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BAS 750F
(Mefentrifluconazole)

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Version History

When	What
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B.7. RESIDUE DATA

B.7.1. STORAGE STABILITY OF RESIDUES

B.7.1.1. Stability of BAS 750 F

Report: CA 6.1/1
Guedez-Orozco A.-A.,Eilers B., 2015 a
Storage stability of BAS 750 F in plant matrices
2015/1106709
Guidelines: OECD 506, EPA 860.1380, EEC 7032/VI/95 rev. 5
GLP: yes

Materials and Methods

The frozen storage of BAS 750 F in crop matrices for at least 24 months was studied in tomato fruit, apple fruit, grape fruit, lemon fruit, wheat grain, dried bean seed, dried pea seed, soybean seed, rape seed, wheat whole plant no roots, wheat straw and potato tuber.

Untreated sample matrices (5 g per sample) were fortified with BAS 750 F at a level of 0.1 mg/kg. Five samples (two fortified and three non-fortified) per commodity matrix were kept in PE-containers at ≤-18°C in the dark for up to 551 days. Additional reserve samples for each matrix were also stored. After time intervals of approximately 0, 30, 90, 180, 360, 550 and 730 days, samples were removed from storage and were analysed for BAS 750 F. Procedural (fresh) recoveries were undertaken on samples fortified at 0.1 mg/kg

Extraction and analysis was using method BASF L0076/09. Full details and validation data for this method can be found in section CA B.5.1.2.5. In brief, BAS 750 F was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg.

Results and discussion

The recoveries of BAS 750 F from plant matrices after the various storage periods are summarized in Table 7.1.1-1. All compounds remained stable over the whole storage period of about 24 months (~730 days) in every matrix examined.

The analytical method (BASF L0076/09) was validated at the level used for fortification with every series of analysis. The average of all recoveries for BAS 750 F was between 70 and 110% for all samples. The procedural recoveries (freshly spiked samples at 0.1 mg/kg) were in the range of 70 and 110%.

Table 7.1.1-1: Storage stability of BAS 750 F in a range of crops

Commodity	**Level** (mg/kg)	**Storage interval** (days)	**Residues after storage** (mg/kg)		**Mean stored recovery** (% of nominal)	**Fresh recovery** (%)
			Individual values	mean		
Wheat, whole plant	0.1	0	0.092, 0.088	0.090	90.3	90.5
		31	0.092, 0.094	0.093	93.3	95.0
		85	0.087, 0.088	0.088	87.8	90.0
		177	0.088, 0.096	0.092	92.1	94.5
		369	0.084, 0.083	0.084	83.3	94.5
		550	0.097, 0.094	0.096	95.5	90.0
		730	0.090, 0.089	0.090	89.5	89.3

Commodity	**Level** (mg/kg)	**Storage interval** (days)	**Residues after storage** (mg/kg)		**Mean stored recovery** (% of nominal)	**Fresh recovery** (%)
			Individual values	mean		
Wheat, straw	0.1	0	0.085, 0.082	0.084	83.3	85.5
		29	0.093, 0.091	0.092	91.8	94.0
		86	0.090, 0.092	0.091	90.8	84.0
		184	0.094, 0.091	0.093	92.3	92.5
		363	0.090, 0.086	0.088	87.8	85.5
		551	0.100, 0.097	0.099	99.5	96.0
		734	0.094, 0.094	0.094	93.8	90.8
Wheat, grain	0.1	0	0.094, 0.095	0.095	94.9	95.1
		30	0.096, 0.093	0.095	94.5	98.5
		85	0.096, 0.099	0.098	97.0	87.0
		182	0.094, 0.089	0.092	91.5	98.0
		361	0.098, 0.104	0.101	101	105
		547	0.091, 0.096	0.094	93.0	96
		733	0.091, 0.093	0.092	92.0	103
Soybean seed	0.1	0	0.092, 0.088	0.090	90.3	90.0
		30	0.089, 0.092	0.091	90.3	90.5
		85	0.086, 0.086	0.086	85.8	80.5
		182	0.089, 0.094	0.092	91.5	98.0
		361	0.093, 0.088	0.091	90.5	96.0
		550	0.084, 0.093	0.089	88.0	86.0
		734	0.082, 0.089	0.086	85.3	99.3
Rape, seed	0.1	0	0.091, 0.090	0.091	90.3	86.0
		31	0.089, 0.082	0.086	85.8	87.0
		85	0.093, 0.090	0.092	91.6	93.5
		177	0.081, 0.090	0.086	86.0	100
		369	0.073, 0.080	0.077	76.3	88.0
		*378	0.091, 0.096	0.094	93.5	93.5
		550	0.094, 0.095	0.095	94.3	95.5
		735	0.093, 0.091	0.092	91.8	92.3
Potato, tuber	0.1	0	0.089, 0.094	0.092	91.3	88.0
		29	0.091, 0.079	0.085	84.8	89.5
		83	0.092, 0.088	0.090	89.5	97.0
		184	0.065, 0.074	0.070	60.8	65.0
		369	0.080, 0.072	0.076	76.0	93.0
		*378	0.092, 0.091	0.092	91.5	97.0
		551	0.074, 0.079	0.077	76.3	83.5
		735	0.082, 0.077	0.080	79.3	85.3
Apple, fruit	0.1	0	0.087, 0.082	0.085	84.8	91.0
		30	0.088, 0.090	0.089	89.0	92.0
		85	0.093, 0.090	0.092	91.5	77.5
		182	0.083, 0.081	0.082	81.8	98.5
		358	0.085, 0.077	0.081	80.8	93.0
		547	0.078, 0.079	0.079	78.0	89.0
		733	0.082, 0.083	0.083	82.5	94.1
Lemon, fruit	0.1	0	0.090, 0.090	0.090	90.0	89.0
		29	0.094, 0.094	0.094	94.4	97.5
		83	0.094, 0.092	0.093	92.5	97.0
		182	0.096, 0.095	0.096	95.5	97.0
		358	0.094, 0.093	0.094	93.4	92.3
		547	0.10, 0.096	0.098	98.3	91.0
		733	0.093, 0.092	0.093	92.3	98.3

Commodity	**Level** (mg/kg)	**Storage interval** (days)	**Residues after storage** (mg/kg)		**Mean stored recovery** (% of nominal)	**Fresh recovery** (%)
			Individual values	mean		
Dried bean, seed	0.1	0	0.093, 0.093	0.093	93.1	91.5
		30	0.096, 0.092	0.094	94.0	97.0
		85	0.094, 0.096	0.095	94.8	88.5
		182	0.098, 0.097	0.098	97.3	94.5
		358	0.09, 0.087	0.089	88.3	98.0
		547	0.10, 0.104	0.102	102	92.0
		733	0.098, 0.103	0.101	100	97.1
Grape, fruit	0.1	0	0.092, 0.086	0.089	89.3	88.5
		31	0.092, 0.094	0.093	93.1	98.5
		85	0.085, 0.085	0.085	85.3	98.0
		177	0.088, 0.086	0.087	87.3	83.5
		358	0.089, 0.084	0.087	86.1	88.0
		547	0.087, 0.095	0.091	91.0	98.0
		733	0.087, 0.090	0.089	88.3	94.3
Tomato, fruit	0.1	0	0.09, 0.089	0.090	89.8	87.5
		31	0.093, 0.094	0.094	93.8	95.5
		85	0.086, 0.088	0.087	87.3	93.5
		177	0.078, 0.071	0.075	74.9	80.0
		358	0.079, 0.075	0.077	76.6	93.3
		546	0.081, 0.074	0.078	77.0	90.0
		732	0.082, 0.067	0.075	74.3	92.8
Dried pea, seed	0.1	0	0.091, 0.089	0.090	89.5	90.5
		29	0.094, 0.096	0.095	94.8	93.5
		83	0.089, 0.094	0.092	91.3	96.0
		182	0.099, 0.10	0.100	101	104
		361	0.096, 0.098	0.097	96.5	91.0
		550	0.099, 0.098	0.099	98.3	95.0
		734	0.089, 0.086	0.088	87.3	95.8

* *reserve samples*

Conclusions

BAS 750 F is stable in tomato fruit, apple fruit, grape fruit, lemon fruit, wheat grain, dried bean seed, dried pea seed, soybean seed, rape seed, wheat whole plant no roots, wheat straw and potato tuber matrices for at least 730 days when stored under deep frozen conditions.

As at least one crop has been considered in all five crop groups; high water (tomato fruit, apple fruit), high oil (soybean seed, rape seed), high protein (dried pea seed, dried bean seed), high starch (wheat grain, potato tuber) and high acid (grape fruit, lemon fruit), it can be considered that sufficient data is available to support the storage stability of BAS 750 F in all plant commodities for at least 730 days.

Report:	CA 6.1/3 Heger N.,Guedez-Orozco A.-A., 2015 b Storage stability of BAS 750 F in animal matrices 2015/1106711
Guidelines:	EEC 7032/VI/95 rev. 5, EEC 91/414 (1607/IV/97 Rev. 2), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EPA 860.1380,
OECD	506
GLP:	yes

Materials and Methods

The frozen storage of BAS 750 F in animal matrices for at least 5 months was studied in bovine tissues, milk and cream as well as hen egg.

Untreated sample matrices (5 g per sample) were fortified with BAS 750 F at a level of 0.1 mg/kg. Five samples (two fortified and three non-fortified) per commodity matrix were kept in PE-containers at ≤-18°C in the dark for up to 182 days. Additional reserve samples for each matrix were also stored. After time intervals of approximately 0, 30, 90, 120 and 180 days, samples were removed from storage and were analysed for BAS 750 F. Procedural (fresh) recoveries were undertaken on samples fortified at 0.1 mg/kg

Extraction and analysis was using method BASF L0272/01. Full details and validation data for this method can be found in section CA B.5.1.2.5. In brief, BAS 750 F was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg.

Results and discussion

The recoveries of BAS 750 F from animal matrices after the various storage periods are summarized in Table 7.1.1-2. All compounds remained stable over the whole storage period of about 5 months (172-180 days) in every matrix examined.

The analytical method (BASF L0272/01) was validated at the level used for fortification with every series of analysis. The average of all recoveries for BAS 750 F was between 70 and 110% for all samples. The procedural recoveries (freshly spiked samples at 0.1 mg/kg) were in the range of 70 and 110%.

Table 7.1.1-2: Storage stability of BAS 750 F in a range of animal matrices

Commodity	**Level** (mg/kg)	**Storage interval** (days)	**Residues after storage** (mg/kg)		**Stored Recovery** (%)	**Fresh recovery** (mean, %)
			Individual values*	mean		
Cow liver	0.1	0	0.11 0.10	0.105	104	94.4
		28	0.09 0.09	0.09	92	99.0
		120	0.09 0.09	0.09	90	94.5
		177	0.1 0.11	0.105	105	104
Cow kidney	0.1	0	0.11 0.10	0.105	104	113
		29	0.09 0.09	0.09	90.5	97.5
		90	0.10 0.10	0.10	96.7	105
		120	0.09 0.09	0.09	88	95.0
		182	0.10 0.10	0.10	99.5	102
Cow muscle	0.1	0	0.09 0.11	0.10	99.5	101
		30	0.09 0.09	0.09	91.3	92.5
		89	0.10 0.10	0.10	99.2	105
		120	0.09 0.09	0.09	87.3	98.5
		182	0.09 0.10	0.095	94.3	110
Cow fat	0.1	0	0.09 0.09	0.09	90.8	89.8
		32	0.08 0.08	0.08	83.7	87.0
		85	0.09 0.09	0.09	93.0	95.4
		117	0.09 0.08	0.085	84.8	93.8
		180	0.08 0.09	0.085	87.5	80.5
Cow milk	0.1	0	0.10 0.10	0.10	97.6	96.1
		29	0.08 0.08	0.08	83.8	87.0
		84	0.10 0.10	0.10	98.2	96.0
		116	0.08 0.08	0.08	83.5	88.6
		177	0.09 0.10	0.095	96.0	96.5
Cow cream	0.1	0	0.10 0.10	0.10	96.9	97.0
		32	0.09 0.08	0.085	88.9	94.8
		83	0.10 0.10	0.10	99.6	100
		118	0.09 0.09	0.09	89.9	99.0
		177	0.09 0.09	0.09	91.5	99.0
Hen egg	0.1	0	0.10 0.11	0.105	107	114
		28	0.09 0.09	0.09	88	81.0
		83	0.11 0.11	0.11	105	103
		118	0.09 0.09	0.09	87.8	95.0
		180	0.11 0.10	0.105	105	108

* *to 3s.f.*

Conclusions

BAS 750 F is stable in cow tissue, milk and cream and hen egg for at least 177 days when stored under deep frozen conditions. It can be considered that sufficient data is available to support the storage stability of BAS 750 F in all animal commodities for at least 177days

B.7.1.2. Stability of BAS 750 F metabolites

Report:	CA 6.1/2 Heger N., Taraschewski I., 2015 a Storage stability of Reg.No. 6011210 in animal matrices 2015/1106710
Guidelines:	OECD 506 (Oct. 2007), EPA 860.1380, EEC 7032/VI/95 rev. 5
GLP:	yes

Materials and Methods

The frozen storage of metabolite M750F022 in animal matrices for at least 5 months was studied in bovine tissues, milk and cream as well as hen egg.

Untreated sample matrices (5 g per sample) were fortified with M750F022 at a level of 0.1 mg/kg. Five samples (two fortified and three non-fortified) per commodity matrix were kept in PE-containers at ≤-18°C in the dark for up to 183 days. Additional reserve samples for each matrix were also stored. After time intervals of approximately 0, 30, 90, 120 and 180 days, samples were removed from storage and were analysed for M750F022. Procedural (fresh) recoveries were undertaken on samples fortified at 0.1 mg/kg

Extraction and analysis was using method BASF L0309/01. Full details and validation data for this method can be found in section CA B.5.1.2.5. In brief, M750F022 was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. After drying the sample is dissolved in alkaline solution and subjected to SPE, before the final determination was performed by GC/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg.

Results and discussion

The recoveries of M750F022 from animal matrices after the various storage periods are summarized in Table 7.1.2-1. All compounds remained stable over the whole storage period of about 5 months (179-183 days) in every matrix examined.

The analytical method (BASF L0309/01) was validated at the level used for fortification with every series of analysis. The average of all recoveries for M750F022 was between 70 and 110% for all samples. The procedural recoveries (freshly spiked samples at 0.1 mg/kg) were in the range of 70 and 110% in almost all cases.

Table 7.1.2-1: Storage stability of M750F022 in a range of animal matrices

Commodity	Level (mg/kg)	Storage interval (days)	Residues after storage (mg/kg)		Stored Recovery (%)	Fresh recovery (%)
			Individual values	mean		
Cow liver	0.1	0	0.083 0.081	0.082	82.2	82.6
		28	0.072 0.068	0.070	70.0	79.3
		86	0.09 0.081	0.086	85.7	87.0
		114	0.086 0.08	0.083	82.9	78.9
		183	0.092 0.077	0.085	84.4	94.0
Cow kidney	0.1	0	0.067 0.066	0.067	66.5	73.6
		0	0.085 0.082	0.084	83.5	74.6
		31	0.076 0.077	0.077	76.8	82.3
		90	0.083 0.077	0.080	80.3	89.0
		114	0.11 0.096	0.103	102	79.4
		178	0.1 0.084	0.092	93.4	96.7
Cow muscle	0.1	0	0.07 0.07	0.070	70.1	71.0
		29	0.093 0.074	0.084	83.7	80.1
		91	0.074 0.08	0.077	77.2	76.4
		115	0.093 0.098	0.096	95.3	65.0
		181	0.1 0.11	0.105	106	70.3
Cow fat	0.1	0	0.1 0.1	0.100	101	107
		28	0.088 0.087	0.088	87.0	99.5
		87	0.093 0.1	0.097	98.5	108
		115	0.091 0.094	0.093	92.0	102
		180	0.089 0.089	0.089	88.8	88.5
Cow milk	0.1	0	0.086 0.083	0.085	84.3	84.5
		28	0.084 0.08	0.082	81.8	81.5
		84	0.071 0.084	0.078	77.5	86.5
		113	0.078 0.079	0.079	78.3	85.0
		179	0.077 0.083	0.080	80.0	79.0
Cow cream	0.1	0	0.084 0.086	0.085	85.0	106
		27	0.096 0.097	0.097	96.3	99.0
		84	0.11 0.095	0.103	100	89.5
		115	0.092 0.096	0.094	93.8	99.0
		179	0.093 0.093	0.093	93.0	90.5
Hen egg	0.1	0	0.092 0.088	0.090	89.6	73.8
		28	0.082 0.071	0.077	76.3	84.5
		85	0.087 0.094	0.091	90.3	93.5
		113	0.081 0.076	0.079	78.4	53.9
		178	0.082 0.078	0.080	79.9	80.6

** reserve samples*

Conclusions

M750F022 is stable in cow tissue, milk and cream and hen egg for at least 178 days when stored under deep frozen conditions. It can be considered that sufficient data is available to support the storage stability of M750F022 in all animal commodities for at least 178 days.

Report: CA 6.1/4
Perez R., 2015 a
Freezer storage stability of Triazolyl lactic acid in plant samples
2015/7005764
Guidelines: OECD 506 (Oct. 2007), EPA 860.1380
GLP: yes

Materials and Methods

The frozen storage of triazolyl lactic acid (TLA) in crop matrices for at least 48 months was studied in wheat grain, navy bean, orange, canola seed, and lettuce.

Untreated sample matrices (5 g per sample) were fortified with TLA at a level of 0.1 mg/kg. Five samples (two fortified and three non-fortified) per commodity matrix were kept in PE-containers at ≤-18°C in the dark for up to 48 months/1440 days. After time intervals of approximately 0, 47, 97, 181, 362, 548, 736, 915, 1097, 1310 and 1461 days, samples were removed from storage and were analysed for TLA. Procedural (fresh) recoveries were undertaken on samples fortified at 0.1 mg/kg.

Extraction and analysis was using method BASF Method No D0905. Full details and validation data for this method can be found in section CA B.5.1.2.5. In brief, TLA was extracted with a mixture of acetonitrile and water. An aliquot of the extract was acidified and filtered. The final determination was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg.

Results and discussion

The recoveries of TLA from plant matrices after the various storage periods are summarized in Table 7.1.2-2. All compounds remained stable over the whole storage period of about 48 months in every matrix examined.

The analytical method (D0905) was validated at the level used for fortification with every series of analysis. The average of all recoveries for TLA was between 70 and 110% for the majority of samples. The majority of procedural recoveries (freshly spiked samples at 0.01 mg/kg and 0.1 mg/kg) were in a range of 70 and 110%. It is noted that for all matrices except wheat grain, high recoveries were obtained at the 97-548 day storage times (as well as the corresponding procedural recoveries), this consistency indicates a potential issue with the running of the method or sample extraction used at these time points, as recoveries within the acceptable range are determined at later time points. As such, it is not considered these high recoveries impact on the validity of this study to determine the storage stability of TLA over the 48 month period, as multiple acceptable recoveries have been determined after this period.

Table 7.1.2-2: Storage stability of TLA in a range of crops

Commodity	Level (mg/kg)	Storage interval (days)	Procedural recovery (mg/kg)			Fresh recovery (%)	Residues after storage (mg/kg)			Stored Recovery (%)
			Individual values		Mean		Individual values		mean	
Wheat grain	0.1	0	0.096	0.091	0.094	93.5	0.010	0.083	0.092	91.5
		47	0.096	0.091	0.094	93.5	0.072	0.087	0.080	79.5
		97	0.096	0.091	0.094	93.5	0.111	0.109	0.110	110
		181	0.091	0.083	0.087	87	0.097	0.100	0.099	98.5
		362	0.115	0.111	0.113	113	0.112	0.094	0.103	103
		548	0.106	0.107	0.107	106.5	0.117	0.120	0.119	118.5
		736	0.083	0.080	0.082	81.5	0.092	0.087	0.090	89.5
		915	0.089	0.090	0.090	89.5	0.091	0.100	0.096	95.5
		1097	0.082	0.082	0.082	82	0.057	0.051 0.081	0.063	63
		1310	0.077	0.086	0.082	81.5	0.077	0.086	0.082	81.5

Commodity	Level (mg/kg)	Storage interval (days)	Procedural recovery (mg/kg)			Fresh recovery (%)	Residues after storage (mg/kg)			Stored Recovery (%)
			Individual values		Mean		Individual values		mean	
		1461	0.086	0.091	0.089	88.5	0.084	0.087	0.086	85.5
Navy bean	0.1	0	0.095	0.087	0.091	91	0.105	0.099	0.102	102
		47	0.095	0.087	0.091	91	0.102	0.098	0.100	100
		97	0.095	0.087	0.091	91	0.116	0.111	0.114	113.5
		181	0.103	0.070	0.087	86.5	0.116	0.099	0.108	107.5
		362	0.105	0.115	0.110	110	0.119	0.103	0.111	111
		548	0.113	0.105	0.109	109	0.116	0.110	0.113	113
		736	0.097	0.095	0.096	96	0.091	0.096	0.094	93.5
		915	0.099	0.105	0.102	102	0.116	0.122	0.119	119
		1097	0.102	0.092	0.097	97	0.079	0.088	0.084	83.5
		1310	0.114	0.114	0.114	114	0.098	0.118	0.108	108
		1461	0.094	0.091	0.093	92.5	0.096	0.099	0.098	97.5
Orange	0.1	0	0.117	0.113	0.115	115	0.082	0.096	0.089	89
		47	0.117	0.113	0.115	115	0.108	0.107	0.108	107.5
		97	0.117	0.113	0.115	115	0.114	0.125	0.120	119.5
		181	0.104	0.114	0.109	109	0.111	0.106	0.109	108.5
		362	0.106	0.110	0.108	108	0.120	0.120	0.120	120
		548	0.117	0.105	0.111	111	0.119	0.119	0.119	119
		736	0.107	0.110	0.109	108.5	0.109	0.105	0.107	107
		915	0.113	0.094	0.104	103.5	0.111	0.108	0.110	109.5
		1097	0.113	0.114	0.114	113.5	0.113	0.114	0.114	113.5
		1310	0.102	0.102	0.102	102	0.090	0.097	0.094	93.5
		1461	0.093	0.100	0.097	96.5	0.109	0.105	0.107	107
Canola seed	0.1	0	0.093	0.106	0.100	99.5	0.111	0.102	0.107	106.5
		47	0.093	0.106	0.100	99.5	0.136	0.116	0.126	126
		97	0.106	0.128	0.117	117	0.127	0.136	0.132	131.5
		181	0.107	0.108	0.108	107.5	0.134	0.117	0.126	125.5
		362	0.119	0.116	0.118	117.5	0.121	0.118	0.120	119.5
		548	0.099	0.098	0.099	98.5	0.117	0.118	0.118	117.5
		736	0.091	0.091	0.091	91	0.114	0.115	0.115	114.5
		915	0.105	0.102	0.104	103.5	0.103	0.094	0.099	98.5
		1097	0.082	0.099	0.091	90.5	0.094	0.099	0.097	96.5
		1310	0.109	0.107	0.108	108	0.097	0.100	0.099	98.5
		1461	0.09	0.127	0.109	108.5	0.106	0.090	0.098	98
Lettuce	0.1	0	0.107	0.115	0.111	111	0.109	0.093	0.101	101
		47	0.107	0.115	0.111	111	0.117	0.114	0.116	115.5
		97	0.107	0.115	0.111	111	0.117	0.117	0.117	117
		181	0.101	0.108	0.105	104.5	0.114	0.118	0.116	116
		362	0.103	0.117	0.110	110	0.111	0.113	0.112	112
		548	0.107	0.1	0.104	103.5	0.117	0.119	0.118	118
		736	0.086	0.089	0.088	87.5	0.095	0.098	0.097	96.5
		915	0.097	0.093	0.095	95	0.118	0.114	0.116	116
		1097	0.097	0.097	0.097	97	0.113	0.102	0.108	107.5
		1310	0.094	0.103	0.099	98.5	0.099	0.096	0.098	97.5
		1461	0.095	0.067	0.081	81	0.098	0.106	0.102	102

Conclusions

TLA is stable in wheat grain, navy bean, orange, canola seed, and lettuce matrices for at least 48 months when stored under deep frozen conditions. As one crop has been considered in all five crop groups; high water (lettuce), high oil (canola seed), high protein (navy bean), high starch (wheat

grain) and high acid (orange), it is considered that sufficient data is available to support the storage of TLA in all plant commodities for at least 48 months.

B.7.1.3. Overall conclusions on storage stability

BAS 750 F

BAS 750 F has been demonstrated to be stable in all five crop groups; high water (tomato fruit, apple fruit), high oil (soybean seed, rape seed), high protein (dried pea seed, dried bean seed), high starch (wheat grain, potato tuber) and high acid (grape fruit, lemon fruit) for a period of 730 days (~24 months) when stored at ≤-18 °C.

As at least one crop has been considered in all five crop groups, it can be considered that sufficient data is available to support the storage stability of BAS 750 F in all plant commodities for at least 730 days. Additionally, as there is no observed decline in residues across these commodities, specific storage stability data is not required for processed commodities.

BAS 750 F has been demonstrated to be stable in cow tissue, milk and cream and hen egg for at least 177 days when stored under deep frozen conditions.

Metabolites

M750F022 is a metabolite formed at relatively high levels in animal commodities (see section 7.2.2 and 7.4.1). M750F022 has been demonstrated to be stable in cow tissue, milk and cream and hen egg for at least 178 days when stored under deep frozen conditions.

Triazole derivative metabolites (TDMs) are formed during the metabolism of BAS 750 F in plant and animal commodities. The TDMs are 1,2,4-triazole, triazole alanine, triazole acetic acid and triazole lactic acid. Frozen storage stability of these metabolites was considered as part of the TDM review (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015) in which BASF were one of the members of the TDM group who submitted the studies. A summary of the results of the studies is given in Table 7.1.3-1. These studies were considered acceptable in the ongoing TDM review. This table includes the studies in which the longest storage period was considered (other studies covering shorter time scales were also presented in the review).

During the TDM review only an interim storage stability study was available for triazole lactic acid (TLA). To support the duration of sample storage in studies considered for BAS 750 F, the full study for TLA has been submitted, and is evaluated in section B.7.1.2. This study demonstrates that TLA is stable in wheat grain, navy bean, orange, canola seed, and lettuce matrices for at least 48 months when stored under deep frozen conditions. As at least one crop has been considered in all five crop groups, it can be considered that sufficient data is available to support the storage stability of TLA in all plant commodities for at least 48 months.

Table 7.1.3-1: Stability of Triazole Metabolites in Crop and Animal Commodities Following Freezer Storage

Commodities[1]	Crop	Commodity	Nominal period of stability demonstrated (months)			
			1,2,4-Triazole	Triazole alanine	Triazole acetic acid	Triazole lactic acid
Crops – high starch	Wheat	Grain	≥ 54	≥ 54	≥ 26	≥ 12
		Straw[2]	40	≥ 54	40	--
		Flour	≥ 54	≥ 54	≥ 54	--
		Bran[2]	NC[3]	≥ 54	40	--
	Barley	Grain	--	≥ 36	36	--
		Straw[2]	--	≥ 36	≥ 36	--
	Turnip	Root	40	≥ 54	≥ 54	--
	Sugar beet	Root	--	≥ 15	≥ 25	--
	Radish	Root	12	≥ 26	≥ 26	--
Crops – high oil	Oilseed rape	Seed	NC[3]	≥ 15	≥ 54	≥ 12
		Oil	≥ 54	NC[3]	≥ 54	--
		Meal[2]	≥ 54	≥ 54	≥ 54	--
	Soybean	Seed	12	≥ 26	≥ 26	--
	Peanut	Butter	≥ 12	≥ 12	≥ 12	--
Crops – high acid	Orange	Fruit	--	--	--	≥ 12
Crops – high water	Wheat	Forage	NC[3]	≥ 54	≥ 54	--
	Mustard	Leaves	NC[3]	≥ 54	≥ 54	--
	Tomato	Fruit	40	≥ 54	≥ 54	--
		Paste	≥ 54	≥ 54	≥ 54	--
	Apple	Fruit	≥ 12	≥ 12	≥ 12	--
	Cabbage	Head	--	≥ 15	≥ 24	--
	Radish	Tops	≥ 26	≥ 26	12	--
	Lettuce	Lettuce	--	--	--	≥ 12
Crops – high protein	Pea	Dry Seed	--	≥ 15	≥ 25	--
	Navy Bean	Dry Bean	--	--	--	≥ 12
Animal	na	Milk	≥ 18	≥ 12	≥ 12	--
	na	Liver	≥ 12	--	--	--
	na	Muscle	≥ 12	--	--	--
	na	Fat	≥ 12	--	--	--
	na	Eggs	≥ 12	≥ 12	≥ 12	--

[1] Crop commodities according to the categories described in OECD guideline 506.
[2] Commodities not included in the crop categories described in OECD guideline 506.
[3] Not conclusive : for these compounds / commodities the study suggested a noticeable degradation of residues upon storage but it is unclear whether this is attributable to the study design or demotes a real stability issue.

B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

The BAS 750 F molecule is a three ring structure, as shown in Figure 7.2-1. It consists of the triazole ring (T-ring) characteristic for the group of azoles, the chlorophenyl-ring (C-ring) at the opposite side of the molecular backbone as well as the trifluoromethylphenyl-ring (TFMP-ring) in between.

The test items were a mixture of ^{14}C-BAS 750 F, ^{13}C-BAS 750 F and unlabelled BAS 750 F. The molecular structures and the positions of the labels are shown in Figure. 7.2-2.

The metabolism and distribution of BAS 750 F in plants was investigated using the active substance radiolabelled in the Chlorophenyl ring (C-label) or in the 3(5)-position of the Triazole ring (T-label). This labelling positions are considered appropriate to provide sufficient information, as discussed in section B.7.2.1.5. These studies on wheat and soybean were undertaken in green house conditions (or similar). It should be noted that BAS 750 F demonstrates limited absorbance at wavelengths >290 nm, therefore there is some minor potential for photolytic degradation (see section CA B.2.4). However, the large majority of absorbance is at wavelengths <290 nm, therefore it is not considered that photolytic degradation will have any significant effect on BAS 750 F when used in the field, and hence studies under glass are considered relevant to the use of this active substance in the field. This is confirmed by the study on grape which was conducted outdoors.

The metabolism and distribution of BAS 750 F in animals was investigated using the active substance radiolabelled in the chlorophenyl-ring (C-ring), the trifluoromethylphenyl-ring (TFMP-label) or in the 3(5)-position of the Triazole ring (T-label).

In all metabolism studies, the levels extracted, characterised, identified and unextracted residues were determined using different subsamples, as such, the total radioactive residues (TRR) do not in most cases add up to exactly 100%. This is due to the experimental procedure, rather than any error in the calculation, and does not impact adversely upon the study outcomes

BAS Code:	**BAS 750 F**
Registry No.:	5834378
CAS No.:	1417782-03-6
Chemical name (IUPAC):	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol

Figure 7.2-1: Structure of non-radiolabelled BAS 750 F

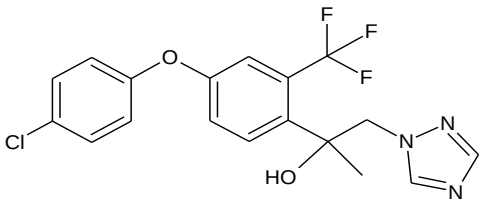

Figure 7.2-2: Structures of radiolabelled BAS 750 F

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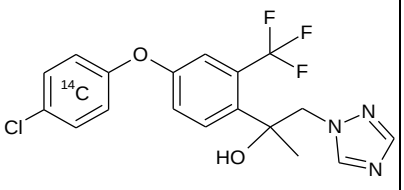
<td> 
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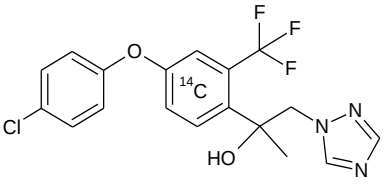
<td> 
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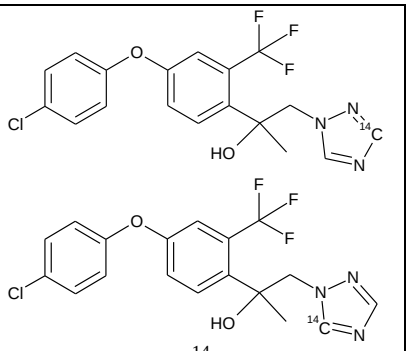
<td> 
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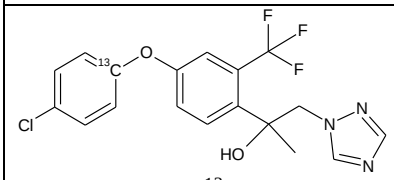
<td> 
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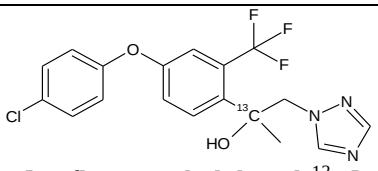
<td> 
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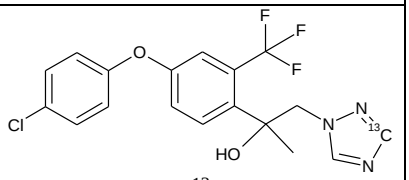
BAS 750 F</td>
<td> 
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B.7.2.1. Plants

B.7.2.1.1. Wheat

Report:	CA 6.2.1/1 Rabe U.,Bogen C., 2015 a Metabolism of 14C LS 5834378 in wheat 2015/1001872
Guidelines:	EPA 860.1000, EPA 860.1300: Nature of the Residue in Plants Livestock, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants, JMAFF 59 NohSan No 4200, Test No. 501: Metabolism in crops
GLP:	yes

Materials and methods

Materials

1. C-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Chlorophenyl-U-C14 (spec. activity 7.88 MBq/mg) added to a 1:1 (w:w) mixture of Chlorophenyl-1-C13-labelled test item	
Lot/Batch #:	Chlorophenyl-U-C14:	CFQ41561
	Chlorophenyl-1-C13:	RS4-2012-173A2
Purity:	Chlorophenyl-U-C14:	99.1% (radiochem 98.9%)
	Chlorophenyl-1-C13:	97.7%

2. T-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Triazole-3(5)-C14 (spec. activity 5.46 MBq/mg) added to 2:1 (w:w) mix of Triazole-3(5)-C13-labelled test item	
Lot/Batch #:	Triazole-3(5)-C14:	1062-2001
	Triazole-3(5)-C13:	1077-1001
Purity:	Triazole-3(5)-C14:	98.8% (radiochem 98.8%)
	Triazole-3(5)-C13:	97.1%

Methods

A metabolism study on spring wheat (variety *Thassos*) grown indoors in Limburgerhof, Germany was carried out in 2013-2015. Spring wheat plants were cultivated using normal agricultural practices in twenty containers (2.4 m^2) with sandy loam soil. Containers were initially located in a vegetation hall/greenhouse prior to transfer to climatic chambers (phytotrons) for application of the BAS 750F.

Two foliar spray applications (21 day interval) of either triazole or chlorophenyl labelled BAS 750F were made to ten containers per label. The structural formulae of the labelled BAS 750F molecules are given in Figure 7.2-2.

For the each application, tank mixes of the test items (taken up in blank EC formulation and water) were prepared. Applications were made at a target rate of 150 g a.s./ha (1N for cereal crops) per application at BBCH49 and 69 (timings in line with the proposed GAP) . A summary of the applications in the study are given in Table 7.2.1.1-1.

Table 7.2.1.1-1: Study design: plant uptake part (wheat)

Label	C-label		T-label	
intended use rate [g a.s./ha]	150		150	
application number	2		2	
application interval [days]	21		21	
application growth stages	BBCH49, BBCH69		BBCH49, BBCH69	
sampled matrices	forage, grain, straw		forage, grain, straw	
sampling [DALA] 1)	forage	-6 (=15 DAT) 2)	forage	-6 (=15 DAT) 2)
	grain	35	grain	35
	straw	35	straw	35

1) days after last application, 2) only one application: 15 days after the first application (= 15 DAT) corresponding to 6 days prior to last application (DALA=-6)

Samples of forage (BBCH61) were taken 15 days after the first application (corresponding to 6 days prior to the second application). Samples of grain and straw (both BBCH 89) were collected 35 days after the last application. Samples of straw were cut with scissors and the straw was minced prior to separation of the ears in to chaff and grain using a thresher and the chaff mixed with straw. Samples were then stored in a freezer at ≤-18°C. The maximum time of frozen storage between sampling and analysis was 281 days (extraction to analysis up to 197 days). Stability data to support this duration of storage is presented in the results section.

For TRR determination and the measurement of solid residues following solvent extraction (RRR) or solubilisation procedures (final residue), homogenized subsamples were combusted using a sample oxidizer. The resultant ^{14}C-CO_2 was absorbed, mixed with scintillation fluid and radioactivity was determined by liquid scintillation counting (LSC). For liquid samples scintillation fluid was added, and subjected to LSC measurement.

Prior to solvent extraction plant samples (forage, grain and straw) were homogenized. Forage and straw were then extracted with methanol (3x) and water (2x). After each extraction step, solid material was separated from extract by centrifugation and filtration, and the supernatants of methanol and water extracts were each combined.

Grain was extracted three times with a mixture of acetonitrile and isohexane (1:1). After a centrifugation step, the acetonitrile and isohexane phase were separated using a separatory funnel. Obtained acetonitrile and isohexane phases were combined, respectively. Acetonitrile/isohexane extraction was followed by extraction with water (two times). The solid material was separated from the aqueous extract by centrifugation and filtration. The residue after solvent extraction was dried in a fume hood, homogenized and radio assayed.

The aqueous extract was concentrated to dryness, subsequently taken up in water and adjusted to pH 4 with formic acid. For protein precipitation, acetone was added, the sample incubated in a refrigerator followed by centrifugation to obtain the precipitate and supernatant for further analysis.

Solubilisation steps were performed for forage, straw and grain (both labels). For forage and straw, a four step treatment was carried out, with macerozymes, α amylase/β amylase/amyloglucosidase, glucosidase/hesperidinase and then laccase/tyrosinase. During each treatment the sample was incubated for 1-3 days, and post treatment acetonitrile was added prior to centrifuging and filtering.

For grain, a two step treatment was carried out, with protease and then α amylase/β amylase/amyloglucosidase. During each treatment the sample was incubated for 1-2 days, and post treatment acetonitrile was added prior to centrifuging and filtering.

Components of the residue were identified by HPLC-MS as well as by co-chromatography and comparison of retention times. In addition, for the parent BAS 750 F enantiomer-specific HPLC analyses were performed in samples of the application solution, as well as extracts of forage and straw (samples purified by SPE and HPLC fractionation).

Results and discussion

Total radioactive residue

The calculated total radioactive residues (TRR) with the C-label were highest in straw (DALA 35) at 24.38 mg/kg, lower in forage at 2.38 mg/kg, and lowest in grain with 0.062 mg/kg.

A similar distribution was seen with the T-label (TRR highest in straw: 13.98 mg/kg, lower in forage at 2.31 mg/kg, and grain at 0.62 mg/kg). Notably the TRR in grain was much higher with the T-label compared with the C-label, indicating a difference in composition of the detectable residue. This is discussed further below. A summary of the TRRs are presented in Table 7.2.1.1-2.

Table 7.2.1.1-2: Total radioactive residue after foliar spray application of BAS 750 F

Matrix [BBCH]	**DALA** *1)*	**TRR measured (LSC)** *2) [mg/kg]*	**TRR calculated** *3) [mg/kg]*
C-label			
forage [61]	-6 (=15 DAT) *4)*	2.472	2.378
grain [89]	35	0.065	0.062
straw [89]	35	24.305	24.380
T-label			
forage [61]	-6 (=15 DAT) *4)*	2.634	2.310
grain [89]	35	0.619	0.620
straw [89]	35	14.339	13.984

1) days after last application, 2) TRR measured directly via combustion LSC, 3) TRR calculated as the sum of ERR(extractable radioactive residue) and RRR (residual radioactive residue)after extraction of the residues 4) 15 days after the first application (=15DAT) corresponding to 6 days prior to last application (DALA= -6)

Extractability

The extractabilities of ^{14}C residues from wheat forage, straw and grain are summarized in Table 7.2.1.1-3 and Table 7.2.1.1-4.

High extractability of ^{14}C residue was seen in forage (>95% TRR for total extract for both labels) and straw (>83% TRR for total extract for both labels). The majority of the radioactivity was extracted with methanol (>73% TRR) while with subsequent water extraction resulted in additional extraction of <10% TRR. Solvent extraction left a RRR (residual radioactive residue) in forage of <5% TRR, while the RRR in straw amounted to 17% TRR (4.14 mg/kg, C-label) and 13.6 % TRR (1.9 mg/kg, T-label). The RRR was therefore further investigated by enzyme treatment as discussed below. No significant label specific differences were seen for forage and straw.

Extractability in grain was significantly different for both labels, indicative of different composition of the radioactive residue. For the C-label 43.9 % TRR was extractable, 24.8% TRR of which by water extraction and 17.4% TRR by acetonitrile. In contrast, for the T-label 77.9% TRR was extracted with the majority (74.2%) released by water extraction, acetonitrile had removed only 3.6% TRR.

The RRR after solvent extraction amounted to 56.1% TRR (C-label, 0.035 mg/kg) and 22.1% TRR (T-label, 0.137 mg/kg) and thus was subject to further investigation by enzyme treatment.

Table 7.2.1.1-3: Extractability of radioactive residue from forage and straw

Matrix	DALA [1]	TRR [2]	distribution of radioactive residues							
			methanol extracts [3]		water extracts [3]		ERR [2]		RRR [2]	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
C-label										
forage	-6 (=15DAT)	*2.378*	**94.0**	*2.236*	**1.2**	*0.029*	**95.2**	*2.264*	**4.8**	*0.114*
straw	35	*24.38*	**73.8**	*17.99*	**9.3**	*2.255*	**83.0**	*20.24*	**17.0**	*4.139*
T-label										
forage	-6 (=15DAT)	*2.310*	**94.7**	*2.188*	**1.3**	*0.030*	**96.0**	*2.218*	**4.0**	*0.092*
straw	35	*13.98*	**77.7**	*10.87*	**8.7**	*1.213*	**86.4**	*12.08*	**13.6**	*1.901*

[1] days after last application, for forage sampling was 15 days after the first application (DAT=15) corresponding to 6 days prior to last application (DALA=-6). [2] TRR was calculated as the sum of ERR and RRR with ERR=Extractable Radioactive Residue, RRR=Residual Radioactive Residue (after solvent extraction) [3] pool of combined repetitive extracts

Table 7.2.1.1-4: Extractability of radioactive residue from grain

Matrix	DALA[1]	TRR[2]	distribution of radioactive residues									
			acetonitrile phases[3]		isohexane phases [3]		water extracts [4]		ERR[2]		RRR[2]	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
C-label												
grain	35	*0.062*	**17.4**	*0.011*	**1.7**	*0.001*	**24.8**	*0.015*	**43.9**	*0.027*	**56.1**	*0.035*
T-label												
grain	35	*0.620*	**3.6**	*0.022*	**0.2**	*0.001*	**74.2**	*0.460*	**77.9**	*0.483*	**22.1**	*0.137*

[1] days after last application. [2] TRR was calculated as the sum of ERR and RRR with. ERR=Extractable Radioactive Residue, RRR=Residual Radioactive Residue (after solvent extraction), [3] separated phases of acetonitrile/isohexane extracts, [4] pool of combined repetitive extracts

The results of the ammonia incubations and enzyme solubilisations of the residue after solvent extraction are summarized in Table 7.2.1.1-5. Generally, low amounts of radioactive residues were released from forage and straw (both labels) by ammonia and enzyme incubations, which accounted in sum for up to 8.2 % TRR, respectively. These residues are likely to be associated into the cell structure (e.g. starch or lignin associated).

For C-labelled wheat grain, 0.026 mg/kg (41.6% TRR) were released by ammonia and amylase/amyloglucosidase, the largest portion of which was released by incubation with amylase /amyloglucosidase (24.5 % TRR), which indicates the presence of starch-associated radioactive residues.

For T-labelled wheat grain, 0.123 mg/kg (19.8 % TRR) were released by ammonia and amylase incubation, the largest portion of which was released by incubation with ammonia (15.3 % TRR).

Table 7.2.1.1-5: Summary of solubilised components in wheat

Distribution of radioactive residues	C-Label						T-Label					
	forage		grain		straw		forage		grain		straw	
	%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR	**100**	**2.378**	**100**	**0.062**	**100**	**24.38**	**100**	**2.310**	**100**	**0.620**	**100**	**13.98**
RRR	**4.8**	**0.114**	**56.1**	**0.035**	**17.0**	**4.139**	**4.0**	**0.092**	**22.1**	**0.137**	**13.6**	**1.901**
Ammonia	1.0	0.023	17.1	0.011	4.2	1.026	0.7	0.017	15.3	0.095	3.6	0.506
Macerozyme	0.5	0.012	-	-	1.7	0.421	0.5	0.010	-	-	1.3	0.184
Amylase/ amyloglucosidase	0.3	0.008	24.5	0.015	0.8	0.198	0.3	0.006	4.6	0.028	0.7	0.098
Glucosidase/ hesperidinase	0.3	0.007	-	-	0.7	0.167	0.2	0.005	-	-	1.1	0.148
Laccase/ tyrosinase	0.3	0.008	-	-	0.8	0.191	0.3	0.006	-	-	0.5	0.070
Sum solubilised	**2.4**	**0.057**	**41.6**	**0.026**	**8.2**	**2.003**	**1.9**	**0.045**	**19.8**	**0.123**	**7.2**	**1.007**
Unextractable	**2.0**	**0.047**	**15.4**	**0.010**	**7.9**	**1.924**	**1.9**	**0.043**	**1.9**	**0.012**	**5.8**	**0.804**

Characterisation and Identification

The parent compound BAS 750 F and the metabolites in wheat straw, forage and grain were identified by HPLC-MS analysis. An overview over the components of the extractable residue is given below in Table 7.2.1.1-7. Structures of the metabolites are outlined in Appendix 1.

Chiral analysis of both forage and straw samples (C-label and T-label) confirmed that the racemic mixture (1:1 ratio of S-enantiomer and R-enantiomer) of the application formulation is essentially maintained, and hence that there is no significant change in BAS 750 F enantiomers. Chiral analysis was not conducted for grain since BAS 750 F was not present in quantifiable amounts. Details of the isomer ratio are given in Table 7.2.1.1-6.

Table 7.2.1.1-6: Determination of isomer ratio of BAS 750 F in wheat matrices

Matrix	S-enantiomer [%]	R-enantiomer [%]
C-label		
application formulation	53.6	46.4
forage	47.5	52.5
straw	48.2	51.8
T-label		
application formulation	48.5	51.5
forage	49.0	51.0
straw	47.8	52.2

Forage

For forage, similar results were observed with both labels. Unchanged parent represented > 84% TRR (2.0 and 2.1 mg/kg for C- and T-label) and was the predominant component of the residue. The only other components identified were present in amounts of 2% TRR (0.05 mg/kg) or less and were structurally related to the parent (sugar conjugates of the parent molecule and a hydroxylated parent molecule).

For C-label and T-label, the malonylglucosyl-O-conjugate of BAS 750 F metabolite M750F012, (including M750F021) accounted for <2% (0.05 mg/kg), M750F018/M750F020 (malonylglucosyl-O-conjugate of BAS 750 F and hydroxylated parent) accounted for 1.6% (0.037 mg/kg), and M750F019 (glucosyl-O-conjugate of hydroxylated parent) accounted for 0.1% (0.003 mg/kg).

The RRR after solvent extraction (4.8% and 4.0% TRR (0.11 mg/kg and 0.09 mg/kg) was treated sequentially with ammonia and various enzymes allowing a solubilisation of 2.4% and 1.9 % TRR corresponding to approximately 0.05 mg/kg (per treatment at maximum 1% TRR were solubilised).

In total, for C-label and T-label, identification amounted to 88% and 92% of TRR, considering also amounts characterized by solubilisation and HPLC an amount of 93.5% and 94.7% TRR were identified/characterized, leaving the final unextracted residue at <2% TRR (<0.05 mg/kg).

Straw

For straw, similar results were observed with both labels, although a minor difference was seen for metabolites M750F009/M750F010 (see below). Unchanged parent represented 58.6% and 68.5% TRR (14.3 and 9.6 mg/kg for C- and T-label) and was the predominant component of the residue, similar to forage. The only other components identified were present in amounts of 7% TRR (1.7 mg/kg) or less. Notably, for C-label and T-label,

- M750F018/F020 (a fraction including malonylglucosyl-O-conjugate of BAS 750 F and hydroxylated parent) accounted for 6.9% TRR (1.68 mg/kg) and 4.3% TRR (0.60 mg/kg)
- M750F019 (glucosyl-O-conjugate of hydroxylated parent) accounted for 5.8% (1.41 mg/kg) and 4.8% (0.67 mg/kg)
- M750F018 (malonylglucosyl-O-conjugate of hydroxylated parent) accounted for 2.9% (0.72 mg/kg) and 5.5% (0.77 mg/kg).
- M750F012 (malonylglucosyl-BAS 750 F) accounted for 5.5% TRR (1.34 mg/kg) and 3.5% TRR (0.48 mg/kg). (Note, that amounts of M750F021, a putative artefact are included).

Label-specific components were detected only in low amounts, namely the cleavage product M750F003 (non-quantifiable amounts), as well its conjugated forms M750F009 (glucosyl-O-conjugate) and M750F010 (malonylglucosyl-O-conjugate), both also at low amounts (1.3% TRR, 0.18 mg/kg). These metabolites result from cleavage at the ether bridge thus containing the T-label while the C-label is absent.

The RRR after solvent extraction (17.0% and 13.6% TRR (4.2 mg/kg and 1.9 mg/kg) was treated sequentially with ammonia and various enzymes allowing a solubilisation of 8.2% and 7.2% TRR (2.0 and 1.0 mg/kg). The solubilised fraction comprised 5-7 identifiable components, including parent. No individual component was present at >2% TRR (0.5 mg/kg) for the C-label and >2.1%TRR (0.29mg/kg) for the T-label.

In total, for C-label and T-label, identification amounted to 88% and 92% of TRR, considering also amounts characterized by solubilisation and HPLC an amount of 85.4% and 91.8% TRR were identified/characterized, leaving the final uncharacterised residue at 7.9% TRR (1.9 mg/kg) and 5.8% TRR (0.8 mg/kg).

Grain

For grain, unchanged parent BAS750F was not detected with the C-label nor the T-label. The radioactive residue detected with the C-label and with the T-label was significantly different both in quantity as well as in composition.

For the C-label the TRR was low (0.06 mg/kg), and 43.9 % (0.027 mg/kg) could be extracted, most of which was characterized by HPLC (42.4% TRR, 0.026 mg/kg) but not identified at the molecular level. The RRR of 56.1%TRR was further investigated allowing characterization of 41.6% TRR with

17% TRR (0.011 mg/kg) as releasable by ammonia, 24.5 % TRR (0.015 mg/kg) as releasable by amylase/amyloglucosidase treatment. Thus, the final uncharacterised residue was reduced to 0.010 mg/kg (15% TRR). In total, most of the C-labelled residue in grain (84 % of 0.05 mg/kg) was characterized by solvent extraction or enzyme treatment.

In contrast, for the T-label the TRR was significantly higher (0.62 mg/kg). 78% of this could be extracted by solvents (see above). Two metabolites were present in major amounts, triazole alanine (TA, M750F029) at 45.6% TRR (0.282 mg/kg) and triazole acetic acid (TAA, M750F030) at 21.4% TRR (0.133 mg/kg). In addition, 1,2,4-triazole (M750F001) was identified albeit only at very low amounts (1.0% TRR, 0.006 mg/kg). Most of the RRR with 22.1% TRR (0.14 mg/kg) was further characterized (19.8% TRR, 0.123 mg/kg) with ammonia releasing 15.3% TRR (0.10 mg/kg) and amylase/amyloglucosidase releasing 4.6% (0.03 mg/kg). Thus, the final uncharacterised residue was reduced to 0.012 mg/kg (1.9% TRR). In total, most of the T-labelled residue in grain was identified (67.9% TRR), considering also amounts characterized by solubilisation and/or HPLC an amount of 93.1% TRR were identified/characterized.

The results from both labels taken together, indicate that most of the BAS 750 F residue in grain is carrying only the T-label (TA and TAA approximately 0.4 mg/kg) while the components carrying only the C-label (approximately 0.05 mg/kg) account for only a minor part of the total treatment related residue of BAS 750 F.

Table 7.2.1.1-7: Summary of identified/characterized components in wheat

Labelled radioactive component (min) [4] in ERR & RRR	C-label						T-label					
	forage		grain		straw		forage		grain		straw	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR	**100.0**	*2.378*	**100.0**	*0.062*	**100.0**	*24.380*	**100.0**	*2.310*	**100.0**	*0.620*	**100.0**	*13.984*
ERR	**95.2**	*2.264*	**43.9**	*0.027*	**83.0**	*20.241*	**96.0**	*2.218*	**77.9**	*0.483*	**86.4**	*12.083*
BAS 750 F	**84.4**	*2.007*	-	-	**58.6**	*14.297*	**89.3**	*2.062*	-	-	**68.5**	*9.573*
M750F001 (1,2,4-T)	-	-	-	-	-	-	-	-	**1.0**	*0.006*	-	-
M750F029 (TA)	-	-	-	-	-	-	-	-	**45.6**	*0.282*	-	-
M750F030 (TAA)	-	-	-	-	-	-	-	-	**21.4**	*0.133*	-	-
M750F009	-	-	-	-	-	-	-	-	-	-	**1.3**	*0.178*
M750F010	-	-	-	-	-	-	-	-	-	-	**1.3**	*0.180*
M750F018 (20.3)	-	-	-	-	**2.9**	*0.716*	-	-	-	-	**5.5**	*0.767*
M750F019 (20.9, 20.5, 21.9)	**0.1**	*0.003*	-	-	**5.8**	*1.407*	-	-	-	-	**4.8**	*0.671*
M750F018/ M750F020 (22.5)	**1.6**	*0.037*	-	-	**6.9**	*1.682*	**1.6**	*0.037*	-	-	**4.3**	*0.603*
M750F012/ M750F021 (25.6)	**2.0**	*0.049*	-	-	**4.9**	*1.184*	**1.1**	*0.025*	-	-	**3.4**	*0.471*
M750F012 (26.9)	-	-	-	-	**0.6**	*0.158*	-	-	-	-	**0.1**	*0.008*
ID [1]	**88.1**	*2.096*	-	-	**75.2**	*18.344*	**92.0**	*2.125*	**67.9**	*0.421*	**83.5**	*11.677*
CHAR [1]	**2.9**	*0.070*	**42.4**	*0.026*	**1.9**	*0.468*	**0.8**	*0.018*	**5.4**	*0.033*	**1.1**	*0.151*
sum of ID/CHAR [1]	**91.1**	*2.166*	**42.4**	*0.026*	**77.2**	*18.812*	**92.8**	*2.142*	**73.3**	*0.454*	**84.6**	*11.828*
RRR	**4.8**	*0.114*	**56.1**	*0.035*	**17.0**	*4.139*	**4.0**	*0.092*	**22.1**	*0.137*	**13.6**	*1.901*
BAS 750 F	-	-	-	-	**2.0**	*0.500*	-	-	-	-	**1.2**	*0.173*
M750F009	-	-	-	-	-	-	-	-	-	-	**0.4**	*0.061*
M750F018 (20.3)	-	-	-	-	**0.4**	*0.099*	-	-	-	-	**2.1**	*0.294*
M750F019 (20.9, 21.5, 21.9)	-	-	-	-	**1.4**	*0.330*	-	-	-	-	**1.2**	*0.164*
M750F012/ M750F021 (25.6)	-	-	-	-	**0.7**	*0.172*	-	-	-	-	**0.5**	*0.072*
M750F012 (26.9)	-	-	-	-	-	-	-	-	-	-	**0.1**	*0.008*
ID [1]	-	-	-	-	**4.5**	*1.101*	-	-	-	-	**5.5**	*0.772*
CHAR [1]	**2.4**	*0.057*	**41.6**	*0.026*	**3.7**	*0.901*	**1.9**	*0.045*	**19.8**	*0.123*	**1.7**	*0.235*
sum of ID/CHAR [1]	**2.4**	*0.057*	**41.6**	*0.026*	**8.2**	*2.003*	**1.9**	*0.045*	**19.8**	*0.123*	**7.2**	*1.007*
SUM ID/CHAR in ERR/RRR [1]	**93.5**	*2.224*	**83.9**	*0.052*	**85.4**	*20.814*	**94.7**	*2.187*	**93.1**	*0.577*	**91.8**	*12.835*
Uncharacterised residue [2]	**2.0**	*0.047*	**15.4**	*0.010*	**7.9**	*1.924*	**1.9**	*0.043*	**1.9**	*0.012*	**5.8**	*0.804*
Total [3]	**95.5**	*2.271*	**99.3**	*0.061*	**93.3**	***22.738***	**96.5**	***2.230***	**95.0**	***0.589***	**97.5**	***13.639***

[1] ID=amount identified, CHAR=amount characterized (information on number and quantities of peaks provided in study report), sum ID/CHAR= sum of amounts identified and/or characterized, sum ID/CHAR in ERR/RRR= sum of amounts identified and/or characterized in ERR and in RRR, [2] final residue after solvent extraction and solubilisation [3] sum of amounts characterized and identified as well as final residue, [4] Retention times are provided in brackets, M750F019 eluted in three distinct peaks, the peak at 22.5 min and the peak at 25.6 min each did contain two compounds. Note M750F021 is considered an artefact.

Storage stability

Analysis of the storage stability confirmed the stability of radioactive residues over the period of the study, both in the frozen matrix (prior to extraction) and in extracts. Details of the storage periods are given in Table 7.2.1.1-8.

Stability during storage of matrix at ≤-18°C was investigated in C- and T-labelled wheat matrices, by comparing the extractability as well as the resulting metabolic HPLC profiles after extended storage of the plant sample. Methanol and aqueous extracts of the matrices were analysed. The stability of C and T-labelled BAS 750 F in forage was demonstrated for up to 539 days.

Stability during storage of extract was investigated by comparing the metabolic HPLC profiles after extended storage of the extract. Methanol and aqueous extracts were analysed. The stability of methanol and aqueous forage extract was demonstrated for up to 540 and 595 days respectively for both labels. The stability of aqueous extracts in grain was demonstrated for 13 and 62 days for C-label and T-labelled BAS 750 F respectively, and stability in straw in methanol and aqueous extracts was demonstrated for 195/197 days for both labels.

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the triazole label, the residues were stable for a period of at least 528 days (concentrated methanol extract) and 559 days (concentrated water extract). For the chlorophenyl label, the residues were stable for a period of at least 345 days (concentrated methanol extract) and 559 days (concentrated water extract). The storage stability of residues in the corresponding homogenized samples of forage (stability in matrix) was confirmed for a period of 539 days (sampling to extraction period, both labels).

Table 7.2.1.1-8: Storage intervals of plant samples and extract samples (spring wheat)

Matrix	Storage of matrix			Storage of extract		
	storage interval (analysis 1) [1)]	*storage interval (analysis 2)* [1)]	*Storage period*	*storage interval (analysis 1)* [2)]	*storage interval (analysis 2)* [2)]	*Storage period*
	[days]	*[days]*	*[days]*	*[days]*	*[days]*	*[days]*
C-label						
forage (methanol)	85	624	539	195	540	345
forage (water)	85	624	539	36	595	559
grain (water)	183	- [3)]	-	13	- [3)]	-
straw (methanol)	43	- [3)]	-	197	- [3)]	-
straw (water)	43	- [3)]	-	195	- [3)]	-
T-label						
forage (methanol)	84	623	539	12	540	528
forage (water)	84	623	539	36	595	559
grain (water)	182	- [3)]	-	62	- [3)]	-
straw (methanol)	42	- [3)]	-	197	- [3)]	-
straw (water)	42	- [3)]	-	195	- [3)]	-

1) sampling to extraction, 2) extraction to analysis, 3) not analysed

Translocation and proposed metabolic pathway

The unchanged parent BAS 750 F represents the predominant part of radioactive residues in the directly exposed plant parts forage and straw (> 58 % TRR). In contrast, in wheat grain which was not present during the time of application BAS 750 F was not detected. In conclusion, BAS 750 F is not translocated from treated leaves into the cereal grain.

Metabolism was investigated in foliar treated wheat using C- and T-labelled BAS 750 F. When the results from both labels are considered together the data demonstrate consistent metabolic pathways in wheat forage, straw and grain. The proposed metabolic pathway is outlined in Figure 7.2.1.1-1.

For grain, parent BAS 750 F was not detected, the main route of degradation was cleavage of the triazole from the parent compound, with subsequent conjugations forming the metabolites triazole alanine and triazole acetic acid. The TRR level in T-labelled grain is significantly higher than the TRR in C-labelled grain indicating translocation of TDMs into the grain.

Parent BAS 750F was the predominant compound in forage and straw. Metabolic conversion of this compound is by three main reactions.

Initial hydroxylation of the chlorophenyl/propyl-triazole moiety and a subsequent conjugation with glucose, at either the hydroxyl group of the chlorophenyl moiety or at the hydroxyl group of the propyl-triazole moiety generates metabolite M750F019. The exact position of the hydroxyl group in the chlorophenyl/ propyl-triazole moiety is undetermined, therefore the structure of M750F019 is dependent on this. An example structure is given in the figure. Malonylation of the glucose moiety of M750F019 results in metabolite M750F020. Additional hydroxylation of the chlorophenyl ring results in the formation of M750F018. Again these structures are dependent on the initial hydroxylation site. The metabolite M750F021 is generated by conjugation of the hydroxylated parent compound with acetate and likely represents an artefact of sample processing.

Conjugation of the hydroxyl group of the propyl-triazole moiety of BAS 750 F with glucose generates metabolite M750F011. Subsequent malonylation of this compound forms metabolite M750F012. Alternatively, the glucose moiety of M750F011 can be conjugated with another glucose molecule to form metabolite M750F013. An additional conjugation of the disaccharide moiety of M750F013 with malonyl results in the formation of metabolite M750F014.

To a lesser extent, cleavage of the chlorophenyl moiety from the parent compound results in metabolite M750F003, which was only detected in trace amounts by MS. The metabolite M750F003 can be subsequently conjugated with glucose to form metabolite M750F009, which accounted for 1.3 % TRR. The site of conjugation (either at the hydroxyl group of the propyl-triazole moiety or the hydroxyl group of the trifluoromethyl-phenyl moiety) is undetermined. Therefore, a generic structure is provided. Malonylation of the glucose moiety of M750F009 results in metabolite M750F010, which is also provided as generic structure.

In Figure 7.2.1.1-1, for some of the metabolites generic structures are provided in cases when exact position of hydroxyl group or sugar moiety is not known (indicated by a “dotted” line).

