.44	0.44	0.49	88
(fortified,	1	86 90	88	0.42		0.42	0.42	0.48	84
0.50 mg/kg)	3	84 89	87	0.42		0.39	0.41	0.47	82
Tribolet,	6	91 88	90	0.42		0.41	0.42	0.46	84
Report 222/98,	12	95 88	92	0.40		0.37	0.39	0.42	78
2000a	24	91 96	94	0.39		0.41	0.40	0.43	80
Grapes	0	89 88	89	6.05	5.95	5.81	5.94	6.67	89
(field-	1.2	74 74	74	4.25	4.96	4.88	4.70	6.35	52
incurred	3.5	87 81	84	5.89	5.64	5.61	5.71	6.80	86
from study	6.5	93 91	92	5.77	5.75	5.89	5.80	6.31	87
2069/93)	13.3	74 74	74	5.21	6.55	5.79	5.85	7.91	88
	26.0	88 88	88	5.36	4.70	5.25	5.10	5.80	76
	28.9	86 88	87	6.87	7.59	7.88	7.45	8.56	111
Grapes	0	89 88	89	5.81	5.66	5.54	5.67	6.37	89
(field-	1.2	74 74	74	5.20	5.19	4.22	4.87	6.58	76
incurred	3.5	87 81	84	5.98	5.64	5.92	5.85	6.96	92
from study	6.5	93 91	92	6.02	5.71	5.61	5.78	6.28	91
2070/93)	13.3	74 74	74	5.42	5.50	4.59	5.17	6.99	81
Mair, Report	26.0	88 88	88	3.65	4.46	3.49	3.87	4.39	61
131/93, 1996	28.9	86 88	87	5.66	5.98	5.49	5.71	6.56	90

Crop (fortification level, mg/kg) Reference	Storage (months)	Procedural recovery (%)		Mean procedural recovery (%)	Fludioxonil remaining in stored samples					
					Individual samples (mg/kg)			Mean (mg/kg)	Mean corrected (mg/kg)[1]	% of fortification not corrected
Peas	0	81	88	85	0.42	0.40	0.45	0.42	0.50	84
(fortified,	3	81	70	76	0.42	0.40	0.41	0.41	0.54	82
0.50 mg/kg)	6	88	85	87	0.42	0.44	0.42	0.43	0.49	86
Tribolet,	12	84	84	84	0.41	0.41	0.44	0.42	0.50	84
Report 210/00,	18	86	85	86	0.39	0.44	0.41	0.41	0.48	96
2002	24	80	90	85	0.33	0.50	0.44	0.42	0.50	84
Rape seed	0	72	78	75	0.33	0.35	0.37	0.35	0.47	70
(fortified,	3	80	81	81	0.35	0.29	0.35	0.33	0.41	66
0.50 mg/kg)	6	70	76	73	0.29	0.28	0.32	0.30	0.41	60
Tribolet,	12	83	81	82	0.36	0.35	0.38	0.36	0.44	72
Report 210/00,	18	78	80	79	0.32	0.31	0.35	0.33	0.41	66
2002	24	84	75	80	0.27	0.24	0.29	0.27	0.33	54
Maize	0	108	99	104	0.198		0.187	0.19	0.19	95
Forage	1.8	97	89	93	0.187		0.185	0.19	0.20	95
(fortified,	5.7	93	96	95	0.186		0.191	0.19	0.20	95
0.20 mg/kg)	12.3	103	99	101	0.189		0.198	0.19	0.19	95
Eudy, Report	18.1	91	106	99	0.194		0.197	0.20	0.20	100
ABR-97108,	23.8	100	97	99	0.157		0.176	0.17	0.17	85
1997										
Maize	0	76	99	88	0.058		0.097	0.08	0.09	80
grain	1.6	116	89	103	0.084		0.088	0.09	0.09	90
(fortified,	5.9	102	107	105	0.098		0.098	0.10	0.10	100
0.10 mg/kg)	12.5	100	98	99	0.096		0.091	0.09	0.09	90
	18.1	108	108	108	0.103		0.105	0.10	0.10	100
	23.9	97	99	98	0.094		0.093	0.09	0.10	90
Maize ears	0	97	99	98	0.151		0.190	0.17	0.17	85
(fortified,	1.8	97	97	97	0.185		0.179	0.18	0.19	90
0.20 mg/kg)	6.0	113	110	112	0.197		0.204	0.20	0.20	100
Eudy, Report	12.2	101	106	104	0.208		0.199	0.20	0.20	100
ABR-97108,	18.3	112	112	112	0.205		0.199	0.20	0.20	100
1997	23.9	101	104	103	0.197		0.195	0.20	0.20	100
Maize meal	0	105	106	106	0.212		0.213	0.21	0.21	105
(fortified,	2.8	117	128	123	0.235		0.247	0.24	0.24	120
0.20 mg/kg)	3.0	123	122	123	0.225		0.237	0.23	0.23	115
	6.1	92	96	94	0.177		0.165	0.17	0.18	85
Eudy, Report	12.0	101	102	102	0.190		0.191	0.19	0.19	95
ABR-97108,	18.5	110	115	113	0.210		0.207	0.21	0.21	105
1997	26.6	102	108	105	0.206		0.214	0.21	0.21	105
Sorghum	0	100	93	97	0.480		0.488	0.48	0.50	96
Hay	2.1	94	100	97	0.464		0.477	0.47	0.49	94
(fortified,	5.7	99	103	101	0.504		0.472	0.49	0.49	98
0.50 mg/kg)	12.4	100	103	102	0.487		0.497	0.49	0.49	98
Eudy, Report	17.9	100	98	99	0.460		0.482	0.47	0.48	94
ABR-97108,	24.0	98	98	97	0.446		0.463	0.45	0.47	90
1997										
Potato	0	99	97	98	0.099		0.098	0.10	0.10	100
Tubers	1.8	100	96	98	0.090		0.069	0.08	0.08	80
(fortified,	5.6	101	93	97	0.091		0.095	0.09	0.10	90
0.10 mg/kg)	12.1	93	94	94	0.090		0.100	0.10	0.10	100
Eudy, Report	18.2	100	100	100	0.109		0.107	0.11	0.11	110
ABR-97108,	24	96	92	94	0.094		0.093	0.09	0.10	90
1997										
Potato	0	108	113	111	0.228		0.214	0.22	0.22	110
Flakes	2.8	107	98	103	0.167		0.188	0.18	0.18	90
(fortified,	6.2	112	101	107	0.170		0.178	0.17	0.17	85
0.20 mg/kg)	12.2	98	102	100	0.169		0.165	0.17	0.15	85
Eudy, Report	18.6	100	104	102	0.171		na	0.17	0.17	85
ABR-97108,	26.6	104	117	111	0.161		0.167	0.16	0.16	80
1997										