Conclusion

The metabolism of BAS 750 F was investigated in wheat by applying C-labelled or T-labelled BAS 750 F. The overall residue levels (TRR) in the C-labelled forage, grain and straw were 2.47, 0.065 and 24.3 mg/kg respectively. For T-labelled forage, grain and straw the levels were 2.63, 0.619 and 14.3 mg/kg. Hence, TRR for forage and straw were similar, but a 10 fold difference was observed for grain.

For both labels, solvent extractability (ERR) was high for forage and straw (at least 83% TRR), as well as T-labelled grain (78% TRR). For C-labelled grain, solvent extraction retrieved 44% of TRR, with enzyme solubilisation releasing a further 42 %. Enzyme solubilisation of the other commodities released 2-8% of the TRR. The final unextractable residue was between 2-15.4 %TRR (0.01-1.9 mg/kg) for the C label and between 1.9-5.8 7% TRR (0.012-0.804 mg/kg) for the T label. In

accordance with OECD 501, residues at these levels should be characterised as a minimum; which has not been achieved. However, significant attempts to characterise the residues up to this point have been made, and it is unlikely that any additional characterisation would affect the overall conclusions of the study. Therefore, as sufficient information is available to propose a residue definition for plants, the lack of further characterisation information is considered acceptable.

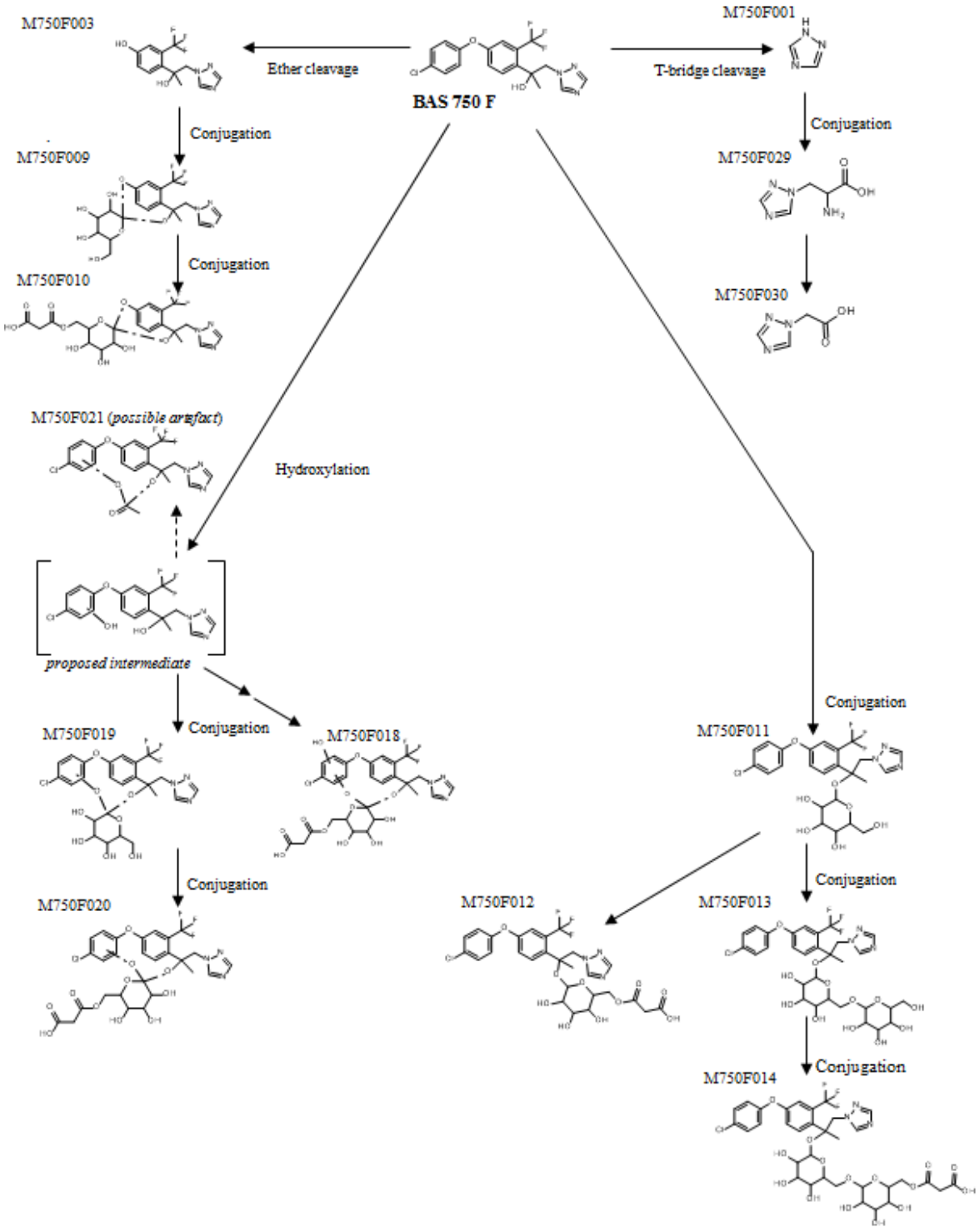
Metabolism of BAS 750 F includes hydroxylation of the parent backbone structure (C-ring) which introduces a second hydroxyl group which is a potential site for conjugation leading to an array of sugar conjugates. Cleavage of BAS 750 F at the T-bridge leads formation of triazole derivative metabolites (TDM) which are common to a range of azole fungicides. Cleavage at the ether bridge does occur, albeit only at minor amounts leading to the metabolites M750F009 and M750F010.

For both labels, unchanged parent BAS 750 F is the only predominant component of the residue in forage and straw representing at least 84% TRR in forage and at least 58% TRR in straw. A higher proportion of sugar conjugates is observed in straw (BBCH89) with <18% TRR compared with forage (BBCH61) with 3-4% TRR.

In contrast, unchanged parent is not detected in grain, where TDM account almost exclusively for the radioactive residue (accounting for 77% TRR).

Other components of the residue were sugar conjugates of parent (unchanged or hydroxylated) individually present <6% TRR as well as minor amounts of two metabolites resulting from ether cleavage (individually present at 1.3% TRR). Data obtained with C-label and T-label taken together; show a consistent picture of the metabolism in foliar applied wheat. Overall, metabolism of BAS 750 F in wheat, and by extrapolation, in the *cereal* crop group is considered well-elucidated.

Figure 7.2.1.1-1: Proposed pathway of BAS 750 F in spring wheat



B.7.2.1.2. Soybean

Report:	CA 6.2.1/2 Thiaener J.,Bogen C., 2015 a Metabolism of 14C-BAS 750 F in soybean 2014/1224012
Guidelines:	EPA 860.1000, EPA 860.1300, EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants, Test No. 501: Metabolism in crops
GLP:	yes

Materials and methods

Materials

1. C-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Chlorophenyl-U-C14 (spec. activity 7.88 MBq/mg) added to a 1:1 (w:w) mixture of Chlorophenyl-1-C13-labelled test item	
Lot/Batch #:	Chlorophenyl-U-C14:	CFQ41561
	Chlorophenyl-1-C13:	RS4-2012-173A2
Purity:	Chlorophenyl-U-C14:	99.1% (radiochem 98.9%)
	Chlorophenyl-1-C13:	97.7%

2. T-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Triazole-3(5)-C14 (spec. activity 5.46 MBq/mg) added to 2:1 (w:w) mix of Triazole-3(5)-C13-labelled test item	
Lot/Batch #:	Triazole-3(5)-C14:	1062-2001
	Triazole-3(5)-C13:	1077-1001
Purity:	Triazole-3(5)-C14:	98.8% (radiochem 98.8%)
	Triazole-3(5)-C13:	97.1%

Methods

A metabolism study on soybean (variety *Sultana*) grown indoors in Limburgerhof, Germany was carried out in 2013-2015. Soybean plants were cultivated using normal agricultural practices in twenty containers (2.4 m^2) with sandy loam soil. Containers were initially located in a vegetation hall prior to transfer to climatic chambers (phytotrons) for application of the BAS 750F.

Three foliar spray applications (18 day interval) of either triazole or chlorophenyl labelled BAS 750F were made to ten containers per label. The structural formulae of the labelled BAS 750F molecules are given in Figure 7.2-2.

For the each application, tank mixes of the test items (taken up in blank EC formulation and water) were prepared. Applications were made at a target rate of 125 g a.s./ha (0.83N with respect to cereal crops) per application at BBCH60/72/77. A summary of the applications in the study are given in Table 7.2.1.2-1.

Table 7.2.1.2-1: Study design: plant uptake part (soybean)

label	C-label		T-label	
intended use rate [g a.s./ha]	125		125	
application number	3		3	
application interval [days]	18±1		18±1	
application growth stages	BBCH60/BBCH72/BBCH77		BBCH60/BBCH72/BBCH77	
sampled matrices	forage, seed, hull, rest-of-plant, green pod		forage, seed, hull, rest-of-plant, green pod	
sampling timepoints [DALA] [1)]	forage	-17 (=19 DAT) [2)]	forage	-17 (=19 DAT) [2)]
	seed	47	seed	48
	hull	47	hull	48
	rest-of-plant	47	rest-of-plant	48
	green pod	47	green pod	48

[1)] days after last application, [2)] 19 days after the first application (19 DAT) corresponding to directly prior to the second application, and 17 days prior to last application (-17 DALA).

Analysis and Identification

Samples of forage (BBCH71/72) were taken 19 days after the first application (directly prior to the second application). Samples of seed, hull, green pod and rest-of-plant were collected at harvest growth stage (BBCH89), which was at 47 - 48 days after the last application. Samples were then stored in a freezer at ≤-18°C. The maximum time of frozen storage between sampling and extraction was 3.5 months forage, seed, pod, rest of plant and 8.5 months for hull. The maximum time between extraction and analysis was 12 months. Stability data to support storage for 11 months is presented below. No significant changes were observed during this time, therefore it is considered that these data demonstrate that acceptable stability for 12 months is expected.

For TRR determination and the measurement of solid residues following solvent extraction (RRR) or solubilisation procedures (final residue), homogenized subsamples were combusted using a sample oxidizer. The resultant ^{14}C-CO_2 was absorbed, mixed with scintillation fluid and radioactivity was determined by liquid scintillation counting (LSC). For liquid samples scintillation fluid was added, and subjected to LSC measurement.

Prior to solvent extraction plant samples (forage, rest-of-plant, hull, green pod and seed) were homogenized and soya bean forage and rest-of-plant were soaked in water for one hour. Forage, hull and rest of plant were then extracted with methanol (3x) and water (2x). After each extraction step, solid material was separated from extract by centrifugation and filtration, and the supernatants of methanol and water extracts were each combined. The methanol extracts were purified by SPE fractionation.

Seed and green pod were extracted three times with a mixture of acetonitrile and isohexane (1:1). After a centrifugation step, the acetonitrile and isohexane phase were separated using a separatory funnel. Obtained acetonitrile and isohexane phases were combined, respectively. Acetonitrile/isohexane extraction was followed by extraction with water (two times). The solid material was separated from the aqueous extract by centrifugation and filtration. The residue after solvent extraction was dried in a fume hood, homogenized and radio assayed.

Solubilisation steps were performed for all commodities except green pod (both labels). For forage hull, and rest of plant, a five step treatment was carried out, with macerozyme/cellulose, glucosidase/hesperidinase, amylase/amyloglucosidase, laccase/tyrosinase and protease. For seed, a four step process, omitting laccase/tyrosinase was undertaken. During each treatment the sample was incubated for 1-3 days, and post treatment extracts were purified by SPE (except hull). Solubilisation of green pod was not undertaken due to the low sample sizes collected.

Components of the residue were identified by HPLC-MS as well as by co-chromatography and comparison of retention times. In addition, for the parent BAS 750 F enantiomer-specific HPLC analyses were performed in samples of the application solution, as well as extracts of forage, rest of plant and hull. (samples purified by flash chromatography).

Results and discussion

Total radioactive residue

The calculated total radioactive residues (TRR) with the C-label were highest in rest of plant (DALA 47) at 16.46 mg/kg, lower in forage at 6.58 mg/kg, green pod (8.72 mg/kg) and hull (3.84 mg/kg) and lowest in seed with 0.129 mg/kg.

A similar distribution was seen with the T-label (TRR highest in rest of plant: 19.3 mg/kg, lower in forage at 4.61 mg/kg and hull at 4.12 mg/kg). Notably the TRR in seed and green pod was much higher with the T-label compared with the C-label (seed; 3.06 mg/kg and green pod 16.00 mg/kg), indicating a difference in composition of the detectable residue. This is discussed further below. A summary of the TRRs are presented in Table 7.2.1.2-2.

Table 7.2.1.2-2: Total radioactive residue after foliar spray application of BAS 750 F

Matrix [BBCH]		**DALA** [1)]	**TRR measured (LSC)** *[mg/kg]*[2)]	**TRR calculated** *[mg/kg]* [3)]
C-label				
forage	[71-72]	-17 (=19 DAT) [4)]	6.516	6.575
rest-of-plant	[89]	47	16.016	16.459
hull	[89]	47	3.735	3.838
green pod		47	8.857	8.721
seed	[89]	47	0.109	0.129
T-label				
forage	[71-72]	-17 (=19 DAT) [4)]	4.416	4.609
rest-of-plant	[89]	48	19.934	19.264
hull	[89]	48	3.890	4.122
green pod		48	16.005	16.006
seed	[89]	48	2.592	3.063

1) days after last application, 2) TRR measured directly via combustion LSC, 3) TRR calculated as the sum of ERR(extractable radioactive residue) and RRR (residual radioactive residue)after extraction of the residues, 4) 19 days after the first application (=19DAT) corresponding to 17 days prior to last application (=-17DALA).

Extractability

The extractabilities of ^{14}C residues from soybean forage, rest of plant, hull, green pod and seed are summarized in Table 7.2.1.2-3 and Table 7.2.1.2-4.

High extractability of ^{14}C residue was seen in forage (>91% TRR for both labels), rest of plant (>87% TRR), green pod (>78% TRR) and hulls (>68% TRR). The majority of the radioactivity was extracted with methanol (>66% TRR) while with subsequent water extraction resulted in additional extraction of <5% TRR. Solvent extraction left a RRR (residual radioactive residue) in forage of <9% TRR, rest of plant of <13% TRR, hull of <32 % TRR and green pod of <22% TRR. The RRR was therefore further investigated by enzyme treatment as discussed below. No significant label specific differences were seen for these commodities.

Extractability in seed was significantly different for both labels, indicative of different composition of the radioactive residue. For the C-label 56.6% TRR was extractable, 32.5% TRR of which by water extraction, 17.4% TRR by isohexane and 4% by acetonitrile. In contrast, for the T-label 75.6% TRR

was extracted with the majority (74.2%) released by water extraction, acetonitrile and isohexane had removed only 1.4% TRR. The RRR after solvent extraction amounted to 43.4% TRR (C-label, 0.056 mg/kg) and 24.4% TRR (T-label, 0.747 mg/kg) and thus was subject to further investigation by enzyme treatment.

Table 7.2.1.2-3: Extractability of radioactive residue from forage, rest-of-plant, hull

matrix	DALA[1)]	TRR [2)]	distribution of radioactive residues							
			methanol extracts [3)]		water extracts [3)]		ERR [2)]		RRR [2)]	
		mg/kg	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
C-label										
forage	-17 (=19 DAT)	*6.575*	**89.7**	*5.898*	**1.4**	*0.090*	**91.1**	*5.988*	**8.9**	*0.587*
plant [4)]	47	*16.46*	**83.4**	*13.73*	**3.7**	*0.601*	**87.1**	*14.33*	**12.9**	*2.126*
hull	47	*3.838*	**66.7**	*2.558*	**2.0**	*0.078*	**68.7**	*2.637*	**31.3**	*1.201*
T-label										
forage	-17 (=19 DAT)	*4.609*	**92.2**	*4.249*	**1.2**	*0.054*	**93.3**	*4.302*	**6.7**	*0.307*
plant [4)]	47	*19.26*	**83.9**	*16.17*	**3.9**	*0.757*	**87.8**	*16.92*	**12.2**	*2.342*
hull	47	*4.122*	**70.4**	*2.903*	**3.8**	*0.156*	**74.2**	*3.059*	**25.8**	*1.063*

[1)] days after last application, for forage sampling was 19 days after the first application (=19DAT) which was 17 days prior to last application (= -17DALA).[2)] TRR was calculated as the sum of ERR and RRR with. ERR= Extractable Radioactive Residue, RRR=Residual Radioactive Residue

Table 7.2.1.2-4: Extractability of radioactive residue from seed and green pod

matrix	DALA[1)]	TRR [2)]	distribution of radioactive residues									
			acetonitrile phase		isohexane phase		water extracts [3)]		ERR [2)]		RRR [2)]	
		mg/kg	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
C-label												
seed	47	*0.129*	**4.0**	*0.005*	**17.4**	*0.022*	**35.2**	*0.045*	**56.6**	*0.073*	**43.4**	*0.056*
green pod	47	*8.721*	**74.6**	*6.503*	**1.2**	*0.108*	**7.6**	*0.660*	**83.4**	*7.271*	**16.6**	*1.451*
T-label												
seed	48	*3.063*	**0.6**	*0.019*	**0.8**	*0.025*	**74.2**	*2.272*	**75.6**	*2.316*	**24.4**	*0.747*
green pod	48	*16.01*	**67.4**	*10.78*	**0.9**	*0.141*	**9.8**	*1.564*	**78.0**	*12.49*	**22.0**	*3.518*

[1)] days after last application, for forage sampling was 19 days after the first application (=19DAT) which was 17 days prior to last application (= -17DALA). [2)] TRR was calculated as the sum of ERR and RRR with. ERR= Extractable Radioactive Residue, RRR=Residual Radioactive Residue (after solvent extraction), [3)] pool of combined repetitive extracts

The results of the enzyme solubilisations of the residue after solvent extraction are summarized in Table 7.2.1.2-5. Generally, low amounts of radioactive residues were released from forage and rest of plant (both labels) by enzyme incubations, which accounted in sum for up to 8.3 % TRR, respectively. These residues are likely to be associated into the cell structure (e.g. starch or lignin associated).

For C-labelled seed, 0.049 mg/kg (32.8% TRR) were released by enzyme incubation, the largest portion of which was released by incubation with macerozyme/cellulase (15.5 % TRR), which indicates the presence of cellulose-associated radioactive residues.

For T-labelled seed, 0.824 mg/kg (26.9 % TRR) were released by ammonia and amylase incubation, the largest portion of which was released by incubation with macerozyme/cellulase (23.1 % TRR), which indicates the presence of cellulose-associated radioactive residues.

Table 7.2.1.2-5: Summary of solubilised components in soybean

Distribution of radioactive residues	C-Label								T-Label							
	Forage		Plant		Hull		Seed		Forage		Plant		Hull		Seed	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR	**100.0**	*6.58*	**100.0**	*16.46*	**100.0**	*3.84*	**100.0**	*0.129*	**100.0**	*4.61*	**100.0**	*19.26*	**100.0**	*4.12*	**100.0**	*3.06*
RRR	**8.9**	*0.587*	**12.9**	*2.126*	**31.3**	*1.201*	**43.4**	*0.056*	**6.7**	*0.307*	**12.2**	*2.342*	**25.8**	*1.063*	**24.4**	*0.747*
Macerozyme /cellulase	**1.1**	*0.075*	**2.1**	*0.352*	**9.0**	*0.346*	**15.5**	*0.020*	**0.7**	*0.033*	**1.8**	*0.340*	**11.0**	*0.452*	**23.1**	*0.706*
Amylase/ amyloglucosidase	**0.5**	*0.032*	**2.5**	*0.407*	**3.6**	*0.137*	**5.0**	*0.006*	**0.5**	*0.023*	**1.4**	*0.264*	**2.7**	*0.109*	**3.0**	*0.093*
Glucosidase/ hesperidinase	**0.4**	*0.026*	**1.0**	*0.170*	**2.8**	*0.106*	**4.0**	*0.005*	**0.3**	*0.016*	**0.8**	*0.148*	**2.6**	*0.108*	**0.5**	*0.016*
Laccase/ tyrosinase	**0.3**	*0.023*	**0.7**	*0.113*	**1.5**	*0.059*	**-**	**-**	**0.4**	*0.016*	**0.7**	*0.141*	**1.0**	*0.042*	**-**	**-**
Protease	**1.2**	*0.076*	**2.0**	*0.332*	**1.9**	*0.074*	**8.3**	*0.011*	**1.0**	*0.044*	**2.3**	*0.434*	**0.8**	*0.032*	**0.3**	*0.009*
Sum solubilised	**3.5**	*0.232*	**8.3**	*1.374*	**18.8**	*0.722*	**32.8**	*0.049*	**2.9**	*0.133*	**6.9**	*1.328*	**18.0**	*0.743*	**26.9**	*0.824*
Unextractable	**4.8**	*0.316*	**5.8**	*0.954*	**11.0**	*0.420*	**5.2**	*0.007*	**3.5**	*0.16*	**5.7**	*1.096*	**7.5**	*0.309*	**0.2**	*0.005*

Characterisation and Identification

The parent compound BAS 750 F and the metabolites in soybean matrices were identified by HPLC-MS analysis. An overview over the components of the extractable residue is given below in Tables 7.2.1.2-7 and 7.2.1.2-8. Structures of the metabolites are outlined in Appendix 1.

Chiral analysis of forage, hull and rest of plant samples (C-label and T-label) confirmed that the racemic mixture (1:1 ratio of S-enantiomer and R-enantiomer) of the application formulation is essentially maintained, and hence that there is no significant change in BAS 750 F enantiomers. Chiral analysis was not conducted for seed/green pod since BAS 750 F was not present in quantifiable amounts. Details of the isomer ratio are given in Table 7.2.1.2-6.

Table 7.2.1.2-6: Determination of isomer ratio of BAS 750 F in soybean matrices

matrix	S-enantiomer [%]	R-enantiomer [%]
C-label		
application formulation	50.5	49.5
forage	45.5	54.5
hull	45.6	54.4
rest-of-plant	46.4	53.4
T-label		
forage	51.3	48.7
hull	48.1	51.9
rest-of-plant	42.7	57.3

Table 7.2.1.2-7: Summary of identified/characterized components in soybean (C-label)

C-labelled radioactive component (min) [4] ERR & RRR	forage		rest-of-plant		hull		green pod		seed	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR	**100.0**	*6.58*	**100.0**	*16.46*	**100.0**	*3.84*	**100.0**	*8.72*	**100.0**	*0.129*
ERR	**91.1**	*5.988*	**87.1**	*14.333*	**68.7**	*2.637*	**83.4**	*7.271*	**56.6**	*0.073*
BAS 750 F	**77.2**	*5.078*	**54.0**	*8.886*	**67.0**	*2.572*	**68.5**	*5.978*	**4.0**	*0.005*
M750F018/M750F020	**1.9**	*0.123*	**4.5**	*0.748*	**< 0.1**	*0.001*	**3.9**	*0.338*	-	-
M750F012 (25.6)	**3.9**	*0.256*	**4.0**	*0.651*	**0.2**	*0.008*	**2.2**	*0.188*	-	-
M750F012 (26.9)	**< 0.1**	*0.003*	**2.0**	*0.321*	**0.2**	*0.006*	-	-	-	-
"region 1"	**0.3**	*0.023*	**4.1**	*0.678*	**0.5**	*0.020*	-	-	-	-
"region 2"	-	-	**5.0**	*0.830*	**0.3**	*0.012*	-	-	-	-
"peak 29.8"	**0.9**	*0.060*	**2.5**	*0.415*	**2.2**	*0.085*	-	-	-	-
"peak 33.9"	**0.5**	*0.032*	**2.3**	*0.379*	-	-	-	-	-	-
ID [1]	**82.9**	*5.454*	**64.0**	*10.531*	**67.4**	*2.587*	**74.6**	*6.503*	**4.0**	*0.005*
CHAR [1]	**1.8**	*0.116*	**14.7**	*2.425*	**1.3**	*0.051*	**8.8**	*0.767*	**48.5**	*0.063*
sum ID/CHAR	**84.7**	*5.570*	**78.7**	*12.955*	**68.7**	*2.638*	**83.4**	*7.271*	**52.5**	*0.068*
RRR	**8.9**	*0.587*	**12.9**	*2.126*	**31.3**	*1.201*	**16.6**	*1.451*	**43.4**	*0.056*
BAS 750 F	**2.7**	*0.179*	**5.8**	*0.962*	**15.8**	*0.607*	-	-	-	-
M750F018/-F020	**< 0.1**	*<0.001*	-	-	-	-	-	-	-	-
M750F012	**1.1**	*0.006*	**0.5**	*0.075*	-	-	-	-	-	-
"peak 29.8"	**0.1**	*0.008*	**0.5**	*0.088*	**2.0**	*0.078*	-	-	-	-
"peak 33.9"	**< 0.1**	*0.003*	**0.2**	*0.039*	-	-	-	-	-	-
other	**0.3**	*0.022*	**0.8**	*0.132*	**0.9**	*0.036*	-	-	**32.1**	*0.041*
ID [1]	**2.8**	*0.185*	**6.3**	*1.037*	**15.8**	*0.607*	-	-	-	-
CHAR [1]	**0.5**	*0.034*	**1.6**	*0.259*	**3.0**	*0.115*	-	-	**32.1**	*0.041*
sum ID/CHAR	**3.3**	*0.218*	**7.9**	*1.296*	**18.8**	*0.722*	-	-	**32.1**	*0.041*
SUM ID/CHAR in ERR/RRR [1]	**88.0**	*5.789*	**86.6**	*14.252*	**87.5**	*3.359*	**83.4**	*7.271*	**85.5**	*0.110*
Unextracted residue [2]	**4.8**	*0.316*	**5.8**	*0.954*	**11.0**	*0.420*	**16.6**	*1.451*	**5.2**	*0.007*
Grand Total [3]	**92.8**	***6.104***	**92.4**	***15.206***	**98.5**	***3.780***	***100.0***	***8.721***	**90.7**	***0.117***

[1] ID=amount identified, CHAR=amount characterized, sum ID/CHAR= sum of amounts identified and/or characterized, sum ID/CHAR in ERR/RRR= sum of amounts identified and/or characterized in ERR or in RRR
[2] final residue after solvent extraction and solubilisation, [3] Grand Total=sum of amounts characterized and identified as well as final residue, [4] Retention times are provided in parenthesis.

Table 7.2.1.2-8: Summary of identified/characterized components in soybean (T-label)

T-labelled radioactive component (min) [4] ERR & RRR	forage		rest-of-plant		hull		green pod[5]		seed	
	%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR	**100.0**	*4.61*	**100.0**	*19.26*	**100.0**	*4.12*	*100.0*	*16.01*	**100.0**	*3.06*
ERR	**93.3**	*4.302*	**87.8**	*16.922*	**74.2**	*3.059*	-	-	**75.6**	*2.316*
BAS 750 F	**76.6**	*3.53*	**66.0**	*12.706*	**70.2**	*2.893*	-	-	**0.4**	*0.013*
M750F001 (1,2,4-T)	-	-	-	-	-	-	-	-	**0.3**	*0.008*
M750F029 (TA)	-	-	-	-	-	-	-	-	**47.7**	*1.461*
M750F031 (TLA)	-	-	-	-	-	-	-	-	**1.3**	*0.040*
M750F018/M750F020	**2.1**	*0.096*	**3.8**	*0.735*	-	-	-	-	-	-
M750F012 (25.6)	**3.1**	*0.141*	**2.9**	*0.554*	**0.7**	*0.030*	-	-	-	-
M750F012 (26.9)	**0.5**	*0.022*	**0.2**	*0.043*	**0.5**	*0.022*	-	-	-	-
"region 1"	-	-	**2.7**	*0.513*	-	-	-	-	-	-
"region 2"	-	-	**2.4**	*0.455*	-	-	-	-	-	-
"peak 6.6 min"	-	-	-	-	-	-	-	-	**33.6**	*1.029*
"peak 9.1 min"	-	-	-	-	-	-	-	-	**0.3**	*0.008*
"peak 29.8 min"	**0.7**	*0.034*	**2.6**	*0.500*	**1.3**	*0.055*	-	-	-	-
"peak 33.9 min"	**0.7**	*0.032*	**1.1**	*0.220*	-	-	-	-	-	-
ID [1]	**82.2**	*3.788*	**72.7**	*14.003*	**70.2**	*2.893*	-	-	**33.0**	*1.012*
CHAR [1]	**2.3**	*0.106*	**9.2**	*1.781*	**3.5**	*0.145*	-	-	**35.7**	*1.095*
sum ID/CHAR[1]	**84.5**	*3.894*	**81.9**	*15.783*	**73.7**	*3.038*	-	-	**68.8**	*2.107*
RRR	**6.7**	*0.307*	**12.2**	*2.342*	**25.8**	*1.063*	-	-	**24.4**	*0.747*
BAS 750 F	**2.5**	*0.117*	**5.1**	*0.991*	**8.8**	*0.364*	-	-	-	-
M750F018/-F020	-	-	-	-	-	-	-	-	-	-
M750F012	**< 0.1**	*0.001*	**0.2**	*0.035*	**1.2**	*0.052*	-	-	-	-
M750F029	-	-	-	-	-	-	-	-	**16.7**	*0.510*
"peak 29.8"	**0.1**	*0.003*	**0.6**	*0.119*	**1.3**	*0.055*	-	-	-	-
"peak 33.9"	-	-	**0.2**	*0.03*	-	-	-	-	-	-
other	**0.1**	*0.005*	**0.6**	*0.101*	**6.6**	*0.271*	-	-	**8.3**	*0.254*
ID [1]	**2.6**	*0.118*	**5.3**	*1.026*	**10.1**	*0.417*	-	-	**16.7**	*0.510*
CHAR [1]	**0.2**	*0.007*	**1.3**	*0.251*	**7.9**	*0.326*	-	-	**8.3**	*0.254*
sum ID/CHAR[1]	**2.8**	*0.126*	**6.6**	*1.277*	**18.0**	*0.743*	-	-	**25.0**	*0.764*
SUM ID/CHAR in ERR/RRR[1]	**87.2**	*4.019*	**88.6**	*17.06*	**91.7**	*3.871*	-	-	**93.7**	*2.871*
Unextracted residue [2]	**3.5**	*0.160*	**5.7**	*1.096*	**7.5**	*0.309*	-	-	**0.2**	*0.005*
Grand Total [3]	**90.7**	*4.179*	**94.3**	*18.157*	**99.2**	*4.090*	-	-	**93.9**	*2.877*

[1] ID=amount identified, CHAR=amount characterized, sum ID/CHAR= sum of amounts identified and/or characterized, sum ID/CHAR in ERR/RRR= sum of amounts identified and/or characterized in ERR or in RRR
[2] final residue after solvent extraction and solubilisation, [3] Grand Total=sum of amounts characterized and identified as well as final residue, [4] Retention times are provided in parenthesis.[5] Not analysed

Forage

For forage, similar results were observed with both labels. Unchanged parent represented >79% TRR (5.3 and 3.6 mg/kg for C- and T-label) and was the predominant component of the residue (and only component present in major amounts). The only other components identified were present in amounts of <4% TRR (0.26 mg/kg) and were structurally related to the parent (sugar conjugates of the parent molecule and of a hydroxylated parent molecule).

For C-label and T-label, the malonylglucosyl-O-conjugate of BAS 750 F metabolite M750F012, accounted for 3.9% and 3.6% TRR (0.26 mg/kg and 0.16 mg/kg), M750F018/M750F020 (malonylglucosyl-O-conjugate of BAS 750 F and hydroxylated parent) accounted for 1.9% and 2.1% TRR (0.12 mg/kg and 0.10 mg/kg). In addition, several fractions of <1% TRR (<0.06 mg/kg) represented by peaks in HPLC chromatograms were observed.

The RRR after solvent extraction (8.9% TRR/0.59 mg/kg and 6.7% TRR/0.31 mg/kg) was treated sequentially with various enzymes allowing a solubilisation of 3.5% and 2.9% TRR corresponding to 0.23 mg/kg and 0.13 mg/kg (protease treatment solubilized 1.2 and 1.0% TRR, macerozyme/cellulase solubilized 1.1 and 0.7% TRR corresponding to amounts of 0.03 to 0.08 mg/kg). The solubilized residue consisted mainly of the components identified in the ERR - BAS 750 F and minor amounts of metabolites M750F018, M750F020 and M750F012.

In total, for C-label and T-label, identification amounted to 86% and 85% of TRR, considering also amounts characterized by solubilisation and HPLC an amount of 88% and 87% TRR were identified/characterized, leaving the final unextractable residue at 4.8% and 3.5%TRR (<0.32 mg/kg).

Rest of plant

For the rest of the plant, similar results were observed with both labels. Unchanged parent represented 59.8% and 71.1% TRR (9.8 and 13.7 mg/kg for C- and T-label) and was the predominant component of the residue (and only component present in major amounts). The only other components identified were present in amounts of <4.5% TRR (0.75 mg/kg) and were structurally related to the parent (sugar conjugates of the parent molecule and a hydroxylated parent molecule).

For C-label and T-label, the malonylglucosyl-O-conjugate of BAS 750 F metabolite M750F012 accounted for 6.0% and 3.1% TRR (0.97 mg/kg and 0.59 mg/kg), M750F018/M750F020 (malonylglucosyl-O-conjugate of BAS 750 F and hydroxylated parent) accounted for 4.5% and 3.8% TRR (0.75 mg/kg and 0.74 mg/kg). In addition, three fractions of 5% TRR (0.83 mg/kg) or less represented by peaks in HPLC chromatograms were observed.

The RRR after solvent extraction was 12.9% and 12.2% TRR (1.23 mg/kg and 2.34 mg/kg) and was treated sequentially with various enzymes allowing a solubilisation of 8.3% and 6.9% TRR corresponding to 1.37 mg/kg and 1.33 mg/kg (solubilisation of 2% TRR or higher was achieved with macerozyme/cellulase, with glucosidase/hesperinidase and with protease). The solubilized residue consisted mainly of the components identified in the ERR - predominantly BAS 750 F and minor amounts of metabolite M750F012.

In total, for C-label and T-label, identification amounted to 70% and 78% of TRR, considering also amounts characterized by solubilisation and HPLC an amount of 87% and 89% TRR were identified/characterized, leaving the final unextractable residue at 5.8% and 5.7%TRR (0.95 mg/kg and 1.10 mg/kg).

Hull

For hull, similar results were observed with both labels. Unchanged parent represented >79% TRR (3.2 mg/kg for C- and T-label) and was the predominant component of the residue (and only component present in major amounts) thus similar to forage and rest-of-plant. The only other components identified were present in amounts of <1.2% TRR (0.05 mg/kg) and were structurally related to the parent (sugar conjugates of the parent molecule and a hydroxylated parent molecule).

For C-label and T-label, M750F012 (malonylglucosyl-O-conjugate of BAS 750 F) accounted for 1.2% TRR (0.05 mg/kg) or less, while M750F018/F020 (malonylglucosyl-O-conjugate of BAS 750 F and hydroxylated parent) accounted for <0.1% TRR (<0.001 mg/kg). In addition, three fractions of 2.2% TRR (0.09 mg/kg) or less represented by peaks in HPLC chromatograms were observed.

The RRR after solvent extraction was 31.3% and 25.8% TRR (1.2 mg/kg and 1.1 mg/kg) and thus treated sequentially with various enzymes allowing a solubilisation of >18.8% TRR (>0.74 mg/kg). Solubilisation of up to 11% TRR (up to 0.45 mg/kg), was achieved with macerozyme/cellulase, of up to 3.6% TRR (up to 0.14 mg/kg) was achieved with glucosidase/hesperinidase. The solubilized residue consisted mainly of the components identified in the ERR, thus predominantly BAS 750 F and minor amounts of metabolite M750F012.

In total, for C-label and T-label, identification amounted to >80% of TRR, considering also amounts characterized by solubilisation and HPLC an amount of 87% TRR or higher was identified/characterized, leaving the final unextractable residue at 11.0% and 7.5%TRR (0.42 mg/kg and 0.31 mg/kg).

Green pod

In addition to the soybean matrices, forage, rest-of-plant and seeds, also the immature growth stage green pod (C-label, DALA 47) were sampled and analysed to provide further information.

Overall, similar results were observed as with rest-of-plant (DALA 47). Unchanged parent was with 69% TRR (5.98 mg/kg) the predominant component of the residue (and only component present in major amounts). The only other components identified were present in amounts of 4% TRR (0.34 mg/kg) or less and were structurally related to the parent (sugar conjugates of the parent molecule and a hydroxylated parent molecule).

M750F012 (malonylglucosyl-O-conjugate of BAS 750 F) accounted for 2.2% TRR (0.19 mg/kg), M750F018/M750F020 (malonylglucosyl-O-conjugate of BAS 750 F and hydroxylated parent) accounted for 3.9% TRR (0.34 mg/kg).

In total, identification amounted to 75% of TRR, when the amount characterized by HPLC is also considered 83% TRR was identified/characterized.

Seed

Unchanged parent was detected only in low amounts of 0.01 mg/kg (C-label, 4% TRR and T-label, 0.4% TRR). The radioactive residue detected with the C-label and with the T-label was significantly different both in quantity as well as in composition.

For the C-label the TRR was low (0.13 mg/kg), and 56.6 % (0.073 mg/kg) could be extracted, most of which was characterized by HPLC (48.5% TRR, 0.063 mg/kg), identification was 4% TRR (unchanged parent BAS 750 F). The RRR of 43.4%TRR (0.056 mg/kg) was further investigated allowing characterization of 32.8% TRR (with 15.5% TRR, corresponding to 0.02 mg/kg, as releasable by macerozyme/cellulase, 8.3 % TRR corresponding to 0.01 mg/kg as releasable by protease treatment). Thus, the final unextractable residue was reduced to 0.007 mg/kg (5.2% TRR).

In total, most of the C-label residue in seed (80% of 0.13 mg/kg) was characterized by solvent extraction or enzyme treatment. A smaller portion of the residue was identified (4% TRR, 0.005 mg/kg, BAS 750 F).

In contrast, with the T-label the TRR was significantly higher (3.06 mg/kg), of which 76% could be extracted by solvents. Two fractions were observed to be present in major amounts, one was identified as triazole alanine (TA, M750F029) at 47.7% TRR (1.46 mg/kg), the other one was characterized as "triazole-like compound" since exact molecular structure identification was not possible (33.6% TRR, 1.03 mg/kg). Significant efforts were made to characterise and identify this compound, gel permeation chromatography (GPC) of the extract demonstrated that the residue is polar, and elutes at

approximately the same time as triazole alanine. Additional GPC of the concentrated protease solubilisate again showed the residue eluting at a similar time to triazole alanine. Based on this it was possible to determine the residue is hydrophilic (confirmed by its extractability in water), and that it is likely to have a similar structure to TA based on retention times. In addition, as the residue was solubilised by protease treatment, this indicates the residue is in some way associated with amino acids, which is corroborated by analysis using HPLC with an ion exchange column. Whilst these steps have not lead to identification of the residue as required in OECD 501 for residues at this magnitude, it is considered that appropriate steps have been taken in an attempt to identify the residue, and that the characterisation achieved is acceptable, and feasible given that TA is present at high levels in seed, and hence the presence of high levels of TA bound to amino acids within the seed is plausible.

In addition, 1,2,4-triazole (M750F001) was identified at very low amounts (0.3% TRR, 0.008 mg/kg) as well as triazole lactic acid (M750F031) also at low amounts (1.3% TRR, 0.040 mg/kg). The RRR with 24.4% TRR (0.75 mg/kg) was in its entirety characterized (nominal 26.9% TRR) with macerozyme/cellulase releasing 23% TRR (0.71 mg/kg). Thus, the final unextractable residue was reduced to 0.005 mg/kg (0.2% TRR).

In total, a large proportion of the T-label residue in seed was identified (49.7% TRR, or 83.3%TRR including the "triazole-like compound" discussed above), and almost all the remaining residue was characterised.

The results from both labels taken together, indicate that most of the BAS 750 F residue in seed is carrying only the T-label and thereby resulting in a higher TRR with the T-label than in a comparable application experiment with the C-label (3.1 mg/kg versus 0.1 mg/kg). This difference in the detectable portion of the BAS 750 F residue in soybean seed is attributed largely to triazole alanine (TA 1.5 mg/kg) and the "triazole-like compound" ("6.6 min peak" with 1.0 mg/kg). Unchanged parent BAS 750 F was detected only in very small amounts (0.01 mg/kg) with both C-label or T-label.

Storage stability

Analysis of the storage stability confirmed the stability of radioactive residues over the period of the study, both in the frozen matrix (prior to extraction) and in extracts. Details of the storage periods are given in Table 7.2.1.2-9.

Stability during storage of the matrix at ≤-18°C was investigated in C- and T-labelled soybean matrices, by comparing the extractability as well as the resulting metabolic HPLC profiles after extended storage of the plant sample. The stability of C and T-labelled BAS 750 F in forage was demonstrated for up to 288 days and in rest of plant for up to 330 days.

Stability during storage of extract was investigated in C- and T-labelled forage, seed and rest of plant, by comparing the metabolic HPLC profiles after extended storage of the extract. Methanol and aqueous extracts of the samples were analysed. The stability of methanol forage extract was demonstrated for up to 267 days for both labels, and the stability of methanol rest of plant extract was up to 335 days for both labels. The stability of aqueous extracts in seed was demonstrated for 245 and 330 days for C-label and T-labelled BAS 750 F respectively.

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the triazole label, the residues were stable in extracts for a period of at least 245 days, and for the chlorophenyl label, the residues were stable in extracts for at least 266 days. The storage stability of residues in the corresponding homogenized samples (stability in matrix) was confirmed for a period of at least 287 days (sampling to extraction period, both labels).

Table 7.2.1.2-9: Storage intervals of plant samples and extract samples (soybean)

Matrix	Storage of matrix			Storage of extract		
	storage interval (analysis 1) 1)	*storage interval (analysis 2) 1)*	*Storage period*	*storage interval (analysis 1) 2)*	*storage interval (analysis 2) 2)*	*Storage period*
	[days]	*[days]*	*[days]*	*[days]*	*[days]*	*[days]*
C-label						
Forage (methanol)	86	374	288	28	295	267
Rest-of-plant (methanol)	22	352	330	28	363	335
Seed (water)	34	-*3)*	-	85	330	245
T-label						
Forage (methanol)	86	373	287	28	294	266
Rest-of-plant (methanol)	21	350	329	28	362	334
Seed (water)	32	-*3)*	-	17	330	313

1) sampling to extraction, 2) extraction to analysis, 3) not analysed

Translocation and proposed metabolic pathway

The unchanged parent BAS 750 F represents the predominant part of radioactive residues in the directly exposed plant parts forage, green pod and rest of plant (> 59 % TRR). In contrast, in soybean seed which was not present during the time of application BAS 750 F was only detected as very low levels (≤0.01 mg/kg). In conclusion, BAS 750 F is translocated only in trace amounts from treated green plant parts into the soybean seed.

Metabolism was investigated in foliar treated soybean using C- and T-labelled BAS 750 F. When the results from both labels are considered together the data demonstrate consistent metabolic pathways in soybean matrices. The proposed metabolic pathway is outlined in Figure 7.2.1.2-1.

For seed, parent BAS 750 F was only detected at trace levels, the main route of degradation was cleavage of the triazole from the parent compound, with subsequent conjugations forming the metabolites triazole alanine and triazole acetic acid as well as a further triazole characterised component.

Parent BAS 750F was the predominant compound in forage, hull and the rest of the plant. Metabolic conversion of this compound is by three main reactions.

Initial hydroxylation of the chlorophenyl/propyl-triazole moiety and a subsequent conjugation with glucose, at either the hydroxyl group of the chlorophenyl moiety or at the hydroxyl group of the propyl-triazole moiety generates metabolite M750F019. The exact position of the hydroxyl group in the chlorophenyl/ propyl-triazole moiety is undetermined, therefore the structure of M750F019 is dependent on this. An example structure is given in the figure. Malonylation of the glucose moiety of M750F019 results in metabolite M750F020. Additional hydroxylation of the chlorophenyl ring results in the formation of M750F018. Again these structures are dependent on the initial hydroxylation site. The metabolite M750F021 is generated by conjugation of the hydroxylated parent compound with acetic acid and likely represents an artefact of sample processing.

Conjugation of the hydroxyl group of the propyl-triazole moiety of BAS 750 F with glucose generates metabolite M750F011. Subsequent malonylation of this compound forms metabolite M750F012. Alternatively, the glucose moiety of M750F011 can be conjugated with another glucose molecule to form metabolite M750F013. An additional conjugation of the disaccharide moiety of M750F013 with malonyl results in the formation of metabolite M750F014.

In Figure 7.2.1.2-1, for some of the metabolites generic structures are provided in cases when exact position of hydroxyl group or sugar moiety is not known (indicated by a “dotted” line).

Conclusion

The metabolism of BAS 750 F was investigated in soybean by applying C-labelled or T-labelled BAS 750 F. The overall residue levels (TRR) in the C-labelled forage, rest of plant and hull were 6.52, 16.0 and 3.74 mg/kg respectively. For T-labelled forage, rest of plant and hull the levels were 4.42, 19.9 and 3.89mg/kg. Hence, the TRR for these commodities was similar, but a significant difference was seen for green pod and hull, with residues for the C-label 8.85 and 0.109 mg/kg respectively, compared with 16.0 and 2.59 mg/kg for the T-label.

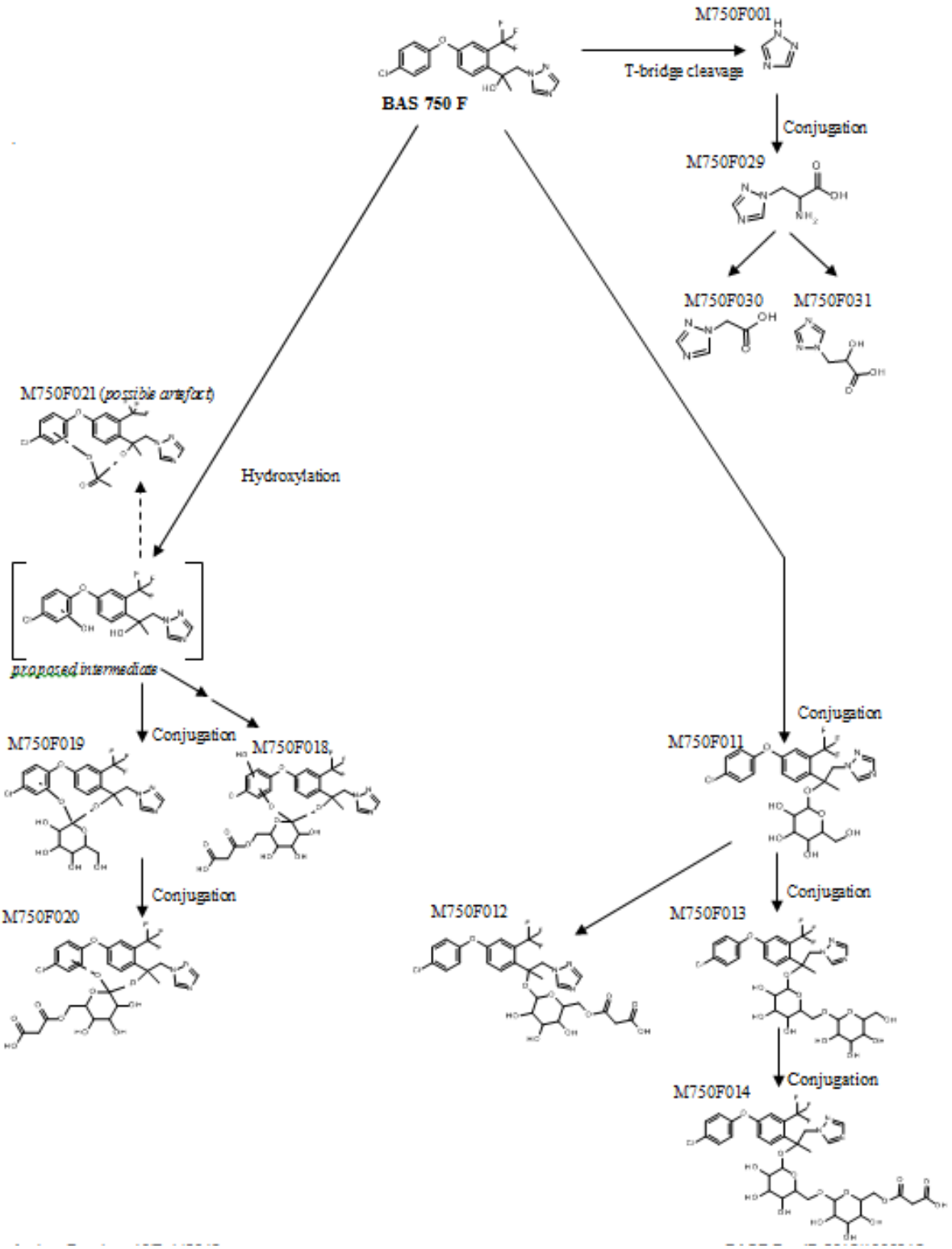
For both labels, solvent extractability (ERR) was high for forage, plant hill and green pod (at least 69% TRR), as well as T-labelled seed (76% TRR). For C-labelled seed, solvent extraction retrieved 57% of TRR, with enzyme solubilisation releasing a further 33 %. Enzyme solubilisation of the other commodities released 3-27% of the TRR. The final unextractable residue was between 0.2-7.5% TRR (0.005-1.1 mg/kg) for the C label. No further efforts were made to characterise the final residues (for the C or T label) as a significant proportion of the residue was available for characterisation/identification.

Metabolism of BAS 750 F includes hydroxylation of the parent backbone structure (C-ring) which introduces a second hydroxyl group which is a potential site for conjugation leading to an array of sugar conjugates. Cleavage of BAS 750 F at the T-bridge leads formation of triazole derivative metabolites (TDM) which are common to a range of azole fungicides.

For both labels, unchanged parent BAS 750 F is the only predominant component of the residue in forage rest of plant and hull representing at least 60% TRR. In contrast, unchanged parent is not detected in grain, where TDM account almost exclusively for the radioactive residue (accounting for 82% TRR).

Other components of the residue were sugar conjugates of parent (unchanged or hydroxylated) individually present <5% TRR. Data obtained with C-label and T-label taken together, show a consistent picture of the metabolism in foliar applied soybean. Overall, metabolism of BAS 750 F in soybean, and by extrapolation, in the *pulses and oilseed* crop group is considered well-elucidated.

Figure 7.2.1.2-1: Proposed pathway of BAS 750 F in soybean



B.7.2.1.3. Grape

Report:	CA 6.2.1/3 Birk B.,Bogen C., 2015 a Metabolism of 14C-BAS 750 F in grape 2015/1073822
Guidelines:	EPA 860.1000, EPA 860.1300: Nature of the Residue in Plants Livestock, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants, JMAFF 59 NohSan No 4200, OECD 501 - Metabolism in crops (adopted January 8 2007)
GLP:	yes

Materials and methods

Materials

1. C-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Chlorophenyl-U-C14 (spec. activity 7.88 MBq/mg) added to a 1:1 (w:w) mixture of Chlorophenyl-1-C13-labelled test item	
Lot/Batch #:	Chlorophenyl-U-C14:	CFQ41561
	Chlorophenyl-1-C13:	RS4-2012-173A2
Purity:	Chlorophenyl-U-C14:	99.1% (radiochem 98.9%)
	Chlorophenyl-1-C13:	97.7%

2. T-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Triazole-3(5)-C14 (spec. activity 5.46 MBq/mg) added to 2:1 (w:w) mix of Triazole-3(5)-C13-labelled test item	
Lot/Batch #:	Triazole-3(5)-C14:	1062-2001
	Triazole-3(5)-C13:	1077-1001
Purity:	Triazole-3(5)-C14:	98.8% (radiochem 98.8%)
	Triazole-3(5)-C13:	97.1%

Methods

A metabolism study on grape (variety *Muller-Thurgau*) grown outdoors in Limburgerhof, Germany was carried out in 2014-2015. Three grapevines were cultivated under natural climatic conditions using normal agricultural practices in an area approximately 0.4 m^2.

Three foliar spray applications (10±1 day interval) of either triazole or chlorophenyl labelled BAS 750F were made to ten containers per label. The structural formulae of the labelled BAS 750F molecules are given in Figure 7.2-2.

For the each application, spray flask mixes of the test items (taken up in blank EC formulation and water) were prepared. Applications were made at a target rate of 150 g a.s./ha (1N with respect to cereal crops) per application with the last application 12 days before harvest (harvest at BBCH89). A summary of the applications in the study are given in Table 7.2.1.3-1.

Table 7.2.1.3-1: Study design: plant uptake part (grapevine)

label	C-label		T-label	
intended use rate [g a.s./ha]	150		150	
application number	3		3	
application interval [days]	10±1		10±1	
sampled matrices	leaf, stalk, grape		leaf, stalk, grape	
sampling [DALA] [1)]	leaf	"-0" (21 DAT) [2)]	leaf	"-0" (21 DAT) [2)]
		12		12
	stalk	"-0" (21 DAT) [2)]	stalk	"-0" (21 DAT) [2)]
		12		12
	grape	"-0" (21 DAT) [2)]	grape	"-0" (21 DAT) [2)]
		12		12

1) days after last application, 2) immediately prior to the last (=third) application (DALA"-0") corresponding to 21 after the first application

Analysis and Identification

Samples of leaves and grape clusters (including stalks) were taken 21 days after the first application (just prior to the third application). Further samples of both were collected 12 days after the last application (BBCH 89). The grapes and stalks were separated and the samples stored separately in a freezer at ≤-18°C. The maximum time of frozen storage between sampling and analysis was 3 months; therefore stability data is not required. However, stability data to support this duration of storage is presented in the results section.

For TRR determination and the measurement of solid residues following solvent extraction (RRR) or solubilisation procedures (final residue), homogenized subsamples were combusted using a sample oxidizer. The resultant ^{14}C-CO_2 was absorbed, mixed with scintillation fluid and radioactivity was determined by liquid scintillation counting (LSC). For liquid samples scintillation fluid was added, and subjected to LSC measurement.

Prior to solvent extraction plant samples (leaves, stalks and grapes) were homogenized. The samples were then extracted with methanol (3x) and water (2x). After each extraction step, solid material was separated from extract by centrifugation and filtration, and the supernatants of methanol and water extracts were each combined. The methanol extracts of all matrices and the water extracts of leaves were purified by SPE fractionation prior to quantitative HPLC analysis. The residue after solvent extraction was dried in a fume hood, homogenized and radio assayed.

Solubilisation steps were performed for leaves, stalks and grapes (both labels). A six step treatment was carried out, with ammonia, macerozymes, α amylase/β amylase/amyloglucosidase, glucosidase/hesperidinase, laccase/tyrosinase and then protease. During each treatment the sample was incubated for 1-3 days, and post treatment acetonitrile was added prior to centrifuging and filtering.

Components of the residue were identified by HPLC-MS as well as by co-chromatography and comparison of retention times. In addition, for the parent BAS 750 F enantiomer-specific HPLC analyses were performed in samples of the application solution, as well as extracts of grape (C-label) and leaf (T-label).

Results and discussion

Total radioactive residue

The total ^{14}C residue (TRR) was measured in grapevine leaf (BBCH71-72), as well as grape and stalk (both BBCH89). The calculated total radioactive residues (TRR) with the C-label were highest in leaf (DALA 12) at 7.37 mg/kg, lower in stalk at 0.65 mg/kg, and lowest in grape with 0.35 mg/kg.

A similar distribution was seen with the T-label (TRR highest in leaf: 7.31 mg/kg, lower in stalk at 1.14 mg/kg, and grape at 0.43 mg/kg). A summary of the TRRs are presented in Table 7.2.1.3-2.

Table 7.2.1.3-2: Total radioactive residue after foliar spray application of BAS 750 F

Matrix [BBCH]	DALA [1)]	TRR measured (LSC) [mg/kg][2)]	TRR calculated [mg/kg] [3)]
C-label			
leaf [71-72]	12	8.860	7.371
stalk [89]	12	0.674	0.648
grape [89]	12	0.435	0.349
T-label			
leaf [71-72]	12	7.245	7.312
stalk [89]	12	1.214	1.136
grape [89]	12	0.400	0.428

1) days after last application, 2) TRR measured directly via combustion LSC, 3) TRR calculated as the sum of ERR(extractable radioactive residue) and RRR (residual radioactive residue)after extraction of the residues

Extractability

The extractabilities of ^{14}C residues from grape leaf, stalk and fruit are summarized in Table 7.2.1.3-3. High extractability of ^{14}C residue was seen all matrices (≥87% TRR for total extract for both labels). The majority of the radioactivity was extracted with methanol (≥87% TRR) while with subsequent water extraction resulted in additional extraction of ≤1.6% TRR. Solvent extraction left a RRR (residual radioactive residue) in leaf of 11% and 9% TRR (C- and T-label), in stalk 5.9% and 7.4% TRR (C- and T-label), and in grape 12.6% TRR (0.048 mg/kg, C-label) and 12.5% TRR (0.045 mg/kg, T-label). The RRR was therefore further investigated by enzyme treatment as discussed below. No significant label specific differences were seen for all matrices.

Table 7.2.1.3-3 Extraction efficiency in grapevine matrices

matrix	DALA[1)]	TRR [2)]	distribution of radioactive residues							
			methanol extracts [3)]		water extracts [3)]		ERR [2)]		RRR [2)]	
		mg/kg	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
C-label										
leaf	12	*7.371*	**87.6**	*6.456*	**1.4**	*0.102*	**89.0**	*6.558*	**11.0**	*0.813*
stalk	12	*0.648*	**93.5**	*0.606*	**0.6**	*0.004*	**94.1**	*0.610*	**5.9**	*0.038*
grape [4)]	12	*0.349*	**88.3**	*0.308*	**0.4**	*0.001*	**88.7**	*0.310*	-	-
	12	*0.349*	**87.1**	*0.331*	**0.2**	*0.001*	**87.4**	*0.332*	**12.6**	*0.048*
T-label										
leaf	12	*7.312*	**89.4**	*6.539*	**1.6**	*0.119*	**91.0**	*6.657*	**9.0**	*0.654*
stalk	12	*1.136*	**91.8**	*1.042*	**0.8**	*0.009*	**92.6**	*1.051*	**7.4**	*0.084*
grape [4)]	12	*0.428*	**89.6**	*0.384*	**0.4**	*0.002*	**90.1**	*0.385*	-	-
	12	*0.428*	**87.2**	*0.316*	**0.3**	*0.001*	**87.5**	*0.318*	**12.5**	*0.045*

[1)] days after last application [2)] TRR was calculated as the sum of ERR and RRR with ERR=Extractable Radioactive Residue, RRR=Residual Radioactive Residue (after solvent extraction), [3)] pool of combined

repetitive extracts, [4] For grape only limited sample material was available. Therefore, a first extraction was done without combustion analysis of the resulting RRR. Later, a second extraction was done with the purpose to determine the RRR for grape.

The results of the ammonia incubations and enzyme solubilisations of the residue after solvent extraction are summarized in Table 7.2.1.3-4. Generally, low amounts of radioactive residues were released from all matrices (both labels) by ammonia and enzyme incubations, which accounted in sum for up to 3.9 % TRR, respectively. These residues are likely to be associated into the cell structure (e.g. starch or lignin associated).

Table 7.2.1.3-4: Summary of solubilised components in grape

Distribution of radioactive residues	C-Label						T-Label					
	Leaf		Stalk		Grape		Leaf		Stalk		Grape	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TRR	**100.0**	*7.371*	**100.0**	*0.648*	**100.0**	*0.349*	**100.0**	*7.312*	**100.0**	*1.136*	**100.0**	*0.428*
RRR	**11.0**	*0.813*	**5.9**	*0.038*	-	- [4]	**9.0**	*0.654*	**7.4**	*0.084*	-	- [4]
Ammonia	**1.5**	*0.108*	**0.7**	*0.005*	**0.9**	*0.003*	**1.1**	*0.079*	**1.1**	*0.013*	**1.2**	*0.005*
Macerozyme	**0.7**	*0.051*	**0.4**	*0.003*	**0.4**	*0.001*	**0.6**	*0.040*	**0.6**	*0.007*	**0.5**	*0.002*
Amylase/ amyloglucosidase	**0.4**	*0.028*	**0.2**	*0.001*	**0.3**	*0.001*	**0.4**	*0.028*	**0.3**	*0.004*	**0.3**	*0.001*
Glucosidase/ hesperidinase	**0.3**	*0.023*	**0.1**	*0.001*	**0.2**	*0.001*	**0.2**	*0.018*	**0.3**	*0.004*	**0.1**	*0.001*
Laccase/ tyrosinase	**0.5**	*0.038*	**0.2**	*0.002*	**0.2**	*0.001*	**0.4**	*0.027*	**0.4**	*0.004*	**0.2**	*0.001*
Protease	**0.5**	*0.035*	**0.3**	*0.002*	**0.1**	*<0.001*	**0.4**	*0.028*	**0.3**	*0.003*	**0.1**	*<0.001*
Sum solubilised	**3.9**	*0.285*	**2.0**	*0.013*	**2.1**	*0.007*	**3.0**	*0.221*	**3.1**	*0.035*	**2.4**	*0.010*
Unextractable	**6.1**	*0.448*	**2.9**	*0.019*	**9.2**	*0.032*	**5.1**	*0.375*	**3.4**	*0.038*	**7.6**	*0.032*

Characterisation and Identification

The parent compound BAS 750 F and the metabolites grape leaf, stalk and fruit were identified by HPLC-MS analysis. An overview over the components of the extractable residue is given below in Table 7.2.1.3-5. Structures of the metabolites are outlined in Appendix 1.