[1]corrected for control and procedural recoveries <100%
na: not analysed

Table 59. Storage stability of fludioxonil residues in frozen crop substrates (single period studies).

Crop, fortification, mg/kg, Reference	Storage (months)	Procedural recoveries (%)	Mean procedural recovery (%)	Recovery from individual stored samples (%)	Mean uncorrected recovery (%)
Peach 4.0 Thompson & Ediger, Report A6934, 1999a	3.3	Not determined	na	87 94 106	96
Plum 4.0 Thompson & Ediger, Report 06943, 1999b	2	Not determined	na	93 102 104	100
Cherry 4.0 Thompson and Ediger, Report 06933, 1999c	3.5	Not determined	na	103 86 89	93
Raspberry 2.0 Starner, Report 06838, 2001	4.5	89 93 86	93	74 68 69	70
Blueberry 2.0 Thompson, Report 06724, 2001	23 days 57 days	89 87 88	88	86 91 117 108 117 103	95 108
Cabbage 1.0 Arsenovic, Report 07121, 2002a	12	81 78 72 83	79	65 64 72	67
Broccoli 1.0 Arsenovic, Report 07122, 2002b	12	97 86 110 74	92	78 69 75	74
Carrot 1.0 Hong Chen, Report 07090, 2002	12	66 82 73	74	86 85 84	85
Chives 1.0 Hong Chen, Report 07126, 2002	9.4	78 85 79 88	83	92 87 104	94

na: not applicable

The storage stability of fludioxonil in beef muscle, beef liver, milk and eggs under freezer storage was reported to the Meeting (Eudy, Report ABR-97055, 1997). Samples of beef muscle, beef liver, milk and eggs fortified with fludioxonil were stored at -20°C for up to about 19 months. The storage conditions were chosen to represent those under which residue samples from animal studies are stored before analysis. Samples were analysed by method AG-616 with minor modifications for some samples.

No significant deterioration of fludioxonil residues with time was observed in the samples. Results are summarised in Table 60.

Table 60. Storage stability of fludioxonil residues in frozen animal tissues (Eudy, Report ABR-97055, 1997).

Tissue (fortification, mg/kg)	Storage (momths)	Procedural recoveries (%)		Mean procedural recovery (%)	Residue[1] Remaining in Store Sample				
					Individual stored samples (mg/kg)		Mean (mg/kg)	Mean corrected[2] (mg/kg)	% of fortification (not corrected)
Beef muscle									
	0	78	76	77	0.40	0.42	0.41	0.53	82
(fortified,	3.8	80	80	80	0.35	0.39	0.37	0.46	74
0.5 mg/kg)	11.2	68	136	102	**	0.51	0.51	0.50	100
	19.3	76	63	70	0.30	**	0.30	0.42	60
Beef liver	0	86	86	86	0.92	0.92	0.92	1.07	192
(fortified,	3.3	78	78	78	0.68	0.68	0.68	0.87	68
1.0 mg/kg)	11.7	96	90	93	0.76	0.63	0.70	0.75	70
	19.2	121	96	109	0.89	0.88	0.88	0.81	88
Milk	0	80	82	81	0.40	0.38	0.39	0.48	78
(fortified,	3.1	79	67	73	0.31	0.38	0.35	0.48	70
0.5 mg/kg)	11.2	61	73	67	0.32	0.33	0.32	0.48	96
	18.8	76	67	72	0.35	0.37	0.36	0.50	72
Eggs	0	89	92	91	0.95	0.87	0.91	1.00	91
(fortified,	3.1	74	79	77	0.77	0.81	0.79	1.03	79
1.0 mg/kg)	12.0	76	79	78	0.73	0.74	0.73	0.94	73
	18.7	77	78	78	0.70	0.82	0.76	0.97	76

[1]method determines fludioxonil and metabolites as CGA 192155 (2,2-difluoro-1,3-benzodioxole-4-carboxylic acid).
[2]corrected for control and procedural recovery <100%

USE PATTERNS

Fludioxonil is registered globally as a fungicide and is used as a seed treatment, as a foliar treatment, and post-harvest application on a wide variety of crops. The information available to the Meeting on registered uses relevant to the supervised field trial data is summarized in Tables 61–63. It is based on the labels or translations of labels provided by the manufacturer.

Table 61. Registered foliar uses of fludioxonil.[1]

Crop	Country	Formulation, ai %	Application rate		No. per season	PHI days
			kg ai/hl	kg ai/ha		
Fruit						
Blackberry	Switzerland	WG, 25	0.025	0.3	2	14
Blackberry	USA	WG, 25		0.25	4	0
Blueberry	USA	WG, 25		0.25	4	0
Cherry (stone fruit)	Switzerland	WG, 25		0.30	2	
Currant	USA	WG, 25		0.25	4	0
Grape	Austria	WG, 25	0.025	0.25	2	35
Grape	Chile	WG, 25		0.25	2	7
Grape	France	WG, 25		0.3	2	50
Grape	Germany	WG, 25	0.015	0.24	2	35
Grape	Italy	WG, 25	0.02	0.2	2	21
Grape	Spain	WG, 25		0.25	2	21
Grape	Switzerland	WG, 25		0.3	1	Before grape close
Lychee	USA	WG, 25		0.25	4	0
Peach	France	WG, 25	0.015			14
Peach	Italy	WG, 25	0.015	0.25	2	14
Peach	Switzerland	WG, 25	0.015	(0.3)	2	
Pear	Italy	WG, 25	0.02	0.25	3	14