Table 7.2.1.3-5: Summary of identified/characterized components in grape

Labelled radioactive component (min) [4] in ERR & RRR	C-label						T-label					
	Leaf		Stalk		Grape		Leaf		Stalk		Grape	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR	**100.0**	*7.371*	**100.0**	*0.648*	**100.0**	*0.349*	**100.0**	*7.312*	**100.0**	*1.136*	**100.0**	*0.428*
ERR	**89.0**	*6.558*	**94.1**	*0.610*	**88.7**	*0.310*	**91.0**	*6.657*	**92.6**	*1.051*	**90.1**	*0.385*
BAS 750 F	**60.1**	*4.432*	**85.8**	*0.556*	**64.1**	*0.224*	**69.9**	*5.110*	**91.5**	*1.039*	**70.3**	*0.301*
M750F019 (22.7, 23.2)	**21.1**	*1.554*	**2.3**	*0.015*	**7.0**	*0.024*	**14.5**	*1.058*	-	-	**6.1**	*0.026*
M750F026	**1.3**	*0.097*	-	-	-	-	-	-	-	-	-	-

Table 7.2.1.3-5: Summary of identified/characterized components in grape

Labelled radioactive component (min) [4] in ERR & RRR	C-label						T-label					
	Leaf		Stalk		Grape		Leaf		Stalk		Grape	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
ID [1]	**82.5**	*6.082*	**88.1**	*0.571*	**71.0**	*0.248*	**84.4**	*6.169*	**91.5**	*1.039*	**76.4**	*0.327*
CHAR [1]	**2.8**	*0.204*	**0.6**	*0.004*	**5.7**	*0.020*	**4.0**	*0.290*	**0.8**	*0.009*	**8.0**	*0.034*
sum of ID/CHAR [1]	**85.3**	*6.286*	**88.8**	*0.575*	**76.7**	*0.268*	**88.3**	*6.458*	**92.3**	*1.048*	**84.4**	*0.361*
RRR	**11.0**	*0.813*	**5.9**	*0.038*	**-**	-	**9.0**	*0.654*	**7.4**	*0.084*	**-**	-
ID [1]	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*
CHAR [1]	**3.9**	*0.285*	**2.0**	*0.013*	**2.1**	*0.007*	**3.0**	*0.221*	**3.1**	*0.035*	**2.4**	*0.010*
sum of ID/CHAR [1]	**3.9**	*0.285*	**2.0**	*0.013*	**2.1**	*0.007*	**3.0**	*0.221*	**3.1**	*0.035*	**2.4**	*0.010*
SUM ID/CHAR in ERR/RRR [1]	**89.1**	*6.571*	**90.8**	*0.588*	**78.8**	*0.275*	**91.4**	*6.680*	**95.3**	*1.083*	**86.7**	*0.371*
Unextractable residue [2]	**6.1**	*0.448*	**2.9**	*0.019*	**9.2**	*0.032*	**5.1**	*0.375*	**3.4**	*0.038*	**7.6**	*0.032*
Total [3]	**95.2**	*7.019*	**93.7**	*0.607*	**88.0**	*0.307*	**96.5**	*7.055*	**98.7**	*1.121*	**94.3**	*0.403*

1) ID=amount identified, CHAR=amount characterized, sum ID/CHAR= sum of amounts identified and/or characterized, SUM ID/CHAR in ERR/RRR= sum of amounts of ERR and of RRR which were identified and/or characterized, 2) final residue after solvent extraction and solubilisation, 3) Grand Total=sum of amounts characterized and identified as well as final residue, 4) Retention times (in minutes) are provided in parenthesis, M750F019 eluted in two distinct peaks.

Chiral analysis of grape (fruit) samples (C-label and T-label) confirmed that the racemic mixture (1:1 ratio of S-enantiomer and R-enantiomer) of the application formulation is essentially maintained, and hence that there is no significant change in BAS 750 F enantiomers. Details of the isomer ratio are given in Table 7.2.1.3-6.

Table 7.2.1.3.6: Determination of isomer ratio of BAS 750 F in grapevine matrices

Matrix	S-enantiomer [%]	R-enantiomer [%]
C-label		
application formulation	48.9	51.1
grape	46.7	53.3
T-label		
application formulation	47.4	52.6
leaf	48.1	52.0

Grape leaf

For leaf, similar results were observed with both labels. Unchanged parent represented > 60% TRR (4.4 and 5.1 mg/kg for C- and T-label) and was the predominant component of the residue. The other identified components were structurally related to the parent. The second most abundant component was metabolite M750F019 (O-glucosyl-conjugate of C-ring hydroxylated BAS 750 F) with 21.1% and 14.5% TRR (1.55 mg/kg and 1.06 mg/kg). In addition, metabolite M750F026 (O-di-glucosyl-conjugate of C-ring hydroxylated BAS 750 F) was found with the C-label, albeit at only small amounts with 1.3% TRR (0.097 mg/kg).

The RRR after solvent extraction (11.0% and 9.0% TRR corresponding to 0.81 mg/kg and 0.65 mg/kg) was treated sequentially with various enzymes allowing a solubilisation of 3.9% and 3.0% TRR corresponding to 0.29 mg/kg and 0.22 mg/kg (ammonia treatment solubilized 1.5 and 1.1 % TRR, macerozyme/cellulase solubilized 0.7% and 0.6% TRR, other treatments were less effective). No identification of residues from the RRR was undertaken.

In total, for C-label and T-label, identification amounted to 83% and 84% of TRR, considering also amounts characterized by solubilisation and HLPC an amount of 89% and 91% TRR were identified/characterized, leaving the final unextractable residue at 6.1% and 5.1%TRR (0.45 mg/kg and 0.38 mg/kg).

Grape stalk

For stalk, similar results were observed with both labels. Unchanged parent represented >85% TRR (0.56 and 1.04 mg/kg for C- and T-label) and was the predominant component of the residue. The only other identified component was structurally related to the parent: M750F019 (O-glucosyl-conjugate of C-ring hydroxylated BAS 750 F) with 2.3% TRR (0.02 mg/kg).

The RRR after solvent extraction (5.9% and 7.4% TRR corresponding to 0.04 mg/kg and 0.08 mg/kg) was treated sequentially with various enzymes allowing a solubilisation of 2.0% and 3.1% TRR corresponding to 0.01 mg/kg and 0.04 mg/kg (ammonia treatment alone solubilized 0.7 and 1.1 % TRR, other treatments were less effective). No identification of residues from the RRR was undertaken.

In total, for C-label and T-label, identification amounted to 88% and 92% of TRR, considering also amounts characterized by solubilisation and HLPC an amount of 91% and 95% TRR were identified/characterized, leaving the final unextractable residue at 3%TRR (<0.04 mg/kg).

Grape fruit

For grapes, similar results were observed with both labels. Unchanged parent represented > 64% TRR (0.22 and 0.30 mg/kg for C- and T-label) and was the predominant component of the residue. The only other identified component was structurally related to the parent: M750F019 (O-glucosyl-conjugate of C-ring hydroxylated BAS 750 F) with 7.0% and 6.1% TRR (0.024 mg/kg and 0.026 mg/kg).

The RRR after solvent extraction was treated sequentially with various enzymes allowing a solubilisation of 2.1% and 2.4% TRR corresponding to 0.007 mg/kg and 0.010 mg/kg (ammonia treatment solubilized 0.9% and 1.2% TRR, other treatments were less effective). No identification of residues from the RRR was undertaken.

In total, for C-label and T-label, identification amounted to 71% and 76% of TRR, considering also amounts characterized by solubilisation and HLPC an amount of 79% and 87% TRR were identified/characterized, leaving the final unextractable residue at 9.2% and 7.6%TRR (0.032 mg/kg for both labels).

Storage stability

Analysis of the storage stability confirmed the stability of radioactive residues over the period of the study, both in the frozen matrix (prior to extraction) and in extracts. Details of the storage periods are given in Table 7.2.1.3-7.

Stability during storage of matrix at ≤-18°C was investigated in C- and T-labelled in grape leaf and fruit matrices, by comparing the extractability as well as the resulting metabolic HPLC profiles after extended storage of a plant sample. Methanol and aqueous extracts of grape matrices were analysed. The stability of C and T-labelled BAS 750 F in leaf was demonstrated for up to 195 days and in grape for up to 148days.

Stability during storage of extract was investigated by comparing the metabolic HPLC profiles after extended storage of the extract. Methanol and aqueous extracts of grape matrices were analysed. The stability of aqueous and methanol leaf extract was demonstrated for up to 211 days for both labels, and the stability of methanol grape extract was up to 211 days for both labels.

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the triazole and chlorophenyl label, the residues were stable in methanol extract for a period of at least 210 days and 204 days in water extract. The storage stability of residues in the corresponding homogenized samples (stability in matrix) was confirmed for a period of at least 147days for both labels.

Table 7.2.1.3-7: Storage intervals of plant samples and extract samples (grape)

Matrix	Storage of matrix			Storage of extract		
	storage interval (analysis 1) [1)]	*storage interval (analysis 2)* [1)]	*Storage period*	*storage interval (analysis 1)* [2)]	*storage interval (analysis 2)* [2)]	*Storage period*
	[days]	*[days]*	*[days]*	*[days]*	*[days]*	*[days]*
C-label						
leaf (MeOH extract)	57	252	195	19	230	211
leaf (water extract)	57	252	195	26	231	205
grape (MeOH extract)	56	203	147	21	231	210
leaf (MeOH extract)	56	251	195	20	230	210
leaf (water extract)	56	251	195	26	230	204
grape (MeOH extract)	55	203	148	20	231	211

1) sampling to extraction, 2) extraction to analysis

Translocation and proposed metabolic pathway

The unchanged parent BAS 750 F represents the predominant part of radioactive residues in the directly exposed plant parts. Since the application formulation was applied over the entire plant including leaf, stalk and grape, no precise information regarding translocation of BAS 750 F within the plant can be derived from this study.

Metabolism was investigated in foliar treated grapevines using C- and T-labelled BAS 750 F. When the results from both labels are considered together the data demonstrate consistent metabolic pathways in grape matrices. The proposed metabolic pathway is outlined in Figure 7.2.1.3-1.

Parent BAS 750F was the predominant compound in in all matrices. Metabolic conversion of this compound is by two main reactions. Initial hydroxylation of the chlorophenyl/propyl-triazole moiety and a subsequent conjugation with glucose, at either the hydroxyl group of the chlorophenyl moiety or at the hydroxyl group of the propyl-triazole moiety generates metabolite M750F019. The exact position of the hydroxyl group in the chlorophenyl/ propyl-triazole moiety is undetermined, therefore the structure of M750F019 is dependent on this. An example structure is given in the figure. Further glucosidisation of the glucose moiety of M750F019 results in metabolite M750F026, while conjugation of the glucose moiety to a pentose moiety results in metabolite M750F027

Conjugation of the hydroxyl group of the propyl-triazole moiety of BAS 750 F with glucose generates metabolite M750F011. Subsequent conjugation of M750F011 with a pentose moiety forms metabolite M750F028.

Conclusion

The metabolism of BAS 750 F was investigated in grape by applying C-labelled or T-labelled BAS 750 F. The overall residue levels (TRR) in the C-labelled leaf, stalk and grape were 8.86, 0.67 and 0.44 mg/kg respectively. For T-labelled leaf, stalk and grape the levels were 7.25, 1.21 and 0.40 mg/kg. Hence, TRR for each commodity was similar were similar.

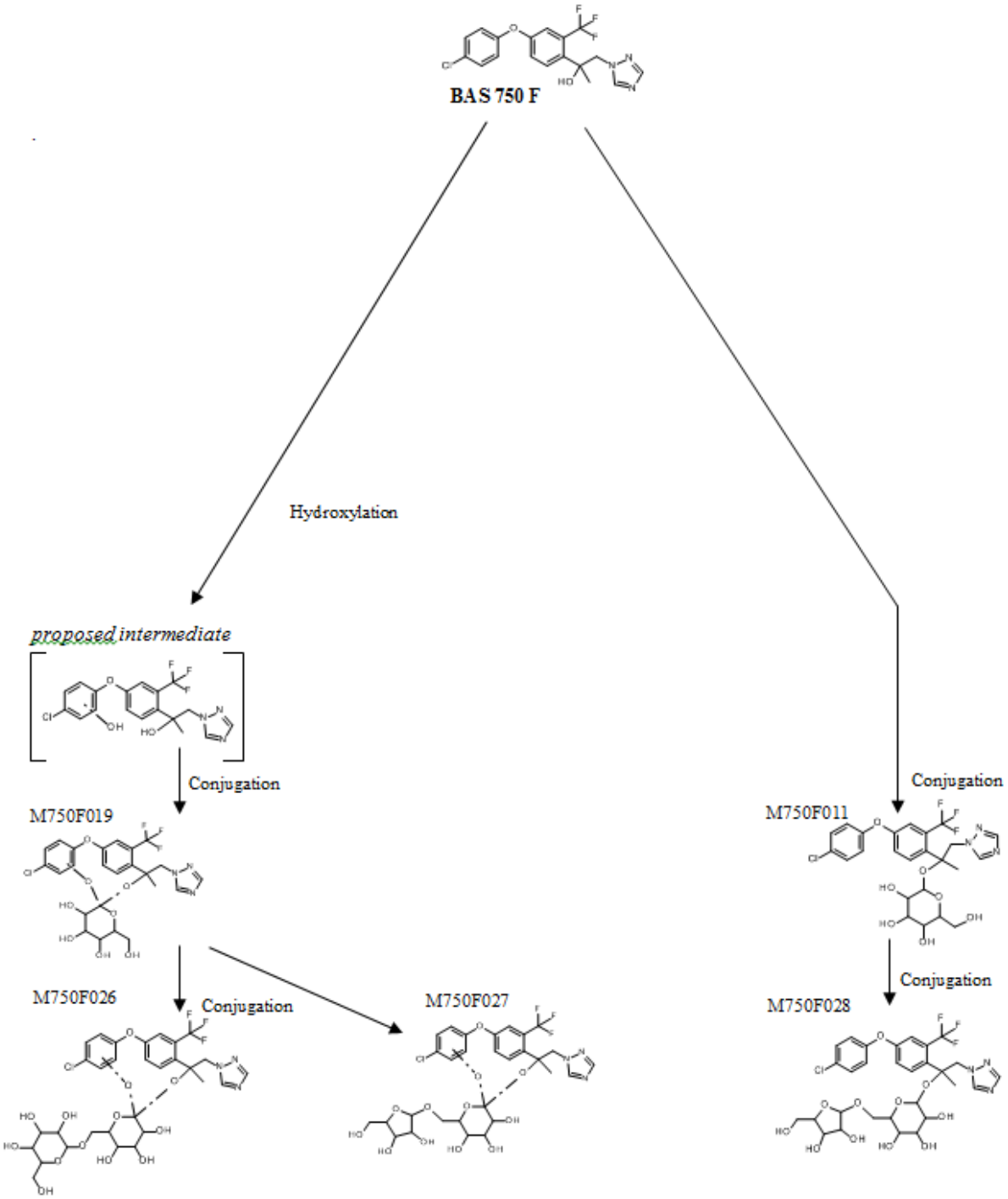
For both labels, solvent extractability (ERR) was high for all grape commodities (at least 88% TRR). Enzyme solubilisation released a further 2-4% of the TRR. The final unextractable residue was between 3-9% TRR (0.019-0.448 mg/kg). No further efforts were made to characterise these residues as the contribution to the TRR is < 10%, and a significant proportion of the residue was available for characterisation/identification, therefore further characterisation is not considered to impact significantly on the study results.

Metabolism of BAS 750 F includes hydroxylation of the parent backbone structure (C-ring) which introduces a second hydroxyl group which is a potential site for conjugation leading to an array of sugar conjugates. Cleavage of BAS 750 F at the T-bridge leads formation of triazole derivative metabolites (TDM) which are common to a range of azole fungicides.

For both labels, unchanged parent BAS 750 F is the only predominant component of the residue in all grape commodities representing at least 60% TRR. Other components of the residue are sugar conjugates of parent (unchanged or C-ring hydroxylated parent), of which two were present in quantifiable amounts, M750F019 (up to 7% in grape and stalk, up to 21% in leaf) and M750F026 (up to 1.3% in leaf). Other sugar conjugates, namely M750F011, M750F027, M750F028 were detected in non-quantifiable amounts.

Data obtained with C-label and T-label taken together, show a consistent picture of the metabolism in foliar applied grapevine. Overall, metabolism of BAS 750 F in grape, and by extrapolation, in the *fruit* crop group is considered well-elucidated.

Figure 7.2.1.3-1: Proposed pathway of BAS 750 F in grape



B.7.2.1.4. Extraction Efficiency

Report:	CA 6.2.1/4 Birk B. et al., 2015 b Investigation of the extractability of BAS 750 F in samples from 14C plant metabolism studies 2014/1261057
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2007)17, OECD 501
GLP:	yes

Materials and methods

Samples from metabolism studies in wheat, soybean and grapevine (forage (wheat), straw (wheat), soybean (green pod), grapevine (grape)) were used to investigate extraction efficiency of radiolabelled BAS 750 F.

Extraction procedures used in analytical methods, namely BASF method 535/1 (see section CA 4.3), as well as plant multi-methods were compared to the extraction procedure used in the wheat, soybean, and grapevine metabolism studies. The plant multi-methods were:

QueEChERs	*(Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method. EN 15662:2008)*
DFG S 19	*(Foods of plant origin - Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS - Part 1: General considerations. EN 12393-1:2013)*
SweET	(*Foods of plant origin - Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS - Part 2: Methods for extraction and cleanup. EN 12393-2:2013)*

A summary of the extraction procedure for each method is detailed below. In each case the analysis was performed using HPLC. A Phenomenex Luna, 5 µm, 250 x 4.6 mm column was used with a water:acetonitrile:formic acid mobile phase at 25 °C.

BAS method 535/1

5 g of homogenised plant sample was extracted once with 100 mL of methanol / water / 2 N HCl (70/25/5, v/v/v) using a homogeniser. 10 mL of the extract were subjected to centrifugation and the resulting supernatant was filtered, diluted with methanol and analysed by LSC and HPLC. For wheat straw, the extraction was repeated to achieve sufficient extractability.

QuEChERS

In the standard QuEChERS procedure, for water-rich matrices (forage, grapes), 10 g of homogenised material is used for extraction. For dry matrices (grain, straw), 5 g of homogenised material is used and 10 g water is added prior to extraction. For fat-rich matrices (soybean green pod), 10 g of homogenised material is used and 3 g water is added prior to extraction. Samples are extracted with 10 mL acetonitrile, centrifuged, filtered, diluted with acetonitrile and analysed by LSC and HPLC.

For the extraction of wheat forage and wheat straw, in addition 60 mL acetonitrile/water (1/1, v/v) was added to achieve a suitable solvent volume for extraction. For soybean green pod, 5 g homogenised sample was taken in order to obtain a sufficient ratio of solid material to extraction solvent. After centrifugation, the clear supernatant was subjected to HPLC preparation without further clean-up or dilution steps. Grapevine grape was extracted as described above. After centrifugation, the clear supernatant was subjected to HPLC preparation without further cleanup or dilution steps.

DFG S 9

For water-rich matrices (forage, grapes), 25 g material was homogenized after addition of 10 g water and 200 mL acetone, centrifuged, filtered, diluted with acetone and analysed by LSC and HPLC.

For dry matrices (straw), 19.96 g of homogenised material was added to 95 g water (40 °C) and homogenised by mixing. After swelling for 20 min, 200 mL acetone was added for extraction using a homogeniser. After centrifugation, the resulting supernatant was filtered, diluted with acetone and analysed by LSC and HPLC.

For fat-rich matrices (soybean), 25 g of homogenised material was mixed with 225 mL acetonitrile and 25 mL acetone and extracted using a homogeniser. After centrifugation, the supernatant was filtered, and analysed by LSC and HPLC.

SweEt

In the standard SweET procedure, 10 g of a homogenised plant sample is mixed with 3 g sodium bicarbonate and at least 10 g sodium sulphate. 20 mL ethyl acetate is added for extraction and further ultra-sonication for 1 min. After centrifugation, the resulting supernatant is filtered, diluted with ethyl acetate and analysed by LSC and HPLC.

For wheat forage, 10 g homogenised material was added to 3 g mixture of sodium bicarbonate and sodium sulphate (3/10, w/w). After adding 50 mL ethyl acetate, due to insufficient solvent volume, the sample was processed as described above.

For wheat straw, 5 g homogenised material was added to 3 g mixture of sodium bicarbonate and sodium sulphate (3/10, w/w). After adding 50 mL ethyl acetate, due to insufficient solvent volume, the sample was processed as described above.

For soybean green pod, 5 g homogenised material was mixed with 1.5 g sodium bicarbonate and 5 g sodium sulphate to obtain a sufficient ratio of solid material to extraction solvent. The mixture was processed as described above. After centrifugation, the supernatant was subjected to LSC analysis and HPLC sample preparation without further clean-up and dilution steps.

Grapevine grape was extracted as described in the standard procedure. After centrifugation, the supernatant was diluted with ethyl acetate without prior filtration.

Results and Discussion

The extractability of radioactive residues from wheat forage, wheat straw, soybean green pod and grapevine grape using four different extraction protocols is summarised in Table 7.2.1.4-1. The amount of radioactive residue extracted in the plant metabolism studies (ERR value) was taken as reference value for extraction efficiency (thus, ERR was set to 100%). The amount extracted with an analytical method was compared to this value, and expressed in percentage of the reference value.

For all matrices, the highest overall extractability was obtained by applying method BASF method 535/1, with extractability ranging from 76 9 % TRR for wheat straw to 93 6 % TRR for wheat forage (except for soybean green pod: 535/1: 80.1 % TRR, QuEChERS: 81.2% TRR). Similar or lower ERR values were obtained using the QuEChERS method (51 4 % TRR to 85.5 % TRR). The DFG S 19 and SWeEt methods led to lower extractabilities ranging from 36.2 % TRR to 81.2 % TRR and from 37.4 % TRR to 73.7 % TRR, respectively.

Table 7.2.1.4-1: Summary of extractability: radioactive residues and parent BAS 750 F

Extraction procedure	TRR	Radioactive residue (in ERR)			BAS 750 F		
	mg/kg	*mg/kg*	*%TRR*	***extraction efficiency (%)*** [1)]	*mg/kg*	*%TRR*	***extraction efficiency (%)*** [1)]
forage (wheat, T-label)							
wheat metabolism study	2.31	*2.218*	96.0	100.0	*2.062*	89.3	100.0
BASF Method 535/1 (1a)		*2.161*	93.6	**97.4**	*2.012*	87.1	**97.6**
QuEChERS (2)		*1.769*	76.6	**79.8**	*1.656*	71.7	**80.3**
DFG S 19 (3)		*1.743*	75.5	**78.6**	*1.307*	56.6	**63.4**
SweEt (4)		*1.476*	63.9	**66.6**	*1.149*	49.7	**55.7**
straw (wheat, C-label)							
wheat metabolism study	24.38	*20.241*	83.0	100.0	*13.798*	56.6	100.0
BASF Method 535/1		*18.758*	76.9	**92.7**	*15.351*	63.0	**111.3**
2 QuEChERS		*12.530*	51.4	**61.9**	*8.174*	33.5	**59.2**
3 DFG S 19		*9.310*	38.2	**46.0**	*7.143*	29.3	**51.8**
4 SweEt		*9.123*	37.4	**45.1**	*8.996*	36.9	**65.2**
green pod (soybean, C-label)							
soya metabolism study	8.72	*7.271*	83.4	100.0	*5.978*	68.5	100.0
1a BASF Method 535/1		*6.989*	80.1	**96.1**	*6.101*	70.0	**102.1**
2 QuEChERS		*7.081*	81.2	**97.4**	*5.900*	67.7	**98.7**
3 DFG S 19		*6.006*	68.9	**82.6**	*5.913*	67.8	**98.9**
4 SweEt		*5.890*	67.5	**81.0**	*5.838*	66.9	**97.7**
grape (T-label)							
grape metabolism study	0.42	*0.385*	90.1	100.0	*0.301*	70.3	100.0
1a BASF Method 535/1		*0.366*	85.5	**95.0**	*0.281*	65.8	**93.4**
2 QuEChERS		*0.366*	85.5	**95.0**	*0.296*	69.1	**98.3**
3 DFG S 19		*0.347*	81.2	**81.2**	*0.301*	70.3	**100.0**
4 SweEt		*0.315*	73.7	**73.7**	*0.267*	62.4	**88.7**

1) extraction efficiency = amounts extracted with analytical method compared with amount extracted in metabolism study (set to 100%).

For all matrices extracted by BASF method 535/1, very similar amounts of ERR were determined in comparison to the relevant metabolism study ranging from 92.7 % extraction efficiency (wheat straw) to 97.4 % extraction efficiency (wheat forage). The multi methods led to similar extraction efficiencies for soybean green pod (81.0 % to 97.4 % extraction efficiency) and grapevine grape (73.7 % to 95.0 % extraction efficiency), whereas for wheat matrices lower extraction efficiencies were determined, ranging from 66.6 % to 79.8% extraction efficiency for wheat forage and from 45.1 % to 61.9% extraction efficiency for wheat straw.

The identification of BAS 750 F was based on retention time comparison of the ^{14}C signals of the quantitative HPLC analyses with those of the reference item.

For all matrices, the highest extractability of BAS750F was obtained by applying method BASF method 535/1, with extractability ranging from 63. % TRR for wheat straw to 87.1 % TRR for wheat forage. The only exception was grape for which the multi-methods lead to similar or higher

extractability. Generally, lower ERR values were obtained using the multi-methods (QuEChERS 33.5 % to 71.7 % TRR, DFG S 19 29.3% to 70.3% TRR and SWeEt 36.9 % to 66.9 % TRR).

For all matrices extracted by BASF method 535/1, very similar amounts of BAS 750 F were determined in comparison to the relevant metabolism study ranging from 93.4 % extraction efficiency (grape) to 111.3 % extraction efficiency (wheat straw). The multi methods led to similar extraction efficiencies for soybean green pod (97.7 % to 98.9 % extraction efficiency) and grapevine grape (88.7 % to 100 % extraction efficiency), whereas for wheat matrices lower extraction efficiencies were determined, ranging from 55.7 % to 80.3 % extraction efficiency for wheat forage and from 51.8 % to 65.2 % extraction efficiency for wheat straw.

Conclusion

Efficient extraction for the analytical method, BASF method 535/1 was confirmed by comparison of residue amounts extracted in the metabolism study with the amounts extracted according to extraction procedures of a residue analytical method.

Extraction efficiencies generally were 90% or higher for all matrices investigated, namely wheat forage (98%), wheat straw (111%), soybean green pod (102%) and grapevine grape (93%).

In contrast, with the multi-methods, extraction efficiency was lower for forage (QuEChERS 80%, DFG S 19 63%, SweEt 56%), and for straw (QuEChERS 59%, DFG S 19 52%, SweEt 65%) while similar high extraction efficiency was observed for soybean green pod and grapevine grape (88% or higher).

B.7.2.1.5. Overall conclusion on metabolism in plant

Metabolism was investigated using two radiolabels (BAS 750 F labelled in the C-ring or in the T-ring). Results obtained with both labels show a consistent picture of BAS 750 F metabolism. Investigations were done in three plant species, wheat (cereal crop group), soybean (pulses and oilseed crop group), and grapevine (fruits/fruiting vegetable crop group), foliar applied with BAS 750 F reflecting the cGAP. Comparable results were obtained for all three crop groups.

In most matrices the unchanged parent is the predominant component of the residue (>60% of the radioactive residue), notably in forage (wheat, soybean), leaf/stalk (grapevine), straw/hull/chaff (wheat, soybean), green pod (soybean) and grape (grapevine). The enantiomer ratio of the two BAS 750 F isomers remains unchanged (racemic mixture).

In wheat grain and soybean seed, the predominant component of the residue is the group of TDM with triazole alanine as the most abundant compound (formed via cleavage of the T-bridge). In these matrices unchanged parent is present at very low levels if at all.

Other metabolites were formed via two main pathways:

- Initial hydroxylation of the chlorophenyl or propyl-triazole moiety and a subsequent conjugation with glucose, followed by malonylation of the glucose moiety or additional hydroxylation of the chlorophenyl ring (M750F018, 019, 020, 026, 027).
- Conjugation of the hydroxyl group of the propyl-triazole moiety of BAS 750 followed by malonylation or conjugation with another glucose molecule (M750F011, 012, 013, 014, 028).

Absence of detectable cleavage at the ether bridge between C-ring and TFMP-ring (trifluoromethylphenyl-ring, linking C-ring and T-ring) confirms that results obtained with C-labelled samples also provide comprehensive information on the metabolic fate of the TFMP-ring.

Figure 7.2.1.5-1 shows the key metabolism pathways determined for the plant commodities.

Metabolites occurring in plant matrices in major amounts (<10% TRR) and in minor amounts (<10% TRR) are listed in the table below. This table groups the metabolites according to their chemical structure together with their corresponding conjugates. The non-conjugated metabolites that were identified in plant matrices are highlighted (underlined).

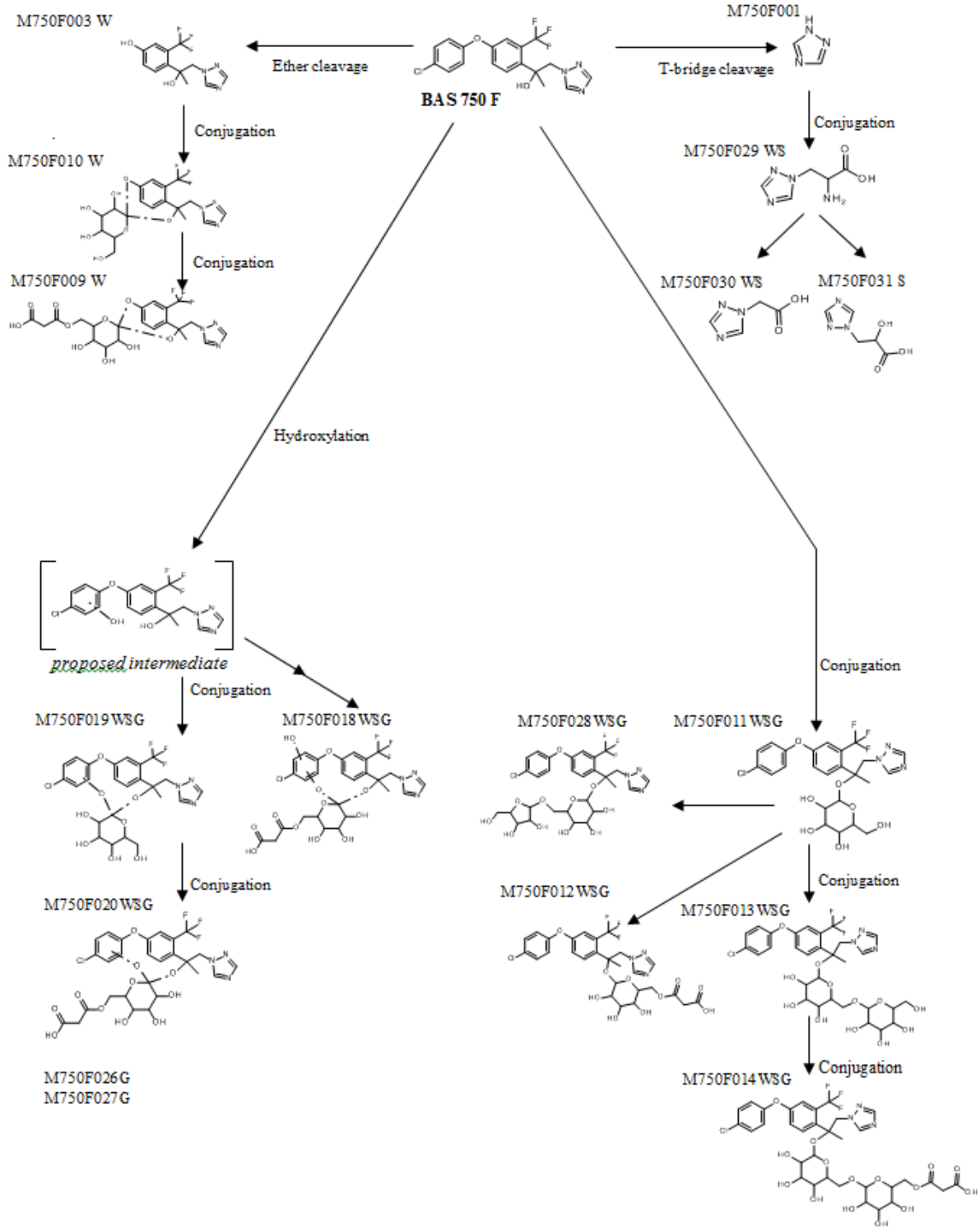
A detailed discussion of residue definitions for plant commodities is provided in section B.7.1.2.6.

Table 7.2.1.5-1: Residue components identified in plant matrices

Group definition	Residue	
	≥ 10% TRR*	**< 10% TRR**
a) parent and conjugates	**<u>BAS 750 F</u>**	M750F011 M750F012 M750F013 M750F014 M750F028
b) "C-Ring"-hydroxylation of non-cleaved molecule & downstream metabolites / conjugates	M750F018 M750F019 M750F020 M750F026 M750F027	
c) cleavage products & downstream metabolites / conjugates metabolites without the C-Ring		M750F009 M750F010
1,2,4-triazole and triazole-derived metabolites (TDM)	**<u>1,2,4-T, TA, TAA</u>**	**<u>TLA</u>**

**In at least one plant commodity*

Figure 7.2.1.5-1 BAS 750 F: metabolic routes in plant (W wheat, S soybean, G grape)



Definition of the residue in plant
For commodities of plant origin, including processed fractions, the following residue definitions are proposed:

Residue definition for MRL enforcement/monitoring (RD-Mo):

- *parent BAS 750 F*

Residue definition for risk assessment (RD-RA):

- *parent BAS 750 F*
- *triazole derivative metabolites (provisional, pending the definition of a common and harmonised approach for all the active substances of the triazole chemical class)*

Based on the evaluation of study results provided in the present dossier, the residue components in plant commodities relevant for consideration in the residue definitions include parent BAS 750 F, as well as the group of triazole derivative metabolites (TDM) and sugar conjugates and cleavage products.

RD-Mo

According to *OECD Guidance on the definition of residue (ENV/JM/MONO(2009)30*), the residue definition for monitoring/MRL enforcement (RD-Mo) should focus on residue components suitable as analyte for multi-residue methods, as well as suitable as general marker compound in food commodities concerned.

Parent BAS 750 F fulfils these criteria since its compatibility with multi-residue methods has been confirmed (see section B.7.2.1.4) and it is a characteristic component of the residue typically accounting for a large proportion of the residue. Although it is either not detected or only present at very low levels in wheat grain and soybean seed, BAS 750 F remains the most appropriate residue component to analyse for in monitoring, as the predominant TDM components in these matrices are not suitable as BAS 750 F specific marker molecules as they are common to a range of pesticides.

In conclusion, the proposed residue for monitoring in plants is BAS 750 F.

RD-RA

According to *OECD Guidance on the definition of residue (ENV/JM/MONO(2009)30)*, the residue definition for risk assessment (RD-RA) should take into account the contribution of residue components to the potential dietary risk considering both the potential for exposure as well as the toxicity relative to the parent compound.

Parent BAS 750 F is the predominant component of the residue in all plant matrices considered, with the exception of wheat grain and soybean seed in which the TDMs (in particular TA) are the most abundant compounds.

TDMs contribute to a large proportion of the residue in all matrices; however these metabolites are common to a range of azole fungicides. Based on different toxicological properties, a separate definition of residue relevant for risk assessment is expected as part of a common approach for azole fungicides. Therefore, TDMs are provisionally included in the RD-RA as part of the present dossier, pending the outcome the EU review of TDMs. At the time of writing, no decision has been made with respect to an EU agreed approach on how to assess TDMs.

Other components present at >10 % TRR are sugar conjugates of BAS 750 F; however as they are present at significantly lower levels than parent, and do not present an increased toxicological risk in comparison to parent, it is not considered necessary to include these metabolites in the residue definition for risk assessment.

In conclusion, the proposed residue for risk assessment in plants is BAS 750 F.

B.7.2.2. Poultry

Report:	CA 6.2.2/1 [redacted] 2015 a The metabolism of 14C-Reg. No 5834378 (BAS 750 F) in laying hens 2015/1001001
Guidelines:	OECD Test Guideline 503 - Metabolism in livestock, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), JMAFF 59 NohSan No 4200, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada)
GLP:	yes

Materials and methods

Materials

1. C-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Chlorophenyl-U-C14 (spec. activity 7.88 MBq/mg) added to a 1:2 (w:w) mixture of Chlorophenyl-1-C13-labelled and unlabelled test item	
Lot/Batch #:	Chlorophenyl-U-C14:	CFQ41561
	Chlorophenyl-1-C13:	RS4-2012-173A2
	Unlabelled:	COD-001740
Purity:	Chlorophenyl-U-C14:	99.1% (radiochem 98.9%)
	Chlorophenyl-1-C13:	97.7%
	Unlabelled:	98.8%

2. TFMP-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Trifluoromethylphenyl-U-C14 (spec. activity 8.265 MBq/mg) added to a 2:2 (w:w) mixture of Propyl-2-C13-labelled and unlabelled test item	
Lot/Batch #:	Trifluoromethylphenyl-U-C14:	CFQ42039
	Propyl-2-C13:	1126-1006
	Unlabelled:	COD-001740
Purity:	Trifluoromethylphenyl-U-C14:	96.3% (radiochem) 98.3%
	Propyl-2-C13:	99.5%
	Unlabelled:	98.8%

3. T-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Triazole-3(5)-C14 (spec. activity 5.46 MBq/mg) added to 1:1 (w:w) mix of Triazole-3(5)-C13-labelled and unlabelled test item	
Lot/Batch #:	Triazole-3(5)-C14:	1062-2001
	Triazole-3(5)-C13:	1077-1001
	Unlabelled:	COD-001740
Purity:	Triazole-3(5)-C14:	98.8% (radiochem 98.8%)
	Triazole-3(5)-C13:	97.1%
	Unlabelled:	98.8%

Methods

The metabolism of BAS 750 F was investigated in laying hens (breed *Lohmann Brown*) following repeated oral administration of ^{14}C-BAS 750 F, labelled either in the Chlorophenyl ring (C-ring), in the Trifluoromethylphenyl ring (TFMP-ring) or in the triazole ring (T-ring) (see Figure 7.2-2).

A total of 30 hens were dosed with radiolabelled BAS 750 F at nominal doses of 12 mg/kg feed (5.5 N for laying hens) for 14 consecutive days (10 birds for each label). Each ^{14}C-labelled test item was mixed with ^{13}C-labelled test item and unlabelled test item (ratios are given in Table 7.2.2-1). The test items were prepared in gelatine capsules, orally administered (once daily). The actual dose was based on the average feed consumption (day 1-14). The mean achieved daily dose administered was 15.0-16.8 mg/kg food consumed (dry weight equivalent) corresponding to 1.11-1.15 mg/kg bw/d. Details of the study outline are summarized in Table 7.2.2-1.

Table 7.2.2-1: Dosing of laying hen with BAS 750 F

Animal no. (10 birds per label)	**Treat-ment period (days)**	**Isotope ratio ^{14}C:^{13}C:^{12}C**	**Mean daily feed consump-tion**	**Mean animal weight (day 1)**	**Nominal daily dose**	**Actual daily dose [1)]**			**Time of sacrifice [2)] (hours)**
			[g/animal]	*[kg]*	*[mg/kg feed]*	*[mg/animal]*	*[mg/kg feed]*	*[mg/kg bw/d]*	
C-label: 1-10	14	1 : 1 : 2	120.2	1.795	12	2	16.8	1.11	3-6
TFMP-label: 21-30	14	1 : 2 : 2	135.2	1.728	12	2	15.9	1.15	3-6
T-label: 11-20	14	1 : 1 : 1	120.5	1.804	12	2	15.0	1.11	3-6

1) based on mean body weights on study day1 2) hours after last dose

Excreta were collected for a 24 h interval prior to first dose and subsequently every 24h until sacrifice. Daily excreta for each label was pooled and weighed, and stored at ≤- 18 °C prior to analysis.

Following each excreta collection, the cage was washed with the minimum amount of methanol:water (1:1, v:v) and rinsings retained. Daily cage wash samples for each label were pooled and weighed, and stored at ambient temperature prior to processing.

Eggs were collected for a 24h interval prior to first dose and twice daily during the subsequent treatment period of 14 days until sacrifice. Eggs were stored at 4 ^{o}C prior to processing.

The birds were sacrificed 3-6 h after the final dose and edible tissues (liver, kidney, muscle (breast, thigh), fat (omental, subcutaneous, and renal)) as well as bile, blood, undeveloped eggs and the GI tract collected, and stored at ≤- 18 °C prior to processing.

All samples (except cage wash) were homogenised prior to analysis. For tissues, this was via blending and mincing, and for blood and bile with was via mixing. For eggs, they were weighed, broken, and separated into whites and yolk, and the whites and yolks were pooled from each day and weighed, and homogenized, then water and liquid scintillant were then added prior to analysis. For excreta, the samples were mixed with water during homogenisation, and subsamples were solubilized in an NaOH:H_2O:MeOH:Triton X405 solution for 48 hours at 55 ^{o}C. Methanol and liquid scintillant were then added prior to analysis. The maximum time of frozen storage between sampling and analysis is 176 days. Stability data to support frozen storage of at least 315 days is presented in the results section.

Prior to extraction, samples were combusted to verify the total radioactive residues (TRR). The homogenised samples were then extracted and worked up as outlined in Table 7.2.2-2.

Table 7.2.2-2: Details of sample extraction and work up

	C-label	T-label	TFMP-label
Muscle	1. 3 x methanol 2. Concentration OR protease solubilisation	1. 3 x methanol, 2 x water 2. Concentration 3. Partition of water against acetonitrile/isohexane OR SPE clean up	1. 3 x methanol 2. Concentration **(EXTR_1)** OR 1. 3 x methanol, 2 x water OR 3 x methanol 2. Concentration **(EXTR_2)**
Egg white		1. 3 x methanol, 2 x water 2. Concentration, 3. Partition of water against acetonitrile/ isohexane OR SPE clean up	
Egg yolk	1. 3 x methanol, 2 x water 2. Concentration OR protease solubilisation **(EXTR_1)** OR 1. 3 x methanol 2. Concentration OR chromatography and fractionation **(EXTR_2)**	1. 3 x methanol, 2 x water 2. Concentration OR partition against acetonitrile/isohexane OR 1. 3 x methanol 2. Partition against acetonitrile/isohexane 3. Fractionation OR concentration to water 4. SPE clean up and concentration **(Chiral)**	1. 3 x methanol, 2 x water 2. Protease solubilisation OR concentration, chromatography and fractionation
Fat	1. 3 x acetonitrile/Isohexane 2. Concentration **(WU_1)** OR 1. 2 x acetonitrile/Isohexane 2. Partition of isohexane against acetonitrile (x3) 3. Concentration OR chromatography and fractionation **(WU_2)** OR 1. 2 x acetonitrile/Isohexane 2. Partition of isohexane against acetonitrile (x3) 3. Fractionation OR chromatography, partition against acetonitrile (x3) and concentration **(WU_3)** OR 1. 2 x acetonitrile/Isohexane 2. Partition of isohexane against acetonitrile (x3) 3. Concentration **(WU_4)**	1. 3 x methanol/isohexane 2. Concentration OR SPE clean up OR fractionation and SPE clean up	1. 3 x methanol/isohexane 2. Partition against methanol (x3) 3. Lipase treatment OR alkaline hydrolysis and partition against THF **(WU_1)** OR 1. 3 x acetonitrile/Isohexane 2. Concentration OR partition against acetonitrile (x3) **(WU_2)**

Liver	1. 3x methanol, 2 x water 2. Protease solubilisation OR partition against acetonitrile/isohexane and concentration **(WU_1)** OR 1. 3x methanol, 2 x water 2. Concentration OR protease solubilisation (**WU_2)**	1. 3x methanol, 2 x water 2. Protease solubilisation OR SPE clean up and concentration	1. 3x methanol, 2 x water 2. Concentration OR protease solubilisation **(WU_1)** OR 1. 3 x methanol 2. Chromatography and fractionation **(WU_2)**
Kidney	1. 3x methanol, 2x dichloromethane, 1x isohexane 2. Concentration **(WU_1)** OR 1. 3x methanol, 2x water 2. Concentration OR protease solubilisation **(WU_2)**	1. 3x methanol, 2 x water 2. Protease solubilisation OR SPE clean up and concentration	1. 3x methanol, 2 x water 2. Concentration OR protease solubilisation
Excreta	1. 3x methanol, 2 x water 2. Concentration	1. 3x methanol, 2 x water 2. Concentration 3. Chromatography and fractionation	

Solubilisation of proteins was undertaken using a protease treatment (incubation for 1-7 days at 37 °C). During the work up of fat (TMFP label) fatty acid conjugates were cleaved enzymatically using a lipase treatment (incubation for 1 day at 37 °C) or using alkaline hydrolysis (incubated with NaOH for 1 hour at ambient temperature).

Components of the residue were identified by HPLC-MS as well as by co-chromatography and comparison of retention times. After individual analysis samples of each matrix and label were pooled (for egg white, egg yolk and excreta this was during the dosing interval 162-288 h).

Results and discussion

Total radioactive residue

When levels of ^{14}C-BAS 750 F were determined via combustion analysis and LSC, recoveries generally were good and similar for the three labels (80% or higher), with most of the radioactivity being excreta-related (76-91% of dose), only low amounts recovered in GI tract (1-3% dose) or cagewash (2-3% dose). Generally, only low amounts of dose were retained in tissues. The total radioactive residues (TRR), expressed as mg/kg BAS 750 F and % administered dose are summarized for each label in Table 7.2.2-3.

The three labels result in largely similar residue levels in kidney (0.43-0.61 mg/kg) while for the other matrices clear differences between the labels are seen. Residues obtained with the C- and TFMP-labelled exceed the residues compared with the T-label for fat (0.68-1.23 mg/kg compared with 0.18 mg/kg), liver (0.31-0.61 mg/kg compared with 0.15 mg/kg) and for egg yolk (0.48-0.62 mg/kg compared with 0.27 mg/kg). Residues obtained with the T-label exceed the residues compared with the C-/TFMP-label for egg white (0.36 mg/kg compared with <0.01 mg/kg) and for muscle (0.38 mg/kg compared with <0.08 mg/kg). The TRR data obtained with all three labels taken together, indicates absence of significant cleavage of the ether bridge (between positions of radiocarbon in C- and TFMP-label) while the T-bridge (between positions of radiocarbon in TFMP- and T-label) appears to be effectively cleaved.

Table 7.2.2-3: TRR after administration of ^{14}C-BAS 750 F to hen

Matrix	C-label		TFMP-label		T-label	
	% of dose	**TRR** *measured [mg/kg]*	**% of dose**	**TRR** *measured [mg/kg]* [4)]	**% of dose**	**TRR** *measured [mg/kg]*
excreta	75.30	*2.92*	86.59	-	88.91	*6.34*
egg white	0.01	*0.009* [1)]	0.02	*0.005* [1)]	0.55	*0.357* [1)]
egg yolk	0.22	*0.477* [1)]	0.28	*0.618* [1)]	0.17	0.269 [1)]
partially formed eggs	0.08	-	0.14	-	0.09	-
muscle [2)]	0.03	*0.054*	0.05	*0.078*	0.23	*0.377*
liver	0.06	*0.307*	0.13	*0.611*	0.03	*0.146*
kidney	0.01	*0.431*	0.01	*0.612*	0.01	*0.590*
fat [3)]	0.13	*0.679*	0.10	*1.227*	0.01	*0.183*
GI tract, contents	1.14	-	2.41	-	1.62	-
bile	0.01	-	0.02	-	< 0.00	-
blood	< 0.00	-	< 0.00	-	< 0.00	-
subtotal organs /tissues	1.38	-	2.72	-	1.90	-
cage wash	2.53	-	2.61	-	2.37	-
Total recovery	**79.52**	-	**92.36**	-	**93.99**	-

1) refer to table 7.2.2-4 for detailed information, 2) muscle pool of breast and thigh muscle, 3) fat pool of omental, renal, and subcutaneous fat, 4) TRR (measured/LSC) for TFMP label was not determined

Daily egg samples obtained on 14 consecutive days, separated into yolk and egg white, were measured for total radioactive residues (TRR). Only very low proportions of the administered dose were found (<1% dose). With C-label and TFMP-label, dose retained was higher in egg yolk compared to egg white (factor of >10X), while for the T-label, dose retained was about 3X higher in egg white compared to egg yolk. Plateau levels of radioactive residues in egg were reached within 5-7 days of dosing (see Table 7.2.2-4) indicating absence of accumulation of residues both in yolk and in egg white.

Table 7.2.2-4: TRR in egg white and yolk after administration of ^{14}C-BAS 750 F

	C-label *TRR measured [mg/kg]*		**TFMP-label** *TRR measured [mg/kg]*		**T-label** *TRR measured [mg/kg]*	
Application day	**white**	**yolk**	**white**	**yolk**	**white**	**yolk**
1	*0.004*	*0.001*	*0.003*	*0.001*	*0.119*	*0.052*
2	*0.009*	*0.043*	*0.008*	*0.039*	*0.260*	*0.138*
3	***0.013***	*0.121*	*0.009*	*0.138*	*0.300*	*0.178*
4	***0.012***	*0.244*	*0.009*	*0.227*	*0.323*	*0.215*
5	***0.012***	*0.334*	***0.013*** [1)]	*0.384*	*0.314*	*0.234*
6	***0.012***	*0.472*	***0.007***	*0.460*	*0.359*	*0.277*
7 [1)]	***0.011***	***0.571*** [1)]	***0.011***	***0.617*** [1)]	***0.387*** [1)]	***0.301*** [1)]
8	***0.009***	***0.595***	***0.014***	***0.622***	***0.363***	***0.301***
9	***0.006***	***0.556***	***0.009***	***0.666***	***0.415***	***0.322***
10	***0.009***	***0.424***	***0.008***	***0.658***	***0.384***	***0.308***
11	***0.008***	***0.471***	***0.010***	***0.639***	***0.366***	***0.302***
12	***0.007***	***0.454***	***0.010***	***0.665***	***0.390***	***0.311***
13	***0.008***	***0.448***	***0.010***	***0.648***	***0.344***	***0.292***
pool sample	*0.009*	*0.477*	*0.005*	*0.618*	*0.357*	*0.269*

[1)] *start of plateau phase,* ***(bold typing)***

Extractability

The extractabilities (ERR – extractable radioactive residues) of ^{14}C residue from hen matrices, yolk, egg white, muscle, liver, kidney, and fat are summarized in Table 7.2.2-5 (C-label), Table 7.2.2-6 (TFMP-label), and Table 7.2.2-7 (T-label).

High extractability of ^{14}C residue was seen for egg, tissues and excreta (83% TRR or higher).

For all three labels, most of the radioactivity was extracted with methanol (88% TRR or higher) for egg yolk, egg white (T-label only), muscle, liver and kidney while subsequent water extraction resulted in additional 5% TRR or less. In C-and TFMP-labelled egg white residues were low (TRR <0.01 mg/kg), thus no extraction was done. Methanol extraction also retrieved about 100 % TRR of T-labelled fat, while for C- and TFMP-labelled fat, acetonitrile extraction (83-112% TRR) followed by isohexane extraction (11-18% TRR) was done to achieve complete extraction of the ^{14}C-residue.

Solvent extraction left a RRR (residual radioactive residues) of <5% TTR in egg white, fat, T-labelled yolk, TFMP-/T-labelled muscle and thus was not investigated further. Protease treatment allowed to further characterized the RRR of yolk (C-/TFMP-label), of muscle (C-label), of liver and kidney reducing the final unextractable residue further to <5% TRR for yolk and kidney, to 8% TRR (0.004 mg/kg) for muscle and to 7.3% TRR (0.02 mg/kg) for liver. The results of further characterization by protease treatment are outlined in Table 7.2.2-8.

Table 7.2.2-5: TRR and extractability of residues of ^{14C}-BAS 750 F (C-label) in egg, tissues and excreta

Matrix [1] **(C-label)**	**TRR** *(measured)*	**Methanol extract**		**Isohexane extract**		**Water extract**		**DCM extracts**		**ERR** [1]		**RRR** [1]		**TRR** [1] *(calculated)*	**Recovery** [2] **(extraction)**
	mg/kg	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*[mg/kg]*	*%*
egg white [3]	*0.009*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
egg yolk	*0.477*	**90.2**	*0.430*	-	-	**1.8**	*0.009*	-	-	**92.0**	*0.439*	**6.1**	*0.029*	*0.468*	**98.1**
egg yolk	*0.477*	**88.3**	*0.421*	-	-	**1.1**	*0.005*	-	-	**89.4**	*0.426*	**9.1**	*0.043*	*0.469*	**98.4**
muscle	*0.050*	**85.0**	*0.043*	-	-	-	-	-	-	**85.0**	*0.043*	**21.5**	*0.011*	*0.053*	**106.4**
liver (WU_1)	*0.320*	**80.6**	*0.258*	-	-	**3.4**	*0.011*	-	-	**84.0**	*0.269*	**14.6**	*0.047*	*0.316*	**98.7**
liver (WU_2)	*0.320*	**79.0**	*0.253*	-	-	**3.7**	*0.012*	-	-	**82.7**	*0.265*	**14.5**	*0.046*	*0.311*	**97.2**
kidney (WU_1)	*0.427*	**82.1**	*0.350*	**0.4**	*0.002*	-	-	**1.2**	*0.005*	**83.7**	*0.357*	**14.5**	*0.062*	*0.419*	**98.1**
kidney (WU_2)	*0.427*	**87.2**	*0.372*	-	-	**5.2**	*0.022*	-	-	**92.4**	*0.394*	**12.9**	*0.055*	*0.449*	**105.3**
excreta	*2.924*	**83.5**	*2.440*	-	-	**5.2**	*0.151*	-	-	**88.6**	*2.592*	**29.0**	*0.847*	*3.439*	**117.6**
	TRR *(measured)*	**acetonitrile extract**		**isohexane extract**		-		-		**ERR** [1]		**RRR** [1]		**TRR** [1] *(calculated)*	**recovery** [2] **(extraction)**
fat (WU_1)	*0.702*	**35.4**	*0.248*	**64.8**	*0.455*	-	-	-	-	**100.2**	*0.703*	**0.7**	*0.005*	*0.708*	**100.9**
fat (WU_2)	*0.702*	**36.0**	*0.253*	**55.8**	0.392	-	-	-	-	**91.9**	*0.645*	**1.1**	*0.007*	*0.652*	**92.9**
fat (WU_3)	*0.702*	**82.6**	*0.580*	**18.2**	*0.128*	-	-	-	-	**100.8**	*0.707*	**1.4**	*0.010*	*0.717*	**102.2**
fat (WU_4)	*0.702*	**83.7**	*0.587*	**14.0**	*0.098*	-	-	-	-	**97.7**	*0.685*	**1.1**	*0.007*	*0.693*	**98.8**

DCM denotes dichloromethane. EXTR_1, EXTR_2 denote extraction 1 and extraction 2, WU_1, WU_2, WU_3, WU_4 denote workup1, workup 2 etc.
[1] The TRR is calculated as the sum of ERR and RRR. The ERR is calculated as the sum of the respective solvent extracts. [2] recovery is calculated as "TRR calculated" divided by "TRR measured" [3] For egg white residue levels are very low, thus no extraction was performed.

Table 7.2.2-6: TRR and extractability of residues of ^{14C}-BAS 750 F (TFMP-Label) in egg, tissues and excreta

Matrix [1] **(TFMP-label)**		**TRR** *(measured)*	**Methanol extract**		**Isohexane extract**		**Water extract**		**DCM extracts**		**ERR** [1]		**RRR** [1]		**TRR** [1] *(calculated)*	**Recovery** [2] **(extraction)**
		mg/kg	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*[mg/kg]*	*%*
egg white [3]		*0.005*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
egg yolk		*0.618*	**93.7**	*0.578*	-	-	**0.5**	*0.003*	-	-	**94.2**	*0.582*	**5.1**[4]	*0.032*	*0.613*	**99.3**
muscle	(EXTR_1)	*0.066*	**109.2**	*0.072*	-	-	**1.1**	*0.001*	-	-	**110.3**	*0.073*	**2.8**	*0.002*	*0.074*	**113.2**
muscle	(EXTR_2)	*0.066*	**114.6**	*0.075*	-	-	-	-	-	-	**114.6**	*0.075*	**3.5**	*0.002*	*0.078*	**118.2**
liver	(WU_1)	*0.582*	**92.3**	*0.537*	-	-	**1.8**	*0.010*	-	-	**94.0**	*0.547*	**5.6**[4]	*0.033*	*0.580*	**99.6**
liver	(WU_2)	*0.582*	**90.1**	*0.525*	-	-	-	-	-	-	**90.1**	*0.525*	**7.4**	*0.043*	*0.567*	**97.4**
kidney		*0.610*	**97.4**	*0.594*	-	-	**1.6**	*0.009*	-	-	**98.9**	*0.603*	**6.3**[4]	*0.038*	*0.642*	**105.2**
fat	(WU_1)	*0.893*	**109.8**	*0.980*	**12.9**	*0.115*	-	-	-	-	**122.7**	*1.095*	**0.1**	*0.001*	*1.096*	**122.8**
			acetonitrile extract		**isohexane extract**		-		-		**ERR** [1]		**RRR** [1]		**TRR** [1] *(calculated)*	**recovery** [2] **(extraction)**
fat	(WU_2)	*0.893*	**112.1**	*1.001*	**11.0**	*0.099*	-	-	-	-	**123.1**	*1.100*	**0.3**	*0.002*	*1.102*	**123.4**

DCM denotes dichloromethane. EXTR_1, EXTR_2 denote extraction 1 and extraction 2, WU_1, WU_2, WU_3, WU_4 denote workup1, workup 2 etc.
[1] The TRR is calculated as the sum of ERR and RRR. The ERR is calculated as the sum of the respective solvent extracts. [2] recovery is calculated as "TRR calculated" divided by "TRR measured", [3] For egg white residue levels are very low, thus no extraction was performed. [4] Samples further investigated by protease treatment.

Table 7.2.2-7: TRR and extractability of residues of ^{14C}-BAS 750 F (T-Label) in egg, tissues and excreta

Matrix (T-label)	TRR *(measured)*	Methanol extract		Isohexane extract		Water extract		DCM extracts		ERR [1)]		RRR [1)]		TRR [1)] *(calculated)*	Recovery [2)] (extraction)
	mg/kg	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*mg/kg*	*%*
egg white	*0.357*	**97.0**	*0.346*	-	-	**1.2**	*0.004*	-	-	**98.2**	*0.350*	**0.2**	*0.001*	*0.351*	*98.3*
egg yolk	*0.269*	**107.9**	*0.290*	-	-	**1.0**	*0.003*	-	-	**108.9**	*0.293*	**2.6**	*0.007*	*0.300*	*111.4*
muscle	*0.353*	**98.8**	*0.349*	-	-	**2.2**	0.008	-	-	**101.0**	*0.356*	**1.4**	*0.005*	*0.361*	*102.4*
liver	*0.480*	**99.2**	*0.476*	-	-	**1.3**	*0.006*	-	-	**100.5**	*0.482*	**3.3**[4)]	*0.016*	*0.498*	*103.8*
kidney	*0.565*	**98.9**	*0.559*	-	-	**1.1**	*0.006*	-	-	**100.0**	*0.565*	**2.0**	*0.011*	*0.577*	*102.0*
fat	*0.190*	**101.8**	*0.194*	**5.0**	*0.010*	-	-	-	-	**106.8**	*0.203*	**3.2**[4)]	*0.006*	*0.209*	*110.0*
excreta	*6.341*	**99.3**	*6.296*	-	-	**2.3**	*0.148*	-	-	**101.6**	*6.444*	**15.2**	*0.962*	*7.405*	*116.8*

DCM denotes dichloromethane

[1)] The TRR is calculated as the sum of ERR and RRR. The ERR is calculated as the sum of the respective solvent extracts. [2)] recovery is calculated as "TRR calculated" divided by "TRR measured". [3)] For egg white residue levels are very low, thus no extraction was performed. [4)]Samples further investigated by protease treatment.

Table 7.2.2-8: Characterization of RRR by enzyme treatment (C-/ TFMP-/ T-label)

	RRR		**CHAR** [1] (protease digestion)		**Unextractable residue** [2]	
C-label	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
yolk	**-** [3]	**-** [3]	**2.1**	*0.023*	**4.0**	*0.019*
muscle	**21.5**	*0.011*	**7.2**	*0.004*	**8.2**	*0.004*
liver	**-** [3]	**-** [3]	**7.2**	*0.023*	**7.3**	*0.023*
kidney	**12.9**	*0.055*	**10.4**	*0.044*	**2.2**	*0.010*
TFMP-label	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
yolk	**5.1**	*0.032*	**2.5**	*0.015*	**2.2**	*0.013*
muscle	**-**	**-**	**-**	**-**	**-**	**-**
liver	**5.6**	*0.033*	**2.8**	*0.016*	**2.4**	*0.014*
kidney	**6.3**	*0.038*	**4.2**	*0.026*	**1.7**	*0.011*
T-label	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
yolk	**-**	**-**	**-**	**-**	**-**	**-**
muscle	**-**	**-**	**-**	**-**	**-**	**-**
liver	**3.3**	*0.016*	**1.8**	*0.009*	**1.0**	*0.005*
kidney	**2.0**	*0.011*	**1.3**	*0.008*	**0.8**	*0.005*

[1] CHAR = characterized by protease digestion. [2] final residue after solvent extraction and solubilization
[3] due to limited sample size, the RRR after solvent extraction was not determined. As indicative information refer to data in extractability table (C-label)

Characterisation and Identification

The parent compound BAS 750 F and the metabolites in hen matrices were identified by HPLC-MS analysis. An overview over the components of the extractable residue is given below in Tables 7.2.2-10 to 7.2.2-12. Structures of the metabolites are outlined in Appendix 1. Overall, largely similar composition of the radioactive residue is found for C-label and TFMP-label, and a significantly different composition with the T-label. Cleavage products account for large proportion of the residue, namely M750F022 and its conjugates in C-/TFMP-labelled samples, and 1,2,4-triazole in T-labelled samples.

Chiral analysis of BAS 750 F residue in representative samples of the hen metabolism study was restricted to samples where BAS 750 F levels were high enough to allow chiral HPLC analysis - egg yolk and fat. The ratio of enantiomers in both egg yolk and fat was 43:56, thus comparable to the ratio in the dose administered, as shown in Table 7.2.2-9.

Table 7.2.2-9: Determination of isomer ratio of BAS 750 F in hen matrices

Matrix	**S-enantiomer [%]**	**R-enantiomer [%]**
T-label		
administered dose	50.4	49.6
egg yolk	43.0	57.0
fat	43.1	56.9

1) Assignment of the two HPLC peaks to the R- and the S-enantiomer was done based on comparison of elution profiles

Egg yolk

Total identified and characterised extractable residue (ERR) in the pooled sample (day 7-12) accounted for 90.5, 83.7, 91.4 % of TRR (C-, TFMP-, T-label) with the residue after extraction (RRR) representing 9.1%, 2.2% and 2.6% TRR (C-TFMP-, T-label) corresponding to 0.04, 0.013 and <0.01

mg/kg. Protease treatment of C- and TFMP-labelled yolk reduced the final unextractable residue further to maximum of 4% TRR (<0.02 mg/kg).

The total identified residue in egg yolk accounted for 59-85% TRR. Additional characterization by chromatographic and extraction properties accounted for 6-31% TRR.

For the C- and TFMP-label, metabolite M750F022 was the most abundant component of the residue (39% TRR and 47% TRR, equivalent to 0.19 mg/kg and 0.29 mg/kg). The second most abundant residue components were fatty acid conjugates of M750F022 (M750F023-25), together accounting for 25.3% TRR for both labels (0.12 mg/kg and 0.16 mg/kg). Parent BAS 750 F was detected, albeit at lower amounts (6.5 and 11.5% TRR, equivalent to 0.03 and 0.07 mg/kg). The position of the radiocarbon in the C-label and TFMP-label explains why M750F001 is not detected in C-labelled or TFMP-label samples.

In contrast, with the T-label, BAS 750 F was present as the most abundant component (44% TRR, 0.12 mg/kg), while M750F001 (1,2,4-triazole) is found at 41% TRR (0.11 mg/kg). The position of the T-label radiocarbon in the T-ring explains why M750F022 is not detected in T-label samples.

Egg white

Total identified and characterised extractable residue (ERR) in the pooled sample (day 7-12) accounted for 98.2% of TRR (only T-label, residues for both C-, TFMP-label were <0.01 mg/kg, and thus not further investigated). The residue after extraction (RRR) represented 0.2% and was thus not further analysed.

The total identified residue in T-labelled egg white accounted for 83% TRR, all of it M750F001 (0.30 mg/kg). No other component was identified, additional characterization by extraction properties accounted for 15% TRR resulting in amounts identified and characterized of 98% TRR. The position of the T-label radiocarbon in the T-ring explains why M750F022 is not detected in T-label samples.

Muscle

Total identified and characterised extractable residue (ERR) accounted for 87.8, 100.0, 101.4% of TRR (C-, TFMP-, T-label). The residue after extraction (RRR) represented 21.5% (0.011 mg/kg, C-label), 2.8% (0.002 mg/kg, TFMP-label) and 1.4% (0.005 mg/kg, T-label). Protease treatment of C-labelled muscle reduced the final unextractable residue further to maximum of 8.2% TRR (0.004 mg/kg).

The total identified residue in muscle accounted for 75-94.3 %. Additional characterization by chromatographic and extraction properties accounted for 5.7-20.1% TRR.

For the C- and TFMP-label, metabolite M750F022 was the most abundant component of the residue (50% TRR and 77% TRR, equivalent to 0.03 mg/kg and 0.05 mg/kg). The second most abundant residue components were fatty acid conjugates of M750F022 (M750F023-25), together accounting for 19.5% and 9.8% TRR (equivalent to 0.01 mg/kg). Parent BAS 750 F was detected, albeit at lower amounts (5.6 and 7.4% TRR, equivalent to <0.01 mg/kg).

In contrast, with the T-label, BAS 750 F was not present at detectable amounts. M750F001 was the only component identified with 91.4% TRR (0.32 mg/kg). The position of the T-label radiocarbon in the T-ring explains why M750F022 is not detected in T-label samples.

Liver

Total identified and characterised extractable residue (ERR) accounted for 81.4, 86.1, 97.0% of TRR (C-, TFMP-, T-label). The residue after extraction (RRR) represented 7.3%, 5.6% and 3.3% (corresponding to 0.023 mg/kg, 0.033 mg/kg and 0.016 mg/kg). A significant amount of the RRR is bound to liver protein as confirmed by release upon up to 7% TRR by protease treatment. For C-

labelled liver the final unextractable residue was reduced to 7.3% (0.023 mg/kg), <5% for the TFMP- and the T-label.

The total identified residue in liver accounted for 55%, 67% and 96% TRR. Additional characterization by chromatographic and extraction properties accounted for 26.5, 19.1 and 1.3%.

For the C- and TFMP-label, metabolite M750F022 was the most abundant component of the residue in the C-label and TFMP-label (36.7% TRR and 29.3% TRR, equivalent to 0.12 mg/kg and 0.18 mg/kg). Fatty acid conjugates of M750F022 (M750F023-25), taken together were found at 6.9% and 11.6% TRR (equivalent to 0.02 and 0.6 mg/kg). Parent BAS 750 F was detected, albeit at lower amounts (4.0 and 5.8% TRR, equivalent to <0.03 mg/kg mg/kg).

In contrast, with the T-label, M750F001 was the predominant component identified with 85.2% TRR (0.41 mg/kg). BAS 750 F was present at 3.7% TRR (0.02 mg/kg). The position of the T-label radiocarbon in the T-ring explains why M750F022 is not detected in T-label samples.

In addition, a liver-specific metabolite was identified in all three labels, M750F034 accounting in the three labels for 4.3%, 20.1%, and 6.7% TRR (up to 0.03 mg/kg).

Kidney

Total identified and characterised extractable residue (ERR) accounted for 80.1, 87.6, 93.2% of TRR (C-, TFMP-, T-label). The residue after extraction (RRR) represented 14.5%, 6.3% and 2.0% (corresponding to 0.062 mg/kg, 0.038 mg/kg and 0.011 mg/kg). Protease treatment (not included in Table 7.2.2-10) reduced the final unextractable residue further to a maximum of 2% TRR (0.01 mg/kg).

The total identified residue in kidney accounted for 28%, 24%, and 66% TRR. Additional characterization by chromatographic and extraction properties accounted for 52.1, 63.8 and 27.6% TRR.

For the C- and TFMP-label, metabolite M750F022 was the most abundant component of the residue (20% TRR, equivalent to 0.09 mg/kg and 0.12 mg/kg). In addition, fatty acid conjugates of M750F022 (M750F023-25) were found at 4% TRR (C-label only, 0.02 mg/kg). Parent BAS 750 F was detected, albeit at lower amounts (4.0 and 3.7% TRR, equivalent to 0.02 mg/kg).

In contrast, with the T-label BAS 750 F was not present at detectable amounts. M750F001 was the only component identified with 66% TRR (0.37 mg/kg). The position of the T-label radiocarbon in the T-ring explains why M750F022 is not detected in T-label samples.

Fat

Total identified and characterised extractable residue (ERR) accounted for about 99.3, 115.2, 98.2% of TRR (C-, TFMP-, T-label). The residue after extraction (RRR) was ≤0.01 mg/kg and was thus not further analysed.

The total identified residue in fat accounted for 81.9%, 101.8% and 93.2% TRR. Additional characterization by chromatographic and extraction properties accounted for 17.3, 13.3 and 5.0% TRR.

For the C- and TFMP-label, metabolite M750F022 was the most abundant component of the residue (25% TRR and 41% TRR, equivalent to 0.18 mg/kg and 0.37 mg/kg). The second most abundant residue components were fatty acid conjugates of M750F022 (M750F023-25), together accounting for 78.6% (C-) and 49.1 (TFMP-) % TRR (equivalent to > 0.44 mg/kg). Treatment with lipase as well as alkaline treatment resulted in quantitative cleavage of the conjugate, thus releasing M750F022 (performed with TFMP-labelled fat). Parent BAS 750 F was detected, albeit at lower amounts (5.4 and 3.7% TRR, equivalent to 0.04 and 0.10 mg/kg).

In contrast, with the T-label, M750F001 was the most abundant component identified with 73.1% TRR (0.14 mg/kg). BAS 750 F was present at 20.1% TRR (0.04 mg/kg). The position of the radiocarbon in the T-label explains why M750F022 and its conjugates is not detected in T-label samples.

Excreta

Total identified and characterised extractable residue (ERR) accounted for 87.6 TRR (C-label only). The total identified residue accounted for 31.6% TRR. Additional characterization by chromatographic and extraction properties accounted for 51% TRR resulting in amounts identified and characterized of 88% TRR. Parent BAS 750 F was the most abundant component of the residue (28.6% TRR) while M750F022 was present at 3.1% TRR.

Table 7.2.2-10: Summary of BAS 750 F and metabolites and of characterized fractions in hen matrices (C-label)

C-labelled residue component		Egg yolk		Egg white[4)]		Muscle		Liver		Kidney		Fat		Excreta	
		%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR		**100**	*0.477*	-	-	**100**	*0.05*	**100**	*0.32*	**100**	*0.427*	**100**	*0.702*	**100**	*2.924*
BAS 750 F		**6.5**	*0.031*	-	-	**5.6**	*0.003*	**7.2**	*0.023*	**4.0**	*0.017*	**5.4**	*0.038*	**28.6**	*0.835*
M750F034		-	-	-	-	-	-	**4.3**	*0.014*	-	-	-	-	-	-
M750F022		**39.0**	*0.186*	-	-	**49.9**	*0.025*	**36.7**	*0.118*	**20.1**	*0.086*	**25.4**	*0.178*	**3.1**	*0.090*
M750F023		**2.6**	0.012	-	-	**8.0**	*0.004*	**2.0**	*0.006*	**1.7**	*0.007*	**23.7**	*0.166*	-	-
M750F024		**10.6**	0.051	-	-	-	-	**1.1**	*0.003*	**0.8**	*0.004*	**13.3**	*0.093*	-	-
M750F024 / M750F025[1)]		**11.4**	0.054	-	-	**11.5**	*0.006*	-	-	-	-	**27.4**	*0.193*	-	-
M750F025		**0.7**	0.003	-	-	-	-	**3.8**	*0.012*	**1.4**	*0.006*	**14.2**	*0.099*	-	-
ERR	**ID**	**59.4**	*0.283*	-	-	**75.0**	*0.038*	**55.0**	*0.176*	**28.0**	*0.120*	**81.9**	*0.575*	**31.6**	*0.925*
	CHAR (HPLC)	**30.0**	*0.143*	-	-	**12.9**	*0.006*	**22.7**	*0.072*	**50.5**	*0.215*	**14.7**	*0.103*	**50.8**	*1.485*
	CHAR (other)	**1.1**	*0.005*	-	-	-	-	**3.8**	*0.012*	**1.6**	*0.007*	**2.6**	*0.018*	**5.2**	0.1513
	sum ID/CHAR	**90.5**	*0.431*	-	-	**87.8**	*0.044*	**81.4**	*0.260*	**80.1**	*0.342*	**99.3**	*0.696*	**87.6**	*2.561*
RRR		**n.d**[2)]**.**	n.d. [2)]	-	-	**21.5**	*0.011*	**n.d.** [2)]	n.d. [2)]	**12.6**[3)]	*0.054*[3)]	-	-	-	-
CHAR		**2.1**[3)]	*0.010*[3)]	-	-	**7.2**	*0.004*	**7.2**	*0.023*	**10.4**[3)]	*0.044*[3)]	-	-	-	-
Final Residue (measured)		**9.1**	*0.043*	-	-	**8.2**	*0.004*	**7.3**	*0.023*	**14.5**	*0.062*	**1.4**	*0.010*	**29.0**	0.847
Grand Total		**99.6**	*0.475*	-	-	**103.2**	*0.052*	**95.9**	*0.307*	**94.6**	*0.404*	**100.6**	*0.706*	**116.6**	3.409

ID identification, CHAR characterization, sum ID/CHAR= sum of amounts identified and/or characterized, Final unextractable residue = unextractable residue after solubilization by protease treatment

[1)] The two metabolites M750F024 and M750F025 were not separated using HPLC.

[2)] Residue after solvent extraction was not measured due to low sample amount

[3)] RRR determined using a separate work up, hence these values are not related to the final residues measured in the ERR experiments for egg yolk and kidney

[4)] Residues were <0.01 mg/kg and therefore not further investigated

Table 7.2.2-11: Summary of BAS 750 F and metabolites and of characterized fractions in hen matrices (TFMP-label)

TFMP-labelled residue component		Egg yolk		Egg white [3]		Muscle		Liver		Kidney		Fat		Excreta	
		%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR		**100**	*0.618*	-	-	**100**	*0.066*	**100**	*0.582*	**100**	*0.610*	**100**	*0.0893*	-	-
BAS 750 F		**11.5**	*0.071*	-	-	**7.4**	*0.005*	**5.8**	*0.034*	**3.7**	*0.022*	**11.7**	*0.104*	-	-
M750F034		-	-	-	-	-	-	**20.1**	*0.117*	-	-	-	-	-	-
M750F022		**46.7**	*0.288*	-	-	**77.1**	*0.051*	**29.3**	*0.171*	**20.1**	*0.123*	**41.1**	*0.367*	-	-
M750F023		**5.3**	*0.032*	-	-	**5.8**	*0.004*	**3.6**	*0.021*	-	-	**27.5**	*0.245*	-	-
M750F024		**9.0**	*0.056*	-	-	-	-	-	-	-	-	**6.1**	*0.054*		
M750F024 / M750F025 [1]		-	-	-	-	**4.0**	*0.003*	**8.0**	*0.047*	-	-	-	-	-	-
M750F024, F025, other		**10.4** [1]	*0.064* [1]	-	-	-	-	-	-	-	-	-	-		
M750F025		**0.6**	*0.003*	-	-	-	-	-	-	-	-	**15.5**	*0.138*		
ERR	**ID**	**73.0**	*0.451*	-	-	**94.3**	*0.062*	**66.9**	*0.389*	**23.8**	*0.145*	**101.8**	*0.909*	-	-
	CHAR (HPLC)	**10.2**	*0.063*	-	-	**4.6**	*0.003*	**17.4**	*0.102*	**62.2**	*0.380*	**12.2**	*0.109*	-	-
	CHAR (other)	**0.6**	*0.004*	-	-	**1.1**	*0.001*	**1.7**	*0.010*	**1.6**	*0.010*	**1.1**	*0.010*	-	-
	sum ID/CHAR	**83.7**	*0.517*	-	-	**100.0**	*0.066*	**86.1**	*0.501*	**87.6**	*0.534*	**115.2**	*1.028*	-	-
RRR		**5.1**	0.032	-	-	-	-	**5.6**	*0.033*	**6.3**	*0.038*	-	-	-	-
CHAR		**2.5**	0.015			-	-	**2.8**	*0.016*	**4.2**	*0.026*	-	-		
Final Residue (measured)		**2.2**	*0.013*	-	-	**2.8**	*0.002*	**2.4**	*0.014*	**1.7**	*0.011*	**0.3**	*0.002*	-	-
Grand Total		**88.3** [2]	*0.545* [2]	-	-	**102.8**	*0.068*	**91.3**	*0.532*	**93.6**	*0.570*	**115.4**	*1.031*	-	-

ID identification, CHAR characterization, sum ID/CHAR= sum of amounts identified and/or characterized, Final unextractable residue = residue after solubilization by protease treatment

[1] The two metabolites M750F024 and M750F025 were not separated using HPLC.

[2] Approximately 10.5 % TRR were lost within a concentration step of the pooled methanol extract (Poo0029) to the HPLC sample (Lab0347).

[3] Residues were <0.01 mg/kg and therefore not further investigated

Table 7.2.2-12: Summary of BAS 750 F and metabolites and of characterized fractions in hen matrices (T-label)

T-labelled residue component		Egg yolk		Egg white		Muscle		Liver		Kidney		Fat		Excreta	
		%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR		**100**	*0.269*	**100**	*0.357*	**100**	*0.353*	**100**	*0.480*	**100**	*0.565*	**100**	*0.190*		
BAS 750 F		**43.7**	*0.117*	**-**	-	**-**	-	**3.7**	*0.018*	**-**	-	**20.1**	*0.038*	**-**	**-**
M750F034		**-**	-	**-**	-	**-**	-	**6.7**	*0.032*	-	-	**-**	-	-	-
M750F001		**41.4**	*0.111*	**83.2**	*0.297*	**91.4**	*0.322*	**85.2**	*0.409*	**65.6**	*0.371*	**73.1**	*0.139*	**-**	**-**
ERR	**ID**	**85.1**	*0.229*	**83.2**	*0.297*	**91.4**	*0.322*	**95.7**	*0.459*	**65.6**	*0.371*	**93.2**	*0.177*	**-**	**-**
	CHAR (HPLC)	**-**	-	-	-	**-**	-	**-**	-	**26.5**	*0.150*	**-**	-	**-**	**-**
	CHAR (other)	**6.2**	*0.017*	**14.8**	*0.053*	**10.0**	*0.035*	**1.3**	*0.006*	**1.1**	*0.007*	**5.0**	*0.010*	-	-
	sum ID/CHAR	**91.4**	*0.246*	**98.2**	*0.350*	**101.5**	*0.358*	**97.0**	*0.465*	**93.2**	*0.527*	**98.2**	*0.187*	**-**	**-**
RRR		-	-	-	-	**-**	-	**3.3**	*0.016*	**2.0**	*0.011*	**-**	-	**-**	**-**
CHAR		-	-	-	-	-	-	**1.8**	*0.009*	**1.3**	*0.008*	-	-	-	-
Final Residue (measured)		**2.6**	*0.007*	**0.2**	*<0.001*	**1.4**	*0.005*	**1.0**	*0.005*	**0.8**	*0.005*	**3.2**	*0.006*	**-**	**-**
Grand Total		**93.9**	*0.253*	**98.3**	*0.351*	**102.9**	*0.363*	**99.8**	*0.479*	**95.3**	*0.539*	**101.3**	*0.193*	**-**	**-**

ID identification, CHAR characterization, sum ID/CHAR= sum of amounts identified and/or characterized, Final unextractable residue = residue after solubilization by protease treatment

Storage stability

Analysis of the storage stability confirmed the stability of radioactive residues over the period of the study, both in the frozen matrix (prior to extraction) and in extracts.

Stability during storage of the matrix at ≤-18°C was investigated in C- and T-labelled poultry matrices, by comparing the resulting metabolic HPLC profiles after extended storage of the animal samples. The stability of C-labelled BAS 750 F in poultry matrices was demonstrated for at least 315 days.

Stability during storage of extract was investigated in C- and T-labelled poultry matrices, by comparing the metabolic HPLC profiles after extended storage of the extract. Methanol and acetonitrile extracts of the samples were analysed. Details of the extract storage times are given in Table 7.2.2-13.

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the triazole label, the residue extracts were stable for a period of at least 342 days. For the chlorophenyl label, the residue extracts were stable for a period of at least 127 days. The storage stability of residues in the corresponding homogenized samples of forage (stability in matrix) was confirmed for a period of at least 315 days for the C-label.

Table 7.2.2-13: Storage stability investigations in hen matrices

Matrix	Storage of matrix			Storage of extract		
	1st storage interval (analysis 1)	2nd storage interval (analysis 2)	Storage period	1st storage interval (analysis 1)	2nd storage interval (analysis 2)	Storage period
	[days]	*[days]*	*[days]*	*[days]*	*[days]*	*[days]*
C-label						
muscle (MeOH extract)	84	399	315	13	140	127
egg yolk (MeOH extract)	184	560	376	77	314	237
fat (ACN phase)	195	561	366	3	346	343
liver (ACN phase)	175	553	378	-	-	
liver (MeOH extract)	274	-		48	274	336
T-label						
egg white (MeOH extract)	150	-		13	363	350
egg yolk (MeOH extract)	149	492	343	18	364	346
fat (MeOH phase)	161	-		3	345	342
liver (MeOH extract)	141	-		15	369	354

ACN denotes acetonitrile phase (of acetonitrile extracts or methanol extracts), MeOH denotes methanol,
For egg white and yolk, the sampling date encompasses the treatment interval of 168-188h, the longest storage interval is represented.

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the triazole label, the residues in the matrix were stable for a period of at least 343 days. For the chlorophenyl label, the residues were stable for a period of at least 315 days. Stability of TFMP-labelled samples can be assumed for this time interval based on absence of TFMP-specific residue components. The storage stability of residues in the extracts was confirmed for a period of at least 127 days.

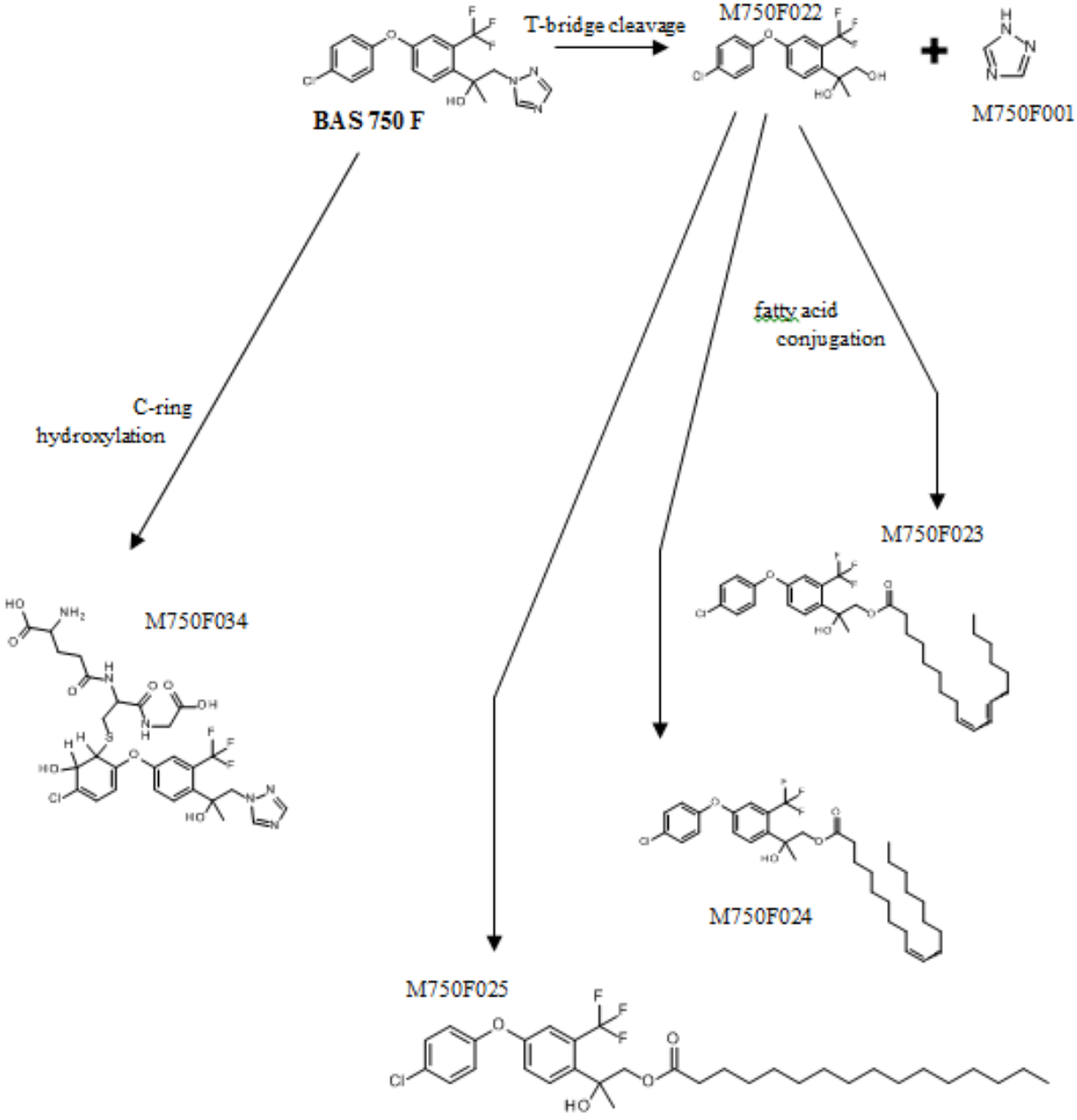
Proposed metabolic pathway

Metabolism was investigated in poultry using C-, TFMP- and T-labelled BAS 750 F. When the results from all labels are considered together the data demonstrate consistent metabolic pathways in poultry matrices. The proposed metabolic pathway is outlined in Figure 7.2.2-1.

The main metabolic step in all matrices is the cleavage of BAS 750 F at the triazole bridge to form the metabolites M750F001 and M750F022. Metabolite M750F022 is further conjugated by fatty acids to from M750F023, M750F024 (conjugated with unsaturated fatty acid), and M750F025 (conjugated with saturated fatty acid).

In the liver only, hydroxylation of BAS 750 F followed by epoxidation and conjugation with glutathione leads to the formation of metabolite M750F034.

Figure 7.2.2-1: Proposed pathway of BAS 750 F in poultry



Conclusion

The metabolism of BAS 750 F was investigated in poultry by dosing laying hens with C-, TFMP- or T-labelled BAS 750 F. For the three labels, the overall accountability of the study was good. The radioactive residue was rapidly and extensively excreted. Until sacrifice, the radioactive residues in excreta amounted to 75-89% of the total radioactivity administered. For all labels, only low portions of the administered dose (≤ 0.4 %) were retained in edible tissues or in egg (<1% of dose).

Label-specific differences were seen for TRR from tissues except kidney. C-label and TFMP-label were generally comparable, but distinct from T-label. In muscle and egg white, TRR for C-/TFMP-label (<0.08 mg/kg) was lower than for the T-label (>0.36 mg/kg). In contrast in liver and fat, TRR for C-/TFMP-label (>0.31 mg/kg) was higher than for the T-label (<0.18 mg/kg).

^{14}C residues in egg (sampled on 14 consecutive days) reached a plateau concentration within 5-7 days confirming absence of accumulation of residues in egg. Plateau levels in egg yolk were at 0.5 mg/kg, 0.65 mg/kg, and 0.3 mg/kg (C-, TFMP-, T-label). In egg white residues were <0.1 mg/kg (C-and TFMP-label) and 0.35 mg/kg for T-label.

The extractability of radioactive residues from all edible matrices (egg white and yolk, muscle, liver, kidney and fat was high (>83 %). Protease treatment released a further 2-10% TRR, with the final unextractable residue was between 1-8% TRR (0.004-0.023 mg/kg). No further efforts were made to characterise these residues as the contribution to the TRR is < 10%/<0.05, and a significant proportion of the residue was available for characterisation/identification, therefore further characterisation is not considered to impact significantly on the study results.

Metabolism of BAS 750 F in laying hen includes two main transformation reactions, cleavage of the parent backbone at the T-bridge generating 1,2,4-triazole (M750F001) as well as the two-ring metabolite M750F022, which itself is subject to conjugation by fatty acids (M750F023/M750F024/M750F025). In addition, a transformation reaction seen in liver is the C-ring oxygenation followed by glutathione conjugation (M750F034).

For the metabolite profile, label-dependent differences similar to the observations at TRR level were seen. Generally, results of C-label and TFMP-label were comparable, and distinct from the T-label. Parent BAS 750 F was present in all matrices investigated except egg white. Significant amounts of BAS 750 F were determined in in egg yolk (7-44% TRR) and fat (5-20% TRR), while proportions were low in muscle, liver, and kidney (<7% TRR).

For both C- and TFMP-label, the cleavage metabolite M750F022 together with its fatty acid conjugates was the predominant component of the residue, together accounting for >69% TRR in yolk, muscle, and fat, and 20-44% TRR in liver and kidney. M750F022 was the most abundant compound in muscle, liver, kidney and yolk, while in fat it's conjugates were present at up to 3x higher amounts.

The cleavage metabolite 1,2,4-triazole (M750F001) was found at a high level in all matrices, proportions were >65% TRR in egg white, muscle, liver, kidney and fat, and 41% TRR in egg yolk.

The only other component identified is the liver-specific metabolite M750F034, a glutathione conjugate of parent found at 4-20% TRR (0.01-0.12 mg/kg). Overall, metabolism of BAS 750 F in laying hen can be considered well-elucidated.

B.7.2.3. Lactating ruminants

Report:	CA 6.2.3/1 [redacted] 2015 a The metabolism of 14C-Reg. No. 5834378 (BAS 750 F) in lactating goats 2015/1078841
Guidelines:	OECD Test Guideline 503 - Metabolism in livestock, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), JMAFF 59 NohSan No 4200
GLP:	yes

Materials and methods

Materials

1. C-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Chlorophenyl-U-C14 (spec. activity 7.88 MBq/mg) added to a 1:2 (w:w) mixture of Chlorophenyl-1-C13-labelled and unlabelled test item	
Lot/Batch #:	Chlorophenyl-U-C14:	CFQ41561
	Chlorophenyl-1-C13:	RS4-2012-173A2
	Unlabelled:	COD-001740
Purity:	Chlorophenyl-U-C14:	99.1% (radiochem 98.9%)
	Chlorophenyl-1-C13:	97.7%
	Unlabelled:	98.8%

2. TFMP-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Trifluoromethylphenyl-U-C14 (spec. activity 8.265 MBq/mg) added to a 2:2 (w:w) mixture of Propyl-2-C13-labelled and unlabelled test item	
Lot/Batch #:	Trifluoromethylphenyl-U-C14:	CFQ42039
	Propyl-2-C13:	1126-1006
	Unlabelled:	COD-001740
Purity:	Trifluoromethylphenyl-U-C14:	96.3% (radiochem) 98.3%
	Propyl-2-C13:	99.5%
	Unlabelled:	98.8%

3. T-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Triazole-3(5)-C14 (spec. activity 5.46 MBq/mg) added to 1:1 (w:w) mix of Triazole-3(5)-C13-labelled and unlabelled test item	
Lot/Batch #:	Triazole-3(5)-C14:	1062-2001
	Triazole-3(5)-C13:	1077-1001
	Unlabelled:	COD-001740
Purity:	Triazole-3(5)-C14:	98.8% (radiochem 98.8%)
	Triazole-3(5)-C13:	97.1%
	Unlabelled:	98.8%

Methods

The metabolism of BAS 750 F was investigated in lactating goats (breed *British Saanen*) following repeated oral administration of ^{14}C-BAS 750 F, labelled either in the Chlorophenyl ring (C-ring), in the Trifluoromethylphenyl ring (TFMP-ring) or in the triazole ring (T-ring) (see Fig 7.2-2).

A total of 5 goats were dosed with radiolabelled BAS 750 F at nominal doses of 12 mg/kg feed (~2N for dairy cattle) for 12-14 consecutive days (2 goats for C-label, 2 for T-label and 1 for TFMP-label). Each ^{14}C-labelled test item was mixed with ^{13}C-labelled test item and unlabelled test item (ratios are given in Table 7.2.3-1). The test items were prepared in gelatine capsules, orally administered (once daily). The actual dose was based on the average feed consumption of (day 1-14). The mean achieved daily dose administered was 13.7-23.4 mg/kg food consumed (dry weight equivalent) corresponding to 0.36-0.43 mg/kg bw/d. Details of the study outline are summarized in Table 7.2.3-1.

Table 7.2.3-1: Dosing of lactating goats with BAS 750 F

Animal no.	**Treat-ment period (days)**	**Isotope ratio ^{14}C : ^{13}C : ^{12}C**	**Daily feed consump-tion**	**Animal weight (day 1)**	**Nominal daily dose**	**Actual daily dose [1)]**			**Time of sacrifice [2)] (hours)**
			[g/animal]	*[kg]*	*[mg/kg feed]*	*[mg/animal]*	*[mg/kg feed]*	*[mg/kg bw/d]*	
C-label: 1, 2	14	1 : 1 : 2	1548, 1270	59.5, 62.0	12	21.11	13.7, 17.3	0.36	23
TFMP-label: 5	12	1 : 2 : 2	897	52.5	12	20.721	23.4	0.40	23
T-label: 3, 4	14	1 : 1 : 1	1359, 1246	53.0, 53.5	12	21.234	15.7, 19.3	0.43	23

1) based on mean body weights on study day1 2) hours after last dose

Blood samples were taken prior to first dose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after the start of dosing.

Excreta, urine and faeces, were collected for a 24 h interval prior to first dose and subsequently every 24h until sacrifice. Daily excreta for each label was weighed, and stored at ≤- 18 °C prior to analysis.

Following each excreta collection, the cage was washed with the minimum amount of methanol:water (1:1, v:v) and rinsings retained. Daily cage wash samples for each label were pooled and weighed, and stored at ambient temperature prior to processing.

Each goat was milked twice daily (AM and PM) with the last sample taken immediately prior to sacrifice. The total volume of each milk sample was recorded, and then the AM and PM samples for each 24 hour period were pooled. Milk was stored at 4 ^{o}C prior to processing. Aliquots of each daily milk sample was separated into cream and skimmed milk samples by centrifugation.

The goats were sacrificed 23h after the final dose and edible tissues (liver, kidney, muscle (loin, flank), fat (omental, subcutaneous, and renal) as well as bile, blood and the GI tract collected, and stored at ≤- 18 °C prior to processing.

Tissue were homogenised prior to analysis via blending. For bile, blood, milk, cage wash and urine samples were homogenised via mixing before analysis. and for blood and bile with was via mixing. Faeces was homogenised via mixing with water, and plasma was obtained from blood samples via centrifugation before analysis.

The maximum time of frozen storage between sampling and analysis is 400 days. Stability data to support storage duration of at least 393 days is presented in the results section.

Prior to extraction, samples were combusted to verify the total radioactive residues (TRR). The homogenised samples were then extracted and worked up as outlined in Table 7.2.3-2.

Table 7.2.3-2: Details of sample extraction and work up

	C-label	T-label	TFMP-label
Muscle	1. 3 x methanol, 2 water 2. Centrifugation and concentration	1. 3 x methanol, 2 water 2. Concentration OR SPE cleanup and concentration	1. 3 x methanol, 2 water 2. Concentration OR fractionation and SPE cleanup
Whole milk	1. 1 x acetonitrile/isohexane, 3 x methanol 2. SPE cleanup and concentration	1. 1 x acetonitrile/isohexane 2. SPE cleanup and concentration	1. 1 x acetonitrile/isohexane 2. SPE cleanup and concentration OR 1. Partition isohexane/ethyl acetate 2. Concentration
Skimmed milk	1. 1 x acetonitrile/isohexane, 3 x methanol 2. Centrifugation and concentration 3. Partition with isohexane and concentration	1. 1 x acetonitrile/isohexane 2. Concentration	1. 1 x acetonitrile/isohexane, 3 x methanol 2. Centrifugation and concentration 3. Partition with isohexane and concentration
Cream	1. 3 x acetonitrile/isohexane 2. Concentration OR partition with acetonitrile x2	1. 1 x acetonitrile/isohexane 2. Concentration OR fractionation SPE cleanup	1. 1 x acetonitrile/isohexane 2. Concentration
Bile	No work up	1. Dilution	1. Dilution
Fat	1. 3 x isohexane, 2 x acetonitrile 2. Centrifugation and partition with acetonitrile 3. Partition with isohexane and concentration	1. 3 x isohexane, 2 x acetonitrile 2. Centrifugation and partition with acetonitrile 3. Fractionation and SPE cleanup OR concentration OR 1. 3 x isohexane, 2 x acetonitrile 2. SPE cleanup OR concentration	1. 3 x isohexane, 2 x acetonitrile 2. Partition with acetonitrile 3. Partition with isohexane and concentration
Liver	1. 3x methanol, 2 x water 2. Protease solubilisation OR centrifugation and concentration OR 1. 3x methanol, 2 x water 2. Centrifugation and concentration 3. Partition with isohexane and ethyl acetate and concentration	1. 3x methanol, 2 x water 2. Protease solubilisation OR SPE clean up and concentration OR 1. 3x methanol, 2 x water 2. Fractionation and SPE clean up OR SPE clean up and enzyme incubation/acid hydrolysis	1. 3x methanol, 2 x water 2. Protease solubilisation OR SPE clean up and concentration
Kidney	1. 3x methanol, 2x water 2. Concentration OR	1. 3x methanol, 2 x water 2. Centrifugation and concentration OR SPE cleanup and concentration	1. 3x methanol, 2 x water 2. Centrifugation and concentration OR fractionation and SPE cleanup

	1. 3 x methanol 2. Fractionation OR 1. 3 x methanol 2. SPE clean up and concentration OR fractionation and concentration		OR 1. 3x methanol, 2 x water 2. Partition with isohexane 3. Partition with ethyl acetate and concentration
Urine	No work up	No work up	No work up
Faeces	1. 3x methanol, 2 x water 2. Concentration	1. 3x methanol, 2 x water 2. Concentration OR fractionation of SPE cleanup	1. 3x methanol, 2 x water 2. Concentration

Solubilisation of proteins in liver was undertaken using a protease treatment (incubation overnight at 37 °C and pH 7.5). During the work up of kidney an attempt to cleave glucuronic acid from the conjugates M750F064 and M750F068 was made using with enzymes (β-glucoronidase/arylsufatase and incubated for 24 hours ta 20 °C at pH 5)

Components of the residue were identified by HPLC-MS and comparison of retention times. After individual analysis samples of each matrix and label were pooled (for urine, faeces and milk this was during the dosing interval 144-288 h).

Results and discussion

Total radioactive residue

When levels of ^{14}C-BAS 750 F were determined via combustion analysis and LSC, recoveries generally were good and similar for the three labels. The total radioactive residues (TRR), expressed as mg/kg BAS 750 F and % administered dose are summarized for each label in Table 7.2.3-3 and Table 7.2.3-4.

For the C-Label (goats 1, 2) 79% and 82% of the total dose was recovered. The majority of the radioactivity was excreta-related (in faeces 47%-49% of dose, in urine 25% and 26% of dose). Only low amounts were recovered in the GI tract contents (2.7-4.0% dose) and the cagewash (ca. 1% dose). For the TFMP-label (goat 5) 82% of the total dose was recovered. The majority of the radioactivity was excreta-related (in faeces 35% of dose, in urine 40% dose). In the GI tract contents 3.8% of dose were found, in the cagewash 0.9% of dose. For the T-label (goats 3, 4) 83-84% of the total dose was recovered. The majority of the radioactivity was excreta-related (in faeces 53% and 47% of dose, in urine 24% and 30% of dose). Only low amounts were recovered in the GI tract contents (up to 2.7% dose) and in the cagewash (0.5% dose).

Table 7.2.3-3: Distribution of radioactivity of ^{14}C-BAS 750 F in goats

Matrix	C-label *% of dose*			TFMP-label *% of dose*	T-label *% of dose*		
	goat 1	**goat 2**	***calculated mean***	**goat 5**	**goat 3**	***goat 4***	***calculated mean***
urine	*25.28*	*26.44*	**25.86**	**40.21**	*23.66*	*30.13*	**26.90**
feces	*48.62*	*47.15*	**47.89**	**34.49**	*52.68*	*46.50*	**49.59**
milk	*0.24*	*0.25*	**0.25**	**0.35**	*2.10*	*2.21*	**2.16**
liver	*0.35*	*0.45*	**0.40**	**0.52**	*0.27*	*0.23*	**0.25**
kidney	*0.01*	*0.01*	**0.01**	**0.02**	*0.01*	*0.01*	**0.01**
muscle (flank)	*0.01*	*0.02*	**0.02**	**0.07**	*0.15*	*0.09*	**0.12**
muscle (loin)	*0.01*	*0.01*	**0.01**	**0.03**	*0.05*	*0.06*	**0.06**
fat (subcutan)	*0.03*	*0.04*	**0.04**	**0.22**	*0.03*	*0.02*	**0.03**
fat (omenal)	*0.16*	*0.25*	**0.21**	**0.60**	*0.13*	*0.10*	**0.12**
fat (renal)	*0.05*	*0.12*	**0.09**	**0.16**	*0.03*	*0.02*	**0.03**
G.I. tract contents	*2.66*	*4.04*	**3.35**	**3.76**	*2.66*	*2.60*	**2.63**
G.I. tract	*0.96*	*2.44*	**1.70**	**1.08**	*1.57*	*0.90*	**1.24**
bile	*0.02*	*0.02*	**0.02**	**0.22**	*0.02*	*0.02*	**0.02**
whole blood	*< 0.01*	*< 0.01*	**< 0.01**	**<0.01**	*n.a.*	*n.a.*	**n.a.**
cage wash	*0.73*	*1.15*	**0.94**	**0.87**	*0.58*	*0.47*	**0.53**
Sum excreta	-	-	**77.2**	**78.5**	-	-	**79.1**
Total recovery	*79.13*	*82.39*	**80.76**	**84.91**	*83.94*	*83.36*	**83.65**

Table 7.2.3-4: TRR in tissues, milk, bile and excreta (pool samples)

Matrix	C-Label		TFMP-Label		T-Label	
	TRR *measured*[5] *[mg/kg]*	**TRR** *calculated*[6] *[mg/kg]*	**TRR** *measured*[5] *[mg/kg]*	**TRR** *calculated*[6] *[mg/kg]*	**TRR** *measured*[5] *[mg/kg]*	**TRR** *calculated*[6] *[mg/kg]*
muscle [3]	0.044	0.047	0.099	0.098	0.222	0.223
liver	1.122	1.085	1.468	1.332	0.655	0.650
kidney	0.353	0.352	0.436	0.429	0.386	0.396
fat [4]	0.307	0.309	0.515	0.532	0.215	0.213
whole milk	0.029	0.029	0.065	0.062	0.284	0.273
skim milk [2]	0.016	0.016	0.031	0.036	0.286	0.270
cream [2]	0.204	0.207	0.491	0.521	0.266	0.289
urine	4.154	- [1]	5.329	- [1]	2.941	- [1]
feces	3.823	5.174	4.569	5.543	3.077	3.206
bile	7.393	- [1]	11.687	- [1]	3.974	- [1]

[1] not analysed (sample not subjected to extraction), [2] composite milk sample separated by centrifugation into fat (cream) and aqueous (skim milk) fraction, [3]muscle types pooled, then pools combined in the ratio 2:1 (w:w) loin: flank muscle, [4]fat types pooled, then pools combined in the ratio 2:1:1 (w:w:w) omental: subcutaneous: renal fat,[5] TRR measured directly via combustion LSC, [6] TRR was calculated as the sum of ERR, the radioactivity measured in the residue obtained after protease solubilisation and the radioactivity measured in the protease solubilisate

Daily milk samples were obtained on 12-14 consecutive days. Only very low proportions of the administered dose were found with up to 0.25 % of dose (C-label), 0.35 % of dose (TFMP-label) and up to 2.2 % of dose (T-label). Plateau levels of radioactive residues in milk were reached within 5-8 days of dosing (see Table 7.2.3-5) indicating absence of accumulation of residues in milk.

Table 7.2.3-5: TRR in milk after administration of ^{14}C-BAS 750 F to goats

	C-label *TRR measured [mg/kg]*		**TFMP-label** *TRR measured [mg/kg]*	**T-label** *TRR measured [mg/kg]*	
application day	goat 1	goat 2	goat 5	goat 3	goat 4
1	- [1]	- [1]	- [1]	- [1]	- [1]
2	*0.014*	*0.015*	*0.021*	*0.076*	*0.045*
3	*0.027*	*0.031*	*0.049*	*0.156*	*0.099*
4	*0.029*	*0.030*	*0.058*	*0.220*	*0.262*
5	***0.033*** [2]	*0.037*	***0.074*** [2]	***0.261*** [2]	***0.372*** [2]
6	***0.031***	***0.041*** [2]	***0.075***	***0.273***	***0.347***
7	***0.029***	***0.046***	***0.074***	***0.285***	***0.317***
8	***0.028***	***0.042***	***0.080***	***0.311***	***0.310***
9	***0.028***	***0.040***	***0.074***	***0.289***	***0.284***
10	***0.028***	***0.038***	***0.071***	***0.281***	***0.259***
11	***0.028***	***0.035***	***0.062***	***0.285***	***0.275***
12	***0.025***	***0.039***	***0.061***	***0.279***	***0.254***
13	***0.027***	***0.038***	***0.056***	***0.271***	***0.228***
14	***0.028***	***0.053***	-	***0.263***	***0.224***
15	***0.030***	***0.059***	-	***0.253***	***0.224***
pool sample [1]	*0.020*		*0.065*	*0.284*	

[1] no determination (i.e. values obtained did not exceed background level) [2] start of plateau phase, indicated by bold typing

Overall, low residue levels were found in milk (except T-labelled milk), muscle, kidney and fat while higher levels were found for liver.

For muscle, the TRR was <0.1 mg/kg for the C- /TFMP-label, and higher for the T-label (0.22 mg/k). Similarly, for milk, the TRR was <0.1 mg/kg for the C- /TFMP-label, with predominant partitioning to cream (0.21 and 0.52 mg/kg for C- and TFMP-label) and 0.27 mg/kg for the T-label with similar levels in cream and in skim milk.

For all three labels the TRR in kidney was 0.35-0.42 mg/kg and the TRR in fat 0.21-0.53 mg/kg, hence relatively similar. In contrast, the TRR in liver was generally higher and showed more variation with levels of 1.09 mg/kg, 1.33 mg/kg and 0.65 mg/kg (C-label, TFMP-label, T-label).

For excreta, all three labels (C-, TFMP-, T-label) showed similarly high levels in both urine (4.15 mg/kg, 5.33 mg/kg, 2.94 mg/kg) and faeces (5.17 mg/kg, 5.54 mg/kg, 3.21 mg/kg). The levels in faeces correlate well with high levels found in bile (7.39 mg/kg, 11.69 mg/kg and 3.97 mg/kg, for the C-, TFMP-, T-label, respectively).

Extractability

The extractabilities (ERR – extractable radioactive residues) of ^{14}C residue from goat matrices, milk (including cream and skim milk), from muscle, liver, kidney and faeces are summarized in Table 7.2.3-6 (C-label), Table 7.2.3-7 (TFMP-label), and Table 7.2.3-8 (T-label).

High extractability of ^{14}C residue was seen for milk and tissues (90% TRR or higher) and faeces (84% TRR or higher).

For all three labels, most of the radioactivity was extracted with methanol (88% TRR or higher) for muscle, liver, and kidney while subsequent water extraction resulted in additional 2% TRR or less. Solvent extraction left a RRR of <2% TRR in muscle and <3% TRR in kidney which was not further investigated. In liver, RRR amounted to 7.6%, 6.5% and 10.1% TRR and therefore were subjected to protease treatment releasing additional 1.8%-3.4% TRR.

In fat, the predominant part of the ^{14}C residue was extracted with isohexane (91% TRR or higher), acetonitrile extracted at maximum 5% of TRR. Solvent extraction left a RRR of 4% TRR or less (representing <0.01 mg/kg BAS 750 F equivalents), which was not further investigated.

In whole milk, the predominant part of the ^{14}C residue was extracted with acetonitrile (86% TRR or higher), isohexane extracted a maximum 5% of TRR, and methanol extraction of the C-labelled sample yielded 6% TRR. Similar extraction was seen for skim milk. In contrast, for cream the C- and TFMP-label, isohexane extracted higher amounts of ^{14}C residue (6-13% TRR) compared with milk correlating with the significantly higher TRR in cream compared to milk (an effect not seen with the T-label). RRR in milk fractions were low (for C- and TFMP-label, <5%, corresponding to <0.003 mg/kg, and for the T-label at maximum 7.5 %TRR, corresponding to 0.02 mg/kg) and therefore not further investigated.

Table 7.2.3-6: TRR and extractability of residues of ^{14C}-BAS 750 F (C-label) in milk, tissues and excreta

Matrix (C-label)	TRR (measured)	Acetonitrile extract		Isohexane extract		Methanol extract		ERR		RRR		TRR (calculated)	Recovery [3] (extraction)
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	[mg/kg]	%
milk (whole)	**0.029**	**85.6**	*0.025*	**5.0**	*0.001*	**5.8**	*0.002*	**96.5**	*0.028*	**3.5**	*0.001*	**0.029**	100.0
cream	**0.204**	**85.2**	*0.176*	**13.2**	*0.027*	**-**	**-**	**98.4**	*0.203*	**1.6**	*0.003*	**0.207**	101.5
skim milk [1]	**0.016**	**87.8**	*0.014*	**1.0**	*<0.001*	**5.0**	*0.001*	**95.3**	*0.015*	**4.7**	*0.001*	**0.016**	100.0
fat	**0.307**	**1.1**	*0.003*	**99.5**	*0.306*	**-**	**-**	**100.6**	*0.309*	-[2]	-[2]	**" 0.307 "** [2]	-[2]
		Methanol extract		**Water extract**		**-**		**ERR**		**RRR**		**TRR (calculated)**	**Recovery [3] (extraction)**
muscle	**0.044**	**98.2**	*0.046*	**0.3**	*<0.001*	**-**	**-**	**98.5**	*0.047*	**1.5**	*0.001*	**0.047**	106.8
liver	**1.122**	**91.3**	*0.990*	**1.1**	*0.012*	**-**	**-**	**92.4**	*1.002*	**7.6[4]**	*0.083*	**1.085**	96.7
kidney	**0.353**	**96.4**	*0.340*	**1.0**	*0.003*	**-**	**-**	**97.4**	*0.343*	**2.6**	*0.009*	**0.352**	99.7
faeces	**3.823**	**85.0**	*4.396*	**1.1**	*0.055*	**-**	**-**	**86.0**	*4.452*	**14.0**	*0.723*	**5.174**	135.3

The TRR is calculated as the sum of ERR and RRR. The ERR is calculated as the sum of the solvent extracts. [1] skim milk (aqueous) of the C-label was also extracted with water. An additional amount of <0.001 mg/kg (1.6 % TRR) was extracted and added to the TRR value. [2] no valid measurement for the RRR was obtained, and thus the "TRR calculated" could not be determined. For further calculations, the TRR measured was used instead. [3] recovery was calculated as "TRR calculated" divided by "TRR measured, [4]subject to further protease solubilisation

Table 7.2.3-7: TRR and extractability of residues of ^{14C}-BAS 750 F (TFMP-label) in milk, tissues and excreta

Matrix (TFMP-label)	TRR (measured)	Acetonitrile extract		Isohexane extract		ERR		RRR		TRR (calculated)	Recovery [3] (extraction)
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	mg/kg	%
milk (whole)	**0.065**	**95.9**	*0.059*	**2.3**	*0.001*	**98.1**	*0.061*	**1.9**	*0.001*	**0.062**	95.3
cream	**0.491**	**93.8**	*0.489*	**5.9**	*0.031*	**99.7**	*0.520*	**0.3**	*0.001*	**0.521**	106.1
skim milk [1]	**0.031**	**96.0**	*0.035*	**0.3**	*<0.001*	**96.3**	*0.035*	**3.7**	*0.001*	**0.036**	116.1
fat	**0.515**	**1.1**	*0.006*	**98.4**	*0.524*	**99.5**	*0.530*	**0.5**	*0.003*	**0.532**	103.3
		Methanol extract		**Water extract**		**ERR**		**RRR**		**TRR (calculated)**	**Recovery [3] (extraction)**
muscle	**0.099**	**98.8**	*0.097*	**<0.1**	*<0.001*	**98.8**	*0.097*	**1.2**	*0.001*	**0.098**	99.0
liver	**1.468**	**91.5**	*1.219*	**0.3**	*0.003*	**91.7**	*1.222*	**6.5 [1]**	*0.086*	**1.332 [1]**	90.7
kidney	**0.436**	**97.9**	*0.420*	**0.3**	*0.001*	**98.2**	*0.422*	**1.8**	*0.008*	**0.429**	98.3
faeces	**4.569**	**81.3**	*4.507*	**3.0**	*0.167*	**84.3**	*4.674*	**15.7**	*0.868*	**5.543**	121.3

The TRR is calculated as the sum of ERR and RRR. The ERR is calculated as the sum of the solvent extracts. [1] Final unextractable residue after protease solubilisation as no valid measurement for RR obtained. Consequently, TRR (calculated) was obtained as the sum of ERR & radioactivity of the post-solubilization solid & solubilisate. [3] recovery was calculated as "TRR calculated" divided by "TRR measured"

Table 7.2.3-8: TRR and extractability of residues of ^{14}C-BAS 750 F (T-label) in milk, tissues and excreta

Matrix (T-label)	TRR (measured)	Acetonitrile extract		Isohexane extract		ERR		RRR		TRR (calculated)	Recovery [1] (extraction)
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	mg/kg	%
milk (whole)	**0.284**	**92.4**	*0.252*	**0.1**	*<0.001*	**92.5**	*0.253*	**7.5**	*0.020*	**0.273**	96.1
cream	**0.266**	**96.7**	*0.280*	**0.5**	*0.001*	**97.1**	*0.281*	**2.9**	*0.008*	**0.289**	108.6
skim milk	**0.286**	**98.7**	*0.267*	**<0.1**	*<0.001*	**98.7**	*0.267*	**1.3**	*0.003*	**0.270**	94.4
fat	**0.215**	**5.0**	*0.011*	**91.0**	*0.193*	**96.0**	*0.204*	**4.0**	*0.008*	**0.213**	99.1
		Methanol extract		**Water extract**		**ERR**		**RRR**		**TRR (calculated)**	**Recovery [1] (extraction)**
muscle	**0.222**	**98.7**	*0.220*	**0.7**	*0.002*	**99.3**	*0.221*	**0.7**	*0.001*	**0.223**	100.5
liver	**0.655**	**87.9**	*0.571*	**2.0**	*0.013*	**89.9**	*0.584*	**10.1**[2]	*0.066*	**0.650**	99.2
kidney	**0.386**	**98.3**	*0.390*	**0.4**	*0.002*	**98.8**	*0.391*	**1.2**	*0.005*	**0.396**	102.6
faeces	**3.077**	**83.6**	*2.680*	**1.2**	*0.040*	**84.9**	*2.720*	**15.1**	*0.486*	**3.206**	104.2

The TRR is calculated as the sum of ERR and RRR. The ERR is calculated as the sum of the solvent extracts.[1] recovery was calculated as "TRR calculated" divided by "TRR measured"[2] subject to further protease solubilisation

Characterisation and Identification

The parent compound BAS 750 F and the metabolites in goat matrices were identified by HPLC-MS analysis. An overview over the components of the extractable residue is given below in Tables 7.2.3-10 to 7.2.3-13. Structures of the metabolites are outlined in Appendix 1. Overall, largely similar composition of the radioactive residue is found for C-label and TFMP-label, and a significantly different composition with the T-label.

Chiral analysis of BAS 750 F residue in representative samples of the goat metabolism study revealed a change of the ratio of the S-enantiomer and R-enantiomer in several matrices. While in the dose administered the ratio was approximately 50:50, in all matrices the proportion of R-enantiomer increased significantly, namely in cream (72%), muscle (76%), liver (70%), kidney (80%), and fat (80%), as shown in Table 7.2.3-9. Note, that in faeces the enantiomer ratio was comparable to the administered dose. A similar change was observed in the toxicology studies on rats (see section CA B.6), with an increase in the R enantiomer; however as the S enantiomer is expected to be the more toxicologically active, a reduction in this should not increase any toxicological concerns.

Table 7.2.3-9: Determination of isomer ratio of BAS 750 F in goat matrices

Matrix	S-enantiomer [%]	R-enantiomer [%]
TFMP-label		
administered dose	50.60	49.40
muscle	23.68	76.32
kidney	19.60	80.40
T-label		
administered dose	50.28	49.72
cream	28.34	71.66
liver	30.04	69.96
fat	20.67	79.33
faeces	50.54	49.46

Assignment of the two HPLC peaks to the R- and the S-enantiomer was done based on comparison of elution profiles

Table 7.2.3-10: Summary of BAS 750 F and metabolites and of characterized fractions in goat matrices (C-label)

C-label residue component	Whole milk		Muscle		Liver		Kidney		Fat		Urine		Faeces		Bile	
	%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR	**100**	*0.029*	**100**	*0.047*	**100**	*1.085*	**100**	*0.352*	**100**	*0.309*	**100**	*4.154*	**100**	*5.174*	**100**	*7.393*
BAS 750 F	**47.5**	*0.014*	**87.9**	*0.042*	**49.9**	*0.541*	**28.3**	*0.100*	**84.6**	*0.260*	**3.0**	*0.124*	**57.2**	*2.962*	**2.8**	*0.206*
M750F068			-	-	**3.0**	*0.033*	**17.8**	*0.063*	-	-			-	-		
M750F039	-	-	-	-	-	-	-	-	-	-	**3.7**	*0.153*	**0.7**	*0.035*	**3.0**	*0.221*
M750F072	**5.9**	*0.002*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M750F041	**6.0**	*0.002*	-	-	-	-	-	-	-	-	**3.7**	*0.154*	-	-	-	-
M750F091	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3.3**	*0.244*
M750F015	-	-	-	-	-	-	-	-	-	-	-	-	**4.7**	*0.244*	**5.5**	*0.403*
M750F015 / -F043	-	-	-	-	-	-	-	-	-	-	**10.1**	*0.421*	-	-	-	-
M750F017	-	-	-	-	-	-	-	-	-	-	**4.2**	*0.173*	**1.5**	*0.079*	-	-
M750F017 /-F078	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**2.7**	*0.197*
M750F016	-	-	-	-	**11.8**	*0.128*	-	-	-	-	**7.8**	*0.325*	**3.8**	*0.194*	**6.3**	*0.468*
M750F063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**26.3**	*1.945*
M750F022	**2.2**	*0.001*	**6.7**	*0.003*	**4.8**	*0.052*	**5.8**	*0.021*	**4.5**	*0.014*	**25.4**	*1.055*	**5.5**	*0.285*	**7.5**	*0.557*
M750F043	**14.2**	*0.004*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M750F038	-	-	-	-	**6.5**	*0.070*	-	-	-	-	-	-	**3.6**	*0.189*	**11.3**	*0.839*
M750F038 /-F042	-	-	-	-	-	-	-	-	-	-	**28.1**	*1.168*	-	-	-	-
M750F038 /-F064	-	-	-	-	-	-	**26.6**	*0.094*	-	-	-	-	-	-	-	-
ERR ID-HPLC	**75.7**	*0.022*	**94.6**	*0.045*	**76.0**	*0.824*	**78.5**	*0.277*	**89.1**	*0.274*	**86.0**	*3.573*	**77.1**	*3.988*	**68.7**	*5.080*
ERR CHAR-HPLC	**6.5**	*0.002*	-	-	**8.4**	*0.091*	**19.3**	*0.068*	-	-	**14.0**	*0.581*	**2.5**	*0.127*	**31.3**	*2.313*
ERR CHAR-other	**10.84**	*0.003*	**0.3**	*<0.001*	**1.1**	*0.012*	**1.0**	*0.003*	**1.1**	*0.003*	-	-	**1.1**	*0.055*	-	-
ERR SUM-ID/CHAR	**93.1**	*0.027*	**94.9**	*0.045*	**85.5**	*0.927*	**98.8**	*0.348*	**90.2**	*0.277*	**100.0**	*4.154*	**80.6**	*4.170*	**100.0**	*7.393*
RRR-CHAR	-	-	-	-	**3.4**	*0.037*	-	-	-	-	-	-	-	-	-	-
Unextractable Residue	**3.5**	*0.001*	**1.5**	*0.001*	**4.5**	*0.049*	**2.6**	*0.009*	-	-	-	-	**14.0**	*0.723*	-	-
Grand Total	**96.7**	*0.028*	**96.4**	*0.046*	**93.4**	*1.013*	**101.5**	*0.358*	**90.2**	*0.277*	-	-	**94.6**	*4.892*	-	-

ID-HPLC= amounts identified by HPLC, CHAR-HPLC= amounts characterized by HPLC, CHAR-other=amounts characterized by extraction, SUM-ID/CHAR=sum of amounts identified and/or characterized, RRR-CHAR=amounts of RRR which were characterized.

Table 7.2.3-11: Summary of BAS 750 F and metabolites and of characterized fractions in goat matrices (TFMP-label)

TFMP-label residue component	Whole milk		Muscle		Liver		Kidney		Fat		Urine		Faeces		Bile	
	%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR	**100**	*0.062*	**100**	*0.098*	**100**	*1.332*	**100**	*0.429*	**100**	0.532	**100**	*5.329*	**100**	*5.543*	**100**	*11.687*
BAS 750 F	**44.5**	*0.028*	**95.7**	*0.094*	**46.7**	*0.622*	**46.0**	*0.198*	**88.1**	0.469	-	-	**26.6**	*1.473*	**1.8**	*0.213*
M750F068	-	-	-	-	**4.2**	*0.056*	-	-	-	-	-	-	-	-	-	-
M750F039	-	-	-	-	-	-	-	-	-	-	-	-	**2.1**	*0.117*	-	-
M750F072	**5.8**	*0.004*	-	-	-	-	**3.0**	*0.013*	-	-	-	-	-	-	-	-
M750F041	**7.2**	*0.004*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M750F091	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**2.7**	*0.319*
M750F015	-	-	-	-	-	-	**2.6**	*0.011*	-	-	**2.3**	*0.122*	**8.8**	*0.488*	**0.5**	*0.064*
M750F017	-	-	-	-	-	-	-	-	-	-	-	-	**2.9**	*0.162*	-	-
M750F016	-	-	-	-	**15.0**	*0.200*	**3.7**	*0.016*	-	-	**3.5**	*0.186*	**13.8**	*0.766*	**0.7**	*0.087*
M750F063	-	-	-	-	-	-	-	-	-	-	**26.6**	*1.417*	-	-	**58.3**	*6.808*
M750F022	**1.2**	*0.001*	-	-	**7.6**	*0.101*	**10.7**	*0.046*	**5.8**	0.031	-	-	**8.7**	*0.482*	-	-
M750F043	**25.0**	*0.016*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M750F038	-	-	-	-	**11.2**	*0.149*	**14.0**	*0.060*	-	-	-	-	**6.2**	*0.342*	-	-
M750F038 /-F064	-	-	-	-	-	-	-	-	-	-	**47.1**	*2.511*	-	-	-	-
M750F003	-	-	-	-	-	-	**3.2**	*0.014*	-	-	-	-	**3.0**	*0.166*	-	-
ERR ID-HPLC	**83.7**	*0.052*	**95.7**	*0.094*	**84.7**	*1.128*	**83.3**	*0.357*	**93.9**	*0.500*	**79.5**	*4.24*	**72.1**	*3.996*	**64.1**	*7.491*
ERR CHAR-HPLC	**3.0**	*0.002*	-	-	**4.5**	*0.060*	**11.0**	*0.047*	-	-	**20.5**	*1.093*	**9.1**	*0.504*	**33.9**	*3.957*
ERR CHAR-other	**2.3**	*0.001*	**<0.1**	*<0.001*	**0.3**	*0.003*	**0.3**	*0.001*	**4.5**	*0.024*	-	-	**3.0**	*0.167*	-	-
ERR SUM-ID/CHAR	**88.9**	*0.055*	**95.7**	*0.094*	**89.4**	*1.191*	**94.6**	*0.406*	**98.4**	*0.524*	**100.0**	*5.329*	**84.2**	*4.667*	**98.0**	*11.448*
RRR-CHAR	-	-	-	-	**1.8**	*0.023*	-	-	-	-	-	-	-	-	-	-
Unextractable Residue	**1.9**	*0.001*	**1.2**	*0.001*	**6.5**	*0.086*	**1.8**	*0.008*	**0.5**	*0.003*	-	-	**15.7**	*0.868*	-	-
Grand Total	**90.8**	*0.056*	**96.9**	*0.095*	**97.7**	*1.301*	**96.3**	*0.414*	**99.0**	*0.527*	-	-	**99.9**	*5.535*	-	-

ID-HPLC= amounts identified by HPLC, CHAR-HPLC= amounts characterized by HPLC, CHAR-other=amounts characterized by extraction, SUM-ID/CHAR=sum of amounts identified and/or characterized, RRR-CHAR=amounts of RRR which were characterized.