Crop	Country	Formulation, ai %	Application rate		No. per season	PHI days
			kg ai/hl	kg ai/ha		
Pear	Spain	WG, 25	0.025	0.25	3	7
Plum	France	WG, 25	0.012	0.12 (up to 1000 l/ha)	3	14
Plum	Italy	WG, 25	0.025	0.25	2	14
Plum	Switzerland	WG, 25		0.3	2	
Raspberry	Switzerland	WG, 25	0.025	0.3 2	2	14
Raspberry	USA	WG, 25		0.25	4	0
Strawberry	France (glasshouse and field)	WG, 25		0.25	ca 1[2]	3
Strawberry	Germany	WG, 25	(0.0125)	0.25	3	7
Strawberry	Italy (glasshouse and field)	WG, 25	0.02	0.2	3	7
Strawberry	Spain	WG, 25		0.25	3	7
Strawberry	Switzerland	WG, 25	0.025	0.3	2	14
Strawberry	USA	WG, 25		0.25	4	0
Vegetables						
Asparagus	Austria	WG, 25	(0.042)	0.25	3	NS
Broccoli	USA	WG, 25	(≤0.13)	0.25	4	7
Cabbage	USA	WG, 25	(<0.13)	0.25	4	7
Carrot	USA	WG, 25		0.25	4	7
Cucumber	Italy (glasshouse and field)	WG, 25	0.02	0.20	3	7
Cucumber	Spain (glasshouse and field)	WG, 25	0.025		3	7
Cucumber	Switzerland (glasshouse)	WG, 25	0.025			3
Egg plant (aubergine)	Italy (glasshouse and field)	WG, 25	0.02	0.2	3	7
Egg plant (aubergine)	Spain (glasshouse and field)	WG, 25	0.025		3	7
Egg plant (aubergine)	Switzerland (glasshouse and field)	WG, 25	0.025			
Herbs (chives & basil)	USA	WG, 25		0.25	4	7
Legume (dry seed; pulse)	Austria+ Spain	WG, 25		0.25	2	14
Legume (fresh seed)	France	WG, 25	(0.083)	0.25		14
Legume (bean, n.o.s.)	Switzerland	WG, 25		0.2		
Legume (pod and seed)	Spain (glasshouse and outdoor)	WG, 25	0.025		3	14
Legume (pod and seed)	France	WG, 25	(0.083)	0.25		14
Lettuce, head	France (glasshouse and outdoor)	WG, 25		0.15	4	14
Lettuce, head	Italy (glasshouse and outdoor)	WG, 25	0.018	0.18	3	14
Lettuce, head	Spain (glasshouse and outdoor)	WG, 25		0.15	3	14
Lettuce, head	Switzerland (glasshouse and outdoor)	WG, 25		0.12	2	Early season use
Melon	USA	WP, 50	0.28	Drip irrigation	3	14
Mustard greens	USA	WG, 25		0.25	4	7
Onion	Austria	WG, 25	(0.12)	0.25	3	7

Crop	Country	Formulation, ai %	Application rate		No. per season	PHI days
			kg ai/hl	kg ai/ha		
Onion	Switzerland	WG, 25		0.25	2	
Onion, green and dry bulb	USA	WG, 25		0.25	4	7
Pepper (sweet)	Austria[2]	WG, 25	(0.025)	0.25	3	7
Pepper (sweet)	Italy (glasshouse and outdoor)	WG, 25	0.02	0.2	3	7
Pepper (sweet)	Spain (glasshouse and outdoor)	WG, 25	0.025		3	7
Tomato	Switzerland	WG, 25		0.25	2	3
Tomato	Italy (glasshouse and field)	WG, 25	0.02	0.2	3	7
Tomato	Spain (glasshouse and field)	WG, 25	0.025		3	7
Tomato	Greece[2]	WG, 25	0.025	0.38	2	7
Watercress	USA	WG, 25		0.25	4	0
Summer squash (Zucchini)	Italy (glasshouse and field)	WG, 25	0.02	0.20	3	7
Treenuts						
Pistachio	USA	WG, 25		0.25	4	7

[1] Includes only the registered uses related to the supervised field trial data supplied to the Meeting, not all possible registered national uses. Values in parenthesis are calculated from the spray volume.
[2] Fludioxonil use should not exceed one of each three fungicide uses.

Table 62. Registered post-harvest uses of fludioxonil in the USA.[1]

Crop	Formulation, ai %	Application rate		No. per season	PHI days
		kg/hl	kg/ha		
Fruit					
Apricot	WP 50	0.06	-	1	0
Cherry	WP 50	0.06	-	1	0
Citrus	WP, 50	0.06[2]	-	2	0
Kiwifruit	WP, 50	0.06[3]			
Nectarine	WP, 50	0.06	-	1	0
Pome fruit	WP, 50	0.06[2]	-	2	0
Peach	WP 50	0.06	-	1	0
Plum	WP, 50	0.06	-	1	0
Yam	WP, 50	0.06	-	1	0

[1] Includes only the registered uses related to the supervised field trial data supplied to the Meeting.
[2] Dip treatment for 30 seconds. Spray treatment low volume at 0.86 kg ai/hl or high volume at 0.24 kg/hl, with 0.5 g ai/250 000 kg fruit, or 2 mg/kg.
[3] 2.5 mg/kg fruit.