Table 7.2.3-12: Summary of BAS 750 F and metabolites and of characterized fractions in goat matrices (T-label)

T-label residue component	Whole milk		Muscle		Liver		Kidney		Fat		Urine		Faeces		Bile	
	%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR	**100**	*0.273*	**100**	*0.223*	**100**	*0.650*	**100**	*0.396*	**100**	*0.213*	**100**	*2.941*	**100**	*3.206*	100	3.974
BAS 750 F	**3.0**	*0.008*	**11.9**	*0.027*	**26.2**	*0.170*	**10.3**	*0.041*	**84.9**	*0.180*	-	-	**49.5**	*1.586*	-	-
M750F068	-	-	-	-	**4.4**	*0.028*	-	-	-	-	-	-	-	-		
M750F039	-	-	-	-	-	-	-	-	-	-	-	-	**2.8**	*0.090*	-	-
M750F091	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1.9**	*0.076*
M750F015	-	-	-	-	-	-	-	-	-	-	**7.4**	*0.217*	**8.9**	*0.285*	-	-
M750F017	-	-	-	-	-	-	-	-	-	-	-	-	**2.5**	*0.080*	-	-
M750F016	-	-	-	-	**10.0**	*0.065*	-	-	-	-	**6.9**	*0.202*	**8.3**	*0.266*	-	-
M750F063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**73.5**	*2.920*
M750F001	**78.4**	*0.214*	**87.3**	*0.194*	-	-	**68.1**	*0.270*	**4.7**	*0.010*	**69.2**	*2.036*	**4.6**	*0.147*	**8.0**	*0.319*
M750F001/derivate[1)]	-	-	-	-	**31.8**	*0.207*	-	-	-	-	-	-	-	-	-	-
M750F003	-	-	-	-	-	-	-	-	-	-	**16.5**	*0.486*	**5.2**	*0.167*	-	-
ERR ID-HPLC	**81.4**	*0.222*	**99.2**	*0.221*	**72.4**	*0.470*	**78.4**	*0.311*	**89.6**	*0.190*	**100.0**	*2.94*	**81.7**	*2.621*	**83.4**	*3.316*
ERR CHAR-HPLC	**1.8**	*0.005*	-	-	**8.0**	*0.052*	**9.9**	*0.039*	-	-	-	-	-	-	**19.7**	*0.782*
ERR CHAR-other	**0.1**	*<0.001*	**0.7**	*0.002*	**2.0**	*0.013*	**0.4**	*0.002*	**2.7**	*0.006*	-	-	**1.2**	*0.040*	-	-
ERR SUM-ID/CHAR	**83.3**	*0.228*	**99.9**	*0.223*	**82.4**	*0.536*	**88.7**	*0.352*	**92.2**	*0.196*	**100.0**	*2.941*	**83.0**	*2.661*	**103.1**	*4.098*
RRR-CHAR	-	-	-	-	**2.2**	*0.014*	-	-	-	-	-	-	-	-	-	-
Unextractable Residue (measured)	**7.5**	*0.020*	**0.7**	*0.001*	**6.7**	*0.044*	**1.2**	*0.005*	**4.0**	*0.008*	-	-	**15.1**	*0.486*	-	-
Grand Total	**90.8**	*0.248*	**100.6**	*0.224*	**91.3**	*0.594*	**89.9**	*0.356*	**96.2**	*0.205*	-	-	**98.1**	*3.146*	-	-

ID-HPLC= amounts identified by HPLC, CHAR-HPLC= amounts characterized by HPLC, CHAR-other=amounts characterized by extraction, SUM-ID/CHAR=sum of amounts identified and/or characterized, RRR-CHAR=amounts of RRR which were characterized.. 1) The metabolites 1,24-triazole and a triazole-derivate metabolite were detected in a ratio of 2:1 in the confirmatory HPLC analysis.

Table 7.2.3-13: Summary of BAS 750 F and metabolites and of characterized fractions in milk fractions

Labelled residue component	C-label						TFMP-label						T-label					
	Whole milk		Skim milk		Cream		Whole milk		Skim milk		Cream		Whole milk		Skim milk		Cream	
	%TRR	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*	*%TRR*	*mg/kg*
TRR	**100**	*0.029*	**100**	*0.016*	**100**	*0.207*	**100**	*0.062*	**100**	*0.036*	**100**	*0.521*	**100**	*0.273*	**100**	*0.270*	**100**	*0.289*
BAS 750 F	**47.5**	*0.014*	**23.3**	*0.004*	**75.6**	*0.156*	**44.5**	*0.028*	**13.9**	*0.005*	**80.3**	*0.419*	**3.0**	*0.008*	-	-	**15.8**	*0.046*
M750F072	**5.9**	*0.002*	**10.4**	*0.002*	-	-	**5.8**	*0.004*	**9.8**	*0.004*	-	-	-	-	-	-	-	-
M750F041	**6.0**	*0.002*	**11.2**	*0.002*	-	-	**7.2**	*0.004*	**12.4**	*0.005*	-	-	-	-	-	-	-	-
M750F022	**2.2**	*0.001*	-	-	**4.2**	*0.009*	**1.2**	*0.001*	-	-	**5.2**	*0.027*	-	-	-	-	-	-
M750F043	**14.2**	*0.004*	**35.9**	*0.006*	**5.3**	*0.011*	**25.0**	*0.016*	**36.8**	*0.013*	**12.3**	*0.064*	-	-	-	-	-	-
M750F001	-	-	-	-	-	-	-	-	-	-	-	-	**78.4**	*0.214*	**95.2**	*0.257*	**74.5**	*0.215*
ERR ID-HPLC	**75.7**	*0.022*	**80.8**	*0.013*	**85.1**	*0.176*	**83.7**	*0.052*	**72.9**	*0.026*	**97.9**	*0.510*	**81.4**	*0.222*	**95.2**	*0.257*	***90.3***	*0.261*
ERR CHAR-HPLC	**6.5**	*0.002*	-	-	-	-	**3.0**	*0.002*	14.2	0.005	-	-	**1.8**	*0.005*	-	-	-	-
ERR CHAR-other	**10.84**	*0.003*	**7.5**	*0.001*	**13.2**	*0.027*	**2.3**	*0.001*	**0.3**	*<0.001*	**5.9**	*0.031*	**0.1**	*<0.001*	**<0.001**	*<0.1*	**0.5**	*0.001*
ERR SUM-ID/CHAR	**93.1**	*0.027*	**88.3**	*0.014*	**98.4**	*0.203*	**88.9**	*0.055*	**87.4**	*0.032*	**103.9**	*0.543*	**83.3**	*0.228*	**95.2**	*0.257*	***90.8***	*0.263*
RRR-CHAR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unextractable Residue (measured)	**3.5**	*0.001*	**4.7**	*0.001*	**1.6**	*0.003*	**1.9**	*0.001*	**3.7**	*0.001*	**0.3**	*0.001*	**7.5**	*0.020*	**1.3**	*0.003*	**2.9**	*0.008*
Grand Total	**96.7**	*0.028*	**93.0**	*0.015*	**100.0**	*0.207*	**90.8**	*0.056*	**91.1**	*0.033*	**104.1**	*0.543*	**90.8**	*0.248*	**96.5**	*0.261*	**93.6**	*0.271*

ID-HPLC= amounts identified by HPLC, CHAR-HPLC= amounts characterized by HPLC, CHAR-other=amounts characterized by extraction, SUM-ID/CHAR=sum of amounts identified and/or characterized, RRR-CHAR=amounts of RRR which were characterized

Milk (whole)

Total identified and characterised extractable residue in the pooled sample (days 6-12) accounted for 93.1%, 88.9% and 83.3 %of TRR (C-, TFMP-, T-label). The residue after extraction (RRR) represented 3.5%, 1.9% and 7.5% (max 0.02 mg/kg) and was thus not further analysed. The total identified residue in milk accounted for >75%. Additional characterization by chromatographic and extraction properties accounted for 2-17% TRR.

Parent BAS 750 F was the most abundant component of the residue in the C-label and TFMP-label (47.5% TRR and 44.5% TRR, equivalent to 0.014 mg/kg and 0.028 mg/kg). In contrast, in the T-label BAS 750 F was present only at 0.008 mg/kg (and 3%TRR), while the metabolite 1,2,4-triazole (M750F001) was the predominant (and only other) component (78.4% TRR, 0.21 mg/kg). The position of the radiocarbon in the C-label and TFMP-label explains why M750F001 is not detected in C-labelled or TFMP-label samples. In the C-label and TFMP-label, the second most abundant component was metabolite M750F043 (14.2% and 25.0 % TRR), albeit at only low absolute amounts (0.004 mg/kg and 0.016 mg/kg). In addition, small amounts of M750F072 and M750F041 were identified (maximum 7.2 % TRR, 0.004 mg/kg).

Skim milk

The data for skim milk is highly comparable to whole milk in respect of the metabolites present. Total identified and characterised extractable residues in the pooled sample (days 6-12) accounted for 88.3%, 87.4% and 95.2% of TRR (C-, TFMP-, T-label). The residue after extraction (RRR) represented 4.7%, 3.7% and 1.3% (max 0.003 mg/kg) and was thus not further analysed. The total identified residue in skim milk accounted for >72%. Additional characterization by chromatographic and extraction properties accounted for 0.1-15%

The metabolite pattern obtained from skim milk samples of the C-label and the TFMP-label were almost identical to those for whole milk samples. Metabolites M750F041, M750F072 and M750F043 were detected in somewhat higher quantities (up to 36.8% TRR). The parent compound BAS 750 F was less prominent (23.3% TRR) compared to whole milk and M750F022 was not detected. The only metabolite detected in T-labelled skim milk was the metabolite M750F001 (95.2% TRR).

Cream

Total identified and characterised extractable residues (TFMP-, T-label). The residue after extraction (RRR) represented 1.6%, 0.3% and 2.9% (max 0.008 mg/kg) and was thus not further analysed. The total identified residue in cream accounted for >85 %. Additional characterization by chromatographic and extraction properties accounted for 0.5-13% TRR.

Parent BAS 750 F was the most prominent component (up to 80.3 % TRR) for the C-label and the TFMP-label. In addition M750F043 and M750F022 were detected (up to 12.3 % TRR). Metabolites M750F041 and M750F072 which are present in milk were not detected in cream. For the T-label, M750F001 was the most prominent metabolite (74.5 % TRR) and the parent compound accounted for 15.8 % TRR.

Muscle

Total identified and characterised extractable residue accounted for >94% of TRR for all three labels, the residue after extraction (RRR) was ≤ 1.5% (max 0.002 mg/kg) and was thus not further analysed. The total identified residue in muscle amounted to 95 - 99 % or higher, further characterization did not result a significant increase (<0.002 mg/kg).

Parent BAS 750 F was the most abundant component of the residue in the C-label and TFMP-label (87.9% TRR and 95.7% TRR, equivalent to 0.042 mg/kg and 0.094 mg/kg). In contrast, in the T-label sample, the BAS 750 F amount present (0.027 mg/kg) accounted for only 11.9 % of TRR, while the metabolite 1,2,4-triazole (M750F001) was the predominant (and only other) component (87.3% TRR, 0.19 mg/kg). The position of the radiocarbon in the C-label and TFMP-label explains why M750F001 is not detected in C-labelled or TFMP-label samples. In the C-label, the only other component

identified was metabolite M750F022 (6.7%), albeit at only very low absolute amounts (0.003 mg/kg). In the TFMP-label, no further component was detected.

Liver

Total identified and characterised extractable residue accounted for 85.5%, 89.5% and 82.6% of TRR (C-, TFMP-, T label). For all labels, the residue after extraction (RRR) was further treated with protease which released the TRR bound to proteins, accounting for up to 3.4% TRR or 0.037 mg/kg. Taken together, the RRR represented 7.6% and 10.1% (0.083 mg/kg, 0.066 mg/kg) for the C- and T-label. For the TFMP-label, the RRR was not determined. The total identified residue accounted for 76.0% TRR, 84.7% TRR, and 72.4% TRR. Additional characterization by protease treatment, chromatographic and extraction properties, accounted for 6.6 – 10.2% TRR.

Parent BAS 750 F was the most abundant component of the residue in the C-label and TFMP-label (49.9% TRR and 46.7% TRR, equivalent to 0.54 mg/kg and 0.62 mg/kg). In contrast, in the T-label sample, BAS 750 F was present at lower amounts (0.17 mg/kg, 26.2% TRR), while the metabolite 1,2,4-triazole (M750F001) was slightly higher (31.8% TRR, 0.21 mg/kg). The position of the radiocarbon in the C-label and TFMP-label explains why M750F001 is not detected in C-labelled or TFMP-label samples.

In all three labels, significant amounts of metabolites resulting from glucuronidation (M750F068, 3.0-4.4 %TRR) or C-ring hydroxylation (M750F016, 10.0–11.8 % TRR) of the parent backbone were detected.

In the C-label and TFMP-label, the cleavage metabolite M750F022 was detected (4.8 and 7.6% TRR, 0.05 and 0.10 mg/kg), as well as its derivative M750F038 (6.5 and 11.2% TRR, 0.07 and 0.15 mg/kg).

Kidney

Total identified and characterised extractable residue accounted for 98.8%, 94.6% and 88.7% of TRR (C-, TFMP-, T label). The residue after extraction (RRR) represented at maximum 2.6 % TRR (0.009 mg/kg or lower) and thus was not further analysed. The total identified residue accounted for 78.5, 83.3 and 78.4%. Additional characterization by chromatographic and extraction properties accounted for 10.4 - 20.3% TRR.

Parent BAS 750 F was the most abundant component of the residue in the C-label and TFMP-label (28.3% TRR and 46.0% TRR, equivalent to 0.10 mg/kg and 0.20 mg/kg). In contrast, in the T-label sample, BAS 750 F was present only at 0.041 mg/kg (and 10.3%TRR), while the metabolite 1,2,4-triazole (M750F001) was the predominant (and only other) component identified (68.1% TRR, 0.27 mg/kg. The position of the radiocarbon in the C-label and TFMP-label explains why M750F001 is not detected in C-labelled or TFMP-label samples.

In the C-labelled kidney, 17.8% of M750F068 (glucuronide of parent compound) were detected, whereas this metabolite was not detected in TFMP- and T-labelled kidney.

In the C-label and TFMP-label, the second most abundant component was metabolite M750F038 (26.6% and 14.0 % TRR), at absolute amounts of 0.094 mg/kg and 0.060 mg/kg. For the C-label, metabolite M750F038 co-eluted with metabolite M750F064. HPLC-MS investigations and enzymatic treatment of an aliquot of the methanol extract of kidney resulted in a 1:1 ratio of both metabolites. The enzyme treatment effectively cleaved off the glucuronic acid moiety from both M750F064, thus generating M750F022, as well as off M750F038 generating parent BAS 750 F. In the TFMP-label, several low level metabolites resulting from modification of the parent backbone were identified: the hydroxy-metabolites M750F015 (2.6% TRR) and M750F016 (3.7% TRR) as well as a sulphate conjugate of parent M750F072 (3.0% TRR) at absolute levels of 0.011 – 0.016 mg/kg.

The cleavage metabolite M750F022 was found at low levels of 5.8% TRR (C-label) and 10.7% (TFMP-label), while M750F003 was found at 3.2% TRR (only TFMP-label). Characterization by chromatographic properties detected 2 further peaks accounting for at maximum 8.4% TRR (0.036 mg/kg).

Fat

Total identified and characterised extractable residue accounted for >90% of TRR for all three labels while the residue after extraction (RRR) was a maximum of 4.0% TRR representing 0.008 mg/kg, thus was not further analysed. The total identified residue in fat accounted for >89%.

Parent BAS 750 F was the most abundant component with 84.6% TRR, 88.1% TRR, 84.9% TRR (C-, TFMP-, T-label). In the C- and TFMP-label, small amounts of metabolite M750F022 were found (4.5% and 5.8% TRR, corresponding to 0.014 and 0.031 mg/kg). In the T-label, small amounts of the metabolite 1,2,4-triazole (M750F001) were found (4.7% TRR, 0.010 mg/kg). The position of the radiocarbon in the C-label and TFMP-label explains why M750F001 is not detected in C-labelled or TFMP-label samples. The position of the radiocarbon in the T-label explains why M750F022 is not detected T-labelled samples.

Urine

No extraction was necessary. The total identified residue accounted for 86.0, 79.5 and 100.0% TRR (C-, TFMP-, T-label). Additional characterization by chromatographic and extraction properties accounted for 14.0 and 20.5% TRR for C- and TFMP-label.

According to HPLC analyses, M750F038 (co-eluting with either M750F042 or M750F064 for the C- and the TFMP-label) was one of the main components detected in urine of the C- and the TFMP-label (28.1% and 47.1 % TRR) while undetectable in T-labelled samples due to absence of the T-ring.

The metabolites M750F022, M750F040, M750F041 and M750F043 (up to 25.4% TRR) were only detected in C-labelled urine, while the two glucuronic acid conjugates, M750F063 (26.6% TRR) and M750F064 (co-eluting with metabolite M750F038) were found at high amounts only in TFMP-labelled urine. In T-labelled urine, M750F001 (69.2% TRR) and metabolite M750F003 (16.5 % TRR) were the main components. M750F003 is a cleavage product (ether bridge cleavage, absence of C-ring). The C-ring hydroxyl-metabolites M750F015 (up to 10.1% TRR), M750F016 (up to 10.17.8% TRR), M750F017 (up to 4.2% TRR) were detected with all three labels.

Faeces

Total identified and characterised extractable residue accounted for 77.1, 72.1 and 81.7% of TRR (C-, TFMP-, T label). The residue after extraction (RRR) represented at maximum 15.7% TRR (0.87 mg/kg or lower) and was not further analysed. The total identified residue accounted for 77.1, 72.1 and 81.7% TRR. Additional characterization by chromatographic and extraction properties accounted for 1.2 – 12.1% TRR.

According to HPLC analyses, the metabolite patterns for faeces extracts of all three labels were very similar. The main component was the parent compound BAS 750 F (26.6-57.2% TRR). In addition, the metabolites M750F015 (up to 8.9 % TRR), M750F016 (up to 13.8% TRR), M750F017 (up to 2.9% TRR) and M750F039 (up to 2.8% TRR) were detected in the extracts of all three labels. Metabolites M750F038 (up to 6.2% TRR) and M750F022 (up to 8.7 % TRR) were found in extracts of C- and TFMP-labelled feces. The label specific metabolites M750F001 (T-label) and M750F003 (T- and TFMP-label) were found at minor quantities (up to 5.2% TRR).

Bile

Extraction was not done for bile. The total identified residue accounted for 68.7%, 64.1% and 83.4% TRR. Additional characterization by chromatographic and extraction properties accounted for 31.3%, 33.9% and 19.7% TRR for C-, TFMP- and T-label.

According to HPLC analyses, the main component detected in bile of all three labels was the glucuronic acid conjugate M750F063 (up to 26.3%, 58.3% and 73.5% TRR). In the C-labelled bile the metabolites M750F038 (11.3% TRR) and M750F022 (7.5% TRR) and the metabolites M750F017 and M750F078 (co-eluting in one peak, 2.7 % TRR) were detected. Metabolites M750F091 (up to 3.3% TRR), M750F015 (up to 5.5% TRR), M750F016 (up to 6.3% TRR) and BAS 750 F (up to 2.8% TRR) were found at minor amounts (C-label, TFMP-label). For the T-label, besides M750F063, only M750F001 and M750F091 were found (up to 8.0 % TRR).

Storage stability

Analysis of the storage stability confirmed the stability of radioactive residues over the period of the study, both in the frozen matrix (prior to extraction) and in extracts.

Stability during storage of the matrix at ≤-18°C was investigated in C-labelled goat kidney, by comparing the resulting metabolic HPLC profiles after extended storage of the animal samples (comparison of sample stored for 607 and 214 days). The stability of C-labelled BAS 750 F in goat kidney was demonstrated for at least 393 days. On the basis that stability is demonstrated for this period in goat kidney, and a range of poultry matrices, it is considered that sufficient data is available to support the storage of goat matrices as outlined in the study.

Stability during storage of extracts was investigated in C-, TFMP- and T-labelled goat matrices, by comparing the metabolic HPLC profiles after extended storage of the extract. Methanol and acetonitrile extracts of the samples were analysed. Details of the extract storage times are given in Table 7.2.3-14.

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the triazole label, the residue extracts were stable for a period of at least 182 days. For the chlorophenyl label, the residue extracts were stable for a period of at least 258 days, and for the TFMP-label, the residue extracts were stable for a period of at least 209 days. The storage stability of residues in the corresponding homogenized samples of forage (stability in matrix) was confirmed for a period of at least 315 days for the C-label.

Table 7.2.3-14: Storage stability investigations in goat matrices

Matrix	**Extract 1st storage interval** (analysis 1) *[days]*	**Extract 2nd storage interval** (analysis 2) *[days]*	**Storage period** *[days]*
C-label			
milk (ACN phase)	6	264	258
cream (ACN phase)	10	411	401
liver (MeOH extract)	12	432	420
kidney (MeOH extract)	8	429	421
muscle (MeOH extract)	7	435	428
fat (isohexane phase)	2	400	398
TFMP-label			
milk (ACN phase)	23	178	155
skim milk (ACN phase)	33	176	143
cream (ACN phase)	21	176	155
liver (MeOH extract)	39	183	144
kidney (MeOH extract)	27	183	156
muscle (MeOH extract)	1	171	170
fat (isohexane phase)	38	186	148
T-label			
milk (ACN phase)	131	334	203
skim milk (ACN phase)	148	331	183
cream (ACN phase)	140	329	189
liver (MeOH extract)	134	407	273
kidney (MeOH extract)	103	406	303
muscle (MeOH extract)	95	335	240
fat (isohexane phase)	156	338	182

ACN denotes acetonitrile phase (of acetonitrile extracts or methanol extracts), MeOH denotes methanol

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the chlorophenyl label, the residues were stable for a period of at least 210 days. For the TFMP label, the residues in the matrix were stable for a period of at least 209 days. For the triazole label, the residues in the matrix were stable for a period of at least 197 days. The storage stability of residues in the extracts was confirmed for a period of at least 143 days.

Proposed metabolic pathway

Metabolism was investigated in goat using C-, TFMP- and T-labelled BAS 750 F. When the results from all labels are considered together the data demonstrate consistent metabolic pathways in goat matrices. The proposed metabolic pathway is outlined in Figure 7.2.3-1.

The main metabolic steps are chlorophenyl-ring hydroxylation (followed by conjugation) and cleavage of the parent backbone at the T-bridge (followed by conjugation).

Initial hydroxylation of the chlorophenyl moiety ring leads to the formation of metabolites M750F015, M750F016, M750F017 (M750F017 being a product of Cl shift and hydroxylation). M750F041 (doubly hydroxylated C-ring) and M750F091 (hydroxylated cysteine conjugate) are intermediates of the C-ring hydroxylation reaction leading to the formation of C-ring hydroxyl metabolites (notably M750F016).

Metabolites M750F015, M750F016, M750F017 can be further conjugated with glucuronic acid leading to M750F063. As glucuronidation may take place at the chlorophenyl or propyl-triazole moiety, the structure of M750F063 is depicted as a generic structure.

Cleavage of BAS 750 F at the T-bridge generates 1,2,4-triazole (M750F001) as well as the two-ring metabolite M750F022, which itself is subject to oxidation (M750F038), followed by demethylation (M750F040), as well as to hydroxylation (M750F078), to sulphation (M750F043), and to glucuronidation (M750F064).

A transformation reaction observed only to a minor extent is cleavage of the parent backbone at the ether bridge, generating the two-ring metabolite M750F003. Notably, metabolites consisting of only either the C-ring or the TFMP-ring were not observed in any of the samples of the C-label or the TFMP-label. Another minor pathway is hydroxylation of the propyl-triazole moiety of BAS 750 F to form M750F039 which was further conjugated with sulphate (M750F072) or oxidised (M750F042).

In Appendix 1, for some of the metabolites generic structures are provided in cases when exact position of hydroxyl group or sugar moiety is not known (indicated by a “dotted” line).

Conclusion

The metabolism of BAS 750 F was investigated in ruminants by dosing lactating goats with C-, TFMP- or T-labelled BAS 750 F. The majority radioactive residue was excreted. Until sacrifice, the radioactive residues in excreta amounted to >77% of the total radioactivity administered. For all labels, only low portions of the administered dose (≤ 0.6 %) were retained in edible tissues or in milk (<2.2% of dose).

The parent BAS 750 F was applied as a racemic mixture of two enantiomers. Chiral analysis of BAS 750 F revealed a significant change of the ratio in most matrices, with proportion of the R-enantiomer of 70-80% in cream, muscle, liver, kidney and fat. In contrast, the racemate was maintained in faeces, indicating a preferential metabolism of the S-enantiomer. Such a change was not observed in plants or poultry, but a comparable change was observed in rats (see section CA B.6).

Label-specific differences were seen for TRR from tissues except kidney. C-label and TFMP-label were generally comparable (except fat), but distinct from T-label. In muscle and whole milk, TRR for C-/TFMP-label (<0.1 mg/kg) was lower than for the T-label (>0.22 mg/kg). In contrast in liver, TRR

for C-/TFMP-label (>1.1 mg/kg) was higher than for the T-label (<0.66 mg/kg). In fat residues in T-label were the lowest at <0.22 mg/kg, slightly higher levels were seen in C-label (ca 0.3 mg/kg) and the highest were in TFMP-label at >0.51 mg/kg.

^{14}C residues in milk (sampled on 12-14 consecutive days) reached a plateau concentration within 5 days confirming absence of accumulation of residues in milk. Plateau levels in whole milk were at around 0.03 mg/kg, 0.07 mg/kg, and 0.3 mg/kg (C-, TFMP-, T-label). Overall, the TRR in whole milk and skim milk of the C- and TFMP-label were low and ranged from 0.016 mg/kg to a maximum of 0.065 mg/kg. In T-labelled milk, skim milk and cream of all labels, higher amounts were detected (0.27- 0.52 mg/kg).

The extractability of radioactive residues from all edible matrices was high (>89 %). Further investigation of the remaining residue was undertaken for liver only which realised 1.8-34 %TRR. The final unextractable residue for all edible matrices was between 0-7.5% TRR (<0.086 mg/kg). No further efforts were made to characterise these residues as the contribution to the TRR is < 10%, and a significant proportion of the residue was available for characterisation/identification, therefore further characterisation is not considered to impact significantly on the study results.

Metabolism of BAS 750 F in lactating goat includes, besides O-conjugation of the unchanged parent (M750F068), two main transformation reactions. The first main transformation reaction is oxygenation of the C-ring of the uncleaved parent BAS 750 F (M750F015, M750F016, M750F017), followed by conjugation (M750F063). The metabolite M750F041 and M750F091 are intermediates with a cyclohexadiene structure of the C-ring prior to re-aromatization. A second transformation reaction is cleavage of the parent backbone at the T-bridge generating 1,2,4-triazole (M750F001) as well as the two-ring metabolite M750F022, which itself is subject to oxidation (M750F038), followed by demethylation (M750F040), as well as to C-ring hydroxylation (M750F078), to sulphation (M750F043), and to glucuronidation (M750F064). A transformation observed only to a minor extent is cleavage of the parent backbone at the ether bridge, generating the two ring metabolite M750F003. A further minor transformation is the hydroxylation of the methyl group at the quaternary C-atom of BAS 750 F (M750F039) which can be oxidized further (M750F042) or conjugation with sulphate (M750F072).

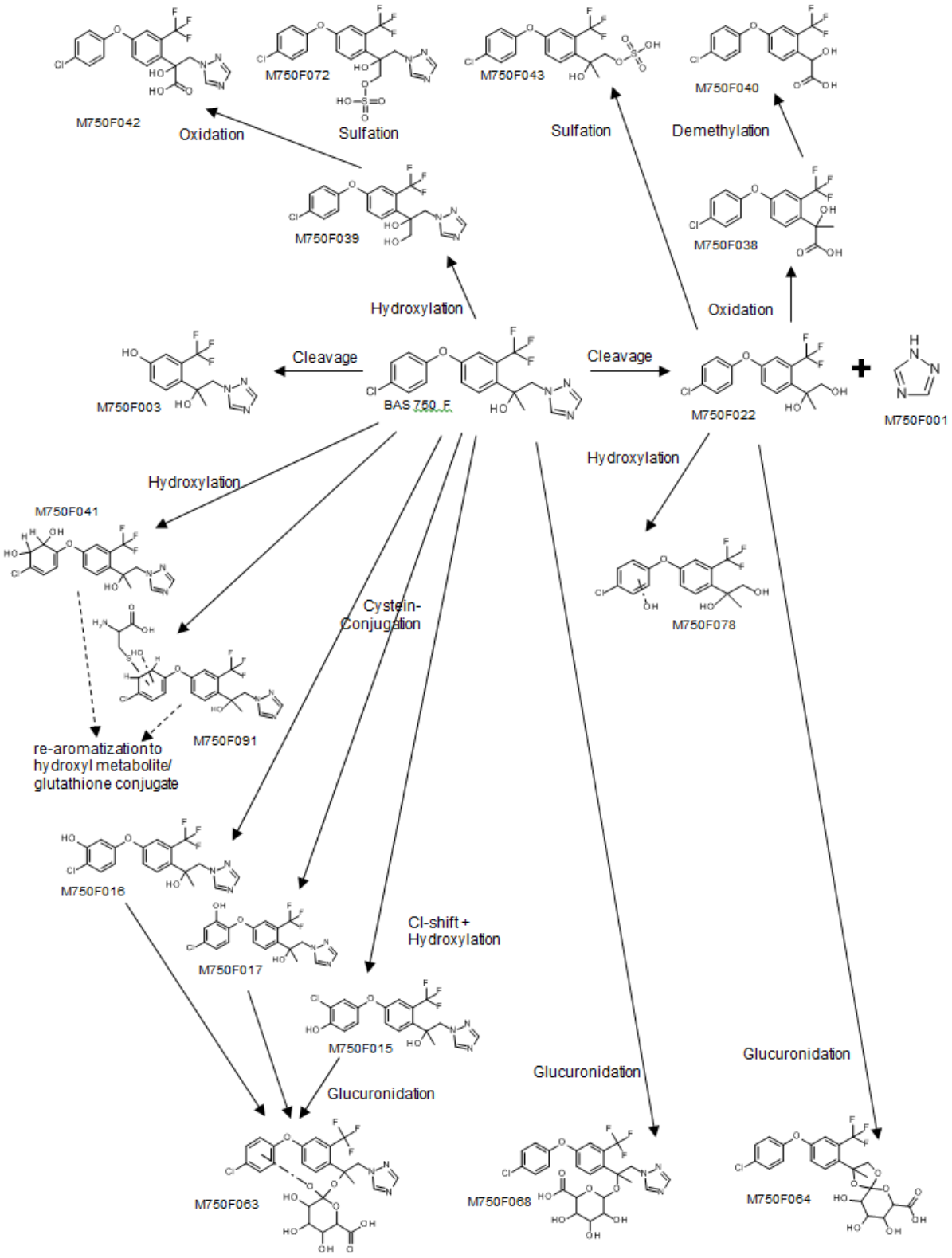
For the metabolite profile, label-dependent differences similar to the observations at TRR level were seen. Generally, results of C-label and TFMP-label were comparable, and distinct from the T-label. For the C- and TFMP-label, parent BAS 750 F was the most abundant component in all matrices investigated (fat/muscle >85% TRR, milk/liver >45% TRR, kidney 28-46% TRR).

The cleavage metabolite M750F022 was seen in all matrices, albeit at much lower levels (<11% TRR) than in poultry. M750F022 appears to be metabolized further in goat, as indicated by its oxidized/glucuronidated products (M750F038/M750F064) present in liver (7-11% TRR) and kidney (14-27% TRR) as well as its sulphation product M750F043 present in milk (14-25% TRR, up to 0.16 mg/kg). In addition, cleavage of the ether bridge occurred to a low extent in kidney only, as indicated by M750F003 (3% TRR, 0.01 mg/kg).

For the T-label the cleavage metabolite 1,2,4-triazole (M750F001) was the predominant residue in milk, muscle and kidney matrices (>68% TRR), and it was present at 32% in liver. A much lower level of 5 % TRR was determined in fat, BAS 750 F, at 85% was the predominant component in fat.

In addition further metabolites were determined, including M750F016, M750F068 which were present at >15% TRR and several further metabolites were present at <7 % TRR. Overall, metabolism of BAS 750 F in lactating goats, and by extrapolation in ruminant livestock, can be considered well-elucidated.

Figure 7.2.3-1: Proposed pathway of BAS 750 F in lactating ruminants



Report:	CA 6.2.2/2 Thiaener J.,Glaessgen W.E., 2015 b Investigation of the extractability of BAS 750 F and M750F022 in samples from 14C animal metabolism studies 2015/1161960
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: EPA Residue Chemistry Test Guidelines, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 91/414 (7030(VI/95 Rev. 3)
GLP:	yes

Materials and methods

Samples from metabolism studies in hen, (fat, liver, muscle and egg yolk) and in goat (cream, whole milk and kidney) were used to investigate extraction efficiency of radiolabelled residues of BAS 750 F.

Extraction procedures used in analytical methods, namely BASF method L0272/01 (for BAS 750 F) and BASF method L0309/01 (for M750F022) were compared to the extraction procedure used in the hen and goat metabolism studies.

A summary of the extraction procedure for each method is detailed below. In each case the analysis was performed using HPLC.

BAS 750 F-method L0272/01

Fat-rich animal matrices (goat cream, goat milk and hen fat) were extracted with acetonitrile/isohexane (100/40, v/v) using a homogeniser. The acetonitrile and isohexane phase of the extract were separated.

Protein-rich animal matrices (goat kidney, hen liver, hen muscle, egg yolk) were extracted with methanol/water/2N HCl (75/25/5, v/v/v). The methanol/water/2N HCl extract was cleaned-up by adding 0.2N HCl to the supernatant after centrifugation prior to partition against cyclohexane (2x).

The acetonitrile phase and the combined cyclohexane phase were concentrated to dryness. The residues were re-dissolved in a mixture containing appropriate ratios of acetonitrile, 20 mmol/L ammonium formate and Triton X-100 prior to LSC and HPLC analyses.

The isohexane phase of the extract and the methanol/water/2N HCl phase after partitioning of the extracts were subjected to LSC analysis. The non-extracted residue was dried and subjected to combustion analysis.

M750F022-method L0309/01

Fat-rich animal matrices (goat cream, goat milk and hen fat) were extracted with acetonitrile/isohexane (50/20, v/v). The acetonitrile and isohexane phase of the extract were separated.

Protein-rich matrices (goat kidney, hen liver, hen muscle, egg yolk) were extracted with methanol/water/2N HCl (75/25/5, v/v/v). The methanol/water/2N HCl extract was purified by adding 0.2N NaOH to the centrifuged supernatant prior to partitioning (2x) against dichloromethane (goat kidney) or cyclohexane (hen liver and muscle).

The acetonitrile phase and the combined DCM or cyclohexane phase were concentrated to dryness and the residues were re-dissolved in a mixture containing appropriate ratios either of acetonitrile, 20 mmol/L ammonium formate and Triton X-100 or methanol, acetonitrile, 20 mmol/L ammonium formate and Triton X-100 prior to LSC and HPLC analyses.

The isohexane phase of the extract and the methanol/water/2N HCl phase after partitioning of the extracts were subjected to LSC analysis. The non-extracted residue was dried and subjected to combustion analysis.

Results and discussion

The extractability of radioactive residues from animal matrices using two different extraction protocols is summarised in Table 7.2.3.1-1 and 7.2.3.1-2. The amount of radioactive residue extracted in the animal metabolism studies (ERR value) was taken as reference value for extraction efficiency (thus, ERR was set to 100%). The amount extracted with an analytical method was compared to this value, and expressed in percentage of the reference value. Note that, in milk residues of M750F022 are very low, thus a precise calculation of extraction efficiency was not feasible.

Table 7.2.3.1-1: Summary of extractability: radioactive residues and parent BAS 750 F

Extraction procedure	TRR	Radioactive residue (in ERR)			BAS 750 F		
	[mg/kg]	*[mg/kg]*	*[% TRR]*	***extraction efficiency (%) 1)***	*[mg/kg]*	*[% TRR]*	***extraction efficiency (%) 1)***
Cream (goat, C-label)							
goat metabolism study	0.207	0.203	98.4	100.0	0.156	75.6	100.0
L0272/01 2)	0.272 3)	0.288	105.8	**107.4**	0.225	82.5	**109.1**
		0.271	99.3	**100.9**	0.207	75.8	**100.2**
Milk (goat, TFMP-label)							
goat metabolism study	0.062	0.061	98.1	100.0	0.028	44.5	100.0
L0272/01		0.050	80.8	**82.3**	0.025	40.1	**90.1**
Kidney (goat, TFMP-label)							
goat metabolism study	0.429	0.422	98.2	100.0	0.198	46.0	100.0
L0272/01		0.386	89.9	**91.5**	0.162	37.7	**82.0**
Fat (hen, C-label)							
hen metabolism study	0.702	0.707	100.8	100.0	0.038	5.4	100.0
L0272/01		0.690	98.4	**97.5**	0.036	5.1	**95.0**
Liver (hen, C-label)							
hen metabolism study	0.320	0.265	82.7	100.0	0.023	7.2	100.0
L0272/01 2)		0.221	69.0	**83.5**	0.011	3.3	**46.4**
		0.225	70.2	**84.9**	0.011	3.4	**47.9**
Muscle (hen, C-label)							
hen metabolism study	0.050	0.043	85.0	100.0	0.003	5.6	100.0
L0272/01		0.033	66.2	**77.9**	0.003	5.2	**94.1**
Egg yolk (hen, C-label)							
hen metabolism study	0.477	0.426	89.4	100.0	0.031	6.5	100.0
L0272/01		0.266	55.7	**62.4**	0.028	6.0	**92.4**

1) *extraction efficiency = amounts extracted with analytical method compared amount extracted in metabolism study (set to 100%). 2) In the case of cream (goat) and liver (hen), two different subsamples from different containers were extracted for confirmation. 3) For cream (goat) extraction efficiency was calculated as the %TRR in metabolism study divided by %TRR extracted with analytical method. TRR determined for present study as TRR determined by analytical methods higher than TRR in metabolism study.*

Table 7.2.3.1-2: Summary of extractability: radioactive residues and M750F022

Extraction procedure	TRR	ERR			M750F022		
	[mg/kg]	*[mg/kg]*	*[% TRR]*	***extraction efficiency (%)*** [1]	*[mg/kg]*	*[% TRR]*	***extraction efficiency (%)*** [1]
Cream (goat, C-label)							
goat metabolism study	0.207	0.203	98.4	100.0	0.009	4.2	100.0
L0309/01 [2]	0.272	0.289	106.0	**107.6**	0.012	4.5	**106.9**
		0.279	102.5	**104.2**	0.008	3.0	**73.1**
Milk (goat, TFMP-label)							
goat metabolism study	0.062	0.061	98.1	100.0	0.001	1.2	100.0
L0309/01		0.050	80.4	**81.9**	0.002	2.5	***(213.6)*** [3]
Kidney (goat, TFMP-label)							
goat metabolism study	0.429	0.422	98.2	100.0	0.046	10.7	100.0
BASF method L0272/01		0.362	84.2	**85.8**	0.042	9.8	**91.7**
Fat (hen, C-label)							
hen metabolism study	0.702	0.707	100.8	100.0	0.178	25.4	100.0
L0309/01		0.639	91.0	**90.3**	0.167	23.8	**93.7**
Liver (hen, C-label)							
hen metabolism study	0.320	0.265	82.7	100.0	0.118	36.7	100.0
L0309/01 [2]		0.161	50.3	**60.8**	0.058	18.3	**49.7**
		0.203	63.4	**76.6**	0.057	17.7	**48.2**
		0.196	61.2	**74.0**	0.054	17.0	**46.3**
Muscle (hen, C-label)							
hen metabolism study	0.050	0.043	85.0	100.0	0.025	49.9	100.0
L0309/01		0.027	53.1	**62.5**	0.015	30.4	**60.9**
Egg yolk (hen, C-label)							
hen metabolism study	0.477	0.426	89.4	100.0	0.186	39.0	100.0
L0309/01 [2]		0.242	50.7	**56.7**	0.123	25.8	**66.2**
		0.211	44.2	**49.5**	0.102 [4]	21.3 [4]	*(54.7)* [4]

1) *extraction efficiency = amounts extracted with analytical method compared to amount extracted in metabolism study (later set to 100%). For cream (goat) extraction efficiency was calculated as the %TRR in metabolism study divided by %TRR extracted with analytical method. 2) As confirmation of the results obtained, for cream (goat) two different subsamples from different containers were extracted for confirmation. For liver (hen) three different subsamples (of the same container) were extracted. For egg yolk (hen), two different subsamples (of the same container) were extracted. 3) The value obtained for milk is indicative only. The value calculated for milk appears to be an overestimation resulting from non-precise data due to low analyte amount. 4) The value calculated for yolk is an underestimation since an additional 0.011 mg/kg (2.4% TRR) was recovers from the contained after extract concentration prior to HPLC analysis.*

Extraction efficiency of method LC0272/01

Concerning "radioactive residue" (ERR), the extraction efficiency achieved with the analytical method L0272/01 was matrix-dependent. For milk, cream, kidney, and fat, the amounts extracted with the analytical method were similar to the amounts extracted in the metabolism study. Thus, extraction efficiency for the analytical method generally was high (82% or higher). Lower extraction efficiencies were determined for liver (84%), for muscle (78%) and for egg yolk (62%).

Analysis specifically of the analyte BAS 750 F, showed that the analytical method L0272/01 achieved high extraction efficiency for all matrices except liver. Comparing amounts of BAS 750 F extracted with the analytical method to the BAS 750 F amounts extracted in the metabolism study, extraction efficiencies of 90% (milk), 100% (cream), 82% kidney, fat (95%), muscle (94%), egg yolk (92%) were obtained while 46-48% was obtained for liver. Overall, the analytical method for BAS 750 F shows good extraction efficiency in all matrices except liver.

Extraction efficiency of method LC0309/01

Concerning "radioactive residue" (ERR), the extraction efficiency achieved with the analytical method L0309/01 was matrix-dependent. For milk, cream, kidney and fat, the amounts extracted with the analytical method were similar to the amounts extracted in the metabolism study. Thus, extraction efficiency for the analytical method was high (82% or higher). Lower extraction efficiencies were determined for liver (61-77%), for muscle (63%) and for egg yolk (50-57%).

Analysis specifically of the analyte M750F022, showed that the analytical method L0309/01 achieved high extraction efficiency for milk (100%) for cream (73-107%), for kidney (92%), and for fat (94%), good extraction efficiency was determined for muscle (61%) and egg yolk (66%) and only moderate extraction efficiency for liver (46-50%). Note that for milk, the data obtained indicates high extraction efficiency of method L0309/01 for M750F022, but a precise determination of extraction efficiency was not feasible due to the very low analyte amounts in milk sample. Overall, the analytical method for M750F022 shows acceptable. extraction efficiency in all matrices except liver.

Conclusion

Comparison of residue amounts extracted in the metabolism study with the amounts extracted by the extraction procedures of a residue analytical method confirms efficient extraction for the analytical methods, method L0272/01 for BAS 750 F and L0309/01 for metabolite M750F022 for all matrices except liver.

For BAS 750 F, extraction efficiencies generally were 80% or higher for most matrices (milk, cream, muscle, kidney, fat, egg yolk), and lower for liver (46%). For M750F022, extraction efficiencies generally were 90% or higher for most matrices (milk, cream, kidney, fat) and lower for egg yolk (66%), for muscle (61%) and for liver (46-50%).

B.7.2.4. Pigs

The metabolism of BAS 750 F in rodents (rats) and ruminants (goats) did not reveal significant qualitative differences. Therefore, investigations of the metabolism in pigs are not required.

B.7.2.5. Fish

At present there are no agreed EU guidance documents or test methods to address these data requirements. At the SCoPAFF (PPP legislation) meeting in October 2014 the COM emphasised, as laid down in document *SANCO/10181/2013 Rev 2.1*, that these data requirements can be waived until test methods or guidance documents are made available. *SANCO/10181/2013 Rev 2.1* states:

*In some cases, agreed **test methods or guidance documents are not yet available for particular data requirements**. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02. Applicants should follow on a routine basis the current developments, e.g. activities of the European Food Safety Authority for guidance documents and in particular publications in the Official Journal.*

Specifically for fish metabolism and fish feeding studies the COM at the SCoPAFF (pesticide residues) meeting in November 2014 stated the following in the minutes of the meeting:

The Commission emphasised that for the time being there are no agreed test guidelines and that hence the pertinent data requirements can be waived. This was also clarified in general at the meeting of them Committee's section on Plant Protection Products - Legislation on 09/10 October 2014, and laid down in document SANCO/10181/2013 Rev 2.1. Such test guidelines must be published in the form of an update of the respective Commission Communications.

Consequently, the above data requirements do not need to be addressed at this time.

B.7.2.6. Overall conclusion on metabolism in livestock

Metabolism was investigated using three radiolabels (BAS 750 F labelled in the C-ring, TFMP-ring or in the T-ring). Results obtained with all labels show a consistent picture of BAS 750 F metabolism. Investigations were done in laying hen and lactating goat, as well as in rat to support toxicology studies (see section CA B.6). For goat and hen the residue was rapidly and extensively eliminated via excreta, and reached a plateau in milk and egg within 7 days. Comparable results were obtained for all three animals, indicating common basic metabolite routes.

In poultry matrices the metabolite M750F022 (and its fatty acid conjugates) is the predominant component of the residue, with unmodified parent BAS 750 F and 1,2,4-triazole also present as significant components. In goat matrices, unmodified parent BAS 750 F and 1,2,4-triazole were the predominant components of the residue, with M750F022 present at much lower levels.

The metabolic pathway is largely based on two main transformation steps in livestock animals:

- hydroxylation at the C-ring (followed by conjugation) (M750F016, 034, 015, 041, 063)
- cleavage at the T-bridge (followed by conjugation) (M750F022-025, 038, 043, 064)

In addition, minor transformation steps were observed in livestock animals:

- cleavage at the ether bridge (followed by conjugation)
- hydroxylation at the T-ring
- hydroxylation of the methyl group (at quaternary C-atom, followed by conjugation)

Differences seen in species and/or matrices are the result of quantitative differences of transformation reactions as well as species-typical conjugation reactions (sulphation, glucuronidation, methylation, glutathione conjugation).

The parent BAS 750 F was applied as a racemic mixture of two enantiomers. Chiral analysis of BAS 750 F revealed a significant change of the ratio in most goat matrices, with proportion of the R-enantiomer of 70-80% in cream, muscle, liver, kidney and fat. In contrast, the racemate was maintained in goat faeces, indicating a preferential metabolism of the S-enantiomer. Such a change was not observed in poultry, but a comparable change was observed in rats (see section CA B.6).

Figure 7.2.4-1 shows the key metabolism pathways determined for the animal commodities.

Metabolites occurring in edible livestock matrices in major amounts (>10% TRR) in minor amounts (<10% TRR) are listed in Table 7.2.4-1. This table groups the metabolites according to their chemical structure together with their corresponding conjugates. The non-conjugated metabolites that were identified in food commodities are highlighted (underlined).

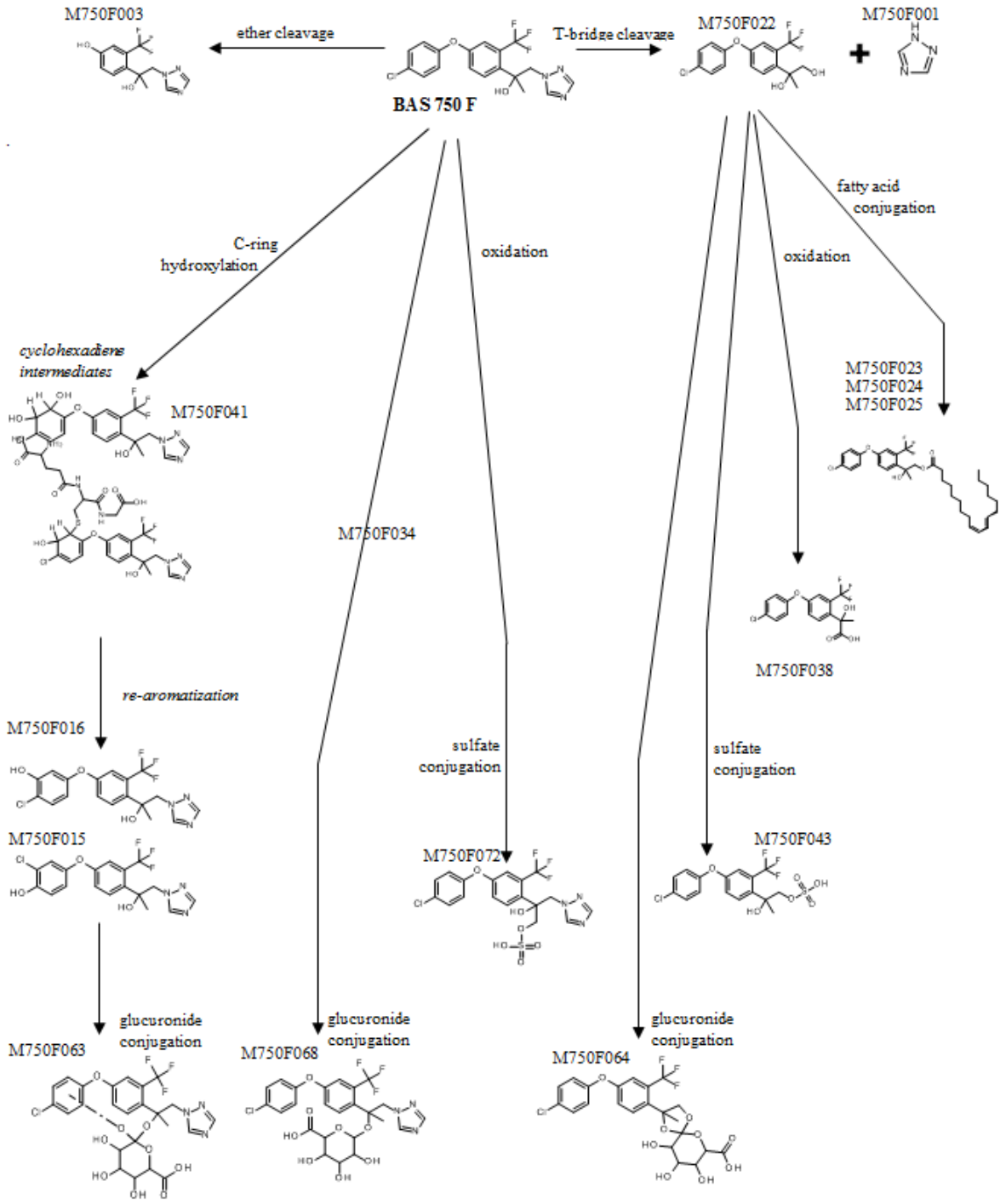
Table 7.2.4-1: Residue components identified in edible livestock commodities

Group definition	Residue	
	≥ 10% TRR*	**< 10% TRR**
a) parent and conjugates	**BAS 750 F** M750F068	M750F072
b) "C-ring"-hydroxylation of non-cleaved molecule & downstream metabolites / conjugates	**M750F016** M750F034	**M750F015** **M750F041** M750F063
c) cleavage products & downstream metabolites / conjugates Metabolites without the C-ring		**M750F003**
Metabolites without 1,2,4-T-ring	**M750F022** M750F023 M750F024 M750F025 **M750F038** M750F043 M750F064	-
1,2,4-triazole and triazole-derived metabolites (TDM)	**1,2,4-T**	-

**In at least one commodity*

Figure 7.2.4-1 BAS 750 F: metabolic routes in ruminant and poultry

The group of M750F023, M750F024, and M750F025 are acyl-conjugates of M750F022, differing only in the fatty acid side chain, thus only one representative molecular structure is provided). Metabolites occurring only in non-edible commodities are not considered.



Definition of the residue in animal

For commodities of animal origin, the following residue definitions are proposed:

Residue definition for MRL enforcement/monitoring (RD-Mo):

- *parent BAS 750 F*

Residue definition for risk assessment (RD-RA):

- *animal except poultry:* - *parent BAS 750 F*
  - *triazole derivative metabolites (provisional, pending the definition of a common and harmonised approach for all the active substances of the triazole chemical class)*
- *poultry:* - *sum of parent BAS 750 F, metabolite M750F022 and fatty acid conjugates of M750F022, expressed as parent equivalents*
  - *triazole derivative metabolites (provisional, pending the definition of a common and harmonised approach for all the active substances of the triazole chemical class)*

Based on the evaluation of study results provided in the present dossier, the residue components in animal commodities relevant for consideration in the residue definitions include parent BAS 750 F, metabolite M750F022 and its fatty acid conjugates as well as the group of triazole derivative metabolites (TDM) and several other metabolites including sugar conjugates and cleavage products.

Residue definition for MRL enforcement/monitoring (RD-Mo)

According to *OECD Guidance on the definition of residue (ENV/JM/MONO(2009)30)* the residue definition for monitoring/MRL enforcement (RD-Mo) should focus on those residue components suitable as analyte of multi-residue methods, as well as suitable as general marker compound in food commodities concerned.

Parent BAS 750 F fulfils these criteria since its compatibility with multi-residue methods has been confirmed (see section B.7.2.1.4) and it is a characteristic component of the residue typically accounting for a significant proportion of the residue.

M750F022 is also present in significant amounts in poultry. However, M750F022 not suitable as analyte for multi-residue methods (due to insufficient ionization properties, GC-MS is required, and due to matrix interference a time-intensive methodology is needed to achieve a quantitation limit of 0.01 mg/kg).

TDMs are also present in significant amounts, but are not considered suitable as BAS 750 F specific marker molecules as they are common to a range of pesticides.

In conclusion, the proposed residue for monitoring in animals is BAS 750 F.

Residue definition for risk assessment (RD-RA)

According to *OECD Guidance on the definition of residue (ENV/JM/MONO(2009)30)*, the residue definition for risk assessment (RD-RA) should take into account the contribution of residue components to the potential dietary risk considering both the potential for exposure as well as the toxicity relative to the parent compound.

In commodities of ruminant origin, and by extrapolation of swine origin, parent BAS 750 F is a characteristic component of the residue and typically represents a predominant proportion of the residue. In conclusion, BAS 750 F is suitable to define the relevant residue in these commodities.

In commodities of poultry origin, in addition to parent BAS 750 F residues, significant amounts of metabolite M750F022 are typically present. In some commodities, namely fat, egg and muscle, a significant proportion of M750F022 is present as fatty acid conjugates. Release of M750F022 by hydrolytic cleavage of fatty acid conjugates in the human digestive tract might contribute significantly to the amount of M750F022. Toxicological investigations show that M750F022 (see section CA5.8) has comparable toxicological properties to BAS 750 F. Therefore, the toxicological reference values derived for BAS 750 F can be applied to M750F022. In order to account for a large proportion of the residue it is therefore proposed to define the sum of BAS 750 F, metabolite M750F022 and its fatty acid conjugates as the relevant component of the residue in poultry commodities for risk assessment. The proposed matrix-specific conversion factors (muscle 6.20, fat 16.33, liver 4.94, egg 4.86) take into account both the matrix-specific contribution of fatty acid conjugates as well as the molecular weight difference between M750F022 and BAS 750 F (details are provided in section B.7.4.5).

In liver of poultry origin only M760F034 was detected at >10% TRR (0.12 mg/kg) for the TFMP label. However as it is only found in one commodity which is a very minor component of the diet, and it is a conjugate of parent BAS 750 F which has low toxicity relative to the expected exposure, it was not considered necessary to include this metabolite in the RD-RA.

TDMs (in particular 1,2,4-triazole) contribute to a large proportion of the residue in all matrices; however these metabolites are common to a range of azole fungicides. Based on different toxicological properties, a separate definition of residue relevant for risk assessment is expected as part of a common approach for azole fungicides. Therefore, TDMs are provisionally included in the RD-RA as part of the present dossier, pending the outcome the EU review of TDMs. At the time of writing, no decision has been made with respect to an EU agreed approach on how to assess TDMs.

Other components present at >10 % TRR in bovine commodities (M750F038, 043, 064, 068) are cleavage products of BAS 750 F and their sugar conjugates, however as they are present at significantly lower levels than parent, are only present in one commodity at >10% TRR, and do not present an increased toxicological risk in comparison to parent, it is not considered necessary to include these metabolites in the residue definition for risk assessment.

In conclusion, the proposed residue for risk assessment in animals except poultry is BAS 750 F, in poultry the proposed residue definition is sum of parent BAS 750 F, metabolite M750F022 and fatty acid conjugates of M750F022, expressed as parent equivalents.

B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

B.7.3.1. Wheat

One of the proposed uses of BAS 750 F in the EU is on wheat. The cGAP for wheat is provided in Table 7.3.1-1.

Table 7.3.1-1: Summary of the critical GAPs for the proposed uses in wheat

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum Application interval (days)	Maximum		Minimum PHI (days) [a)]
					rate (kg as/ha)	water (L/ha)	
Wheat	outdoor	49, 69	2	14	0.15	200	*35*

[a)] Timing of the cGAP determined based on growth stage. PHI of 35 days proposed by the applicant, but has not be used for selection of trials

A total of 17 residues trials have been submitted in support of this use. A summary of the timing, number and location of the residues trials on wheat is given in Table 7.3.1-2.

Table 7.3.1-2: Number of residue trials per geographical region and vegetation period

Crop	Season	Number of trials					Reference
		N-EU	Country	S-EU	Country	Total	
Wheat	2013	4	DE, NL, UK	4	FR, GR, IT, ES	8	6.3.1/1
Wheat	2014	4	DE, FR, NL	5	FR, GR, IT, ES	9	6.3.1/2
Total number of trials per region		**8**	**-**	**9**	**Total number of trials**	**17**	

A summary of the results of the residues trials on wheat is given in Table 7.3.1-3.

Table 7.3.1-3: Residues of BAS 750 F: overall summary from residue trials in wheat

Crop	Region	RAC	n	Residues *[mg/kg]*	STMR *[mg/kg]*	HR *[mg/kg]*
Wheat	N-EU	grain	8	4 x <0.01, 0.011, 0.014, 0.016, 0.024	0.011	0.024
	S-EU		9	7 x <0.01, 0.018, 0.026	0.01	0.026
	N-EU	straw	8	1.9, 2.3, 3.4, 3.6, 3.9, 4.9, 5.5, 10	3.75	10.0
	S-EU		9	0.5, 0.56, 1.6, 2.9, 3.1, 3.8, 4.6, 9.0, 18.0	3.1	18.0

Report:	CA 6.3.1/1 Erdmann H.-P., 2015 a Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in wheat after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013 2014/1010809
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes

Materials and methods

During the growing season of 2013, eight field trials on wheat using the formulated product 'BAS 750 00 F', an EC formulation containing 100 g/L BAS 750F were conducted in Germany, the Netherlands, the United Kingdom, Southern France, Greece, Italy and Spain. The formulation was applied twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha.

Samples of whole plant (root removed) were taken directly before and after the second application. Samples were also taken at three time points after the last application; after 34-35 days, 41-43 days and 46-50 days. Where the crop was unripe, samples consisted of ear and rest-of-plant (roots removed) while for the ripe crop grain and straw were taken. Where BBCH growth stage 89 was not reached after 49 days, an additional sampling time point was added. Control (untreated) specimens were taken at every sampling event. A summary of the application and sampling details is given Table 7.3.1-4.

Table 7.3.1-4 Application and sampling details

Region	No. of trials	No. of appl.	F, G, I[2]	Method	Test item	Active substance	Application		Target timing	
							rate (kg a.s. /ha)	water volume (L/ha)	application (BBCH)	sampling (DALA)[1]
EU-N & EU-S	8	2	F	foliar spray	BAS 750 00 F (EC)	BAS 750 F	0.15	200	1st appl.: BBCH49 2nd appl.: BBCH69	0 34 - 35 41 - 43 46 – 50 54

1) days after last application 2) field, glasshouse or indoor

Weather data were reported for the trials and no exceptional events were noted. Normal agricultural practice was followed during the field stage. Samples weighing at least 1 kg were collected for ears, rest-of-plant (roots removed) and grain, and samples weighing at least 0.5 kg were collected for straw. Samples were stored frozen at ≤-18 °C within 8 hours of harvest, and maintained at this temperature until extraction (with the exception of trial L130170 where the storage temperature rose to -10°C for 24 hours).

The maximum storage interval from harvest until analysis was 626 days for BAS 750 F. The maximum storage interval from harvest until analysis was 477 days for triazole and related analytes.

The level of residues in each processed fraction was determined for BAS 750 F (BASF method L0076/09, LOQ of 0.01 mg/kg) and for the triazole derivative metabolites (TDM) 1,2,4-T, TA, TAA, TLA (BASF method L0170/02, LOQ of 0.01 mg/kg for each of the four analytes). Full details of sample preparation and validation data for these methods is given in section CA B.5.1.2.5. Details of the procedural recoveries are given in Table 7.3.1-5 and 7.3.1-6.

Table 7.3.1-5 Summary of recoveries for BAS 750 F

Matrix	Fortification level (mg/kg)	Summary Recoveries			
		n	mean (%)	SD (+/-)	RSD (%)
Method L0076/09		**BAS 750 F**			
whole plant (no root)	0.01, 0.10, 10	3	95.8	2.1	2.2
ears	0.01, 0.10, 1.0, 10	6	96.0	5.3	5.6
rest of plant (no root)	0.01, 0.10, 1.0, 10, 20	7	92.9	6.4	6.9
grain	0.01, 0.10, 1.0	6	98.6	3.9	4.0
straw	0.01, 0.10, 1.0, 10, 20	7	88.7	8.6	9.7
Overall		**29**	**94.0**	**6.8**	**7.2**

Table 7.3.1-6 Summary of recoveries for 1,2,4-T, TA, TAA and TLA

Matrix	Fortification level (mg/kg)	Summary Recoveries			
		n	mean (%)	SD (+/-)	RSD (%)
Method L0170/02		**1,2,4-triazole (T)**			
whole plant (no root)	0.01, 1.0	12	99.4	10	10
ears	0.01, 1.0	6	94.1	8.5	9.0
grain	0.01, 1.0	6	100	18	18
straw	0.01, 1.0	6	103	12	12
Overall		**30**	**99.3**	**12**	**12**
Method L0170/02		**triazole alanine (TA)**			
whole plant (no root),	0.01, 1.0	12	90.3	12	14
ears	0.01, 1.0	6	91.5	11	12
grain	0.01, 1.0	6	86.3	13	15
straw	0.01, 1.0	6	86.9	20	23
Overall		**30**	**89.0**	**13**	**15**
Method L0170/02		**triazole acetic acid (TAA)**			
whole plant (no root),	0.01, 1.0	12	88.4	15	17
ears	0.01, 1.0	5	85.8	15	17
grain	0.01, 1.0	6	88.8	15	17
straw	0.01, 1.0	6	76.3	15	20
Overall		**29**	**85.5**	**15**	**18**
Method L0170/02		**triazole lactic acid (TLA)**			
whole plant (no root),	0.01, 1.0, 10	10	94.8	15	15
ears	0.01, 1.0	5	93.0	23	25
grain	0.01, 1.0	6	77.3	10	13
straw	0.01, 1.0	4	80.1	7	9
Overall		**25**	**87.9**	**16**	**18**

Results and Discussion

Eight field trials on wheat using the formulated product 'BAS 750 00 F', an EC formulation containing 100 g/L BAS 750F were conducted in NEU and SEU during the growing season of 2013. The formulation was applied twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha. This application rate and timing is in accordance with the proposed GAP for wheat.

Samples were taken at three time points after the last application; after 34-35 days, 41-43 days and 46-50 days Where BBCH growth stage 89 was not reached after 49 days, an additional sample was taken at this stage. Residues trials data are presented in Table 7.3.1-7 (treated samples) and Table 7.3.1-8 (untreated control samples).

Samples were stored frozen for up to 626 days prior to analysis for BAS 750 F and for up to 477 days for triazole and related analytes. Storage stability data is available to support storage of BAS 750 F for up to 730 days in cereals (section B.7.1.1), which is sufficient to support the storage times in this study. Storage stability data is available to support storage of 1,2,4-triazole and TA for 54 months, TTA for 26 months (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015) and TLA for 49 months (section B.7.1.2). This is sufficient to support the storage times in this study.