Table 63. Registered seed treatment uses of fludioxonil.[1]

Crop	Country	Formulation, ai %	Appliacation rate g/100 kg seed
Vegetables			
Bean, pea	USA	FS,48	5.0
Pea	UK	WG,5	10
Potato	Australia	FS,10	2.5
Potato	USA	FS, 48	2.5
Sweet corn	USA	FS, 48	5.0
Oilseeds			
Rape	USA	FS, 48	5.0
Rape	Germany	FS, 2.5	12
Cotton seed	USA	FS, 48	5.0

Crop	Country	Formulation, ai %	Appliacation rate g/100 kg seed
Sunflower	US	FS, 40	5.0
Soya	Argentina Paraguay Brazil	XL, 2,5 XL, 2,5 XL, 2,5	5.0
Cereals			
Barley	Austria	FS, 2.5	5.0
Barley	Belgium	FS, 2.5	5.0
Barley	UK	FS, 2.5	5.0
Barley	USA	FS, 48	5.0
Maize	Argentina	FS, 2.5	3.0
Maize	Brazil	FS, 2.5	3.8
Maize	Mexico	FS, 48	5.8
Maize (field corn)	USA	FS, 48	5.0
Oats	UK	FS, 2.5	5.0
Oats	USA	FS, 48	5.0
Popcorn	USA	FS, 48	5.0
Rye	Austria	FD, 2.5	5.0
Sorghum	USA	FS, 48	5.0
Spelt	Belgium	FS, 2.5	5.0
Sweet corn	USA	FS, 48	5.0
Wheat/Triticale	Austria	FS, 2.5	5.0
Wheat/Triticale	Belgium	FS, 2.5	5.0
Wheat/Triticale	UK	FS, 2.5	5.0
Wheat/Triticale	USA	FS, 48	5.0

[1] Includes only the registered uses related to the supervised field trial data supplied to the Meeting, not all possible registered national uses.

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on fludioxonil supervised field trials on the following crops.

Commodity	Application	Country	Table no.
Citrus (orange, lemon, grapefruit)	Post-harvest	USA	64
Apple	Post-harvest	USA	65
Pear	Post-harvest	USA	66
Pear	Foliar	France, Italy, Spain	67
Peach	Post-harvest	USA	68
Peach	Foliar	France, Italy, Spain	69
Plum	Post-harvest	USA	70
Plum	Foliar	France, Germany, Italy, Switzerland	71
Cherry	Post-harvest	USA	72
Cherry	Foliar	Grance, Germany, Italy, Switzerland	73
Grapes	Foliar	Chile, France, Germany, Greece, Italy, South Africa, Spain, Switzerland	74
Strawberry	Foliar	France, Germany, Italy, Spain, Switzerland, UK, USA	75
Strawberry	Foliar indoor	France, Italy, Spain, UK	76

Commodity	Application	Country	Table no.
Raspberry	Foliar	Germany, US	77
Blueberry and Currant	Foliar	Germany, US	78
Lychee	Foliar	USA	79
Kiwifruit	Post-harvest	USA	80
Pomegranate	Post-harvest	Usa	81
Green Onion and Bulb Onion	Foliar	France, Germany, Italy, Switzerland, USA	82
Broccoli	Foliar	Canada, USA	83
Cabbage	Foliar	USA	84
Cucumber	Foliar (indoor and outdoor)	Greece, Spain, Switzerland	85
Squash (Zucchini)	Foliar (indoor and outdoor)	Italy	86
Melon (Cantaloupe)	Foliar	USA	87
Tomato	Foliar (indoor and outdoor)	Greece, Italy, Spain, Switzerland, UK	88
Bell Pepper	Foliar (indoor and outdoor)	Italy, Spain, Switzerland	89
Egg plant (Aubergine)	Foliar (indoor and outdoor)	Italy, Spain	90
Sweet Corn	Seed treatment	USA	91
Head lettuce	Foliar (indoor and outdoor)	France, Germany, Italy, Spain, Switzerland	92
Watercress	Foliar	USA	93
Mustard Greens	Foliar	USA	94
Bean pods with seeds (succulent)	Foliar (indoor and outdoor)	France, Spain, Switzerland	95
Bean pods with seeds (succulent)	Seed treatment	Denmark, Germany	96
Peas without pod	Foliar	France, Switzerland	97
Peas without pod	Seed treatment	France, UK	98
Dry pea and kidney bean	Foliar	France	99
Dry pea	Seed treatment	France, UK	100
Potato	Seed treatment	USA	101
Yam	Post-harvest	USA	102
Carrot	Foliar	USA	103
Asparagus	Foliar	Germany	104
Wheat	Seed tretment	France, Germany, Switzerland	105
Rye	Seed treatment	Denmark	106
Barley	Seed treatment	France, Germany, Switzerland	107
Maize (Field Corn)	Seed treatment	France, Germany, Greece, Hungary, Spain, South Africa, USA	108
Sorghum	Seed treatment	USA	109
Pistachio	Foliar	USA	110