The methods of analysis for determination of BAS 750 F and TDMs are considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4. Acceptable procedural recovery data, using an appropriate number of samples were presented.

BAS 750 F

In untreated samples no residues of BAS 750 F exceeding the LOQ (0.01 mg/kg) were detected. A summary of the results from the treated samples is presented in Table 7.3.1-9.For grain from treated samples, BAS 750 F residues were below LOQ in six trials, and present in one trial at 0.018 mg/kg and another at 0.024 mg/kg.

For straw from treated samples, BAS 750 F residues showed high variability ranging from 0.5 to 18 mg/kg. Two trials in particular showed high results. For one SEU trial (Spain, L130173), residues amounted to 9.9 mg/kg in straw at DALA43, increasing further to 18 mg/kg at DALA49 (in the corresponding plant sample at DALA35 residues were 12 mg/kg), for one NEU trial (UK, L130169) residues of 10 mg/kg were determined at DALA35, declining further to 6.2 mg/kg at DALA50. No experimental factors are reported which may have led to these higher values.

For plants directly harvested after application (DALA0), BAS 750 F residues were in the range of 2.2 – 3.9 mg/kg (7 trials) and the trial in Spain (L130173) with 6.3 mg/kg. Residues in plant parts (ears, roots removed) declined to levels of 1.6 – 2.7 mg/kg at DALA34-35 and for the trial in Spain (L130173) 12 mg/kg.

Table 7.3.1-9: Summary of BAS 750 F residues in BAS 750 00 F treated wheat

Region	Matrix	DALA[1)]	BBCH	BAS 750 F *[mg/kg]*
N-EU & S-EU	whole plant[2)]	0	69	2.2 – 6.3
	ears	34-35	77-87	0.10 – 1.3
	rest of plant[2)]			0.46 - 12
	grain	35	87-89	0.017
	straw			10
	ears	41-43	83-89	0.15 – 0.71
	rest of plant[2)]			0.58 – 4.8
	grain	42-43	89	< 0.01 – 0.017
	straw			3.9 – 9.9
	ears	49	87	0.34
	rest of plant[2)]			3.8
	grain	46-50	89	< 0.01 – 0.024
	straw			0.50 - 18
	grain	54	89	< 0.01
	straw			3.8

[1)] DALA =days after last application; [2)] no root

TDM

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. Details of the previous treatments to the plots are not available in the study reports; however it is considered that the most likely explanation as to the source of the TDMs in untreated crops, is residual TDMs in the soil from previous applications of a triazole containing pesticide. A comparison of the results for treated and untreated crops is presented in Table 7.3.1-10.

For TA, residue levels in grain were up to 0.76 mg/kg in the treated samples and up to 0.49 mg/kg in untreated samples. Residue levels in straw were up to 0.04 mg/kg in the treated samples and up to 0.33 mg/kg in untreated samples.

For TAA, residue levels in grain were up to 0.2 mg/kg in the treated samples and up to 0.04 mg/kg in untreated samples. Residue levels in straw were up to 0.01 mg/kg in the treated samples and up to 0.03 mg/kg in untreated samples.

For TLA, residue levels in grain were up to 0.09 mg/kg in the treated samples and up to 0.06 mg/kg in untreated samples. Residue levels in straw were up to 1.5 mg/kg in the treated samples and up to 1.1 mg/kg in untreated samples.

As such, it is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar. For further discussion see section 7.3.3.

Table 7.3.1-10: Summary of TDM in untreated wheat (plot 1) as well as after treatment with BAS 750 00 F (plot2)

Region	Matrix	DALA [1)]	BBCH	Plot	1,2,4-triazole [mg/kg]	TA [mg/kg]	TAA [mg/kg]	TLA [mg/kg]
N-EU & S-EU	plant [2)]	0	69	1	<0.01	< 0.01 – 0.051	< 0.01	0.13 – 4.2
				2	<0.01	0.014 – 0.13	< 0.01 – 0.012	0.25 – 4.1
	ears	34-49	77-89	1	<0.01	< 0.01 – 0.28	< 0.01 – 0.028	0.013 – 0.19
				2	<0.01	0.096 - 0.46	<0.01 – 0.045	0.024 – 0.29
	rest-of-plant [2)]			1	<0.01	< 0.01 – 0.024	< 0.01 – 0.017	0.056 – 0.99
				2	<0.01	<0.01 - 0.028	<0.01 – 0.01	0.25 - 2.1
	grain	34-54	87/89	1	<0.01	0.032 – 0.49	< 0.01 – 0.043	< 0.01 – 0.057
				2	<0.01	<0.01 – 0.76	<0.01 – 0.023	<0.01 – 0.092
	straw			1	<0.01	< 0.01 – 0.33	< 0.01 – 0.025	0.030 – 1.1
				2	<0.01	<0.01 – 0.035	<0.01 – 0.014	0.091 – 0.67

[1)] DALA = days after last application; [2)] no root

Table 7.3.1-7: Residues of BAS 750 F and TDM in wheat (treated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433781 2014/1010809 74193 Stetten a. H. Germany (N) L130166	GC 0654 Wheat Asano	1. 01.11.2012 2. 27.05.-18.06.2013 3. 05.08.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 18.06 13	69	plant [1] ears rest of pl. [2] grain straw	0 34 34 43 43	3.6 0.16 2.1 <u>< 0.01</u> <u>3.9</u>	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.047 0.46 0.014 0.51 0.012	< 0.01 0.017 < 0.01 0.023 0.014	0.68 0.064 1.2 0.031 0.63
433781 2014/1010809 16833 Lentzke Germany (N) L130167	GC 0654 Wheat Smaragd	1. 10.10.2012 2. 10.06.-18.06.2013 3. 06.08.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 18.06 13	69	plant [1] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 34 34 43 43 49 49	3.9 0.38 2.7 0.46 4.8 <u>< 0.01</u> <u>5.5</u>	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.022 0.33 < 0.01 0.30 < 0.01 0.21 0.025	< 0.01 0.011 < 0.01 0.019 < 0.01 0.019 < 0.01	0.53 0.080 0.69 0.033 0.40 < 0.01 0.33
433781 2014/1010809 6595 ME Ottersum The Netherlands (N) L130168	GC 0654 Wheat Premio	1. 05.11.2012 2. 05.06.-19.06.2013 3.06.08.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 18.06 13	69	plant [1] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 34 34 41 41 49 49	2.2 0.42 1.6 0.42 1.6 <u>< 0.01</u> <u>2.3</u>	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.022 0.32 < 0.01 0.29 < 0.01 0.11 0.011	< 0.01 < 0.01 < 0.01 0.015 < 0.01 0.022 < 0.01	0.25 0.064 0.48 0.024 0.25 < 0.01 0.15
433781 2014/1010809 CO112NF Lawford/Manningtree, United Kingdom (N) L130169	GC 0654 Wheat Solstice	1. 12.11.2012 2. 24.06-08.07.2013 3. 27.08.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 08.07 13	69	plant [1] grain straw grain straw grain straw	0 35 35 42 42 50 50	2.8 0.017 <u>10</u> 0.015 8.6 <u>0.024</u> 6.2	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.13 0.15 0.014 0.11 < 0.01 0.18 0.027	< 0.01 0.017 < 0.01 0.021 < 0.01 0.016 < 0.01	0.31 < 0.01 0.31 < 0.01 0.44 < 0.01 0.091
433781 2014/1010809 32130 Cazaux-Saves France (S) L130170	GC 0654 Wheat Tiepolo	1. 04.11.2012 2. 22.05.-28.05.2013 3. 12.07.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 27.05 13	69	plant [1] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 35 35 43 43 46 46	2.7 0.10 0.46 0.15 0.58 <u>< 0.01</u> <u>0.5</u>	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.022 0.22 0.011 0.32 < 0.01 0.33 < 0.01	< 0.01 < 0.01 < 0.01 0.014 < 0.01 0.015 < 0.01	0.48 0.072 0.35 0.052 0.26 0.034 0.22

Table 7.3.1-7: Residues of BAS 750 F and TDM in wheat (treated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433781 2014/1010809 58300 Galatades, Greece (S) L130171	GC 0654 Wheat Trofeo	1. 09.11.2012 2. 20.04.-30.04.2013 3. 05.06.-15.06.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 26.04 13	69	plant [1] ears rest of pl. [2] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 35 35 42 42 49 49 54 54	3.6 0.25 1.3 0.35 2.2 0.34 3.8 <u>< 0.01</u> <u>3.8</u>	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.046 0.21 < 0.01 0.18 < 0.01 0.20 < 0.01 0.32 < 0.01	0.012 0.045 < 0.01 0.18 < 0.01 0.023 0.010 0.20 < 0.01	4.1 0.29 0.51 0.22 2.1 0.071 1.2 < 0.01 1.5
433781 2014/1010809 40018 Bologna Italy (S) L130172	GC 0654 Wheat Palaiso	1. 09.10.2012 2. 09.05.-18.05.2013 3. 05.07.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 18.05 13	69	plant [1] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 34 34 42 42 48 48	2.4 0.48 2.3 0.71 2.1 <u>< 0.01</u> <u>2.9</u>	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.014 0.26 0.024 0.34 0.028 0.76 0.035	< 0.01 0.025 < 0.01 0.019 < 0.01 0.023 0.013	0.64 0.19 1.6 0.073 0.64 0.092 0.67
433781 2014/1010809 41710 Utrera Spain (S) L130173	GC 0654 Wheat Artur Nick	1. 28.12.2012 2. 17.04.-22.04.2013 3. 10.06.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 22.04 13	69	plant [1] ears rest of pl. [2] grain straw grain straw	0 35 35 43 43 49 49	6.3 1.3 12 0.017 9.9 <u>0.018</u> <u>18</u>	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.019 0.096 < 0.01 0.072 0.010 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.45 0.12 0.49 < 0.01 0.42 < 0.01 0.54

1) whole plant (no root) 2) rest of plant (no root), 3) time between applications supports the proposed minimum of 14 days, 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid. The underlined values (e.g. <u>0.018</u>) are used for calculation of the STMR, HR and MRL for grain, and the STMR and HR for straw

Table 7.3.1-8: Residues of BAS 750 F and TDM in wheat (untreated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433781 2014/1010809 74193 Stetten a. H. Germany (N) L130166	GC 0654 Wheat Asano	1. 01.11.2012 2. 27.05.-18.06.2013 3. 05.08.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] grain straw	0 34 34 43 43	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.049 0.28 < 0.01 0.31 0.015	< 0.01 0.016 < 0.01 < 0.01 0.019	0.66 0.093 0.98 0.026 0.37
433781 2014/1010809 16833 Lentzke Germany (N) L130167	GC 0654 Wheat Smaragd	1. 10.10.2012 2. 10.06.-18.06.2013 3. 06.08.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 34 34 43 43 49 49	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.023 0.19 < 0.01 0.17 < 0.01 0.11 < 0.01	< 0.01 0.017 < 0.01 0.018 < 0.01 0.023 < 0.01	0.41 0.044 0.76 0.019 0.31 < 0.01 0.18
433781 2014/1010809 6595 ME Ottersum The Netherlands (N) L130168	GC 0654 Wheat Premio	1. 05.11.2012 2. 05.06.-19.06.2013 3.06.08.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 34 34 41 41 49 49	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.024 0.21 < 0.01 0.20 < 0.01 0.14 < 0.01	< 0.01 < 0.01 < 0.01 0.019 < 0.01 0.020 0.013	0.31 0.046 0.37 0.015 0.20 < 0.01 0.18
433781 2014/1010809 CO112NF Lawford/Manningtree, United Kingdom (N) L130169	GC 0654 Wheat Solstice	1. 12.11.2012 2. 24.06-08.07.2013 3. 27.08.2013	-	-	-	-	-	-	-	plant [1] grain straw grain straw grain straw	0 35 35 42 42 50 50	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.044 0.062 < 0.01 0.032 < 0.01 0.033 0.013	< 0.01 0.014 < 0.01 0.013 < 0.01 0.012 < 0.01	0.13 < 0.01 0.16 < 0.01 0.11 < 0.01 0.030

Table 7.3.1-8: Residues of BAS 750 F and TDM in wheat (untreated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433781	GC 0654	1. 04.11.2012	-	-	-	-	-	-	-	plant [1]	0	< 0.01	< 0.01	< 0.01	< 0.01	0.26
2014/1010809	Wheat	2. 22.05.-28.05.2013								ears	35	< 0.01	< 0.01	0.034	< 0.01	0.020
32130 Cazaux-Saves	Tiepolo	3. 12.07.2013								rest of pl. [2]	35	< 0.01	< 0.01	< 0.01	< 0.01	0.061
France (S)										ears	43	< 0.01	< 0.01	0.047	< 0.01	0.013
L130170										rest of pl. [2]	43	< 0.01	< 0.01	< 0.01	< 0.01	0.056
										grain	46	< 0.01	< 0.01	0.056	< 0.01	< 0.01
										straw	46	< 0.01	< 0.01	0.014	< 0.01	0.099
433781	GC 0654	1. 09.11.2012	-	-	-	-	-	-	-	plant [1]	0	< 0.01	< 0.01	0.051	< 0.01	4.2
2014/1010809	Wheat	2. 20.04.-30.04.2013								ears	35	< 0.01	< 0.01	0.11	0.028	0.18
58300 Galatades,	Trofeo	3. 05.06.-15.06.2013								rest of pl. [2]	35	< 0.01	< 0.01	< 0.01	0.014	0.32
Greece (S)										ears	42	< 0.01	< 0.01	0.089	0.026	0.19
L130171										rest of pl. [2]	42	< 0.01	< 0.01	< 0.01	< 0.01	0.99
										ears	49	< 0.01	< 0.01	0.11	0.020	0.061
										rest of pl. [2]	49	< 0.01	< 0.01	< 0.01	0.017	0.95
										grain	54	< 0.01	< 0.01	0.13	0.040	0.019
										straw	54	< 0.01	< 0.01	< 0.01	0.025	1.1
433781	GC 0654	1. 09.10.2012	-	-	-	-	-	-	-	plant [1]	0	< 0.01	< 0.01	0.027	< 0.01	0.43
2014/1010809	Wheat	2. 09.05.-18.05.2013								ears	34	< 0.01	< 0.01	0.21	0.017	0.14
40018 Bologna	Palaiso	3. 05.07.2013								rest of pl. [2]	34	< 0.01	< 0.01	0.024	< 0.01	0.83
Italy (S)										ears	42	< 0.01	< 0.01	0.26	0.015	0.13
L130172										rest of pl. [2]	42	< 0.01	< 0.01	0.023	< 0.01	0.41
										grain	48	< 0.01	< 0.01	0.49	0.015	0.057
			-	-	-	-	-	-	-	straw	48	< 0.01	< 0.01	0.033	< 0.01	0.42
433781	GC 0654	1. 28.12.2012								plant [1]	0	< 0.01	< 0.01	< 0.01	< 0.01	0.28
2014/1010809	Wheat	2. 17.04.-22.04.2013								ears	35	< 0.01	< 0.01	< 0.01	< 0.01	0.014
41710 Utrera	Artur Nick	3. 10.06.2013								rest of pl. [2]	35	< 0.01	< 0.01	< 0.01	< 0.01	0.11
Spain (S)										grain	43	< 0.01	< 0.01	0.13	0.012	0.017
L130173										straw	43	< 0.01	< 0.01	< 0.01	< 0.01	0.095
										grain	49	< 0.01	< 0.01	0.29	0.043	0.032
										straw	49	< 0.01	< 0.01	< 0.01	< 0.01	0.14

Note, for trial L130166 no sampling was done at the last time point DALA49, while for trial L130171 an additional sampling was done at DALA54.
1) whole plant (no root) 2) rest of plant (no root), 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid.

Conclusion

Residue data obtained in 8 independent field trials in wheat (conducted in both N-EU, S-EU with the formulated product BAS 750 00 F according to the critical GAP) showed that the residues of BAS 750 F are <0.01-0.024 mg/kg in grain and 0.5-18 mg/kg in straw.

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. As such, it is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar (see section B.7.3.3).

Report:	CA 6.3.1/2 Ale E., 2015 a Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to wheat in Northern and Southern Europe in 2014 2015/1099704
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, OECD 509 Crop Field Trial (2009), EEC 7525/VI/95 rev. 9 (March 2011), EEC 7029/VI/95 rev. 5 (July 22 1997)
GLP:	yes

Materials and methods

During the growing season of 2014, nine field trials on wheat using either the formulated product 'BAS 750 00 F' or 'BAS 750 00F', both EC formulations containing 100 g/L BAS 750F, were conducted in Germany, the Netherlands, Northern France, Southern France, Greece, Italy and Spain. Each formulation was applied to a separate plot, each formulation was applied twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha.

Samples of whole plant (root removed) were taken directly before and after the second application. Samples were also taken at three time points after the last application; after 34-36 days, 41-43 days and 48-51 days. Where the crop was unripe, samples consisted of ear and rest-of-plant (roots removed) while for the ripe crop grain and straw were taken. Where BBCH growth stage 89 was not reached after 51 days, an additional sampling time point was added. Control (untreated) specimens were taken at every sampling event. A summary of the application and sampling details is given Table 7.3.1-11.

Table 7.3.1-11 Application and sampling details

Region	No. of trials	No. of appl.	F, G, I[2]	Method	Test items	Active substance	Application		Target timing	
							rate (kg a.s. /ha)	water volume (L/ha)	application (BBCH)	sampling (DALA)[1]
EU-N & EU-S	9	2	F	foliar spray	BAS 750 01 F (EC) BAS 750 00 F (EC)	BAS 750 F	0.15	200	1st appl.: BBCH49 2nd appl.: BBCH69	0 34 - 36 41 - 43 48 - 51 51

1) days after last application, 2) field, glasshouse or indoor

Weather data were reported for the trials and no exceptional events were noted. Normal agricultural practice was followed during the field stage. Samples weighing at least 1 kg were collected for ears, rest-of-plant (roots removed) and grain, and samples weighing at least 0.5 kg were collected for straw. Samples were stored were stored at ≤-18 °C within 9.5 hours of harvest, and maintained at this temperature until extraction (with the exception of trials L140173 and L140177 where the storage temperature rose to -10°C for 24 hours).

The maximum storage interval from harvest until analysis was 258 days for BAS 750 F. The maximum storage interval from harvest until analysis was 319 days for triazole and related analytes.

The level of residues in each processed fraction was determined for BAS 750 F (BASF method L0076/09, LOQ of 0.01 mg/kg) and for the triazole derivative metabolites (TDM) 1,2,4-T, TA, TAA, TLA (BASF method L0170/02, LOQ of 0.01 mg/kg for each of the four analytes). Full details of sample preparation and validation data for these methods is given in section CA B.5.1.2.5. Details of the procedural recoveries are given in Table 7.3.1-12 and 7.3.1-13.

Table 7.3.1-12 Summary of recoveries for BAS 750 F

Matrix	Fortification level (mg/kg)	Summary recoveries			
		n	mean (%)	SD (+/-)	RSD (%)
method L0076/09		**BAS 750 F**			
whole plant (no root)	0.01, 10, 100	3	84.5	2.6	3.1
ears	0.01, 0.10, 1.0, 10	8	89.6	5.9	6.6
rest of plant (no root)	0.01, 1.0, 10, 20	9	84.2	8.2	9.8
grain	0.01, 0.10, 1.0	12	87.4	3.4	3.9
straw	0.01, 0.10, 1.0, 10, 20	13	91.1	9.7	11
Overall		**45**	**88.1**	**7.4**	**7.4**

Table 7.3.1-13 Summary of recoveries for 1,2,4-T, TA, TAA and TLA

matrix	fortification level (mg/kg)	summary recoveries			
		n	mean (%)	SD (+/-)	RSD (%)
Method L0170/02		**1,2,4-triazole**			
whole plant (no root)	0.01, 1.0	8	93.7	7.9	8.4
ears	0.01, 1.0	8	97.3	14	14
rest of plant (no root)	0.01, 1.0	8	89.9	13	14
grain	0.01, 1.0	7	99.3	11	11
straw	0.01, 1.0	8	95.8	18	19
Overall		**39**	**95.1**	**13**	**14**
Method L0170/02		**TA**			
whole plant (no root)	0.01, 1.0	7	103	17	17
ears	0.01, 1.0, 1.5	8	89.3	19	21
rest of plant (no root)	0.01, 1.0	7	90.6	17	19
grain	0.01, 1.0, 1.5	8	90.0	15	17
straw	0.01, 1.0	8	92.0	18	20
Overall		**38**	**93.0**	**17**	**19**
Method L0170/02		**TAA**			
whole plant (no root)	0.01, 1.0	7	105	6.8	6.5
ears	0.01, 1.0	7	98.1	18	18
rest of plant (no root)	0.01, 1.0	6	89.6	16	18
grain	0.01, 1.0	7	88.3	16	19
straw	0.01, 1.0	8	95.8	15	15
Overall		**35**	**95.5**	**15**	**16**
Method L0170/02		**TLA**			
whole plant (no root)	0.01, 1.0	8	93.4	9.9	11
ears	0.01, 1.0	7	91.5	18	20
rest-of-plant (no root)	0.01, 1.0	8	79.4	13	16
grain	0.01, 1.0, 1.5	8	79.3	14	18
straw	0.01, 1.0	8	91.1	12	13
Overall		**39**	**87.1**	**14**	**17**

Results and Discussion

Nine field trials on wheat using the formulated products 'BAS 750 00 F' and 'BAS 750 01 F', EC formulations containing 100 g/L BAS 750F were conducted in NEU and SEU during the growing season of 2014. Each formulation was applied to a separate plot twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha. This application rate and timing is in accordance with the proposed GAP for wheat.

Samples were taken at three time points after the last application; after 34-36 days, 41-43 days and 48-51 days Where BBCH growth stage 89 was not reached after 49 days, an additional sample was taken at this stage. Residues trials data are presented in Table 7.3.1-14 (treated samples) and Table 7.3.1-15 (untreated samples).

Samples were stored frozen for up to 258 days prior to analysis for BAS 750 F and for up to 319 days for triazole and related analytes. Storage stability data is available to support storage of BAS 750 F for up to 730 days in cereals (section B.7.1.1), which is sufficient to support the storage times in this study. Storage stability data is available to support storage of 1,2,4-triazole and TA for 54 months, TTA for 26 months (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015) and TLA for 49 months (section B.7.1.2). This is sufficient to support the storage times in this study.

The methods of analysis for determination of BAS 750 F and TDMs are considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4. Acceptable procedural recovery data, using an appropriate number of samples were presented.

BAS 750 F

In untreated samples, no residues of BAS 750 F exceeding the LOQ (0.01 mg/kg) were detected.

For grain from samples treated with 'BAS 750 01 F', BAS 750 F residues were below LOQ in seven trials, and present in one trial at 0.014 mg/kg and another at 0.026 mg/kg. For grain from samples treated with 'BAS 750 00F', BAS 750 F residues were below LOQ in five trials, and present in the others at 0.011-0.025 mg/kg.

For straw from samples treated with 'BAS 750 01F', BAS 750 F residues ranged from 0.56-8.5 mg/kg. For straw from samples treated with 'BAS 750 00F', BAS 750 F residues ranged from 0.5-9.0 mg/kg.

For plants directly after application of 'BAS 750 01 F' (DALA0), BAS 750 F residues were in the range of 2.2 – 6.6 mg/kg. For plants directly after application of 'BAS 750 00 F' (DALA0) residues were in the range of 2.3 - 7.1 mg/kg.

Similar residues in grain and straw were determined in trials with each formulation. A comparison of the results is presented in Table 7.3.1-16. As the trials only differed in formulation type the highest residue for each trial has been used for HR and STMR determinations.

Table 7.3.1-16: Summary of BAS 750 F in wheat after treatment with the formulated products BAS 750 01 F and BAS 750 00 F

Region	Matrix	DALA [1)]	BBCH	n	BAS 750 F [mg/kg]	
					BAS 750 01 F	BAS 750 00 F
N-EU & S-EU	plant [2)]	0	69	9	2.2 – 6.6	2.3 – 7.1
	ears	34 - 36	79 - 87	9	0.063 – 2.8	0.073 – 3.5
	rest-of-plant [2)]			9	0.29 – 10	0.26 – 7.9
	ears	42 - 43	85 - 89	5	0.053 – 2.3	0.061 – 2.6
	rest-of-plant [2)]			5	0.37 – 7.2	0.60 – 8.8
	grain	41 - 42	87 - 89	4	< 0.01 – 0.012	< 0.01 – 0.014
	straw			4	1.9 – 4.6	1.3 – 5.0
	ears	49	89	1	1.2	0.93
	rest-of-plant [2)]			1	6.6	5.3
	grain	48 - 51	89	8	< 0.01 – 0.014	< 0.01 – 0.016
	straw			8	0.52 – 4.4	0.48 – 4.6
	grain	51	89	1	0.026	0.025
	straw			1	8.6	8.8

[1)] DALA = days after last application; [2)] no root

TDM

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. A comparison of the results is presented in Table 7.3.1-17.

For TA, residue levels in grain were up to 1.5 mg/kg in the treated samples and up to 1.0 mg/kg in untreated samples. Residue levels in straw were up to 0.39 mg/kg in the treated samples and up to 0.78 mg/kg in untreated samples.

For TAA, residue levels in grain were up to 0.23 mg/kg in the treated samples and up to 0.29 mg/kg in untreated samples. Residue levels in straw were up to 0.14 mg/kg in the treated samples and up to 0.17 mg/kg in untreated samples.

For TLA, residue levels in grain were <0.01 mg/kg in the treated samples and up to 0.09 mg/kg in untreated samples. Residue levels in straw were up to 0.08 mg/kg in the treated samples and up to 0.09 mg/kg in untreated samples.

As such, it is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar. For further discussion see section 7.3.3.

Table 7.3.1-17: Summary of TDM in untreated wheat (plot 1) as well as after treatment with BAS 750 01 F (plot2) or BAS 750 00 F (plot 3)

Region	Matrix	DALA [1]	BBCH	Plot	1,2,4-triazole *[mg/kg]*	TA *[mg/kg]*	TAA *[mg/kg]*	TLA *[mg/kg]*
N-EU & S-EU	plant [2]	0	69	1	<0.01	0.10 - 0.24	<0.01 - 0.085	<0.01 - 0.42
				2	<0.01	0.043 - 0.17	<0.01 - 0.051	<0.01 - 0.29
				3	<0.01	0.043 - 0.19	<0.01 - 0.074	0.017 - 0.39
	ears	34-36	79/83-87	1	<0.01	0.019 - 0.18	<0.01	0.055 - 1.1
				2	<0.01	0.030 - 0.27	<0.01	0.095 - 1.2
				3	<0.01	0.038 - 0.32	<0.01	0.12 - 1.0
	rest-of-plant [2]			1	<0.01	0.042 - 0.22	0.032 - 0.11	0.053 - 0.23
				2	<0.01	0.019 - 0.12	0.022 - 0.077	0.044 - 0.10
				3	<0.01	0.014 - 0.12	0.018 - 0.075	<0.01 - 0.10
	ears	42-43	85/87-87/89	1	<0.01	0.020 - 0.30	<0.01	0.042 - 0.27
				2	<0.01	0.080 - 0.12	<0.01	0.12 - 0.30
				3	<0.01	0.017 - 0.087	<0.01	0.016 - 0.28
	rest-of-plant [2]			1	<0.01	0.036 - 0.17	0.010 - 0.24	0.086 - 0.26
				2	<0.01	0.030 - 0.070	0.019 - 0.078	0.027 - 0.19
				3	<0.01	0.024 - 0.051	0.021 - 0.075	<0.01 - 0.099
	grain	41-42	87/89-89	1	<0.01	0.072 - 0.19	<0.01	0.14 - 0.96
				2	<0.01	0.085 - 0.23	<0.01	0.18 - 1.3
				3	<0.01	0.067 - 0.36	<0.01	0.21 - 1.2
	straw			1	<0.01	0.026 - 0.076	<0.01	0.22 - 0.72
				2	<0.01	0.058 - 0.085	<0.01 - 0.075	<0.01 - 0.28
				3	<0.01	0.065 - 0.14	0.020 - 0.13	0.014 - 0.079
	ears	49	89	1	<0.01	0.023	<0.01	0.065
				2	<0.01	0.062	<0.01	0.087
				3	<0.01	0.043	<0.01	0.06
	rest-of-plant [2]			1	<0.01	0.098	<0.01	0.062
				2	<0.01	0.044	0.04	0.017
				3	<0.01	0.025	0.027	0.018
	grain	48-50	89	1	<0.01	0.019 - 0.29	<0.01	0.064 - 1.0
				2	<0.01	0.043 - 0.20	<0.01	0.19 - 1.2
				3	<0.01	0.046 - 0.42	<0.01	0.17 - 1.2
	straw			1	<0.01	<0.01 - 0.15	<0.01 - 0.029	0.099 - 0.77
				2	<0.01	<0.01 - 0.14	<0.01 - 0.077	0.090 - 0.43
				3	<0.01	0.032 - 0.16	<0.01 - 0.051	0.020 - 0.11
	grain	51	89	1	<0.01	0.014 - 0.21	<0.01	0.11 - 0.19
				2	<0.01	0.068 - 0.16	<0.01	0.049 - 0.19
				3	<0.01	0.060 - 0.083	<0.01	0.12
	straw			1	<0.01	<0.01 - 0.10	<0.01	0.088 - 0.095
				2	<0.01	0.029 - 0.030	0.017 - 0.037	<0.01 - 0.10
				3	<0.01	0.024 - 0.025	<0.01	0.017 - 0.029

[1] DALA = days after last application; [2] no root

Table 7.3.1-14: Residues of BAS 750 F and TDM in wheat (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783 2015/1099704 74193 Stetten a. H. Germany (N) L140168	GC 0654 Wheat Asano	1. 01.11.2013 2. 21.05.- 02.06.2014 3. 23.07.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 02.06 14	69	plant [1]	0	2.8	<0.01	0.071	0.085	0.020
										ears	35	0.46	<0.01	0.17	0.089	<0.01
										rest of pl. [2]	35	2.8	<0.01	0.047	0.064	0.022
										ears	42	0.45	<0.01	0.12	0 12	<0.01
										rest of pl. [2]	42	4.2	<0.01	0.19	0.055	0.019
										grain	51	<0.01	<0.01	0.19	0 16	<0.01
										straw	51	3.6 (3.6)	<0.01	0.10	0.029	0.017
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 02.06 14	69	plant [1]	0	2.6	<0.01	0.092	0.068	0.012
										ears	35	0.48	<0.01	0.13	0.072	<0.01
										rest of pl. [2]	35	4.1	<0.01	0.034	0.041	0.027
										ears	42	0.41	<0.01	0.12	0.086	<0.01
										rest of pl. [2]	42	4.2	<0.01	0.069	0.051	0.021
										grain	51	0.011	<0.01	0.12	0.083	<0.01
										straw	51	3.6 (3.4)	<0.01	0.029	0.025	<0.01
433783 2015/1099704 47589 Uedem Germany (N) L140169	GC 0654 Wheat Elixier	1. 18.11.2013 2. 04.06.- 16.06.2014 3. 04.08.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 16.06 14	69	plant [1]	0	2.2	<0.01	<0.01	0.057	0.020
										ears	36	0.38	<0.01	0.34	0 10	<0.01
										rest of pl. [2]	36	1.5	<0.01	0.077	0.098	0.044
										grain	42	<0.01	<0.01	0.27	0.085	<0.01
										straw	42	1.9	<0.01	0.15	0.081	0.033
										grain	49	<0.01	<0.01	0.25	0.047	<0.01
										straw	49	1.7	<0.01	0.10	<0.01	0.052
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 16.06 14	69	plant [1]	0	2.5	<0.01	0.043	0.069	0.028
										ears	36	0.46	<0.01	0.18	0.060	<0.01
										rest of pl. [2]	36	0.78	<0.01	0.016	0.064	0.024
										grain	42	<0.01	<0.01	0.30	0.067	<0.01
										straw	42	1.3	<0.01	0.035	0.066	0.020
										grain	49	<0.01	<0.01	0.20	0.063	<0.01
										straw	49	1.6	<0.01	0.030	0.088	<0.01

Table 7.3.1-14: Residues of BAS 750 F and TDM in wheat (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783 2015/1099704 37360 Rouzières de Touraine France (N) L140170	GC 0654 Wheat Altigo	1. 01.10.2013 2. 20.05.-05.06.2014 3. 24.07.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 05.06 14	69	plant [1]	0	2.6	<0.01	0.29	0 17	0.051
										ears	35	0.42	<0.01	1.3 (1.1)	0 28 (0.26)	<0.01
										rest of pl. [2]	35	2.6	<0.01	0.071	0 12	0.070
										grain	42	0.012	<0.01	1.5 (1.2)	0 22	<0.01
										straw	42	2.6	<0.01	0.31 (0.24)	0.085	0.055
										grain	49	0.014	<0.01	1.2	0 20	<0.01
										straw	49	2.3	<0.01	0.33 (0.41)	0 14	0.073
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 05.06 14	69	plant [1]	0	2.3	<0.01	0.39	0 19	0.074
										ears	35	0.60	<0.01	1.1 (0.91)	0 30 (0.34)	<0.01
										rest of pl. [2]	35	3.0	<0.01	0.10	0 12	0.059
										grain	42	0.012	<0.01	1.2	0 36	<0.01
										straw	42	3.4	<0.01	0.079	0 14	0.050
										grain	49	0.016	<0.01	1.2	0.42	<0.01
										straw	49	3.1	<0.01	0.11	0 16	0.045
433783 2015/1099704 6595 ME Ottersum, The Netherlands (N) L140171	GC 0654 Wheat Tabasco	1. 22.11.2013 2. 03.06-16.06.2014 3. 04.08.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 16.06 14	69	plant [1]	0	2.2	<0.01	0.064	0.051	0.024
										ears	36	0.70	<0.01	0.20	0 12	<0.01
										rest of pl. [2]	36	2.5	<0.01	0.066	0.084	0.028
										grain	42	<0.01	<0.01	0.18	0 11	<0.01
										straw	42	4.3	<0.01	0.099	0.071	0.036
										grain	49	<0.01	<0.01	0.25	0 10	<0.01
										straw	49	4.4	<0.01	0.39 (0.47)	0.072	0.041
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 16.06 14	69	plant [1]	0	2.4	<0.01	0.072	0.061	0.026
										ears	36	0.76	<0.01	0.17	0.11	<0.01
										rest of pl. [2]	36	3.5	<0.01	0.056	0.061	0.035
										grain	42	0.014	<0.01	0.21	0.099	<0.01
										straw	42	4.9	<0.01	0.014	0.086	0.030
										grain	49	<0.01	<0.01	0.18	0 10	<0.01
										straw	49	4.6	<0.01	0.021	0.086	0.027

Table 7.3.1-14: Residues of BAS 750 F and TDM in wheat (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783 2015/1099704 16220 Quintanar Del Rey Spain (S) L140173	GC 0654 Wheat Adagio	1. 08.11.2013 2. 25.04.- 10.05.2014 3. 25.06.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 05.05 14	69	plant [1]	0	4.8	<0.01	0.041	0.077	0.023
										ears	35	2.8	<0.01	0.095	0.065	<0.01
										rest of pl. [2]	35	10	<0.01	0.10	0.037	0.058
										ears	42	2.3	<0.01	0.13	0.080	<0.01
										rest of pl. [2]	42	7.2	<0.01	0.096	0.070	0.058
										ears	49	1.2	<0.01	0.087	0.062	<0.01
										rest of pl. [2]	49	6.8	<0.01	0.017	0.044	0.040
										grain	51	0.026	<0.01	0.049	0.068	<0.01
										straw	51	8.5 (8.5)	<0.01	<0.01	0.030	0.037
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 05.05 14	69	plant [1]	0	5.4	<0.01	0.024	0.052	0.018
										ears	35	3.5	<0.01	0.14	0.054	<0.01
										rest of pl. [2]	35	7.9	<0.01	0.027	0.053	0.054
										ears	42	2.6	<0.01	0.081	0.059	<0.01
										rest of pl. [2]	42	8.8	<0.01	<0.01	0.041	0.056
										ears	49	0.93	<0.01	0.060	0.043	<0.01
										rest of pl. [2]	49	5.3	<0.01	0.018	0.025	0.027
										grain	51	0.025	<0.01	0.12	0.060	<0.01
										straw	51	9.0 (8.6)	<0.01	0.017	0.024	<0.01
433783 2015/1099704 32220 St. Soulan France (S) L140174	GC 0654 Wheat Aprilio	1. 29.11.2013 2. 23.05.- 29.05.2014 3. 15.07.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 27.06 14	69	plant [1]	0	3.2	<0.01	0.054	0.055	0.022
										ears	34	0.19	<0.01	0.29	0.095	<0.01
										rest of pl. [2]	34	1.0	<0.01	0.080	0.039	0.043
										ears	42	0.18	<0.01	0.30	0 11	<0.01
										rest of pl. [2]	42	1.3	<0.01	0.11	0.058	0.026
										grain	49	<0.01	<0.01	0.33	0.061	<0.01
										straw	49	1.6 (1.6)	<0.01	0.16	0.060	0.038
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 27.06 14	69	plant [1]	0	2.7	<0.01	0.050	0.047	0.016
										ears	34	0.16	<0.01	0.29	0 10	<0.01
										rest of pl. [2]	34	0.91	<0.01	0.054	0.033	0.049
										ears	42	0.16	<0.01	0.28	0.087	<0.01
										rest of pl. [2]	42	1.1	<0.01	0.080	0.048	0.028
										grain	49	<0.01	<0.01	0.36	0.081	<0.01
										straw	49	1.5 (1.5)	<0.01	0.061	0.043	<0.01

Table 7.3.1-14: Residues of BAS 750 F and TDM in wheat (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783 2015/1099704 58500 Agios Greece (S) L140175	GC 0654 Wheat Trofeo	1. 10.11.2013 2. 15.04.-26.04.2014 3. 13.06.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 24.04 14	69	plant [1]	0	2.6	<0.01	0.034	0.043	<0.01
										ears	35	0.063	<0.01	0.16	0.030	<0.01
										rest of pl. [2]	35	0.29	<0.01	0.044	0.081	0.026
										ears	43	0.053	<0.01	0.15	0.081	<0.01
										rest of pl. [2]	43	0.37	<0.01	0.027	0.046	0.024
										grain	50	<0.01	<0.01	0.19	0.091	<0.01
										straw	50	0.53 (0.56)	<0.01	0.13	0.031	0.024
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 24.04 14	69	plant [1]	0	2.5	<0.01	0.017	0.054	<0.01
										ears	35	0.073	<0.01	0.12	0.038	<0.01
										rest of pl. [2]	35	0.26	<0.01	<0.01	0.044	0.018
										ears	43	0.061	<0.01	0.10	0.046	<0.01
										rest of pl. [2]	43	0.61	<0.01	<0.01	0.033	0.023
										grain	50	<0.01	<0.01	0.22	0.050	<0.01
										straw	50	0.46 (0.45)	<0.01	0.023	0.041	0.027
433783 2015/1099704 20060 S Martino Italy (S) L140176	GC 0654 Wheat Aprilio	1. 20.10.2013 2. 10.05.-17.05.2014 3. 02.07.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 15.05 14	69	plant [1]	0	6.6	<0.01	0.083	0.066	0.045
										ears	34	0.42	<0.01	0.27	0 14	<0.01
										rest of pl. [2]	34	3.2	<0.01	0.096	0.043	0.077
										grain	41	<0.01	<0.01	0.36	0 23	<0.01
										straw	41	4.6	<0.01	0.10	0.058	0.075
										grain	48	<0.01	<0.01	0.28	0 11	<0.01
										straw	48	4.2	<0.01	0.087	0.024	0.077
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 15.05 14	69	plant [1]	0	7.1	<0.01	0.072	0.067	0.035
										ears	34	0.40	<0.01	0.23	0 11	<0.01
										rest of pl. [2]	34	4.0	<0.01	0.022	0.051	0.075
										grain	41	<0.01	<0.01	0.29	0.082	<0.01
										straw	41	3.7	<0.01	0.031	0.065	0.13
										grain	48	<0.01	<0.01	0.31	0 11	<0.01
										straw	48	4.6	<0.01	0.020	0.054	0.051

Table 7.3.1-14: Residues of BAS 750 F and TDM in wheat (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783 2015/1099704 02110 La Gineta Spain (S) L140177	GC 0654 Wheat Califa	1. 17.01.2014 2. 10.05.-25.05.2014 3. 09.07.2014	Foliar application	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 21.05 14	69	plant [1]	0	3.9	<0.01	0.086	0.053	<0.01
										ears	35	0.14	<0.01	0.15	0.066	<0.01
										rest of pl. [2]	35	1.9	<0.01	0.059	0.019	0.040
										ears	42	0.19	<0.01	0.22	0 10	<0.01
										rest of pl. [2]	42	2.6	<0.01	0.067	0.030	0.078
										grain	49	<u><0.01</u>	<0.01	0.25	0.043	<0.01
										straw	49	<u>3.1</u> (3.0)	<0.01	0.18	0.020	0.065
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 21.05 14	69	plant [1]	0	4.3	<0.01	0.11	0.043	<0.01
										ears	35	0.22	<0.01	0.18	0.043	<0.01
										rest of pl. [2]	35	1.7	<0.01	0.045	0.014	0.024
										ears	42	0.45	<0.01	0.016	0.017	<0.01
										rest of pl. [2]	42	2.9	<0.01	0.099	0.024	0.075
										grain	49	<0.01	<0.01	0.17	0.046	<0.01
										straw	49	3.1 (3.0)	<0.01	0.014	0.032	0.022

1) whole plant (no root) 2) rest-of-plant (no root), 3) time between applications supports the proposed minimum of 14 days, 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid. A number in parenthesis indicates re-analysis to confirm analytical results. The highest both values is selected. The underlined values (e.g. <u>0.018</u>) are used for calculation of the STMR, HR and MRL for grain, and the STMR and HR for straw

Table 7.3.1-15: Level of BAS 750 F and TDM in untreated wheat (plot 1)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783	GC 0654	1. 01 11.2013	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.12	0.24	0.057
2015/1099704	Wheat	2. 21.05.-02.06.2014								ears	35	<0.01	<0.01	0.16	0.15	<0.01
74193 Stetten a. H.	Asano	3. 23.07.2014								rest of pl. [2]	35	<0.01	<0.01	0.12	0.11	0.036
Germany (N)										ears	42	<0.01	<0.01	0.27	0.27 (0.26)	<0.01
L140168										rest of pl. [2]	42	<0.01	<0.01	0.13	0.17	0.035
										grain	51	<0.01	<0.01	0.19	0.21	<0.01
										straw	51	<0.01	<0.01	0.088	0.10	<0.01
433783	GC 0654	1. 18 11.2013	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.053	0.13	0.049
2015/1099704	Wheat	2. 04.06.-16.06.2014								ears	36	<0.01	<0.01	0.19	0.11	<0.01
47589 Uedem	Elixier	3. 04.08.2014								rest of pl. [2]	36	<0.01	<0.01	0.20	0.16	0.067
Germany (N)										grain	42	<0.01	<0.01	0.14	0.077	<0.01
L140169										straw	42	<0.01	<0.01	0.78 (0.66)	0.061	<0.01
										grain	49	<0.01	<0.01	0.11	0.088	<0.01
										straw	49	<0.01	<0.01	0.70 (0.83)	0.071	<0.01
433783	GC 0654	1. 01.10.2013	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.42	0.15	0.071
2015/1099704	Wheat	2. 20.05.-05.06.2014								ears	35	<0.01	<0.01	1.3 (0.94)	0.18	<0.01
37360 Rouzières de Touraine	Altigo	3. 24.07.2014								rest of pl. [2]	35	<0.01	<0.01	0.23	0.13	0.055
France (N)										grain	42	<0.01	<0.01	0.96	0.19	<0.01
L140170										straw	42	<0.01	<0.01	0.25 (0.20)	0.17	<0.01
										grain	49	<0.01	<0.01	1.0	0.18	<0.01
										straw	49	<0.01	<0.01	0.34 (0.41)	0.11	0.016
433783	GC 0654	1. 22.11.2013	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.036	0.10	0.023
2015/1099704	Wheat	2. 03.06-16.06.2014								ears	36	<0.01	<0.01	0.29	0.071	<0.01
6595 ME Ottersum,	Tabasco	3. 04.08.2014								rest of pl. [2]	36	<0.01	<0.01	0.064	0.081	0.032
The Netherlands (N)										grain	42	<0.01	<0.01	0.16	0.072	<0.01
L140171										straw	42	<0.01	<0.01	0.24 (0.22)	0.026	<0.01
										grain	49	<0.01	<0.01	0.22	0.043	<0.01
										straw	49	<0.01	<0.01	0.25 (0.23)	0.055	<0.01

Table 7.3.1-15: Level of BAS 750 F and TDM in untreated wheat (plot 1)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783 2015/1099704 16220 Quintanar Del Rey Spain (S) L140173	GC 0654 Wheat Adagio	1. 08.11.2013 2. 25.04.-10.05.2014 3. 25.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.015	0.17	<0.01
										ears	35	<0.01	<0.01	0.055	0.032	<0.01
										rest of pl. [2]	35	<0.01	<0.01	0.14	0.22	0.015
										ears	42	<0.01	<0.01	0.052	0.027	<0.01
										rest of pl. [2]	42	<0.01	<0.01	0.15	0.16	0.016
										ears	49	<0.01	<0.01	0.065	0.023	<0.01
										rest of pl. [2]	49	<0.01	<0.01	0.062	0.098	<0.01
										grain	51	<0.01	<0.01	0.11	0.014	<0.01
										straw	51	<0.01	<0.01	0.095	<0.01	<0.01
433783 2015/1099704 32220 St. Soulan France (S) L140174	GC 0654 Wheat Aprilio	1. 29.11.2013 2. 23.05.-29.05.2014 3. 15.07.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.14	0.21	0.085
										ears	34	<0.01	<0.01	0.48	0.17	<0.01
										rest of pl. [2]	34	<0.01	<0.01	0.16	0.15	0.098
										ears	42	<0.01	<0.01	0.20	0.31 (0.28)	<0.01
										rest of pl. [2]	42	<0.01	<0.01	0.26	0.14	0.081
										grain	49	<0.01	<0.01	0.75	0.29	<0.01
										straw	49	<0.01	<0.01	0.10	0.15	0.029
433783 2015/1099704 58500 Agios Greece (S) L140175	GC 0654 Wheat Trofeo	1. 10.11.2013 2. 15.04.-26.04.2014 3. 13.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	<0.01	0.11	<0.01
										ears	35	<0.01	<0.01	0.11	0.024	<0.01
										rest of pl. [2]	35	<0.01	<0.01	0.053	0.15	0.013
										ears	43	<0.01	<0.01	0.11	0.020	<0.01
										rest of pl. [2]	43	<0.01	<0.01	0.15	0.12	0.010
										grain	50	<0.01	<0.01	0.069	0.030	<0.01
										straw	50	<0.01	<0.01	0.23 (0.13)	<0.01	<0.01
433783 2015/1099704 20060 S Martino Italy (S) L140176	GC 0654 Wheat Aprilio	1. 20.10.2013 2. 10.05.-17.05.2014 3. 02.07.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.061	0.11	0.034
										ears	34	<0.01	<0.01	0.23	0.060	<0.01
										rest of pl. [2]	34	<0.01	<0.01	0.095	0.084	0.047
										grain	41	<0.01	<0.01	0.18	0.13	<0.01
										straw	41	<0.01	<0.01	0.26 (0.24)	0.076	<0.01
										grain	48	<0.01	<0.01	0.16	0.097	<0.01
										straw	48	<0.01	<0.01	0.16	0.028	<0.01

Table 7.3.1-15: Level of BAS 750 F and TDM in untreated wheat (plot 1)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433783	GC 0654	1. 17.01.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.044	0.10	<0.01
2015/1099704	Wheat	2. 10.05.-25.05.2014								ears	35	<0.01	<0.01	0.20	0.019	<0.01
02110 La Gineta	Califa	3. 09.07.2014								rest of pl. [2]	35	<0.01	<0.01	0.16	0.042	0.11
Spain (S)										ears	42	<0.01	<0.01	0.042	0.023	<0.01
L140177										rest of pl. [2]	42	<0.01	<0.01	0.086	0.036	0.24
										grain	49	<0.01	<0.01	0.064	0.019	<0.01
										straw	49	<0.01	<0.01	0.099	0.012	<0.01

1) whole plant (no root) 2) rest-of-plant (no root), 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid. A number in parenthesis indicates re-analysis to confirm analytical results.

Conclusion

Residue data obtained in 9 independent field trials in wheat (conducted in both N-EU, S-EU with the formulated product BAS 750 00 F or BAS 750 01 F according to the critical GAP) showed that the residues of BAS 750 F are <0.01-0.026 mg/kg in grain and 0.5-9 mg/kg in straw.

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. As such, it is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar (see section B.7.3.3).

B.7.3.2. Barley

One of the proposed uses of BAS 750 F in the EU is on barley. The cGAP for barley is provided in Table 7.3.2-1.

Table 7.3.2-1: Summary of the critical GAPs for the proposed uses in barley

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum Application interval (days)	Maximum		Minimum PHI (days)[a)]
					rate (kg as/ha)	water (L/ha)	
Barley	outdoor	49, 69	2	14	0.15	200	35

[a)] Timing of the cGAP determined based on growth stage. PHI of 35 days proposed by the applicant, but has not be used for selection of trials

A total of 18 residues trials have been submitted in support of this use. A summary of the timing, number and location of the residues trials on barley is given in Table 7.3.2-2.

Table 7.3.2-2: Number of residue trials per geographical region and vegetation period

Crop	Season	Number of trials					Reference
		N-EU	Country	S-EU	Country	Total	
barley	2013	4	DE (2x), NL, UK	4	ES, FR, GR, IT	8	6.3.2/1
barley	2014	5	DE (2x), FR, NL, UK	5	ES (2x), FR, GR, IT	10	6.3.2/2
Total number of trials per region		**9**		**9**	**Total number of trials**	**18**	

A summary of the results of the residues trials on barley is given in Table 7.3.2-3.

Table 7.3.2-3: Overall summary of residue data for BAS 750 F from barley residue trials

Crop	Region	RAC	n	Residues *[mg/kg]*	STMR *[mg/kg]*	HR *[mg/kg]*
Barley	N-EU	grain	9	0.014, 0.06, 0.071, 0.087, 0.1, 0.15, 0.15, 0.19, 0.28	0.1	0.28
	S-EU		9	0.03, 0.033, 0.07, 0.1, 0.1, 0.14, 0.16, 0.29, 0.41	0.1	0.41
	N-EU	straw	9	1.0, 1.7, 3.1, 3.9, 4.3, 4.3, 5.6, 6.8, 15.0	4.3	15.0
	S-EU		9	0.39, 2.1, 2.2, 3.3, 4.2, 4.6, 6.4, 11.0, 18.0	4.2	18.0

Report:	CA 6.3.2/1 Erdmann H.-P., 2015 b Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in barley after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013 2014/1010808
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes

Materials and methods

During the growing season of 2013, eight field trials on barley using the formulated product 'BAS 750 00 F', an EC formulation containing 100 g/L BAS 750F were conducted in Germany, the Netherlands, the United Kingdom, Southern France, Greece, Italy and Spain. The formulation was applied twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha.

Samples of whole plant (root removed) were taken directly before and after the second application. Samples were also taken at three time points after the last application; after 27-29 days, 34-36 days and 41-42 days. Where the crop was unripe, samples consisted of ear and rest-of-plant (roots removed) while for the ripe crop grain and straw were taken. Where BBCH growth stage 89 was not reached after 42 days, an additional sampling time point was added. Control (untreated) specimens were taken at every sampling event. A summary of the application and sampling details is given Table 7.3.2-4.

Table 7.3.2-4 Application and sampling details

Region	No. of trials	No. of appl.	F, G, I[2]	Method	Test item	Active substance	Application		Targeted timing	
							rate (kg a.s. /ha)	water volume (L/ha)	application (BBCH)	sampling (DALA)[1]
N-EU & S-EU	8	2	F	foliar spray	BAS 750 00 F (EC)	BAS 750 F	0.15	200	1st appl.: BBCH 49 2nd appl.: BBCH 69	0 28 ± 1 35 ± 1 42 ± 1 48 - 55

1) days after last application, 2) field, glasshouse or indoor

Weather data were reported for the trials and no exceptional events were noted. Normal agricultural practice was followed during the field stage. Samples weighing at least 1 kg were collected for ears, rest-of-plant (roots removed) and grain, and samples weighing at least 0.5 kg were collected for straw. Samples were stored were stored at ≤-18 °C within 6 hours of harvest, and maintained at this temperature until extraction (with the exception of trial L130178 where the storage temperature rose to -10°C for 24 hours).

The maximum storage interval from harvest until analysis was 736 days for BAS 750 F. The maximum storage interval from harvest until analysis was 389 days for triazole and related analytes.

The level of residues in each processed fraction was determined for BAS 750 F (BASF method L0076/09, LOQ of 0.01 mg/kg) and for the triazole derivative metabolites (TDM) 1,2,4-T, TA, TAA, TLA (BASF method L0170/02, LOQ of 0.01 mg/kg for each of the four analytes). Full details of sample preparation and validation data for these methods is given in section CA B.5.1.2.5. Details of the procedural recoveries are given in Table 7.3.2-5 and 7.3.1-6.

Table 7.3.2-5: Summary of recoveries for BAS 750 F

Matrix	Fortification level (mg/kg)	Summary Recoveries			
		n	mean (%)	SD (+/-)	RSD (%)
Method L0076/09		**BAS 750 F**			
whole plant (no root)	0.01, 1.0, 20	3	99.7	4.0	4.0
ears	0.01, 0.10, 1.0, 10	7	96.7	9.0	9.3
rest of plant (no root)	0.01, 1.0, 10, 20	13	88.7	15	16
grain	0.01, 0.10, 1.0	6	94.0	12	12
straw	0.01, 0.02, 1.0, 10, 20	10	91.2	15	16
Overall		**39**	**92.4**	**13**	**14**

Table 7.3.2-6 Summary of recoveries for 1,2,4-T, TA, TAA and TLA

Matrix	Fortification level (mg/kg)	Summary recoveries			
		n	mean (%)	SD (+/-)	RSD (%)
Method L0170/02		**1,2,4-triazole**			
whole plant (no root) rest-of-plant (no root)	0.01, 1.0	10	92.5	9.5	10
ears	0.01, 1.0	5	95.4	6.1	6.3
grain	0.01, 1.0	8	95.8	10	16
straw	0.01, 1.0	8	95.0	15	16
Overall		**31**	**94.5**	**12**	**13**
Method L0170/02		**TA**			
whole plant (no root) rest-of-plant (no root)	0.01, 1.0, 20	9	87.6	17	20
ears	0.01, 1.0, 20	7	91.4	12	13
grain	0.01, 1.0, 20	9	77.3	12	16
straw	0.01, 1.0, 20	8	84.2	20	22
Overall		**33**	**84.8**	**16**	**18**
Method L0170/02		**TAA**			
whole plant (no root) rest-of-plant (no root)	0.01, 1.0	10	91.4	14	16
ears	0.01, 1.0	6	90.0	12	13
grain	0.01, 1.0	8	75.9	13	13
straw	0.01, 1.0	8	83.7	13	15
Overall		**32**	**85.3**	**14**	**16**
Method L0170/02		**TLA**			
whole plant (no root) rest-of-plant (no root)	0.01, 1.0, 20	9	95.2	18	20
ears	0.01, 1.0, 20	7	103	11	11
grain	0.01, 1.0, 20	9	96.8	21	18
straw	0.01, 1.0, 20	9	96.2	6.8	7.1
Overall		**34**	**97.5**	**14**	**14**

Results and Discussion

Eight field trials on barley using the formulated product 'BAS 750 00 F', an EC formulation containing 100 g/L BAS 750F were conducted in NEU and SEU during the growing season of 2013. The formulation was applied twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha. This application rate and timing is in accordance with the proposed GAP for barley.

Samples were taken at three time points after the last application; 27-29 days, 34-36 days and 41-42 days. Where BBCH growth stage 89 was not reached after 42 days, an additional sample was taken at this stage. Residues trials data are presented in Table 7.3.2-7 (treated samples) and Table 7.3.1-8 (untreated samples).

Samples were stored frozen for up to 736 days prior to analysis for BAS 750 F and for up to 389 days for triazole and related analytes. Storage stability data is available to support storage of BAS 750 F for up to 730 days in cereals (section B.7.1.1), which is sufficient to support the storage times in this study. Storage stability data is available to support storage of 1,2,4-triazole and TA for 54 months, TTA for 26 months (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015) and TLA for 49 months (section B.7.1.2). This is sufficient to support the storage times in this study.

The methods of analysis for determination of BAS 750 F and TDMs are considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4. Acceptable procedural recovery data, using an appropriate number of samples were presented.

BAS 750 F

In untreated samples no residues of BAS 750 F exceeding the LOQ (0.01 mg/kg) were detected. A summary of the results from the treated samples is presented in Table 7.3.2-9.

For grain from treated samples, BAS 750 F residues were between 0.014-0.41 mg/kg. For straw from treated samples, BAS 750 F residues showed high variability ranging from 0.39 to 15mg/kg.

For plants directly harvested after application (DALA0), BAS 750 F residues were in the range of 2.3 – 7.4 mg/kg.

Table 7.3.2-9: Summary of BAS 750 F residues in BAS 750 00 F treated barley

Region	Matrix	DALA[1)]	BBCH	BAS 750 F *[mg/kg]*
N-EU & S-EU	plant [2)]	0	69	2.3 – 7.4
	ears	27 - 28	75-87	0.082 – 1.2
	rest-of-plant[3)]			0.49 – 12
	grain	28 - 29	85-89	0.14 – 0.42
	straw			9.3 – 11
	ears	34 - 35	77-87	0.058 – 1.0
	rest-of-plant[3)]			0.20 – 13
	grain	34 - 36	87-89	0.056 – 0.29
	straw			2.9 – 15
	ears	41 - 42	83-87	0.065 – 1.7
	rest-of-plant[3)]			0.18 - 13
	grain	41 - 42	89	0.071 - 0.41
	straw			3.9 – 11
	grain	48 - 55	89	0.014 – 0.16
	straw			0.39 – 6.4

[1)] DALA = days after last application; [2)] whole plant (no roots); [3)] no roots

TDM

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples Details of the previous treatments to the plots are not available in the study reports; however it is considered that the most likely explanation as to the source of the TDMs in untreated crops, is residual TDMs in the soil from previous applications of a triazole containing pesticide. A comparison of the results for treated and untreated crops is presented in Table 7.3.2-10.

For TA, residue levels in grain were up to 2.6 mg/kg in the treated samples and up to 1.9 mg/kg in untreated samples. Residue levels in straw were up to 0.67 mg/kg in the treated samples and up to 0.34 mg/kg in untreated samples.

For TAA, residue levels in grain were up to 0.37 mg/kg in the treated samples and up to 0.36 mg/kg in untreated samples. Residue levels in straw were up to 0.2 mg/kg in the treated samples and up to 0.11 mg/kg in untreated samples.

For TLA, residue levels in grain were up to 1.2 mg/kg in the treated samples and up to 1.1 mg/kg in untreated samples. Residue levels in straw were up to 11 mg/kg in the treated samples and up to 10 mg/kg in untreated samples.

The TLA residue data obtained in one trial (L130176) appears to be treatment-unrelated. While residue data for BAS 750 F is comparable to the data of other residue trials on barley, the residue levels of TLA by far exceed the levels seen in the other trials. Both in inflorescence (unripe ears and mature grain with 1.0-13 mg/kg) as well as plant part (plant DALA0, rest-of-plant, straw with 8-11 mg/kg) TLA residues are extremely high. Compared of the overall data package, higher levels are also seen in this trial for other triazole derivative metabolites. It is assumed that these atypical residue levels are result of a non-documented “triazole-releasing treatment”, such as a fertilizer treatment, specifically at this trial site since the control samples (untreated plot) contained residues in the same order of magnitude. It is not considered that this has an impact upon the residue behaviour of BAS 750 F and hence data for the analyte BAS 750 F, obtained in this trial is valid.

Overall, it is considered that TDM residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar. For further discussion see section 7.3.3.

Table 7.3.2-10: Summary of TDM in untreated barley (plot 1) as well as after treatment with BAS 750 00 F (plot2)

Region	Matrix	DALA [1)]	BBCH	Plot	1,2,4-triazole *[mg/kg]*	TA *[mg/kg]*	TAA *[mg/kg]*	TLA *[mg/kg]*
N-EU & S-EU	plant [2)]	0	69	1	<0.01	< 0.01 - 0.41	< 0.01 - 0.058	0.20 - 7.3
				2	<0.01	< 0.01 - 0.40	< 0.01 - 0.041	0.26 - 9.6
	ears	27-29	73-87	1	<0.01	0.016 – 1.1	<0.01 – 0.34	0.060 – 1.3
				2	<0.01	0.095 – 1.4	< 0.01 – 0.35	0.070 – 1.3
	rest-of-plant [3]			1	<0.01	<0.01 – 0.11	<0.01 – 0.078	0.14 – 6.8
				2	<0.01	< 0.01 – 0.13	< 0.01 – 0.086	0.18 – 7.9
	grain			1	<0.01	0.096 – 0.11	0.021 – 0.034	0.037 – 0.11
				2	<0.01	0.13 – 0.22	0.022 – 0.029	0.087 – 0.16
	straw			1	<0.01	<0.01	<0.01 – 0.18	1.3 – 3.4
				2	<0.01	< 0.01	< 0.01	1.5 – 2.2
	ears	34-36	83-87/89	1	<0.01	< 0.01 – 0.13	< 0.01	0.012 – 0.082
				2	<0.01	0.074 – 0.24	< 0.01 – 0.028	0.081 – 0.20
	rest-of-plant [3]			1	<0.01	< 0.01 – 0.011	< 0.01	0.12 – 0.49
				2	<0.01	< 0.01 – 0.015	< 0.01	0.15 – 1.1
	grain			1	<0.01	0.045 – 1.9	0.015 – 0.28	0.11 – 0.88
				2	<0.01	0.061 – 2.0	0.025 – 0.29	0.076 – 0.96
	straw			1	<0.01	< 0.01 – 0.15	< 0.01 – 0.11	1.2 - 10
				2	<0.01	< 0.01 – 0.16	< 0.01 – 0.11	1.2 - 11
	ears	41-43	87-89/89	1	<0.01	0.023 – 0.43	< 0.01	0.043 – 0.096
				2	<0.01	0.090 – 0.79	< 0.01 – 0.023	0.099 – 0.25
	rest-of-plant [3)]			1	<0.01	<0.01 – 0.028	< 0.01	0.14 – 0.62
				2	<0.01	< 0.01 – 0.064	< 0.01	0.20 – 1.0
	grain			1	<0.01	0.050 – 1.5	0.017 – 0.36	0.10 – 1.1
				2	<0.01	0.054 – 2.6	0.019 – 0.37	0.17 – 1.2
	straw			1	<0.01	< 0.01 - 0.34	< 0.01 – 0.11	0.67 - 10
				2	<0.01	< 0.01 – 0.67	< 0.01 – 0.20	0.83 – 10
	grain	48	89	1	<0.01	0.014 – 0.49	< 0.01 – 0.011	0.044 – 0.052
				2	<0.01	0.052 – 1.1	< 0.01 – 0.021	0.074 – 0.12
	straw			1	<0.01	< 0.01 – 0.030	< 0.01	0.18 – 0.48
				2	<0.01	< 0.01 – 0.11	< 0.01	0.53 – 4.4

[1)] DALA = days after last application; [2)] no roots; [3)] without roots

Table 7.3.2-7: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433782 2014/1010808 67294 Mauchenheim Germany (N) L130174	GC 0640 Barley Propino	1. 30.03.2013 2. 21.06.-28.06.2013 3. 02.08.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 27.06 13	69	plant [1]	0	7.4	< 0.01	0.028	< 0.01	0.68
										grain	28	0.14	< 0.01	0.22	0.022	0.087
										straw	28	9.3	< 0.01	< 0.01	< 0.01	1.5
										grain	35	0.15	< 0.01	0.061	0.025	0.60
										straw	35	15	< 0.01	< 0.01	0.025	1.2
										grain	41	0.13	< 0.01	0.054	0.019	0.46
										straw	41	11	< 0.01	< 0.01	0.025	0.93
433782 2014/1010808 16833 Lentzke Germany (N) L130175	GC 0640 Barley Sandra	1. 12.09.2012 2. 20.05.-24.05.2013 3. 16.07.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 24.05 13	69	plant [1]	0	4.9	< 0.01	0.055	< 0.01	0.34
										ears	27	0.082	< 0.01	0.21	< 0.01	0.070
										rest of pl. [2]	27	0.84	< 0.01	0.022	< 0.01	0.41
										ears	34	0.058	< 0.01	0.24	< 0.01	0.081
										rest of pl. [2]	34	0.52	< 0.01	0.015	< 0.01	0.31
										ears	42	0.065	< 0.01	0.79	0.011	0.13
										rest of pl. [2]	42	0.61	< 0.01	0.064	< 0.01	0.56
										grain	53	0.014	< 0.01	1.1	0.021	0.086
										straw	53	1.0	< 0.01	0.097	< 0.01	0.80
433782 2014/1010808 6595 ME Ottersum The Netherlands (N) L130176	GC 0640 Barley Sequel	1. 02.10.2012 2. 29.05.-12.06.2013 3. 22.07.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 11.06 13	69	plant [1]	0	2.3	< 0.01	0.40	0.041	9.6
										ears	28	1.2	< 0.01	1.4	0.35	1.3
										rest of pl. [2]	28	2.4	< 0.01	0.13	0.086	7.9
										grain	34	0.13	< 0.01	2.0	0.29	0.96
										straw	34	5.3	< 0.01	0.16	0.11	11
										grain	41	0.19	< 0.01	1.4	0.37	1.2
										straw	41	5.6	< 0.01	0.67	0.20	10
433782 2014/1010808 CO112NF Mannigtree, United Kingdom (N) L130177	GC 0640 Barley Cassata	1. 16.10.2012 2. 11.06.-18.06.2013 3. 26.07.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 14.06 13	69	plant [1]	0	3.2	< 0.01	0.029	< 0.01	0.50
										ears	28	0.71	< 0.01	0.19	0.027	0.32
										rest of pl. [2]	28	1.9	< 0.01	0.017	< 0.01	1.1
										grain	35	0.056	< 0.01	0.33	0.030	0.076
										straw	35	2.9	< 0.01	0.030	0.038	1.7
										grain	41	0.071	< 0.01	2.6	0.34	1.2
										straw	41	3.9	< 0.01	0.071	0.027	1.1

Table 7.3.2-7: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433782 2014/1010808 32130 Cazaux-Saves France (S) L130178	GC 0640 Barley Bamboo	1. 20.10.2012 2. 08.05.-17.05.2013 3. 09.07.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 17.05 13	69	plant [1]	0	3.4	< 0.01	0.023	< 0.01	0.75
										ears	28	0.36	< 0.01	0.13	< 0.01	0.23
										rest of pl. [2]	28	0.49	< 0.01	< 0.01	< 0.01	0.27
										ears	34	0.13	< 0.01	0.17	< 0.01	0.11
										rest of pl. [2]	34	0.20	< 0.01	< 0.01	< 0.01	0.15
										ears	41	0.16	< 0.01	0.17	< 0.01	0.099
										rest of pl. [2]	41	0.18	< 0.01	< 0.01	< 0.01	0.20
										grain	55	0.070	< 0.01	0.81	< 0.01	0.11
										straw	55	0.39	< 0.01	0.078	< 0.01	0.61
433782 2014/1010808 58300 Galatades Greece (S) L130179	GC 0640 Barley Moutso	1. 09.12.2012 2. 08.04.-18.04.2013 3. 30.05.-05.06.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 12.04 13	69	plant [1]	0	7.2	< 0.01	0.011	< 0.01	0.44
										ears	28	0.86	< 0.01	0.15	0.033	0.35
										rest of pl. [2]	28	12	< 0.01	< 0.01	< 0.01	1.2
										ears	35	1.0	< 0.01	0.074	0.028	0.20
										rest of pl. [2]	35	12	< 0.01	< 0.01	< 0.01	1.1
										ears	42	1.7	< 0.01	0.090	0.023	0.25
										rest of pl. [2]	42	13	< 0.01	< 0.01	< 0.01	1.0
										grain	54	0.16	< 0.01	0.052	< 0.01	0.074
										straw	54	6.4	< 0.01	< 0.01	< 0.01	0.53
433782 2014/1010808 12050 Castagnito d´Alba Italy (S) L130180	GC 0640 Barley Corneta Delicious	1. 16.10.2012 2. 01.05.-10.05.2013 3. 26.06.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 09.05 13	69	plant [1]	0	3.9	< 0.01	< 0.01	< 0.01	0.26
										ears	27	0.40	< 0.01	0.095	< 0.01	0.11
										rest of pl. [2]	27	1.8	< 0.01	< 0.01	< 0.01	0.18
										ears	34	0.31	< 0.01	0.096	< 0.01	0.12
										rest of pl. [2]	34	13	< 0.01	< 0.01	< 0.01	0.28
										ears	42	0.35	< 0.01	0.098	< 0.01	0.11
										rest of pl. [2]	42	1.9	< 0.01	< 0.01	< 0.01	0.26
										grain	48	0.10	< 0.01	0.12	< 0.01	0.12
										straw	48	4.2	< 0.01	0.11	< 0.01	4.4

Table 7.3.2-7: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433782 2014/1010808 41720 Los Palacios Spain (S) L130181	GC 0640 Barley Prestige	1. 15.12.2012 2. 17.04.-22.04.2013 3. 03.06.2013	Foliar application	BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 22.04 13	69	plant [1]	0	5.7	< 0.01	0.055	< 0.01	1.2
										grain	29	0.42	< 0.01	0.13	0.029	0.16
										straw	29	11	< 0.01	< 0.01	< 0.01	2.2
										grain	36	0.29	< 0.01	0.13	0.028	0.16
										straw	36	11	< 0.01	< 0.01	< 0.01	2.6
										grain	42	<u>0.41</u>	< 0.01	0.10	0.020	0.17
										straw	42	<u>11</u>	< 0.01	0.012	< 0.01	0.83

1) whole plant (no root) 2) rest-of-plant (no root), 3) time between applications supports the proposed minimum of 14 days, 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid. A number in parenthesis indicates re-analysis to confirm analytical results. The underlined values (e.g. <u>0.018)</u> are used for calculation of the STMR, HR and MRL for grain, and the STMR and HR for straw

Table 7.3.2-8: Level of BAS 750 F and TDM in barley (untreated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433782 2014/1010808 67294 Mauchenheim Germany (N) L130174	GC 0640 Barley Propino	1. 30.03.2013 2. 21.06.-28.06.2013 3. 02.08.2013	-	-	-	-	-	-	-	plant [1] grain straw grain straw grain straw	0 28 28 35 35 41 41	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.022 0.11 < 0.01 0.045 < 0.01 0.050 < 0.01	< 0.01 0.021 < 0.01 0.015 < 0.01 0.019 0.017	0.72 0.037 1.3 0.58 1.2 0.54 0.67
433782 2014/1010808 16833 Lentzke Germany (N) L130175	GC 0640 Barley Sandra	1. 12.09.2012 2. 20.05.-24.05.2013 3. 16.07.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 27 27 34 34 42 42 53 53	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.023 0.16 0.020 0.13 0.011 0.43 0.028 0.49 0.028	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 0.011 < 0.01	0.31 0.12 0.38 0.053 0.27 0.096 0.23 0.052 0.48
433782 2014/1010808 6595 ME Ottersum The Netherlands (N) L130176	GC 0640 Barley Sequel	1. 02.10.2012 2. 29.05.-12.06.2013 3. 22.07.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] grain straw grain straw	0 28 28 34 34 41 41	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.41 1.07 0.11 1.9 0.15 1.5 0.34	0.058 0.34 0.078 0.28 0.11 0.36 0.11	7.3 1.3 6.8 0.88 10 1.1 10
433782 2014/1010808 CO112NF Mannigtree, United Kingdom (N) L130177	GC 0640 Barley Cassata	1. 16.10.2012 2. 11.06.-18.06.2013 3. 26.07.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] grain straw grain straw	0 28 28 35 35 41 41	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.013 0.19 0.015 0.22 0.016 0.28 0.061	< 0.01 0.019 < 0.01 0.24 0.014 0.029 0.022	0.20 0.38 0.94 0.11 1.2 0.10 1.1

Table 7.3.2-8: Level of BAS 750 F and TDM in barley (untreated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433782 2014/1010808 32130 Cazaux-Saves France (S) L130178	GC 0640 Barley Bamboo	1. 20.10.2012 2. 08.05.-17.05.2013 3. 09.07.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 28 28 34 34 41 41 55 55	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.012 0.042 < 0.01 0.056 < 0.01 0.071 < 0.01 0.37 0.030	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.49 0.10 0.16 0.071 0.12 0.074 0.14 0.047 0.18
433782 2014/1010808 58300 Galatades Greece (S) L130179	GC 0640 Barley Moutso	1. 09.12.2012 2. 08.04.-18.04.2013 3. 30.05.-05.06.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 28 28 35 35 42 42 54 54	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 0.028 < 0.01 0.027 < 0.01 0.026 < 0.01 0.014 0.017	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.36 0.061 0.55 0.082 0.49 0.093 0.62 0.044 0.24
433782 2014/1010808 12050 Castagnito d´Alba Italy (S) L130180	GC 0640 Barley Corneta Delicious	1. 16.10.2012 2. 01.05.-10.05.2013 3. 26.06.2013	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] ears rest of pl. [2] ears rest of pl. [2] grain straw	0 27 27 34 34 42 42 48 48	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	< 0.01 0.016 < 0.01 < 0.01 < 0.01 0.023 < 0.01 0.034 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.36 0.060 0.14 0.012 0.20 0.043 0.22 0.044 0.25

Table 7.3.2-8: Level of BAS 750 F and TDM in barley (untreated samples)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433782	GC 0640	1. 15.12.2012	-	-	-	-	-	-	-	plant [1]	0	< 0.01	< 0.01	0.023	< 0.01	0.95
2014/1010808	Barley	2. 17.04.-22.04.2013								grain	29	< 0.01	< 0.01	0.096	0.034	0.11
41720 Los Palacios	Prestige	3. 03.06.2013								straw	29	< 0.01	< 0.01	< 0.01	0.18	3.4
Spain (S)										grain	36	< 0.01	< 0.01	0.075	0.045	0.17
L130181										straw	36	< 0.01	< 0.01	< 0.01	< 0.01	3.1
										grain	42	< 0.01	< 0.01	0.060	0.017	0.11
										straw	42	< 0.01	< 0.01	< 0.01	< 0.01	0.87

1) whole plant (no root) 2) rest-of-plant (no root), 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid.

Conclusion

Residue data obtained in 8 independent field trials in barley (conducted in both N-EU, S-EU with the formulated product BAS 750 00 F according to the critical GAP) showed that the residues of BAS 750 F are 0.014-0.41 mg/kg in grain and 0.39-15 mg/kg in straw.

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. As such, it is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar (see section B.7.3.3).

Report:	CA 6.3.2/2 Ale E., 2015 b Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to barley in Northern and Southern Europe in 2014 2015/1099703
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, OECD 509 Crop Field Trial (2009), EEC 7525/VI/95 rev. 9 (March 2011), EEC 7029/VI/95 rev. 5 (July 22 1997)
GLP:	yes

Materials and methods

During the growing season of 2014, ten field trials on barley using either the formulated product 'BAS 750 00 F' or 'BAS 750 00F', both EC formulations containing 100 g/L BAS 750F, were conducted in Germany, the Netherlands, the UK Northern France, Southern France, Greece, Italy and Spain. Each formulation was applied to a separate plot, each formulation was applied twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha.

Samples of whole plant (root removed) were taken directly before and after the second application. Samples were also taken at three time points after the last application; after 27-29 days, 34-36 days and 41-43 days. Where the crop was unripe, samples consisted of ear and rest-of-plant (roots removed) while for the ripe crop grain and straw were taken. Where BBCH growth stage 89 was not reached after 42 days, an additional sampling time point was added. Control (untreated) specimens were taken at every sampling event. A summary of the application and sampling details is given Table 7.3.1-11.

Table 7.3.2-11 Application and sampling details

Region	No. of trials	no. of appl.	F, G, I[2]	Method	Test item	Active substance	Application		Timing target	
							rate (kg a.s. /ha)	water vol. (L/ha)	application (BBCH)	sampling (DALA)[1]
EU North & South	10	2	F	-	BAS 750 01 F (EC) BAS 750 00 F (EC)	BAS 750 F	0.15	200	1st appl.: BBCH 49 2nd appl.: BBCH 69	0 28 ± 1 35 ± 1 42 ± 1 48

1) days after last application, 2) field, glasshouse or indoor

Weather data were reported for the trials and no exceptional events were noted. Normal agricultural practice was followed during the field stage. Samples weighing at least 1 kg were collected for ears, rest-of-plant (roots removed) and grain, and samples weighing at least 0.5 kg were collected for straw. Samples were stored were stored at ≤-18 °C within 24 hours of harvest, and maintained at this temperature until extraction (with the exception of trials L140161, L140166 and L140167 where the storage temperature rose to -11°C for up to 24 hours).

The maximum storage interval from harvest until analysis was 374 days for BAS 750 F. The maximum storage interval from harvest until analysis was 376 days for triazole and related analytes.

The level of residues in each processed fraction was determined for BAS 750 F (BASF method L0076/09, LOQ of 0.01 mg/kg) and for the triazole derivative metabolites (TDM) 1,2,4-T, TA, TAA, TLA (BASF method L0170/02, LOQ of 0.01 mg/kg for each of the four analytes). Full details of

sample preparation and validation data for these methods is given in section CA B.5.1.2.5. Details of the procedural recoveries are given in Table 7.3.2-12 and 7.3.1-13.

Table 7.3.2-12 Summary of recoveries for BAS 750 F

Matrix	Fortification level (mg/kg)	BAS 750 F			
		n	mean (%)	SD (+/-)	RSD (%)
Method L0076/09					
whole plant (no root)	0.01, 1.0, 10, 20, 100	10	96.2	3.5	3.6
ear	0.01, 0,1, 1,0, 5.0, 10	12	92.0	3.9	4.2
rest-of-plant (no root)	0.01, 1.0, 20	14	98.6	7.3	7.4
grain	0.01, 0.1, 1.0	11	96.1	10	11
straw	0.01, 0,1, 1.0, 20	14	93.3	7.7	8.3
Overall		**61**	**95.2**	**7.3**	**7.7**

Table 7.3.2-13 Summary of recoveries for 1,2,4-T, TA, TAA and TLA

Matrix	Fortification level (mg/kg)	Summary recoveries			
		n	mean (%)	SD (+/-)	RSD (%)
Method L0170/02		**1,2,4-triazole**			
whole plant (no root)	0.01, 1.0	10	97.3	11	11
ear	0.01, 1.0	12	89.3	12	13
rest-of-plant (no root)	0.01, 1.0	16	93.9	8.5	9.0
grain	0.01, 1.0	8	87.0	9.2	11
straw	0.01, 1.0	8	80.4	9.4	12
Overall		**54**	**90.5**	**11**	**12**
Method L0170/02		**TA**			
whole plant (no root)	0.01, 1.0	8	93.0	16	17
ear	0.01, 1.0	12	86.2	19	22
rest-of-plant (no root)	0.01, 1.0	8	89.8	18	21
grain	0.01, 1.0	13	90.9	22	24
straw	0.01, 1.0	11	87.6	18	21
Overall		**52**	**89.2**	**19**	**21**
Method L0170/02		**TAA**			
whole plant (no root)	0.01, 1.0	10	93.6	11	11
ear	0.01, 1.0	12	98.5	13	14
rest-of-plant (no root)	0.01, 1.0	16	103	17	16
grain	0.01, 1.0	8	86.6	1	15
straw	0.01, 1.0	7	86.9	19	21
Overall		**53**	**95.7**	**16**	**17**
Method L0170/02		**TLA**			
whole plant (no root)	0.01, 1.0	8	103	10	10
ear	0.01, 1.0	12	89.3	17	19
rest-of-plant (no root)	0.01, 1.0	10	91.4	13	15
grain	0.01, 1.0	13	78.2	8.8	11
straw	0.01, 1.0	12	89.0	19	21
Overall		**55**	**89.0**	**16**	**18**

Results and Discussion

Ten field trials on barley using the formulated products ‘BAS 750 00 F’ and ‘BAS 750 01 F’, EC formulations containing 100 g/L BAS 750F were conducted in NEU and SEU during the growing season of 2014. Each formulation was applied to a separate plot twice as a foliar spray (at BBCH 49 and 69) at a rate of 0.15 kg as/ha in a spray volume of 200 L/ha. This application rate and timing is in accordance with the proposed GAP for barley.

Samples were taken at three time points after the last application; after 27-29 days, 34-36 days and 41-43 days Where BBCH growth stage 89 was not reached after 42 days, an additional sample was taken at this stage. Residues trials data are presented in Table 7.3.1-14 (treated samples) and Table 7.3.1-15 (untreated samples).

Samples were stored frozen for up to 374 days prior to analysis for BAS 750 F and for up to 354 days for triazole and related analytes. Storage stability data is available to support storage of BAS 750 F for up to 730 days in cereals (section B.7.1.1), which is sufficient to support the storage times in this study. Storage stability data is available to support storage of 1,2,4-triazole and TA for 54 months, TTA for 26 months (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015) and TLA for 49 months (section B.7.1.2). This is sufficient to support the storage times in this study.

The methods of analysis for determination of BAS 750 F and TDMs are considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4. Acceptable procedural recovery data, using an appropriate number of samples were presented.

BAS 750 F
In untreated samples, no residues of BAS 750 F exceeding the LOQ (0.01 mg/kg) were detected.

For grain from samples treated with 'BAS 750 01 F', BAS 750 F residues were between 0.018-0.28 mg/kg. For grain from samples treated with 'BAS 750 00F', BAS 750 F residues were between 0.029-0.29 mg/kg.

For straw from samples treated with 'BAS 750 01F', BAS 750 F residues ranged from 1.7-18 mg/kg. For straw from samples treated with 'BAS 750 00F', BAS 750 F residues ranged from 0.99-16 mg/kg.

For plants directly after application of 'BAS 750 01 F' (DALA0), BAS 750 F residues were in the range of 2.6-9.2 mg/kg. For plants directly after application of 'BAS 750 00 F' (DALA0) residues were in the range of 2.5-8.1 mg/kg.

Similar residues in grain and straw were determined in trials with each formulation. A comparison of the results is presented in Table 7.3.2-16. As the trials only differed in formulation composition (the formulation type was EC in both cases) the highest residue for each trial has been used for HR and STMR determinations.

Table 7.3.2-16: Summary of BAS 750 F residues in barley treated with the formulated products BAS 750 01 F (plot 2), or BAS 750 00 F (plot 3)

Region	Matrix	DALA [1)]	BBCH	n	BAS 750 F *[mg/kg]*	
					BAS 750 01 F	BAS 750 00 F
N-EU & S-EU	plant [2)]	0	69	10	2.0 – 9.2	2.5 – 8.1
	ears	27 - 29	73 - 87	10	0.094 – 5.7	0.14 – 3.9
	rest-of-plant [3)]			10	1.0 - 21	0.72 - 20
	ears	34 - 36	83 - 87	5	0.26 – 6.0	0.19 – 5.3
	rest-of-plant [3]			5	1.5 - 21	0.86 - 16
	grain	34 - 36	87 – 89	5	0.085 – 0.28	0.057 – 0.22
	straw			5	2.2 – 6.8	1.6 – 5.9
	ears	41	87 - 89	1	0.40	0.33
	rest-of-plant [3]			1	1.6	1.4
	grain	41 - 43	89	9	0.018 – 0.25	0.030 – 0.29
	straw			9	1.7 - 18	0.99 - 16
	grain	48	89	1	0.033	0.029
	straw			1	2.2	1.9

[1)] DALA = days after last application; [2)] no roots; [3)] without roots

TDM

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. A comparison of the results is presented in Table 7.3.2- 17.

For TA, residue levels in grain were up to 0.97 mg/kg in the treated samples and up to 1.1 mg/kg in untreated samples. Residue levels in straw were up to 0.11 mg/kg in the treated samples and up to 0.11 mg/kg in untreated samples.

For TAA, residue levels in grain were up to 0.55 mg/kg in the treated samples and up to 0.44 mg/kg in untreated samples. Residue levels in straw were up to 0.33 mg/kg in the treated samples and up to 0.19 mg/kg in untreated samples.

For TLA, residue levels in grain were up to 0.22 mg/kg in the treated samples and up to 0.10 mg/kg in untreated samples. Residue levels in straw were up to 0.2 mg/kg in the treated samples and up to 0.58 mg/kg in untreated samples.

As such, it is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar. For further discussion see section 7.3.3.

Table 7.3.2-17: Summary of TDM residues in untreated barley (plot 1) as well as after treatment with BAS 750 01 F (plot 2), or BAS 750 00 F (plot 3)

Region	Matrix	DALA [1)]	BBCH	Plot	1,2,4-triazole *[mg/kg]*	TA *[mg/kg]*	TAA *[mg/kg]*	TLA *[mg/kg]*
N-EU & S-EU	plant [2)]	0	69	1	<0.01	0.029 - 0.11	0.012 - 0.18	<0.01 - 0.25
				2	<0.01	0.020 - 0.098	<0.01 - 0.21	0.012 - 0.27
				3	<0.01	0.013 - 0.14	0.084 - 0.41	<0.01 - 0.36
	ears	27-29	73-87	1	<0.01	0.019 - 0.64	<0.01 - 0.095	<0.01 - 1.0
				2	<0.01	0.032 - 0.48	<0.01 - 0.028	0.016 - 0.84
				3	<0.01	0.028 - 0.38	<0.01 - 0.060	0.050 - 0.77
	rest-of-plant [3]			1	<0.01	<0.01 - 0.090	<0.01 - 0.15	<0.01 - 0.091
				2	<0.01	0.017 - 0.11	<0.01 - 0.21	<0.01 - 0.039
				3	<0.01	0.016 - 0.11	<0.01 - 0.68	<0.01 - 0.19
	ears	34-36	83-87/89	1	<0.01	0.061 - 0.16	<0.01 - 0.075	<0.01 - 0.44
				2	<0.01	0.080 - 0.23	<0.01 - 0.023	0.058 - 0.20
				3	<0.01	0.080 - 0.27	<0.01 - 0.043	0.015 - 0.61
	rest-of-plant [3]			1	<0.01	0.036 - 0.062	0.011 - 0.087	<0.01 - 0.026
				2	<0.01	0.029 - 0.10	0.027 - 0.11	<0.01 - 0.023
				3	<0.01	0.010 - 0.092	<0.01 - 1.1	<0.01 - 0.10
	grain			1	<0.01	0.019 - 0.46	0.023 - 0.047	0.015 - 0.87
				2	<0.01	0.041 - 0.55	<0.01 - 0.015	0.035 - 0.97
				3	<0.01	0.032 - 0.40	0.055 - 0.082	0.037 - 0.74
	straw			1	<0.01	0.027 - 0.28	0.044 - 0.30	<0.01 - 0.30
				2	<0.01	0.022 - 0.28	<0.01 - 0.20	0.016 - 0.38
				3	<0.01	0.033 - 0.21	0.15 - 0.59	<0.01 - 0.090
	ears	41-43	87-89/89	1	<0.01	0.053	0.014	0.026
				2	<0.01	0.085	<0.01	0.11
				3	<0.01	0.087	0.016	0.06
	rest-of-plant [3)]			1	<0.01	0.025	0.019	<0.01
				2	<0.01	0.032	0.019	0.03
				3	<0.01	0.02	0.21	<0.01
	grain			1	<0.01	0.023 - 0.59	<0.01 - 0.080	<0.01 - 1.1
				2	<0.01	0.017 - 0.50	<0.01 - 0.22	0.039 - 0.82
				3	<0.01	0.025 - 0.44	<0.01 - 0.10	0.059 - 1.1
	straw			1	<0.01	0.022 - 0.17	0.038 - 0.62	<0.01 - 0.062
				2	<0.01	0.019 - 0.33	<0.01 - 0.18	0.014 - 0.12
				3	<0.01	0.026 - 0.19	0.15 - 0.58	<0.01 - 0.11
	grain	48	89	1	<0.01	0.088	<0.01	0.035
				2	<0.01	0.091	<0.01	0.078
				3	<0.01	0.079	<0.01	0.05
	straw			1	<0.01	0.045	0.059	0.011
				2	<0.01	0.035	0.031	0.013
				3	<0.01	0.024	0.4	<0.01

[1)] DALA = days after last application; [2)] no roots; [3)] without roots

Table 7.3.2-14: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 67294 Mauchenhein, Germany (N) L140158	GC 0640 Barley Propino	1. 06.03.2014 2. 30.05.-10.06.2014 3. 23.07.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 10.06.14	69	plant [1]	0	3.6	<0.01	0.013	0.045	0.023
										ears	29	0.89	<0.01	0.050	0.080	0.016
										rest of pl. [2]	29	3.0	<0.01	<0.01	0.019	<0.01
										grain	35	0.087	<0.01	0.035	0.069	<0.01
										straw	35	6.8	<0.01	0.14	0.031	<0.01
										grain	43	0.061	<0.01	0.11	0.096	<0.01
										straw	43	5.3	<0.01	0.12	0.035	<0.01
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 10.06.14	69	plant [1]	0	3.6	<0.01	0.034	0.048	0.084
										ears	29	0.71	<0.01	0.021	0.060	<0.01
										rest of pl. [2]	29	3.6	<0.01	<0.01	0.025	0.018
										grain	35	0.057	<0.01	0.059	0.069	0.059
										straw	35	5.9	<0.01	<0.01	0.033	0.15
										grain	43	0.048	<0.01	0.078	0.053	0.077
										straw	43	5.6	<0.01	<0.01	0.030	0.15
433784 2015/1099703 47589 Uedem, Germany (N) L140159	GC 0640 Barley Meridian	1. 01.10.2013 2. 08.-22.05.2014 3. 02.07.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 22.05.14	69	plant [1]	0	2.6	<0.01	0.054	0.058	0.069
										ears	27	0.42	<0.01	0.21	0.11	0.011
										rest of pl. [2]	27	1.2	<0.01	<0.01	0.060	0.095
										grain	36	0.085	<0.01	0.19	0.15	<0.01
										straw	36	2.2	<0.01	0.38	0.12	0.10
										grain	41	0.071	<0.01	0.058	0.11	<0.01
										straw	41	3.1	<0.01	0.093	0.12	0.049
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 22.05.14	69	plant [1]	0	2.7	<0.01	0.13	0.059	0.37
										ears	27	0.33	<0.01	0.29	0.12	0.019
										rest of pl. [2]	27	1.3	<0.01	<0.01	0.058	0.081
										grain	36	0.10	<0.01	0.16	0.098	0.060
										straw	36	2.4	<0.01	<0.01	0.11	0.37
										grain	41	0.077	<0.01	0.35	0.099	0.047
										straw	41	2.5	<0.01	<0.01	0.084	0.33

Table 7.3.2-14: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 6595 ME Ottersum, The Netherlands (N) L140160	GC 0640 Barley Sequel	1. 27.09.2013 2. 08.-21.05.2014 3.01.07.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 21.05.14	69	plant [1]	0	2.7	<0.01	0.27	0.098	0.21
										ears	28	0.53	<0.01	0.86 (0.82)	0.47 (0.49)	0.028
										rest of pl. [2]	28	2.1	<0.01	0.039	0.10	0.21
										grain	35	0.11	<0.01	0.97	0.55	0.015
										straw	35	2.2	<0.01	0.11	0.28	0.20
										grain	41	0.10	<0.01	0.82	0.50	0.22
										straw	41	3.6	<0.01	0.078	0.33	0.18
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 21.05.14	69	plant [1]	0	2.9	<0.01	0.36	0.14	0.29
										ears	28	0.53	<0.01	0.77	0.38	0.060
										rest of pl. [2]	28	1.6	<0.01	<0.01	0.11	0.16
										grain	35	0.15	<0.01	0.74	0.40	0.082
										straw	35	1.6	<0.01	0.090	0.21	0.53
										grain	41	0.10	<0.01	1.1	0.44	0.064
										straw	41	4.3	<0.01	<0.01	0.19	0.38
433784 2015/1099703 CM22 6JD Ugley Green, United Kingdom (N) L140161	GC 0640 Barley Flagon	1. 25.10.2013 2. 10.-28.06.2014 3. 05.08.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 24.06.14	69	plant [1]	0	6.1	<0.01	0.033	0.027	<0.01
										ears	29	1.3	<0.01	0.016	0.032	<0.01
										rest of pl. [2]	29	2.7	<0.01	0.012	0.021	<0.01
										grain	35	0.28	<0.01	0.040	0.041	<0.01
										straw	35	4.3	<0.01	0.016	0.022	<0.01
										grain	42	0.25	<0.01	0.039	0.017	<0.01
										straw	42	3.7	<0.01	0.018	0.019	<0.01
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 24.06.14	69	plant [1]	0	6.0	<0.01	0.061	0.027	0.087
										ears	29	0.93	<0.01	0.055	0.028	<0.01
										rest of pl. [2]	29	3.0	<0.01	0.022	0.027	0.014
										grain	35	0.22	<0.01	0.037	0.032	0.067
										straw	35	3.1	<0.01	0.023	0.037	0.51
										grain	42	0.26	<0.01	0.059	0.025	0.10
										straw	42	2.7	<0.01	<0.01	0.026	0.58

Table 7.3.2-14: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 72500 Saint Pierre de chevillé, France (N) L140162	GC 0640 Barley Sandra	1. 07.10.2013 2. 02.-15.05.2014 3. 25.06.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 15.05.14	69	plant [1]	0	2.0	<0.01	0.048	0.050	0.050
										ears	27	0.20	<0.01	0.22	0.17	0.016
										rest of pl. [2]	27	1.0	<0.01	<0.01	0.041	0.060
										ears	34	0.26	<0.01	0.14	0.23	0.011
										rest of pl. [2]	34	1.5	<0.01	<0.01	0.039	0.064
										grain	41	0.060	<0.01	0.21	0.21	<0.01
										straw	41	1.7	<0.01	0.021	0.099	0.080
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 15.05.14	69	plant [1]	0	2.5	<0.01	0.30	0.055	0.21
										ears	27	0.17	<0.01	0.58	0.23	0.045
										rest of pl. 2	27	0.72	<0.01	0.19	0.034	0.11
										ears	34	0.19	<0.01	0.61	0.27	0.043
										rest of pl. 2	34	0.86	<0.01	0.10	0.036	0.15
										grain	41	0.050	<0.01	0.83	0.30	0.014
										straw	41	0.99	<0.01	0.11	0.11	0.46
433784 2015/1099703 32380 Tournecoupe, France (S) L140163	GC 0640 Barley Ketos	1. 27.10.2013 2. 05.-09.05.2014 3. 20.06.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 09.05.14	69	plant [1]	0	4.5	<0.01	0.21	0.042	0.075
										ears	28	0.43	<0.01	0.24	0.088	0.015
										rest of pl. [2]	28	2.0	<0.01	0.024	0.033	0.083
										ears	35	0.51	<0.01	0.20	0.18	0.023
										rest of pl. [2]	35	2.8	<0.01	0.023	0.053	0.10
										grain	42	0.088	<0.01	0.54	0.20	0.011
										straw	42	2.4	<0.01	0.042	0.050	0.089
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 09.05.14	69	plant [1]	0	4.3	<0.01	0.11	0.059	0.12
										ears	28	0.46	<0.01	0.51	0.14	0.024
										rest of pl. 2	28	2.4	<0.01	<0.01	0.043	0.10
										ears	35	0.65	<0.01	0.49	0.21	0.040
										rest of pl. 2	35	3.3	<0.01	0.032	0.050	0.16
										grain	42	0.10	<0.01	0.69	0.14	<0.01
										straw	42	3.3	<0.01	0.095	0.049	0.37

Table 7.3.2-14: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 57011 Prochoma, Greece (S) L140164	GC 0640 Barley Chill	1. 03.11.2013 2. 10.-23.04.2014 3. 05.06.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 23.04.14	69	plant [1]	0	5.3	<0.01	0.012	0.020	0.028
										ears	28	0.094	<0.01	0.062	0.087	<0.01
										rest of pl. [2]	28	2.0	<0.01	<0.01	0.017	0.022
										ears	36	0.26	<0.01	0.058	0.12	<0.01
										rest of pl. [2]	36	2.8	<0.01	<0.01	0.034	0.035
										grain	43	0.018	<0.01	0.11	0.081	<0.01
										straw	43	2.1	<0.01	0.014	0.045	0.028
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 23.04.14	69	plant [1]	0	5.6	<0.01	<0.01	0.013	0.41
										ears	28	0.14	<0.01	0.050	0.074	0.010
										rest of pl. 2	28	2.8	<0.01	0.016	0.016	0.52
										ears	36	0.31	<0.01	0.088	0.11	0.033
										rest of pl. 2	36	2.8	<0.01	0.017	0.010	<0.01
										grain	43	0.030	<0.01	0.11	0.081	<0.01
										straw	43	1.9	<0.01	<0.01	0.034	0.57
433784 2015/1099703 20062 Cassano d´Adda, Italy (S) L140165	GC 0640 Barley Atomo	1. 15.10.2013 2. 01.-10.05.2014 3. 19.06.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 09.05.14	69	plant [1]	0	4.9	<0.01	0.043	0.057	0.049
										ears	27	0.36	<0.01	0.058	0.071	<0.01
										rest of pl. [2]	27	2.2	<0.01	<0.01	0.041	0.028
										grain	34	0.12	<0.01	0.12	0.12	<0.01
										straw	34	2.8	<0.01	0.025	0.10	0.053
										grain	41	0.14	<0.01	0.14	0.18	<0.01
										straw	41	4.6	<0.01	0.024	0.091	0.055
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 09.05.14	69	plant [1]	0	4.4	<0.01	0.048	0.043	0.13
										ears	27	0.52	<0.01	0.11	0.13	0.017
										rest of pl. [2]	27	2.3	<0.01	<0.01	0.044	<0.01
										grain	34	0.10	<0.01	0.10	0.11	0.055
										straw	34	2.5	<0.01	0.027	0.053	0.59
										grain	41	0.14	<0.01	0.085	0.13	0.093
										straw	41	3.1	<0.01	0.021	0.074	0.52

Table 7.3.2-14: Residues of BAS 750 F and TDM in barley (treated samples)

Report No. Location (EU-region) trial No	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treatments and last date[3]	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 16220 Quintanar del Rey, Spain (S) L140166	GC 0640 Barley Acapulco	1. 21.11.2013 2. 20.04.-05.05.2014 3. 16.06.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 05.05.14	69	plant [1]	0	9.2	<0.01	0.029	0.076	0.028
										ears	28	5.7	<0.01	0.092	0.12	<0.01
										rest of pl. [2]	28	21	<0.01	<0.01	0.11	0.083
										ears	35	6.0	<0.01	0.070	0.11	<0.01
										rest of pl. [2]	35	21	<0.01	<0.01	0.09 (0.11)	0.11
										grain	42	0.058	<0.01	0.088	0.11	<0.01
										straw	42	<u>18</u>	<0.01	0.047	0.11	0.076
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 05.05.14	69	plant [1]	0	8.1	<0.01	0.017	0.046	0.14
										ears	28	3.9	<0.01	0.064	0.10	<0.01
										rest of pl. 2	28	20	<0.01	0.041	0.062	0.68
										ears	35	5.3	<0.01	0.015	0.11	<0.01
										rest of pl. 2	35	16	<0.01	0.046	0.092	1.1
										grain	42	<u>0.29</u>	<0.01	0.090	0.083	<0.01
										straw	42	16	<0.01	<0.01	0.075	0.20
433784 2015/1099703 02110 La Gineta, Spain (S) L140167	GC 0640 Barley Hispanic	1. 17.12.2013 2. 25.04.-05.05.2014 3. 19.06.2014	Boom sprayer	BAS 750 01 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 02.05.14	69	plant [1]	0	4.6	<0.01	0.032	0.049	0.019
										ears	28	0.23	<0.01	0.068	0.055	<0.01
										rest of pl. [2]	28	1.3	<0.01	<0.01	0.025	0.028
										ears	35	0.34	<0.01	0.069	0.080	<0.01
										rest of pl. [2]	35	2.0	<0.01	<0.01	0.029	0.027
										ears	41	0.40	<0.01	0.11	0.085	<0.01
										rest of pl. [2]	41	1.6	<0.01	0.030	0.032	0.019
										grain	48	<u>0.033</u>	<0.01	0.078	0.091	<0.01
										straw	48	<u>2.2</u>	<0.01	0.013	0.035	0.031
				BAS 750 00 F (EC) 100 g/L BAS 750 F	0.075	200	0.15	2 02.05.14	69	plant 1	0	4.3	<0.01	0.038	0.036	0.11
										ears	28	0.29	<0.01	0.087	0.063	<0.01
										rest of pl. 2	28	1.5	<0.01	<0.01	0.020	<0.01
										ears	35	0.43	<0.01	0.12	0.080	<0.01
										rest of pl. 2	35	1.6	<0.01	<0.01	0.028	0.054
										ears	41	0.33	<0.01	0.060	0.087	0.016
										rest of pl. 2	41	1.4	<0.01	<0.01	0.020	0.21
										grain	48	0.029	<0.01	0.050	0.079	<0.01
										straw	48	1.9	<0.01	<0.01	0.024	0.40

1) whole plant (no root) 2) rest-of-plant (no root), 3) time between applications supports the proposed minimum of 14 days, 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid. The underlined values (e.g. <u>0.018)</u> are used for calculation of the STMR, HR and MRL for grain, and the STMR and HR for straw

Table 7.3.2-15: Level of BAS 750 F and TDM in untreated barley (plot 1)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 67294 Mauchenhein, Germany (N) L140158	GC 0640 Barley Propino	1. 06.03.2014 2. 30.05.-10.06.2014 3. 23.07.2014	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] grain straw grain straw	0 29 29 35 35 43 43	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.028 0.090 0.058 0.095 <0.01 0.11 0.017	0.063 0.074 0.034 0.11 0.062 0.066 0.033	0.042 <0.01 0.065 0.047 0.057 0.028 0.11
433784 2015/1099703 47589 Uedem, Germany (N) L140159	GC 0640 Barley Meridian	1. 01.10.2013 2. 08.-22.05.2014 3. 02.07.2014	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] grain straw grain straw	0 27 27 36 36 41 41	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.049 0.13 0.013 0.12 0.029 0.094 <0.01	0.079 0.10 0.058 0.15 0.14 0.11 0.080	0.082 0.071 0.067 0.023 0.19 <0.01 0.39
433784 2015/1099703 6595 ME Ottersum, The Netherlands (N) L140160	GC 0640 Barley Sequel	1. 27.09.2013 2. 08.-21.05.2014 3.01.07.2014	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] grain straw grain straw	0 28 28 35 35 41 41	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.25 1.3 (0.76) 0.019 0.87 0.41 (0.19) 1.1 0.061	0.11 0.60 (0.68) 0.071 0.46 0.28 0.59 0.17	0.18 0.095 0.15 0.045 0.30 0.042 0.19
433784 2015/1099703 CM22 6JD Ugley Green, United Kingdom (N) L140161	GC 0640 Barley Flagon	1. 25.10.2013 2. 10.-28.06.2014 3. 05.08.2014	-	-	-	-	-	-	-	plant [1] ears rest of pl. [2] grain straw grain straw	0 29 29 35 35 42 42	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01	0.010 <0.01 <0.01 0.015 0.046 0.034 <0.01	0.038 0.019 <0.01 0.019 0.027 0.023 0.024	0.012 <0.01 <0.01 0.027 0.044 0.033 0.038

Table 7.3.2-15: Level of BAS 750 F and TDM in untreated barley (plot 1)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 72500 Saint Pierre de chevillé, France (N) L140162	GC 0640 Barley Sandra	1. 07.10.2013 2. 02.-15.05.2014 3. 25.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.051	0.060	0.042
										ears	27	<0.01	<0.01	0.24	0.085	0.01
										rest of pl. [2]	27	<0.01	<0.01	<0.01	0.046	0.042
										ears	34	<0.01	<0.01	0.12	0.16	0.075
										rest of pl. [2]	34	<0.01	<0.01	<0.01	0.057	0.047
										grain	41	<0.01	<0.01	0.18	0.14	0.016
										straw	41	<0.01	<0.01	<0.01	0.098	0.11
433784 2015/1099703 32380 Tournecoupe, France (S) L140163	GC 0640 Barley Ketos	1. 27.10.2013 2. 05.-09.05.2014 3. 20.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.14	0.038	0.070
										ears	28	<0.01	<0.01	0.42	0.14	0.024
										rest of pl. [2]	28	<0.01	<0.01	0.091	0.028	0.069
										ears	35	<0.01	<0.01	0.44	0.15	0.034
										rest of pl. [2]	35	<0.01	<0.01	0.023	0.062	0.087
										grain	42	<0.01	<0.01	0.092	0.18	0.080
										straw	42	<0.01	<0.01	0.062	0.027	0.11
433784 2015/1099703 57011 Prochoma, Greece (S) L140164	GC 0640 Barley Chill	1. 03.11.2013 2. 10.-23.04.2014 3. 05.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	<0.01	0.029	0.012
										ears	28	<0.01	<0.01	<0.01	0.043	<0.01
										rest of pl. [2]	28	<0.01	<0.01	<0.01	0.028	<0.01
										ears	36	<0.01	<0.01	<0.01	0.061	<0.01
										rest of pl. [2]	36	<0.01	<0.01	<0.01	0.036	0.011
										grain	43	<0.01	<0.01	<0.01	0.031	0.074
										straw	43	<0.01	<0.01	<0.01	0.022	0.11
433784 2015/1099703 20062 Cassano d´Adda, Italy (S) L140165	GC 0640 Barley Atomo	1. 15.10.2013 2. 01.-10.05.2014 3. 19.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.029	0.056	0.034
										ears	27	<0.01	<0.01	0.039	0.083	0.065
										rest of pl. [2]	27	<0.01	<0.01	<0.01	0.029	0.026
										grain	34	<0.01	<0.01	0.15	0.091	0.028
										straw	34	<0.01	<0.01	0.033	0.071	0.071
										grain	41	<0.01	<0.01	0.12	0.11	0.018
										straw	41	<0.01	<0.01	0.017	0.057	0.11

Table 7.3.2-15: Level of BAS 750 F and TDM in untreated barley (plot 1)

Report No. Location (EU-region) trial No	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Method of treatment	Formulation	Application rate per treatment			No. of treat-ments and last date	Growth stage at last date	Portion analysed	PHI (days)	Residues (mg/kg)				
					kg as/hL	Water L/ha	kg as/ha					BAS 750 F	1,2,4-T	TA	TAA	TLA
433784 2015/1099703 16220 Quintanar del Rey, Spain (S) L140166	GC 0640 Barley Acapulco	1. 21.11.2013 2. 20.04.-05.05.2014 3. 16.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	<0.01	0.065	0.017
										ears	28	<0.01	<0.01	0.039	0.048	<0.01
										rest of pl. [2]	28	<0.01	<0.01	0.013	0.090	0.053
										ears	35	<0.01	<0.01	0.054	0.084	0.028
										rest of pl. [2]	35	<0.01	<0.01	0.026	0.062	0.083
										grain	42	<0.01	<0.01	<0.01	0.050	0.078
										straw	42	<0.01	<0.01	0.035	0.063	0.62
433784 2015/1099703 02110 La Gineta, Spain (S) L140167	GC 0640 Barley Hispanic	1. 17.12.2013 2. 25.04.-05.05.2014 3. 19.06.2014	-	-	-	-	-	-	-	plant [1]	0	<0.01	<0.01	0.037	0.053	0.034
										ears	28	<0.01	<0.01	0.023	0.074	<0.01
										rest of pl. [2]	28	<0.01	<0.01	0.011	0.062	0.032
										ears	35	<0.01	<0.01	0.038	0.08	<0.01
										rest of pl. [2]	35	<0.01	<0.01	<0.01	0.038	0.027
										ears	41	<0.01	<0.01	0.026	0.053	0.014
										rest of pl. [2]	41	<0.01	<0.01	<0.01	0.025	0.019
										grain	48	<0.01	<0.01	0.035	0.088	<0.01
										straw	48	<0.01	<0.01	0.011	0.045	0.059

1) whole plant (no root) 2) rest-of-plant (no root), 1,2,4-T =1,2,4-triazole , TA = triazole alanine, TAA= triazole acetic acid, TLA=triazole lactic acid.

Conclusion

Residue data obtained in 10 independent field trials in barley (conducted in both N-EU, S-EU with the formulated product BAS 750 00 F or BAS 750 01 F according to the critical GAP) showed that the residues of BAS 750 F are 0.018-0.29 mg/kg in grain and 0.99-18 mg/kg in straw.

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. As such, it is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar (see section B.7.3.3).

B.7.3.3. Conclusions on the magnitude of residues in plants

The proposed uses of BAS 750 F in the EU are on wheat and barley. The cGAP for both commodities is given in Table 7.3.3-1.

Table .7.3-1: Critical GAP for the proposed use in wheat and barley

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)[a)]
					Rate (kg as/ha)	Water (L/ha)	
Cereals	Outdoor	49, 69	2	14	0.15	200	35

[a)] Timing of the cGAP determined based on growth stage. PHI of 35 days proposed by the applicant, but has not be used for selection of trials

In support of the representative uses, 17 cGAP-compliant field trials on wheat and 18 cGAP-compliant field trials on barley have been evaluated. It should be noted that in terms of the timing in the trials, the applications are in line with the BBCH growth stages in the proposed GAP, but in most cases the PHI is longer than the 35 days proposed. This is not considered to be of concern as for cereal applications, as the latest time of application is usually defined by the proposed BBCH. The residue trials were performed in various European Member States in both European regions during two growing seasons. A summary of the residues trials data for BAS 750 F residues are given in Table 7.3.3-2.

Table .7.3-2: Summary BAS 750 F residue data for the proposed uses

Crop	RAC	Region	n	Residues *[mg/kg]*	STMR *[mg/kg]*		HR *[mg/kg]*	
Wheat	Grain	NEU	8	4 x <0.01, 0.011, 0.014, 0.016, 0.024	0.011	0.01	0.024	0.026
		SEU	9	7 x <0.01, 0.018, 0.026	0.01		0.026	
	Straw	NEU	8	1.9, 2.3, 3.4, 3.6, 3.9, 4.9, 5.5, 10	3.75	3.6	10.0	18.0
		SEU	9	0.5, 0.56, 1.6, 2.9, 3.1, 3.8, 4.6, 9.0, 18.0	3.1		18.0	
Barley	Grain	NEU	9	0.014, 0.06, 0.071, 0.087, 0.1, 0.15, 0.15, 0.19, 0.28	0.1	0.1	0.28	0.41
		SEU	9	0.03, 0.033, 0.07, 0.1, 0.1, 0.14, 0.16, 0.29, 0.41	0.1		0.41	
	Straw	NEU	9	1.0, 1.7, 3.1, 3.9, 4.3, 4.3, 5.6, 6.8, 15.0	4.3	4.25	15.0	18.0
		SEU	9	0.39, 2.1, 2.2, 3.3, 4.2, 4.6, 6.4, 11.0, 18.0	4.2		18.0	

STMR and HR values have been calculated for the NEU and SEU region separately; however the U-test confirms that the NEU and SEU data sets in each case are not statistically different and hence the NEU and SEU results can be combined in each case to provide overall STMR and HR values.

For straw, the Dixon's Q-test indicates that the values of 9, 10 and 18 mg/kg in wheat straw and 11, 15 and 18 mg/kg in barley straw are outliers. No specific deviations in these trials were noted to which these elevated values could be attributed to, hence they have not been discarded, and are used in calculations of the STMR and HR. If these values had been discarded these values in straw remained high enough to trigger consideration of livestock feeding studies, and hence would not have impacted upon other areas of the risk assessment.

The final application to cereals in the proposed cGAP is after formation of the edible part of the crop, therefore, in accordance with SANCO 7525/VI/95 rev. 10.2 (Appendix D: Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs), trials on barley can be extrapolated to support oats and trials on wheat can be extrapolated to support rye. Triticale is in the same MRL class as wheat and hence wheat trials can also be used in support of triticale. Therefore, sufficient data is available to support the proposed cGAP on the cereals crop group. The STMR, HR and MRLs for cereal grain and straw are given in Table 7.3.3-3.

Table .7.3-3: STMR, HR and MRL for the proposed cereal uses

Crop	RAC	STMR *[mg/kg]*	HR *[mg/kg]*	MRL[1)] *[mg/kg]*
Wheat	Grain	0.01	0.026	0.04
	Straw	3.6	18.0	*30*
Rye	Grain	0.01	0.026	0.04
	Straw	3.6	18.0	*30*
Triticale	Grain	0.01	0.026	0.04
	Straw	3.6	18.0	*30*
Barley	Grain	0.1	0.41	0.6
	Straw	4.25	18.0	*30*
Oats	Grain	0.1	0.41	0.6
	Straw	4.25	18.0	*30*

[1)] MRLs for straw calculated using the EU-OECD MRL calculator for information only, as MRLs for livestock feed are not currently required

Triazole derivative metabolites were also considered in the residue trials. For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. It is considered that these residues are to a certain extent treatment unrelated as residues in treated and untreated samples are similar. A summary of STMR and HR for TDM residues are given in Table 7.3.3-4. TDMs are common to a range of triazole containing pesticides, and therefore it is likely that they were present in the soil prior to the current study and application of BAS 750 F.

Table .7.3-4: Summary TDM residue data for the proposed uses

Crop	Commodity	TA				TAA				TLA			
		Untreated *[mg/kg]*		Treated *[mg/kg]*		Untreated *[mg/kg]*		Treated *[mg/kg]*		Untreated *[mg/kg]*		Treated *[mg/kg]*	
		STMR	HR	STMR	HR	STMR	HR	STMR	HR	STMR	HR	STMR	HR
Wheat	Grain	0.14	1.0	0.25	1.5	0.03	0.29	0.068	0.36	0.01	0.32	0.01	0.092
	Straw	0.088	0.83	0.035	0.47	0.013	0.76	0.029	0.16	0.029	1.1	0.077	1.5
Barley	Grain	0.101	1.9	0.25	2.6	0.069	0.59	0.081	0.55	0.047	1.1	0.011	1.2
	Straw	0.03	0.83	0.087	0.71	0.022	0.76	0.035	0.33	0.12	10	0.44	11

A summary of the residues of TDMs in treated cereal grain as presented in the TDM review (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015) is given in Table 7.3.3-5.

Table .7.3-5: Summary TDM residue data on cereals from the TDM Review

Crop	Commodity	1,2,4-T		TA		TAA		TLA	
		STMR *[mg/kg]*	HR *[mg/kg]*	STMR *[mg/kg]*	HR *[mg/kg]*	STMR *[mg/kg]*	HR *[mg/kg]*	STMR *[mg/kg]*	HR *[mg/kg]*
Cereal	Grain	0.05	0.08	0.62	2.20	0.79	1.73	0.022	0.16
	Straw	0.05	0.05	0.12	0.65	0.24	0.78	0.37	1.1

Data obtained in the present field study with BAS 750 F is comparable to the TDM data previously submitted by the Triazole Derivative Metabolite Group (TDMG). As the results are comparable, it is not considered necessary to undertake a new risk assessment for TDMs in relation to BAS 750 F, as these are encompassed within the risk assessment in the TDM review.

STMRs for each TDM metabolite are lower in the trials on BAS 750 F, therefore no increase on the chronic risk assessment would be observed (NB: the highest EU MS NEDIs in the TDM review are 14.5% (1,2,4-T), 1.5% (TA), 1.1% (TAA) and 0.2% (TLA) of the respective ADIs (1,2,4-T: 0.05 mg/kg, TA, TAA, TLA: 1 mg/kg) Slightly higher HRs are determined for TA and TLA in barley grain in comparison to the TDM review; however these are not considered to significantly impact the acute risk assessment. The highest EU MS NESTI for wheat is 2.5%, of the ARfD (1 mg/kg) for TAA (cereals contribute <1% of the ARfD for TA and TLA), hence a minor increase in the HR would not significantly impact the acute risk assessment.

B.7.4. FEEDING STUDIES

Dietary Burden

The dietary burden calculation has been performed according to the approach presented in the OECD Guidance document on residues in livestock, series on pesticides No 73 for a total of 9 animal species, fish excluded.

All feed items which might be treated with the active substance under evaluation have been considered (barley and wheat). In this calculation only the proposed uses are considered, as no previous EU evaluations have been made for BAS 750F. Calculations are performed using the Excel calculator proposed by EFSA. Input values are summarized in Table 7.4-1 and the highest and median calculated animal intakes are reported in Table 7.4-2.

Table 7.4-1: Input values for the dietary burden calculation

Feed commodity[1)]	Median dietary burden		Maximum dietary burden	
	(mg/kg)	Comment	(mg/kg)	Comment
RD-RA Plant commodities: BAS 750F				
Wheat grain	0.01	STMR	0.01	STMR
Wheat straw	3.6	STMR	18.0	HR
Wheat gluten meal	0.003	$STMR_P$ (STMR 0.01 x PF 0.3)	0.003	$STMR_P$ (STMR 0.01 x PF 0.3)
Wheat milled by products	0.006	$STMR_P$ (STMR 0.01 x PF 0.6)	0.006	$STMR_P$ (STMR 0.01 x PF 0.6)
Rye grain	0.01	STMR	0.01	STMR
Rye straw	3.6	STMR	18.0	HR
Triticale grain	0.01	STMR	0.01	STMR
Triticale straw	3.6	STMR	18.0	HR
Barley grain	0.1	STMR	0.1	STMR
Barley straw	4.25	STMR	18.0	HR
Barley brewers grain (dried)	0.24	$STMR_P$ (STMR 0.1 x PF 2.4)	0.24	$STMR_P$ (STMR 0.1 x PF 2.4)
Barley distiller's grain (dried)	0.24	$STMR_P$ (STMR 0.1 x PF 2.4)	0.24	$STMR_P$ (STMR 0.1 x PF 2.4)
Oat grain	0.1	STMR	0.1	STMR
Oat straw	4.25	STMR	18.0	HR

[1)] *As the proposed GAP is on cereal 'for grain production' residue data for silage and forage are not included in the calculation*

Table 7.4.-2: Estimated maximum animal intakes (mg/kg bw/day)

Animals	**Median burden** (mg/kg bw)	**Maximum burden** (mg/kg bw)	**Above 0.004 mg /kg bw**	**Maximum burden** (mg/kg DM)	**Highest contributing commodities**
Beef cattle	0.037	0.148	Yes	6.16	Barley straw
Dairy cattle	0.058	0.237	Yes	6.15	Barley straw
Ram/Ewe	0.098	0.407	Yes	12.22	Barley straw
Lamb	0.124	0.518	Yes	12.20	Barley straw
Pig (breeding)	0.003	0.003	No	0.14	Distiller's grain dried
Pig (finishing)	0.004	0.004	Yes	0.14	Distiller's grain dried
Poultry broiler	0.007	0.007	Yes	0.11	Brewer's grain dried
Poultry layer	0.036	0.148	Yes	2.16	Wheat straw
Turkey	0.007	0.007	Yes	0.09	Brewer's grain dried

As the maximum dietary burden is greater than the trigger value for further assessment (0.004 mg/kg bw/day) for all animal species considered (except breeding pig), further consideration of the residues in animal commodities is required.

As discussed in section B.7.3.3, residues of the TDMs TA, TAA and TLA were determined in cereal grain and straw. However, the STMR and HR for these metabolites are largely comparable to the levels determined in cereals in the TDM review (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015) for grain, and for straw the STMR values are comparable, with the HR values for TA and TLA in the current evaluation being slightly higher (TLA HR in barley straw is significantly higher, however it is considered this may be an erroneous result as in the same trial the untreated samples had TLA levels at 10 mg/kg and the next highest level in the trials was 4.4 mg/kg).

In the TDM review, the maximum dietary burden demonstrates residues of TA, TAA and TLA are significant in livestock diet and trigger consideration of feeding studies. Therefore, the slightly higher values in this evaluation would not affect the requirement for feeding studies. As discussed in section B.7.3.3, due to the low contribution of residues of TDM metabolites to the respective ADI/ARfDs, if slightly higher STMR/HR values for animal commodities were determined as a result of the residues of TDMs determined for BAS 750 F, this would have no significant impact on the chronic and acute consumer risk assessments.

As such it is not considered necessary to undertake a new dietary burden calculation for the TDMs in cereals from the BAS 750 F uses.

B.7.4.1. Poultry

Report: CA 6.4.1/1
[redacted] 2015 a
Magnitude of residues in tissues and eggs of laying hens following multiple oral administrations of BAS 750 F
2015/1106667

Guidelines: Commission of the European Communities KOM(2005) 221 2005/0099 (CNS) (30 May 2005), OECD 505 (Jan. 2007), EPA 860.1480, EPA 860.1340, EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2013)8 Guidance Document on Residues in Livestock No. 73

GLP: yes

Materials and methods

69 laying hens (breed: *ISA Brown*) received feed dosed with BAS 750 F daily for 33 days. The hens were divided into 6 groups, one control (9 hens, group A) and 5 dosed (12 hens per group, groups B-F). The dosing levels were nominally 0.15, 1.5, 4.5 and 15 (2 groups) mg/kg feed (predicted dietary burden 2.16 mg/kg feed for laying hens). In each group the hens were divided into equally sized sub groups. For the control group (A) and one of the 15 mg/kg feed groups (F) the subgroups were used to considered depuration on withdrawal at 0/2, 7 and 14 days.

The test items were prepared in gelatine capsules, orally administered (once daily). The actual dose was based on the average feed consumption. The mean achieved daily doses administered were between 0.176-17.2 mg/kg food consumed (dry weight equivalent) corresponding to 0.01-0.98 mg/kg bw/d. Details of the study outline are summarized in Table 7.4.1-1. Feed samples were tested to ensure that no TDMs were present.

Table 7.4.1-1: Dosing of laying hen with BAS 750 F

Dose group	Mean daily food consumption *[kg/animal]*	Mean animal weight [1)] *[kg]*	Nominal dose *[mg/kg feed]*	Actual daily dose (mean) *[mg/animal]*	*[mg/kg dry feed]*	*[mg/kg bw/d]*	Time of sacrifice *[days]*
A (0 X)	0.1	1.80	-	-	-	-	34, 36, 48
B (0.1X)	0.1	1.76	0.15	0.0172	0.176	0.010	34
C (1 X)	0.1	1.79	1.5	0.172	1.743	0.096	34
D (3 X)	0.1	1.77	4.5	0.516	5.124	0.296	34
E (10 X)	0.1	1.78	15	1.72	17.247	0.984	34
F (10 X)	0.1	1.75	15	1.72	17.194	0.978	36, 41, 48

[1)] *the mean bodyweight from week -1 to 5 for each subgroup was used for calculation*

Eggs were collected twice daily. On days -1, 1, 3, 5, 7, 10, 14, 17, 21, 24, 28 and 33 specimens were taken from all treatment groups. To consider depuration additional eggs were collected on study day 35, 40 and 47 from groups A and F. Eggs from the evening and next morning of each subgroup were weighed separately, then pooled, filled into ice cube bags and stored at ≤-18 °C until analysis.

Sacrifice was within 5 hours of administration of the final dose, with the exception of samples in groups A and F which were used in depuration studies. Tissue samples (skin with fat, muscle, liver, abdominal fat) were taken immediately after sacrifice of the animals, pooled for each subgroup, weighed and stored at ≤-18 °C until analysis. The maximum time of frozen storage between sampling and analysis is 97 days for BAS 750 F, 86 days for M750F022 and 82 days for the triazole metabolites.

Analysis of BAS 750 F in eggs and tissues was carried out with BASF method L0272/01. The metabolite M750F022 was analysed with the BASF method L0309/01. The metabolites 1,2,4-T, TA, TAA and TLA were determined in eggs and tissues using the modified BASF method L0263/01. In each case the LOQ is 0.01 mg/kg. Full details of sample preparation and validation data for these methods is given in section CA B.5.1.2.5. Details of the procedural recoveries are given in Table 7.4.1-2 and 7.4.1-3.

Table 7.4.1-2: Summary of procedural recoveries for BAS 750 F and M750F022

Matrix	Fortification level [mg/kg]	L0272/01 mean recovery BAS 750 F [%]	RSD [%] BAS 750 F	L0309/01 mean recovery M750F022 [%]	RSD [%] M750F022
Egg	0.01 / 0.02 / 0.05 / 0.1	97.8	3.9 (n=30)	82.0	12 (n=54)
Muscle	0.01 / 0.05 / 0.1	102	1.5 (n=5)	74.1	6.1 (n=4)
Liver	0.01 / 0.05 / 0.1	93.7	4.4 (n=8)	79.1	5.7 (n=4)
Fat	0.01 / 0.05 / 0.1	84.5	2.2(n=5)	89.7	6.4 (n=5)
Skin with fat	0.01 / 0.05 / 0.1	84.7	9.0(n=7)	85.0	8.8 (n=5)

RSD = relative standard deviation,

Table 7.4.1-3: Summary of procedural recoveries for TDMs

Matrix	Fortification level [mg/kg]	mean recovery 1,2,4-T [%]	RSD [%] 1,2,4-T	mean recovery TA [%]	RSD [%] TA
Egg	0.01 / 0.05 / 0.1	84.9	3.9 (n=36)	97.7	7.0 (n=33)
Muscle	0.01 / 0.05 / 0.1	86.0	7.1 (n=3)	93.2	6.5 (n=3)
Liver	0.01 / 0.05 / 0.1	81.7	6.0 (n=3)	86.0	6.2 (n=3)
Fat	0.01 / 0.05 / 0.1	86.0	3.1(n=3)	92.0	12 (n=3)
Skin with fat	0.01 / 0.05 / 0.1	92.0	9.7(n=3)	84.7	8.7 (n=3)
Matrix	**Fortification level [mg/kg]**	**mean recovery TAA [%]**	**RSD [%] TAA**	**mean recovery TLA [%]**	**RSD [%] TLA**
Egg	0.01 / 0.05 / 0.1	91.8	6.1 (n=33)	91.2	5.8 (n=33)
Muscle	0.01 / 0.05 / 0.1	84.3	10 (n=3)	108	2.1 (n=3)
Liver	0.01 / 0.05 / 0.1	97.7	2.1 (n=3)	95.7	5.4 (n=3)
Fat	0.01 / 0.05 / 0.1	98.0	5.4 (n=3)	97.7	3.9 (n=3)
Skin with fat	0.01 / 0.05 / 0.1	99.3	7.3 (n=3)	93.7	4.3 (n=3)

RSD = relative standard deviation

Results and Discussion

Laying hens were dosed at 0.15-15 mg/kg feed (nominal) per day with BAS 750 F for a 33 day period. The eggs and tissue samples were then analysed for BAS 750 F, M750F022 and TDMs. The maximum time of frozen storage between sampling and analysis is 97 days for BAS 750 F, 86 days for M750F022 and 82 days for the triazole metabolites. Storage stability data is available to support storage of BAS 750 F and M750F022 for at least 177 days in animal commodities, where no significant degradation is observed during this time frame (see section B.7.1). Storage stability data is available to support storage of 1,2,4-triazole, TA and TTA for 12 months (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015). This is sufficient to support the storage times in this study. Storage stability data to support TLA is not available; however no residues of TLA were detected in any poultry commodity.

The methods of analysis for determination of BAS 750 F, M750F022 and TDMs are considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4, or fit for purpose. Acceptable procedural recovery data, using an appropriate number of samples were presented.

Eggs

The residue levels of BAS 750 F, M750F022, and TDMs determined in eggs for each dosing group are presented in Table 7.4.1-4 to Table 7.4.1-6.

In the highest dose group (15 mg/kg feed) BAS 750 F residues above LOQ were found. The group mean reached a plateau level around 0.030 mg/kg (starting day 10). The highest group mean value was 0.035 mg/kg. The highest individual residue value was 0.042 mg/kg (day 14). In the samples of the depuration group (dose of 15 mg/kg feed) residues were below the LOQ after 7 days of depuration (day 40) (after 2 days depuration the group mean was 0.023 mg/kg). Analysis of samples representative for the plateau phase (day 24) showed that residues are predominantly present in egg yolk (group mean 0.076 mg/kg, maximal individual 0.091 mg/kg) while residues in egg white were below the LOQ. In the dose groups D (4.5 mg/kg feed), C (1.5 mg/kg feed) and A (control 0 mg/kg feed) residues were below the LOQ. Consequently, dose group B (0.15 mg/kg feed) can be expected to show no residues above LOQ and therefore was not analysed.

Table 7.4.1-4: Residues of BAS 750 F in egg (including yolk and egg white)

Study day	BAS 750 F : residue in mg/kg					
	Group A *(0 mg/kg)*	**Group B** *(0.15 mg/kg)*	**Group C** *(1.5 mg/kg)*	**Group D** *(4.5 mg/kg)*	**Group E** *(15 mg/kg)*	**Group F** *(15 mg/kg)*
-1	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]
1	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]
3	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.011, <0.01, <0.01 **(0.01)**	**<0.01** [3]
5	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.024, 0.016, 0.011 **(0.017)**	0.019, 0.011, 0.014 **(0.015)**
7	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.038, 0.024, 0.022 **(0.028)**	0.023, 0.016, 0.02 **(0.020)**
10	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.041, 0.02, 0.025 **(0.030)**	0.028, 0.025, 0.024 **(0.026)**
14	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.042, 0.034, 0.030 **(0.035)**	0.03, 0.025, 0.025 **(0.026)**
17	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.035, 0.032, 0.027 **(0.031)**	0.029, 0.02, 0.022 **(0.024)**
21	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.03, 0.021, 0.022 **(0.024)**	0.03, 0.019, 0.022 **(0.024)**
24 [1)]	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	Yolk: 0.091, 0.060, 0.078 **(0.076)** White: **<0.01** [3]	0.032, 0.024, 0.024 **(0.027)**
28	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.037, 0.023, 0.031 **(0.030)**	0.029, 0.022, 0.033 **(0.028)**
33	**<0.01** [3]	-	**<0.01** [3]	**<0.01** [3]	0.036, 0.025, 0.028 **(0.030)**	0.037, 0.017, 0.02 **(0.025)**
35	**<0.01** [2]	-	-	-	-	0.029, 0.02, 0.021 **(0.023)**
40	**<0.01** [1]	-	-	-	-	**<0.01** [2]
47	**<0.01** [1]	-	-	-	-	**<0.01** [1]
mean (14-33)	**<0.01**	-	**<0.01**	**<0.01**	**0.03**	**0.03**

[1)] *on day 24, eggs from group A and E were separated into egg white and yolk, no results for whole egg were determined*

<0.01 denotes less than the LOQ. For calculation of mean < 0.01 was set to 0.01 mg/kg.

In the highest dose group (15 mg/kg feed) M750F022 residues above LOQ were found. The group mean residue reached a plateau level around 0.066 mg/kg in the highest dose (starting day 10). The highest group mean value was 0.083 mg/kg. The highest individual residue value was 0.094 mg/kg (day 14).

In the samples of the depuration group F (dose of 15 mg/kg feed) residues were below the LOQ after 14 days of depuration (having declined from 0.064 mg/kg and 0.014 mg/kg after 2 and 7 days of

depuration). Analysis of samples representative for the plateau phase (day 24) showed that residues are predominantly present in egg yolk (group mean 0.017 mg/kg, maximal individual 0.021 mg/kg), while residues in egg white were below the LOQ.

In the dose group D (4.5 mg/kg feed), residue levels were below or close to LOQ (during day 10 to day 21 group mean was 0.012 – 0.019 mg/kg). For the plateau phase, the difference between the residue level (group E: 0.066 and group D: 0.015 mg/kg) corresponds to the difference in dose level (group E: 15 mg/kg feed and group D: 4.5 mg/kg feed) indicating a linear dose-dependency. In dose groups C (1.5 mg/kg feed), B (0.15 mg/kg feed) and A (control 0 mg/kg feed) residues were < LOQ.

Table 7.4.1-5: Residues of M750F022 in egg

Study day	M750F022 : residue in mg/kg					
	Group A *(0 mg/kg)*	**Group B** *(0.15 mg/kg)*	**Group C** *(1.5 mg/kg)*	**Group D** *(4.5 mg/kg)*	**Group E** *(15 mg/kg)*	**Group F** *(15 mg/kg)*
-1	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]
1	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]
3	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	<0.01 <0.01 0.012 **(0.011)**	**<0.01** [3]
5	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.032 0.020 0.013 **(0.022)**	0.012 0.013 0.013 **(0.012)**
7	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.035 0.021 0.023 **(0.026)**	0.047 0.036 0.030 **(0.038)**
10	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.012 0.01 0.013 **(0.012)**	0.028 0.052 0.059 **(0.046)**	0.049 0.051 0.040 **(0.047)**
14	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.015 0.015 0.015 **(0.015)**	0.094 0.073 0.071 **(0.079)**	0.064 0.070 0.053 **(0.062)**
17	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.016 0.013 0.015 **(0.015)**	0.059 0.061 0.064 **(0.061)**	0.062 0.050 0.049 **(0.054)**
21	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.019 0.02 0.017 **(0.019)**	0.062 0.063 0.051 **(0.059)**	0.054 0.051 0.047 **(0.051)**
24 [1)]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	Yolk: 0.021 0.015, 0.015 **(0.017)** White: **<0.01** [3]	0.066 0.060 0.054 **(0.060)**
28	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.012 0.01 0.012 **(0.012)**	0.061 0.044 0.063 **(0.056)**	0.065 0.051 0.048 **(0.055)**
33	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.016 0.016 0.015 **(0.016)**	0.076 0.073 0.064 **(0.071)**	0.076 0.048 0.052 **(0.059)**
35	**<0.01** [2]	-	-	-	-	0.069 0.056 0.067 **(0.064)**
40	**<0.01** [1]	-	-	-	-	0.014 0.014 **(0.014)**
47	**<0.01** [1]	-	-	-	-	**<0.01** [1]
mean (14-33)	**<0.01**	**<0.01**	**<0.01**	**0.015**	**0.066**	**0.075**

[1)] *On day 24, eggs from group A and E were separated into egg white and yolk. Residues below the LOQ are denoted by <0.01 mg/kg. For calculation of mean, the result of < 0.01 mg/kg was set to 0.01 mg/kg*

In eggs, 1,2,4-triazole was found at levels above LOQ, while residues of TA, TAA and TLA were below the LOQ in all cases (except for one treated sample: separately analysed yolk of the highest dose group, day 24, see below).

In whole eggs, detectable residues of 1,2,4-triazole were seen in the highest dose group (15 mg/kg feed) while for TA, TAA and TLA residues were <LOQ. The group mean residue reached a plateau level around 0.080 mg/kg (starting day 5). The highest group mean value was 0.088 mg/kg (day 14).

The highest individual residue value was 0.099 mg/kg (day 7). In the samples of the depuration group (dose of 15 mg/kg feed) residues were below the LOQ no later than 7 days of depuration.

At day 24 (of a sample representative of the plateau phase) eggs from group A and E were separated into egg white and yolk. Analysis showed that 1,2,4-triazole residues are distributed between egg yolk (group mean residue 0.047 mg/kg, maximal individual residue 0.050 mg/kg) and egg white (group mean residue 0.083 mg/kg, highest individual residue 0.090 mg/kg). Egg yolk (group E) did contain TA at 0.021 mg/kg (group average, individual values: 0.016, 0.023, 0.025 mg/kg) which is considered largely treatment-unrelated as for the corresponding control sample similar TA levels were determined (0.016 mg/kg) (comparable observation for tissues, see chapter 4.2.3 below). Residues of TAA and TLA in egg yolk were below the LOQ. In egg white, for all three analytes, TA, TAA and TLA, residue levels were below LOQ.

In the dose group D (4.5 mg/kg feed), 1,2,4-triazole residue levels were present at detectable amounts reaching a plateau around 0.022 mg/kg at day 5. The difference between the residues (group mean E: 0.080 mg/kg and D: 0.022 mg/kg) corresponds to the difference in dose level (E: 15 mg/kg feed and D: 4.5 mg/kg feed) indicating a linear dose-dependency. In dose groups C (1.5 mg/kg feed), 1,2,4-triazole levels were near or below the LOQ (highest individual 0.012 mg/kg at day 33). In dose groups B (0.15 mg/kg feed) and A (control 0 mg/kg feed) residues were below the LOQ.

In the dose groups D (4.5 mg/kg feed), C (1.5 mg/kg feed) and A (control 0 mg/kg feed) residues of TA, TAA and TLA were below the LOQ. Consequently, dose group B (0.15 mg/kg) can be expected to show no residues above LOQ and therefore was not analysed.

Table 7.4.1-6: Residues of 1,2,4-T in eggs

Study day	1,2,4-T : residue in mg/kg					
	Group A *(0 mg/kg)*	**Group B** *(0.15 mg/kg)*	**Group C** *(1.5 mg/kg)*	**Group D** *(4.5 mg/kg)*	**Group E** *(15 mg/kg)*	**Group F** *(15 mg/kg)*
-1	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]
1	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]
3	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.015 0.013 0.017 **(0.015)**	0.063 0.053 0.061 **(0.059)**	0.063 0.061 0.044 **(0.056)**
5	**<0.01** [3]	**<0.01** [3]	<0.01 0.01 <0.01 **(<0.01)**	0.024 0.021 0.022 **(0.022)**	0.080 0.074 0.089 **(0.081)**	0.072 0.070 0.064 **(0.069)**
7	**<0.01** [3]	**<0.01** [3]	<0.01 0.011 <0.01 **(<0.01)**	0.026 0.018 0.022 **(0.022)**	0.099 0.071 0.088 **(0.086)**	0.066 0.072 0.066 **(0.068)**
10	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.025 0.019 0.021 **(0.022)**	0.090 0.064 0.090 **(0.081)**	0.080 0.083 0.078 **(0.080)**
14	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.028 0.021 0.019 **(0.023)**	0.089 0.090 0.086 **(0.088)**	0.076 0.079 0.094 **(0.083)**
17	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.025 0.019 0.019 **(0.021)**	0.076 0.086 0.091 **(0.084)**	0.068 0.072 0.080 **(0.073)**
21	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.027 0.021 0.019 **(0.022)**	0.082 0.073 0.072 **(0.076)**	0.065 0.068 0.066 **(0.066)**
24[1)]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.025 0.019 0.021 **(0.02)**	Yolk 0.046 0.044 0.05 **(0.047)** White: 0.09 0.077 0.082 **(0.083)**	0.060 0.072 0.063 **(0.065)**
28	**<0.01** [3]	**<0.01** [3]	<0.01 0.011 0.01 **(0.01)**	0.025 0.022 0.018 **(0.021)**	0.086 0.075 0.063 **(0.075)**	0.069 0.071 0.074 **(0.071)**
33	**<0.01** [3]	**<0.01** [3]	<0.01 0.01 0.012 **(0.011)**	0.024 0.021 0.017 **(0.021)**	0.083 0.070 0.081 **(0.078)**	0.07 0.072 0.064 **(0.069)**
35	**<0.01** [2]	-	-	-	-	0.067 0.068 0.061 **(0.066)**
40	**<0.01** [1]	-	-	-	-	**<0.01** [2]
47	**<0.01** [1]	-	-	-	-	**<0.01** [1]
mean (14-33)	**<0.01**	**<0.01**	**<0.01**	**0.022**	**0.080**	**0.071**

[1)] On day 24, eggs from group A and E were separated into egg white and yolk. Residues below the LOQ are denoted by <0.01 mg/kg. For calculation of the mean, the result of < 0.01 mg/kg was set to 0.01 mg/kg.

Tissues

The residue levels of BAS 750 F, M750F022, and TDMs determined in tissues for each dosing group are presented in Table 7.4.1-7 - 7.4.1-10. For the highest dose group (15 mg/kg feed), the group mean residues of BAS 750 F were 0.016 mg/kg in muscle (highest individual 0.027 mg/kg), 0.097 mg/kg in liver (highest individual 0.20 mg/kg), 0.17 mg/kg in fat (highest individual 0.25 mg/kg), and 0.10 mg/kg in skin with fat (highest individual 0.15 mg/kg). In the samples of the depuration group F (dose of 15 mg/kg feed) residues in tissues were below the LOQ latest 2 days after start of depuration.

Residues in other dose groups were lower. Where residues were present at quantifiable levels, group mean levels indicated a linear dose-dependency, specifically for liver (groups E-D-C), in fat (groups E-D) and for skin with fat (groups E-D).

Table 7.4.1-7: Residues of BAS 750 F in tissues

Tissue	BAS 750 F : residues in mg/kg					
	Group A *(0 mg/kg)*	**Group B** *(0.15mg/kg)*	**Group C** *(1.5 mg/kg)*	**Group D** *(4.5 mg/kg)*	**Group E** *(15 mg/kg)*	**Group F**[1)] *(15 mg/kg)*
Muscle	<**0.01** [3]	<**0.01** [3]	<**0.01** [3]	<**0.01** [3]	0.027, 0.01, <0.01 **(0.016)**	<**0.01** [3]
Liver	<**0.01** [3]	<**0.01** [3]	0.017 <0.01 0.011 **(0.013)**	0.021 0.012 0.013 **(0.015)**	0.20 0.06 0.035 **(0.097)**	<**0.01** [3]
Fat	<**0.01** [3]	<**0.01** [3]	<**0.01** [3]	0.019 0.021 0.025 **(0.022)**	0.25 0.15 0.10 **(0.17)**	<**0.01** [3]
Skin with fat	<**0.01** [3]	<**0.01** [3]	<**0.01** [3]	0.010 0.011 0.011 **(0.011)**	0.15 0.08 0.066 **(0.10)**	<**0.01** [3]

[1)] One sample each at 2 ,7 and 14 days withdrawal, Residues below the LOQ are denoted by <0.01 mg/kg. For calculation of mean, the result of < 0.01 mg/kg was set to 0.01 mg/kg

For the highest dose group (15 mg/kg feed), the group mean residues of M750F022 were 0.033 mg/kg in muscle (highest individual 0.037 mg/kg), 0.15 mg/kg in liver (highest individual 0.20 mg/kg), 0.31 mg/kg in fat (highest individual 0.36 mg/kg), and 0.18 mg/kg in skin with fat (highest individual 0.19 mg/kg). In the samples of the depuration group F (dose of 15 mg/kg) residues were below the LOQ in muscle at least 2 days after start of depuration, in liver and skin with fat at least after 7 days, in fat at least after 14 days.

Residues in other dose groups were lower. In muscle, residues were below the LOQ. Where residues were present at quantifiable levels, group mean levels indicated a linear dose-dependency, namely for liver (groups E-D-C), for fat (groups E-D-C) and for skin with fat (groups E-D-C-B).

Table 7.4.1-8: Residues of M750F022 in tissues

Tissue	M750F022 : residues in mg/kg							
	Group A *(0 mg/kg)*	**Group B** *(0.15mg/kg)*	**Group C** *(1.5 mg/kg)*	**Group D** *(4.5 mg/kg)*	**Group E** *(15 mg/kg)*	**Group F**[1)] *(15 mg/kg)*	**Group F**[2)] *(15 mg/kg)*	**Group F**[3)] *(15 mg/kg)*
Muscle	<**0.01** [3]	<**0.01** [3]	<**0.01** [3]	<**0.01** [3]	0.037 0.030 0.031 **(0.033)**	<**0.01** [1]	<**0.01** [1]	<**0.01** [1]
Liver	<**0.01** [3]	<**0.01** [3]	0.018 0.017 0.019 **(0.018)**	0.033 0.020 0.030 **(0.028)**	0.20 0.13 0.12 **(0.15)**	**0.015** [1]	<**0.01** [1]	<**0.01** [1]
Fat	<**0.01** [3]	<**0.01** [3]	0.024 0.044 0.030 **(0.033)**	0.064 0.070 0.071 **(0.069)**	0.36 0.27 0.30 **(0.31)**	**0.061**[1]	**0.013** [1]	<**0.01** [1]
Skin with fat	<**0.01** [3]	0.015 < 0.01 <0.01 **(0.012)**	0.012 0.021 0.018 **(0.017)**	0.036 0.041 0.035 **(0.037)**	0.19 0.18 0.19 **(0.18)**	**0.037**[1]	<**0.01** [1]	<**0.01** [1]

[1)] 2 days withdrawal; [2)] 7 days withdrawal; [3)] 14 days withdrawal, Residues below the LOQ are denoted by <0.01 mg/kg. For calculation of mean, the result of < 0.01 mg/kg was set to 0.01 mg/kg

In tissues, 1,2,4-triazole and TA were present above the LOQ in some samples, while residues of TAA and TLA were below LOQ. Residues of TA in muscle (around 0.020 mg/kg) and liver (0.024 mg/kg) can be considered treatment-unrelated as they were similar in all dose groups including the untreated control group (comparable observation for egg yolk, see above).

For the highest dose group (15 mg/kg feed), the group mean residues of 1,2,4-triazole were in muscle at 0.10 mg/kg (highest individual 0.11 mg/kg), in liver at 0.099 (highest individual 0.12 mg/kg), in fat below LOQ, in skin with fat 0.039 mg/kg (highest individual 0.044 mg/kg).

In the samples of the depuration group (dose of 15 mg/kg feed) residues were below the LOQ in muscle and liver at latest after 7 days of depuration.

Residues in other dose groups were lower. Levels in muscle indicate a linear dose-dependency (group E: 0.10 mg/kg, group D: 0.030 mg/kg, group C: 0.012 mg/kg) as do the levels in liver (group E: 0.099 mg/kg, group D: 0.027 mg/kg, group C: 0.012 mg/kg) and skin with fat (group E: 0.039 mg/kg, group D: 0.012 mg/kg).

Table 7.4.1-9: Residues of 1,2,4-triazole in tissues

Tissue	1,2,4-T : residue in mg/kg							
	Group A *(0 mg/kg)*	**Group B** *(0.15mg/kg)*	**Group C** *(1.5 mg/kg)*	**Group D** *(4.5 mg/kg)*	**Group E** *(15 mg/kg)*	**Group F**[1)] *(15 mg/kg)*	**Group F**[2)] *(15 mg/kg)*	**Group F**[3)] *(15 mg/kg)*
Muscle	**<0.01** [3]	**<0.01** [3]	<0.01 0.014 0.013 **(0.012)**	0.031 0.035 0.025 **(0.03)**	0.11 0.081 0.11 **(0.1)**	**0.024** [1]	**<0.01** [1]	**<0.01** [1]
Liver	**<0.01** [3]	**<0.01** [3]	<0.01 0.012 0.014 **(0.012)**	0.029 0.028 0.024 **(0.027)**	0.096 0.087 0.12 **(0.099)**	**0.023** [1]	**<0.01** [1]	**<0.01** [1]
Fat	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [1]	**<0.01** [1]	**<0.01** [1]
Skin with fat	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.012 0.015 <0.01 **(0.012)**	0.037 0.037 0.044 **(0.039)**	**<0.01** [1]	**<0.01** [1]	**<0.01** [1]

[1)] 2 days withdrawal; [2)] 7 days withdrawal; [3)] 14 days withdrawal. Residues below the LOQ are denoted by <0.01 mg/kg. For calculation of mean, the result of < 0.01 mg/kg was set to 0.01 mg/kg.

Table 7.4.1-10: Residues of TA in tissues

Tissue	TA : residue in mg/kg							
	Group A *(0 mg/kg)*	**Group B** *(0.15mg/kg)*	**Group C** *(1.5 mg/kg)*	**Group D** *(4.5 mg/kg)*	**Group E** *(15 mg/kg)*	**Group F**[1)] *(15 mg/kg)*	**Group F**[2)] *(15 mg/kg)*	**Group F**[3)] *(15 mg/kg)*
Muscle	0.013 0.019 0.011 **(0.014)**	0.014 0.018 0.019 **(0.017)**	0.016 0.018 0.018 **(0.017)**	0.017 0.018 0.017 **(0.017)**	0.015 0.02 0.02 **(0.018)**	**0.020** [1]	**0.014** [1]	**0.017** [1]
Liver	0.022 0.024 0.022 **(0.023)**	0.022 0.026 0.021 **(0.023)**	0.028 0.019 0.017 **(0.021)**	0.029 0.023 0.025 **(0.025)**	0.02 0.03 0.021 **(0.024)**	**0.028** [1]	**0.024** [1]	**0.024** [1]
Fat	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [1]	**<0.01** [1]	**<0.01** [1]
Skin with fat	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	**<0.01** [1]	**<0.01** [1]	**<0.01** [1]

[1)] 2 days withdrawal; [2)] 7 days withdrawal; [3)] 14 days withdrawal. Residues below the LOQ are denoted by <0.01 mg/kg. For calculation of mean, the result of < 0.01 mg/kg was set to 0.01 mg/kg.

Conclusion

Eggs and tissue samples from laying hens that were dosed with BAS 750 F for 34 days were analysed for triazole derived metabolites, for BAS 750 F and for M750F022.

Concerning eggs, generally no residues above the LOQ were found in the two lowest dose groups (B and C). Starting with dose group D, residues of M750F022 and 1,2,4-triazole were detected. Highest levels were found for 1,2,4-triazole (up to 0.028 mg/kg) while residues of M750F022 were slightly lower (up to 0.019 mg/kg). Residues of BAS 750 F were only present in dose group E (and F), at about half the levels of M750F022.

For BAS 750 F, M750F022 and 1,2,4-triazole time dependent plateaus were reached after around 14 days of administration of BAS 750 F. For TA, TAA and TLA no time dependent plateau was obtained, since no residues above the LOQ were found. Upon withdrawal residues rapidly declined to

levels below the LOQ demonstrating that residues do not accumulate in egg (BAS 750 F latest after 7 days, M750F022 latest after 14 days, 1,2,4-triazole latest after 7 days).

In tissues, only the lowest dose group (B) had no residues above the LOQ (except one sample of skin with M750F022 residues slightly above the LOQ). In muscle, residues of BAS 750 F and M750F022 were only present at the highest dose group, where residues of M750F022 were approximately twice that of BAS 750 F (0.033 mg/kg/0.016 mg/kg). In the other tissues, residues were present in dose group C and above, in each case, residues of M750F022 were higher than residues of BAS 750 F.

Residues of 1,2,4-triazole were found only in muscle, liver and skin and were generally in the same range as BAS 750 F residues or lower except for muscle where 1,2,4-triazole residues were higher. Residues of TA were observed only in muscle and liver but were always at the same level for all dose groups including the control group, suggesting that this is a background level coming from ingestion of TA residues present in feedstuff, although no analysis of the feedstuff has been undertaken to confirm this. No residues of the other triazole derived metabolites TAA and TLA were observed. Upon withdrawal, residues in tissues rapidly declined to levels below the LOQ demonstrating absence of accumulation for BAS 750 F (in muscle, liver, and fat latest after 2 days of depuration), for M750F022 (in muscle, latest after 2 days, in liver, latest after 7 days, in fat, latest after 14 days) and for 1,2,4-triazole (in muscle and liver, latest after 7 days, in fat latest after 2 days).

The relative amounts of BAS 750 F and its metabolites (M750F022 and TDM) are seen most clearly in the highest dose group, although comparable relative amounts are seen in the lower dose groups as a result of the observed linear dose-dependency for all three analytes (with the exception of TA where largely dose independent residues were observed). Table 7.4.1-11 shows the mean residues of BAS 7450 F, M750F022, 1,2,4-T and TA in the highest dose group (E) and the group dosed at realistic levels (B).

Table 7.4.1-11: Overview tissue distribution for BAS 750 F, M750F022, 1,2,4-triazole and TA in groups B (0.15 mg/kg feed) and E (15 mg/kg feed).

Commodity	BAS 750 F		M750F022		1,2,4-T		TA	
	B mean (mg/kg)	*E mean (mg/kg)*	*B mean (mg/kg)*	*E mean (mg/kg)*	*B mean (mg/kg)*	*E mean (mg/kg)*	*B mean (mg/kg)*	*E mean (mg/kg)*
egg [1)]	<0.01[2)]	0.03	<0.01	0.07	<0.01	0.08	<0.01[2)]	<0.01
muscle	<0.01	0.02	<0.01	0.03	<0.01	0.10	0.02	0.02
liver	<0.01	0.10	<0.01	0.15	<0.01	0.10	0.02	0.02
fat	<0.01	0.17	<0.01	0.31	<0.01	<0.01	<0.01	<0.01
skin with fat	<0.01	0.1	0.012	0.18	<0.01	0.04	<0.01	<0.01

[1)] *plateau level* [2)] *Determined for group C (1.5 mg/kg feed) as no determination made for group B*

In conclusion, BAS 750 F residues present in feed items are transferred to poultry commodities showing a linear dose-residue-dependency. Residues in eggs reach plateau level within 10 days indicating absence of accumulation for parent BAS 750 F, its metabolite M750F022 and 1,2,4-triazole. Residues in eggs and tissues decline rapidly within a few days after withdrawal of the dose.

Report: CA 6.4.2/2
Guedez Orozco A.A.,Heger N., 2016 a
Determination of the fatty conjugates metabolites of M750F022 (Reg. No. 6011210) in animal matrices
2016/1001326
Guidelines: SANCO/3029/99 rev. 4 (11 July 2000)
GLP: yes

Materials and Methods

Fatty acid conjugates of M750F022 were identified as metabolites of BAS 750 F in the metabolism study on poultry (section 7.1.2). As such, samples from the feeding study on laying hens (study ref 2015/1106667), (which had previously been analysed only for M750F022 using method L0309/01), were analysed for these fatty acid conjugates to determine to total amount of fatty acid conjugates.

Fatty acid conjugates of M750F022 were analysed with the BASF method L0309/02, with an LOQ of 0.01 mg/kg. For the validation of the method, M750F025, a hexadecanoate conjugate of M750F022, was used as a representative fatty acid conjugate. The structure of M750F025 is given in Appendix 1. Full details of sample preparation and validation data for these methods is given in section B.5.1.2.5. Details of the procedural recoveries are given in Table 7.4.1-11.

Table 7.4.1-11: Procedural Recoveries M750F025 (analysed as M750F022 at m/z 295 fragment)

Matrix	Fortification Level [mg/kg]	M750F025 m/z 295			
		Mean [%]	SD [±]	RSD [%]	n
Egg	0.01; 0.1	85.5	5.0	5.8	10
Fat	0.01; 0.1	68.0	4.8	7.1	10
Liver	0.01; 0.1; 1	82.7	11	13	10
Muscle	0.01; 0.1	93.8	7.0	7.4	6
Overall:		82.5	11.8	12.6	40

Results and Discussion

Conjugates of M750F022 carrying various acyl moieties (fatty acids) were quantitated in samples dosed a higher levels which were obtained as part of the hen feeding study (study ref 2015/1106667). The method of analysis (L0309/02) for determination of fatty acid conjugates of M750F022 is considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4, or fit for purpose (depending on the commodity). Acceptable procedural recovery data, using an appropriate number of samples were presented.

Free M750F022 was determined as well as the sum of M750F022 and conjugates, expressed as M750F022, as shown in Table 7.4.1-12. Correction factors calculated for each sample were used as basis to propose matrix-specific correction factors for the purpose of calculating the total content of M750F022 residue (including conjugates) based on residue data for free M750F022.

Table 7.4.1-12: Residues of M750F022 and fatty acid conjugates in hen matrices

Samples [1)]			**Residues [2)] [expressed as M750F022 equivalents]**		***Correction factor* [3)]**	
Matrix	Sample	M750F022 *[mg/kg]*	(a) M750F022 *[mg/kg]*	(b) M750F022&conjugates *[mg/kg]*	factor calculated (b/a)	factor proposed
egg	F	0.053	0.060	0.071	1.18	1.5
	C	< 0.01	<0.010 *(0.008)*[4)]	0.015	1.54	
	D	0.015	0.019	0.023	1.22	
	F	0.052	0.060	0.092	1.54	
	C	< 0.01	0.010	0.014	1.38	
	D	0.016	0.021	0.022	1.07	
fat	C	0.36	0.040	0.094	2.35	4.0
	D	0.071	0.069	0.30	4.28	
	E	0.36	0.36	1.3	3.53	
skin with fat	D	0.041	0.043	0.067	1.55	4.0
	E	0.19	0.20	0.73	3.71	
liver	D	0.2	0.23	0.25	1.10	1.0
	E	0.033	0.046	0.045	1.00	
muscle	D	< 0.01 *(0.0083)*[4)]	0.014	0.017	1.27	1.5
	E	0.037	0.047	0.070	1.48	

[1)] samples from hen feeding study (CA 6.4.1/1, DocID 2015/1106667, analysed by method L0309/1)
[2)] re-analysis using BASF method L0309/2 to determine first, content of free M750F022 and second, content of M750F022&fatty acid conjugates.
[3)] the correction factor is calculated as sum of M750F022&fatty acid conjugates divided by free M750F022.
[4)] Residue level below LOQ is provided in parenthesis.

Conclusion

Based on residue levels of free M750F022 and of the sum of M750F022 releasing compounds (expressed as M750F022) in hen matrices, factors to convert free M750F022 residue to total M750F022 residue were calculated. Matrix-specific correction factors are proposed as outlined in Table 7.4.1-13.

Table 7.4.1-13: Proposed conversion factors for M750F022

Matrix	Correction factor
liver	1.0
muscle	1.5
egg	1.5
fat, skin with fat	4.0

B.7.4.2. Ruminants

Report: CA 6.4.2/1
[redacted] 2015 a
Magnitude of residues in milk and tissues of dairy cows following multiple oral administration of BAS 750 F
2015/1107649

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), EPA 860.1340, EPA 860.1480, OECD 505 (Jan. 2007), OECD Guidance Document on Pesticide Analytical Methods (13 August 2007), OECD Guidance Document on Residue in Livestock (10 July 2013)

GLP: yes

Materials and methods

18 lactating cows (breed: *Holstein/Friesian/Ayrshire cross*) received feed dosed with BAS 750 F daily for 28 days. The cows were divided into 6 groups, one control (3 cows, group A) and 5 dosed (3 cows per group, groups B-F). The dosing levels were nominally 1.5, 7.5, 50, 150 (2 groups) mg/kg feed (predicted dietary burden 6.2 mg/kg feed for cattle). For the control group (A) and one of the 15 mg/kg feed groups (F) the subgroups were used to considered depuration on withdrawal at 3, 7 and 14 days.

The test items were prepared in gelatine capsules, orally administered (once daily). The actual dose was based on the average feed consumption. The mean achieved daily doses administered were between 1.5-147.2 mg/kg food consumed (dry weight equivalent) corresponding to 0.034-3.93 mg/kg bw/d. Details of the study outline are summarized in Table 7.4.2-1.

Table 7.4.2.-1: Dosing of lactating cow with BAS 750 F

Dose group	Mean daily food consumption *[kg/animal]*	Mean animal weight [1)] *[kg]*	Nominal dose *[mg/kg feed]*	Actual daily dose (mean) *[mg/animal]*	*[mg/kg dry feed]*	*[mg/kg bw/d]*	Time of sacrifice *[days]*
A	16.34	617	-	-	-	-	29, 43
B	15.01	672	1.5	23	1.55	0.034	29
C	16.00	620	7.5	122	7.44	0.192	29
D	15.40	720	50	731	48.9	1.037	29
E	14.13	525	150	1906	140.8	3.719	29
F	17.43	745	150	2616	149.2	3.449	31, 35, 42/43

[1)] *the mean bodyweight from week -1 for each subgroup was used for calculation*

The cows were milked twice daily. On days -1, 1, 3, 5, 7, 10, 14, 17, 21, 24 and 28 specimens were taken from all treatment groups. To consider depuration additional samples were collected each day up to day 43 from the remaining cows in groups A and F. Milk from the evening and next morning of each group were weighed separately, then pooled and stored at ≤-18 °C until analysis.

Sacrifice was within 24 hours of administration of the final dose, with the exception of samples in groups A and F which were used in depuration studies. Tissue samples (liver, kidneys, composite muscle, peri-renal fat, mesenterial fat and subcutaneous fat) were taken immediately after sacrifice of the animals, homogenised, weighed and stored at ≤-18 °C until analysis. The maximum time of frozen storage between sampling and analysis is 47 days for BAS 750 F, 158 days for M750F022 and 139 days for the triazole metabolites.

Analysis of BAS 750 F in milk and tissues was carried out with BASF method L0272/01. The metabolite M750F022 was analysed with the BASF method L0309/01. The metabolites 1,2,4-T, TA, TAA and TLA were determined in milk and tissues using the modified BASF method L0263/01. In each case the LOQ is 0.01 mg/kg. Full details of sample preparation and validation data for these

methods is given in section CA B.5.1.2.5. Details of the procedural recoveries are given in Table 7.4.2-2 and 7.4.2-3.

Table 7.4.2-2 Summary of procedural recoveries for BAS 750 F and M750F022

Matrix	Fortification level *[mg/kg]*	BAS 750 F		M750F022	
		mean recovery *[%]*	**RSD** *[%]*	**mean recovery** *[%]*	**RSD** *[%]*
Milk	0.01	86.9	11.3 (n=29)	105	8.5 (n=5)
	0.10	88.9	6.0 (n=29)	93.3	3.8 (n=5)
	overall	**88.0**	**8.7 (n=63)**	**99.1**	**8.9 (n=10)**
Cream	0.01	86.3	7.5 (n=13)	102	4.9 (n=5)
	0.10	90.9	3.0 (n=13)	88.2	12.1 (n=8)
	0.50	89.5	n.a. (n=1)	n.a.	n.a.
	overall	**88.6**	**6.0 (n=27)**	**95.0**	**11.3 (n=16)**
Skim milk	0.01	89.7	5.9 (n=10)	*n.a.*	*n.a.*
	0.10	91.5	2.3 (n=10)		
	overall	**90.6**	**4.4 (n=20)**		
Muscle	0.01	80.1	9.9 (n=5)	75.4	7.3 (n=5)
	0.10	81.0	2.6 (n=5)	74.0	3.3 (n=5)
	overall	**80.5**	**6.8 (n=10)**	**74.7**	**5.5 (n=10)**
Fat	0.01	79.8	8.4 (n=23)	101.0	17.3 (n=14)
	0.10	80.3	9.0 (n=23)	83.9	12.3 (n=14)
	0.30	73.9	n.a. (n=1)	n.a.	n.a.
	2.5	70.0	n.a. (n=1)	n.a.	n.a.
	overall	**80.2**	**8.2 (n=48)**	**92.2**	**18.1 (n=28)**
Liver	0.01	82.4	10.8 (n=13)	96.1	12.5 (n=8)
	0.10	78.3	3.0 (n=13)	73.2	5.2 (n=8)
	0.60	78.3	n.a. (n=1)	n.a.	n.a.
	3.60	72.9	n.a. (n=1)	n.a.	n.a.
	overall	**80.0**	**8.3 (n=28)**	**84.7**	**17.6 (n=16)**
Kidney	0.01	90.2	10.8 (n=13)	83.7	7.0 (n=6)
	0.10	82.6	7.0 (n=13)	76.6	4.5 (n=6)
	1.90	78.5	n.a. (n=1)	*n.a.*	*n.a.*
	overall	**86.1**	**10.1 (n=27)**	**80.1**	**7.4 (n=12)**

RSD = relative standard deviation, n.a. = not analysed

Table 7.4.2-3: Summary of procedural recoveries for TDMs

Matrix	Forti-fication level *[mg/kg]*	1,2,4-T		TA		TAA		TLA	
		mean rec *[%]*	**RSD** *[%]*	**mean rec** *[%]*	**RSD** *[%]*	**mean rec** *[%]*	**RSD** *[%]*	**mean rec** *[%]*	**RSD** *[%]*
Milk	0.01 0.10 **overall**	95.0 82.9 **88.9**	16.3 (n=46) 15.3 (n=47) **17.3 (n=93)**	93.9 83.6 **88.9**	22.5 (n=37) 13.6 (n=35) **20.0 (n=72)**	87.9 90.1 **89.0**	13.8 (n=38) 11.3 (n=38) **12.6 (n=76)**	92.61 97.7 **95.2**	25.75 (n=37) 10.2 (n=38) **19.2 (n=75)**
Cream	0.01 0.10 **overall**	106 102 **104**	5.2 (n=5) 3.5 (n=5) **4.7 (n=10)**	77.4 83.7 **80.6**	17.3 (n=5) 5.8 (n=5) **12.5 (n=10)**	95.9 101 **98.4**	9.6(n=5) 7.4 (n=5) **8.5 (n=10)**	98.2 107 **103**	7.3 (n=5) 2.6 (n=5) **6.8 (n=10)**
Skim milk	0.01 0.10 **overall**	85.3 84.3 **84.9)**	19.0 (n=5) 2.1 (n=4) **13.6 (n=9)**	94.5 89.1 **91.8**	25.9 (n=5) 5.1 (n=5) **18.3 (n=10)**	99.4 110 **105**	14.2 (n=5) 5.3(n=5) **11.0 (n=10)**	99.7 110 **105**	14.8 (n=5) 3.9 (n=5) **11.0 (n=10)**
Muscle	0.01 0.10 **overall**	98.1 90.5 **94.3**	5.3 (n=5) 2.8 (n=5) **5.9 (n=10)**	85.4 73.4 **79.4**	13.0 (n=5) 10.9 (n=5) **14.0 (n=10)**	94.9 97.1 **96.0**	3.8 (n=5) 2.7 (n=5) **3.3 (n=10)**	102 98.3 **99.9**	6.0 (n=5) 2.8 (n=5) **4.8 (n=10)**
Fat	0.01 0.10 **overall**	83.2 70.9 **77.3**	17.8 (n=14) 25.8 (n=13) **22.5 (n=27)**	105 83.8 **94.6**	8.8 (n=11) 7.3 (n=11) **14.2 (n=22)**	87.1 92.5 **89.8**	11.3(n=11) 6.2 (n=11) **9.3 (n=22)**	96.6 98.1 **97.3**	14.4 (n=11) 5.7 (n=11) **10.7 (n=22)**
Liver	0.01 0.10 **overall**	93.2 74.6 **84.9**	9.5 (n=5) 2.0 (n=4) **13.7 (n=9)**	105 91.2 **98.1**	9.5 (n=3) 4.4 (n=3) **10.4 (n=6)**	110 98.9 **104**	5.6 (n=5) 2.6 (n=5) **6.9 (n=10)**	103 102 **103**	6.4 (n=5) 4.3 (n=5) **5.2 (n=10)**
Kidney	0.01 0.10 **overall**	103 75.4 **87.5**	3.9 (n=4) 2.8 (n=5) **16.8 (n=9)**	85.6 89.1 **87.3**	9.5 (n=5) 8.7 (n=5) **8.8 (n=10)**	107 89.7 **98.3**	2.7 (n=5) 2.7 (n=5) **9.6 (n=10)**	87.7 90.7 **89.2**	5.2 (n=5) 3.8 (n=5) **4.6 (n=10)**

mean Rec = mean recovery, RSD = relative standard deviation, n = number of single values, n.a. = not applicable

Results and Discussion

Lactating cows were dosed at 1.5-150 mg/kg feed (nominal) per day with BAS 750 F for a 28 day period. The milk and tissue samples were then analysed for BAS 750 F, M750F022 and TDMs. The maximum time of frozen storage between sampling and analysis is 47 days for BAS 750 F, 158 days for M750F022 and 139 days for the triazole metabolites. Storage stability data is available to support storage of BAS 750 F and M750F022 for at least 177 days in animal commodities, where no significant degradation is observed during this time frame (see section B.7.1). Storage stability data is available to support storage of 1,2,4-triazole, TA and TTA for 12 months (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015). This is sufficient to support the storage times in this study. Storage stability data to support TLA is not available; however no residues of TLA were detected in any ruminant commodity.

The methods of analysis for determination of BAS 750 F, M750F022 and TDMs are considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4, or fit for purpose. Acceptable procedural recovery data, using an appropriate number of samples were presented.

Milk

The residue levels of BAS 750 F, M750F022, and TDMs determined in milk for each dosing group are presented in Table 7.4.2-4 to 7.4.2-6.

The two highest dose groups (E: 150 mg/kg feed and D: 50 mg/kg feed) demonstrated residues of BAS 750 F above LOQ. The group mean reached a plateau level around day 3 (E: 0.20 mg/kg, D: 0.06 mg/kg). The highest group mean value for group E was 0.253 mg/kg. The highest individual residue value was 0.354 mg/kg (day 21).

In the samples of the depuration group (F: dose of 150 mg/kg feed) residues declined rapidly from a level of 0.20 mg/kg to 0.10 mg/kg (3 days), 0.04 mg/kg (7 days) and 0.01 mg/kg (14 days of depuration) indicting absence of accumulation of BAS 750 F in milk.

Analysis of samples representative for the plateau phase (day 21) showed that residues are predominantly located in cream (group mean E: 1.23 mg/kg) with much lower levels in skim milk (0.07 mg/kg).

In the dose groups C (7.5 mg/kg feed), B (1.5 mg/kg feed) and A (control 0 mg/kg) residues were typically below the LOQ.

Table 7.4.2-4: Residues of BAS 750 F in milk (including skim milk and cream)

Study day	BAS 750 F: residue in mg/kg					
	Group A *(Control)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F** *(150 mg/kg)*
-1	ND [3]	ND [3]	ND [3]	ND ND <0.01 **(<0.01)**	ND [3]	ND [3]
1	ND [3]	**<0.01** [3]	**<0.01** [3]	0.022 0.0250.029 **(0.025)**	0.081 0.089 0.099 **(0.090)**	0.073 0.104 0.112 **(0.096)**
3	**<0.01** [3]	**<0.01** [3]	<0.01 <0.01 0.014 **(0.011)**	0.049 0.063 0.064 **(0.058)**	0.178 0.202 0.246 **(0.209)**	0.151 0.166 0.282 **(0.200)**
5	ND [3]	**<0.01** [3]	**<0.01** [3]	0.046 0.047 0.048 **(0.047)**	0.132 0.177 0.203 **(0.171)**	0.184.0.230.0.357 **(0.257)**
7	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.047 0.055 0.058 **(0.053)**	0.127 0.183 0.222 **(0.177)**	0.159 0.224 0.337 **(0.240)**
10	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.052 0.063 0.067 **(0.061)**	0.172 0.207 0.265 **(0.215)**	0.162 0.221 0.337 **(0.240)**
14	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.060 0.071 0.110 **(0.080)**	0.184 0.192 0.273 **(0.216)**	0.229 0.247 0.344 **(0.273)**
17	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.050 0.065 0.078 **(0.064)**	0.110 0.127 0.268 **(0.168)**	0.119 0.157 0.210 **(0.162)**
21	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.050 0.060 0.083 **(0.064)**	0.173 0.233 0.354 **(0.253)**	0.168 0.207 0.368 **(0.248)**
24	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.046 0.058 0.063 **(0.056)**	0.161 0.168 0.280 **(0.203)**	0.205 0.239 0.283 **(0.242)**
28	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.047 0.053 0.059 **(0.053)**	0.169 0.226 0.248 **(0.214)**	0.159 0.187 0.258 **(0.201)**
29	**0.077** [1]	-	-	-	-	0.060 0.124 0.128 **(0.104)**
30	**<0.01** [1]	-	-	-	-	0.024 0.042 0.046 **(0.037)**
31	**<0.01** [1]	-	-	-	-	<0.01 0.015 **(0.012)**
32	**<0.01** [1]	-	-	-	-	**<0.01** [2]
33	**<0.01** [1]	-	-	-	-	**<0.01** [2]
34	**<0.01** [1]	-	-	-	-	**<0.01** [2]
35	**<0.01** [1]	-	-	-	-	**<0.01** [1]
36	**<0.01** [1]	-	-	-	-	**<0.01** [1]
37	**<0.01** [1]	-	-	-	-	**<0.01** [1]
38	**<0.01** [1]	-	-	-	-	**<0.01** [1]
39	**<0.01** [1]	-	-	-	-	**<0.01** [1]
40	**<0.01** [1]	-	-	-	-	**<0.01** [1]
41	**<0.01** [1]	-	-	-	-	**<0.01** [1]
42	**<0.01** [1]	-	-	-	-	-
mean (3-28)	**<0.01**	**<0.01**	**0.010**	**0.060**	**0.203**	**0.229**
cream (21)	-	**<0.01** [3]	0.043 0.052 0.061 **(0.052)**	0.382 0.431 0.459 **(0.424)**	0.563 1.19 1.95 **(1.23)**	0.919 1.29 2.16 **(1.46)**
skim milk (21)	-	**<0.01** [3]	**<0.01** [3]	<0.01 0.01 0.016 **(0.012)**	0.026 0.076 0.103 **(0.069)**	0.028 0.033 0.073 **(0.044)**

‚ND denotes non-detection, "-" denotes non-analysis. Residues below the LOQ are denoted by <0.01 mg/kg.. For calculation of mean, the result of < 0.01 mg/kg was set to 0.01 mg/kg. [1)] 3 days withdrawal, [2)] 7 days withdrawal, [3)] 14 days withdrawal [4)] This determination was confirmed in repeated analyses (2x in duplicates), yet appears unlikely to reflect the residue level.

M750F022 levels were analysed in milk and cream samples (day 21) selected to represent the highest dose groups. In milk, detectable residues were determined at the highest dose group (group F, 150 mg/kg) with group mean residues of 0.021 mg/kg (highest individual 0.022 mg/kg). For group D (50 mg/kg), group mean and highest residue were both at the LOQ (0.01 mg/kg), therefore residues below the LOQ can be assumed for the lower dose groups (thus were not analysed). Residues in cream were about fivefold higher, namely in group F (0.099 mg/kg), group D (0.063 mg/kg), group C (0.011 mg/kg), and in group B <LOQ, taken together reflecting indicating a linear dose dependency. Residues in skim milk can be assumed to be lower than in whole milk, thus were not determined.

Table 7.4.2-5: Residues of M750F022 in milk (including cream and skim milk) collected at study day 21

Study day 21	**M750F022 : residues in mg/kg**					
	Group A *(Control)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F** *(150 mg/kg)*
milk	-	-	-	<0.01 <0.01 0.01 **(0.01)**	-	0.02 0.021 0.022 (**0.021)**
cream	-	**<0.01** [3]	<0.01 0.01 0.014 **(0.011)**	0.054 0.063 0.072 **(0.063)**	-	0.09 0.1 0.108 **(0.099)**
skim milk	-	-	-	-	-	-

"-" denotes non-analysis. Note, for groups A, B, C, D, E the group mean value of "<LOQ" is generally based on n=3 independent samples.

In milk from treated animals, 1,2,4-triazole (1,2,4-T) was the only compound of the TDM group found at levels significantly different from the background (untreated control). The group mean reached a plateau level at day 10 at a level of around 0.27 mg/kg. On day 21, the highest group mean value was reached (0.287 mg/kg) as well as the highest individual residue 0.334 mg/kg. In the samples of the depuration group (dose of 150 mg/kg feed) residues declined rapidly from 0.22 mg/kg (day 29) to 0.15 mg/kg (day 31) and reached background levels of 0.02 mg/kg by day 40 (background levels of 0.01 - 0.02 mg/kg on average).

Analysis of samples representative for the plateau phase (day 21) showed 1,2,4-triazole residues levels are similar in milk (0.287 mg/kg) and fractions produced thereof, namely skim milk (0.242 mg/kg) and cream (0.227 mg/kg).

In the dose group D (50 mg/kg feed), 1,2,4-triazole residue levels were present at detectable amounts reaching a plateau around 0.120 mg/kg at day 14. The difference between the residues (group mean E: 0.274 mg/kg and D: 0.120 mg/kg) correspond well with the difference in dose level (E: 150 mg/kg feed and D: 50 mg/kg feed) indicating a linear dose dependency. In dose group C (7.5 mg/kg feed), 1,2,4-triazole levels reached a plateau at 0.030 mg/kg (starting day 5). In dose group B (1.5 mg/kg feed) levels were comparable to the untreated control (maximal 0.019 mg/kg).

TAA and TLA were generally not detected (or were only detected at <LOQ). Residues of TA, if present in quantifiable amounts (only 9 samples were determined at >LOQ), were comparable to levels of corresponding untreated controls and therefore considered largely treatment-unrelated. The highest level of TA determined in a treated sample was 0.018 mg/kg, and the highest level determined in a control sample was 0.011 mg/kg. TA was determined in skim milk at 0.022 mg/kg (highest individual 0.042 mg/kg) while residues in cream were below the LOQ. The levels of TA were by far exceeded by the level of 1,2,4-triazole (0.242 mg/kg) in this sample.

Table 7.4.2-6: Residues of 1,2,4-T in milk (including cream and skim milk)

Study day	1,2,4-T : residue in mg/kg					
	Group A *(Control)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F** *(150 mg/kg)*
-1	<0.01 0.011 0.014 **(0.012)**	<0.01 0.011 0.014 **(0.012)**	<0.01 0.012 0.014 **(0.012)**	<0.01 <0.01 0.014 **(0.011)**	0.012 0.013 0.014 **(0.013)**	**<0.01** [3]
1	<0.01 0.012 0.014 **(0.012)**	0.010 0.010 0.013 **(0.011)**	0.010 0.016 0.020 **(0.015)**	0.027 0.028 0.033 **(0.029)**	0.050 0.060 0.066 **(0.059)**	0.044 0.050 0.062 **(0.052)**
3	<0.01 0.012 0.016 **(0.013)**	0.012 0.014 0.017 **(0.014)**	0.018 0.026 0.027 **(0.024)**	0.065 0.070 0.071 **(0.069)**	0.124 0.166 0.180 **(0.157)**	0.147 0.160 0.175 **(0.161)**
5	<0.01 0.012 0.016 **(0.013)**	0.014 0.015 0.015 **(0.015)**	0.022 0.031 0.036 **(0.029)**	0.093 0.096 0.099 **(0.096)**	0.227 0.232 0.267 **(0.242)**	0.183 0.199 0.216 **(0.200)**
7	<0.01 0.013 0.015 **(0.013)**	0.015 0.015 0.018 **(0.016)**	0.019 0.032 0.035 **(0.029)**	0.102 0.102 0.112 **(0.106)**	0.221 0.250 0.270 **(0.247)**	0.130 0.200 0.213 **(0.181)**
10	<0.01 0.011 0.013 **(0.011)**	0.014 0.015 0.016 **(0.015)**	0.021 0.030 0.039 **(0.030)**	0.097 0.104 0.110 **(0.104)**	0.219 0.277 0.311 **(0.269)**	0.209 0.213 0.250 **(0.224)**
14	<0.01 0.013 0.013 **(0.012)**	0.016 0.018 0.019 **(0.018)**	0.025 0.036 0.048 **(0.036)**	0.111 0.116 0.137 **(0.121)**	0.252 0.301 0.310 **(0.288)**	0.244 0.268 0.318 **(0.276)**
17	<0.01 <0.01 0.012 **(0.011)**	0.016 0.016 0.017 **(0.017)**	0.024 0.029 0.037 **(0.030)**	0.121 0.130 0.136 **(0.129)**	0.236 0.293 0.329 **(0.286)**	0.237 0.262 0.263 **(0.254)**
21	<0.01 0.012 0.013 **(0.012)**	0.013 0.014 0.017 **(0.015)**	0.023 0.031 0.037 **(0.030)**	0.123 0.127 0.130 **(0.127)**	0.259 0.267 0.334 **(0.287)**	0.231 0.243 0.259 **(0.244)**
24	<0.01 <0.01 0.011 **(0.010)**	0.010 0.013 0.014 **(0.012)**	0.021 0.031 0.034 **(0.028)**	0.114 0.118 0.121 **(0.118)**	0.217 0.222 0.262 **(0.234)**	0.214 0.222 0.228 **(0.221)**
28	<0.01 <0.01 0.012 **(0.011)**	0.011 0.013 0.013 **(0.012)**	0.022 0.031 0.033 **(0.029)**	0.099 0.106 0.118 **(0.108)**	0.223 0.287 0.331 **(0.280)**	0.146 0.192 0.229 **(0.189)**
29	**0.049** [1]	-	-	-	-	0.196 0.210 0.255 **(0.220)**
30	**0.015** [1]	-	-	-	-	0.133 0.166 0.220 **(0.173)**
31	**0.013** [1]	-	-	-	-	0.133 0.166 **(0.149)**
32	**0.012** [1]	-	-	-	-	0.094 0.102 **(0.098)**
33	**0.015** [1]	-	-	-	-	0.064 0.072 **(0.068)**
34	**0.015** [1]	-	-	-	-	0.035 0.051 **(0.043)**
35	**0.016** [1]	-	-	-	-	**0.047** [1]
36	**0.016** [1]	-	-	-	-	**0.025** [1]
37	**0.016** [1]	-	-	-	-	**0.023** [1]
38	**0.015** [1]	-	-	-	-	**0.02** [1]
39	**0.016** [1]	-	-	-	-	**0.018** [1]
40	**0.017** [1]	-	-	-	-	**0.016** [1]

Table 7.4.2-6: Residues of 1,2,4-T in milk (including cream and skim milk)

Study day	1,2,4-T : residue in mg/kg					
	Group A *(Control)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F** *(150 mg/kg)*
41	**0.021** [1]	-	-	-	-	**0.016** [1]
42	**0.015** [1]	-	-	-	-	-
mean (3-28)	**0.012**	**0.015**	**0.030**	**0.113**	**0.267**	**0.224**
cream (21)	-	<0.01 0.011 0.012 (**0.011**)	0.017 0.024 0.027 (**0.023**)	0.089 0.092 0.094 (**0.092**)	0.193 0.198 0.290 (**0.227**)	0.163 0.168 0.205 (**0.179**)
skim milk (21)	-	0.014 0.016 0.018 (**0.016**)	0.023 0.029 0.035 (**0.029**)	0.120 0.125 0.129 (**0.125**)	0.194 0.227 0.305 (**0.242**)	0.215 0.226 0.234 (**0.225**)

Tissues

The residue levels of BAS 750 F, M750F022, and TDMs determined in tissues for each dosing group are presented in Table 7.4.2-7 to 7.4.2-10.

For the highest dose group (150 mg/kg feed), the group mean residues of BAS 750 F in muscle were at 0.16 mg/kg (highest individual 0.22 mg/kg), in liver at 3.03 mg/kg (highest individual 3.58 mg/kg), in perirenal fat at 1.71 mg/kg (highest individual 2.29 mg/kg, the levels in other fat types were lower). In the samples of the depuration group (dose of 150 mg/kg feed) residues in tissues declined rapidly, with levels <LOQ latest after 7 days (muscle, kidney), after 14 days (liver, perirenal and mesenterial fat). For subcutaneous fat, levels at withdrawal day 14 day had declined to 0.023 mg/kg (from 1.47 mg/g at withdrawal day 3).

Residues in other dose groups were lower, if present above the LOQ, levels did indicate a linear dose dependency, namely for liver, kidney, fat (groups E-D-C-B) and for muscle (E-D).

Table 7.4.2-7: BAS 750 F residues in tissues

Tissue	BAS 750 F : residues in mg/kg							
	Group A *(0 mg/kg)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F1**[1)] *(150 mg/kg)*	**Group F1**[2)] *(150 mg/kg)*	**Group F1**[3)] *(150 mg/kg)*
muscle	**<0.01** [3]	**<0.01** [3]	**<0.01** [3]	0.051 0.063 0.105 (**0.073**)	0.128 0.141 0.221 (**0.163**)	**0.063** [1]	**<0.01** [1]	**ND** [1]
liver	**<0.01** [3]	0.029 0.031 0.034 (**0.031**)	0.112 0.155 0.182 (**0.150**)	0.643 0.936 1.40 (**0.993**)	2.50 3.01 3.58 (**3.03**)	**0.885** [1]	**0.021** [1]	**<0.01** [1]
kidney	**<0.01** [3]	<0.01 0.013 0.014 (**0.012**)	0.028 0.043 0.074 (**0.048**)	0.047 0.320 0.505 (**0.291**)	0.944 1.06 1.88 (**1.29**)	**0.275** [1]	**<0.01** [1]	**ND** [1]
fat (perir.)	**<0.01** [3]	0.016 0.017 0.018 (**0.017**)	0.029 0.058 0.059 (**0.049**)	0.461 0.586 0.900 (**0.649**)	0.942 1.90 2.29 (**1.71**)	**0.536** [1]	**0.017** [1]	**<0.01** [1]
fat (mesen.)	**<0.01** [3]	0.018 0.018 0.018 (**0.018**)	0.030 0.051 0.077 (**0.053**)	0.456 0.563 0.566 (**0.528**)	0.652 0.961 1.87 (**1.16**)	**2.25** [1]	**0.023** [1]	**<0.01** [1]
fat (subcut.)	**<0.01** [3]	0.012 0.016 0.017 (**0.015**)	<0.01 0.017 0.041 (**0.023**)	0.171 0.493 0.784 (**0.483**)	0.019 0.562 1.200 (**0.594**)	**1.47** [1]	**0.322** [1]	**0.023** [1]

Note, <0.01 denotes residues below LOQ, ND denotes non-detection, "-" denotes non-analysis. Note, for groups A, B, C, D, E the group mean value of "<LOQ" is generally based on n=3 independent samples. For groups F1, F2, F3 n=1 independent sample.[1)] 3 days withdrawal, [2)] 7 days withdrawal, [3)] 14 days withdrawal

For the highest dose group (150 mg/kg feed), the group mean residues of M750F022 in muscle were at 0.016 mg/kg (highest individual 0.018 mg/kg), in liver at 0.038 mg/kg (highest individual

0.044 mg/kg), in perirenal fat at 0.16 mg/kg (highest individual 0.21 mg/kg, note that residues in mesenterial fat and subcutaneous fat generally was lower).

In the samples of the depuration group (dose of 150 mg/kg feed) residues declined rapidly, with residues <LOQ in muscle latest after 3 days of depuration, residues at or below the LOQ in liver latest after 3 days of depuration, in kidney after 7 days of depuration, in perirenal and mesenterial fat latest 14 days of depuration. In subcutaneous fat, decline was also rapid from 0.11 mg/kg (3d-withdrawal-animal) to 0.023 mg/kg (14d-withdrawal-animal).

Residues in other dose groups were lower. In muscle, residues were below the LOQ for group D. Levels in liver, kidney and fat indicate a linear dose dependency (groups E-D-C).

Table 7.4.2-8: M750F022 residues in tissues

Tissue	M750F022 : group mean (maximum individual) residues in mg/kg							
	Group A *(0 mg/kg)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F1**[1)] *(150 mg/kg)*	**Group F1**[2)] *(150 mg/kg)*	**Group F1**[3)] *(150 mg/kg)*
muscle	-	-	-	**<0.01** [3]	0.014 0.016 0.018 **(0.016)**	**<0.01** [1]	**<0.01** [1]	**<0.01** [1]
liver	-	-	**<0.01** [3]	0.019 0.022 0.022 **(0.021)**	0.031 0.039 0.044 **(0.038)**	**0.016** [1]	**ND** [1]	**0.010** [1]
kidney	-	-	**<0.01** [3]	0.018 0.020 0.020 **(0.019)**	0.040 0.041 0.043 **(0.041)**	**0.012** [1]	**<0.01** [1]	**<0.01** [1]
fat (perir.)	-	-	**<0.01** [3]	0.075 0.083 0.089 **(0.082)**	0.134 0.143 0.212 **(0.163)**	**0.096** [1]	**0.051** [1]	**<0.01** [1]
fat (mesen.)	-	-	<0.01 <0.01 0.011 **(0.01)**	0.075 0.086 0.090 **(0.083)**	0.058 0.114 0.203 **(0.125)**	**0.149** [1]	**0.054** [1]	**<0.01** [1]
fat (subcut.)	-	-	**<0.01** [3]	0.026 0.053 0.077 **(0.052)**	0.027 0.061 0.130 **(0.073)**	**0.109** [1]	**0.068** [1]	**0.023** [1]

Note, <0.01 denotes residues below LOQ, ND denotes non-detection, "-" denotes non-analysis. For groups F1, F2, F3 n=1 independent sample.[1)] 3 days withdrawal, [2)] 7 days withdrawal, [3)] 14 days withdrawal

In tissues, TDM residues were present above the LOQ in the case of 1,2,4-triazole and TA, with 1,2,4-triazole as the predominant component. For both these compounds, residues are also found in untreated control samples.

For the highest dose group (150 mg/kg feed), the group mean residues of 1,2,4-triazole in muscle were at 0.28 mg/kg (highest individual 0.33 mg/kg), in liver at 0.26 mg/kg (highest individual 0.30 mg/kg), in kidney at 0.28 mg/kg (highest individual 0.39 mg/kg). In different fat types, the group mean were at 0.11-0.17 mg/kg (highest individual residues was in subcutaneous fat at 0.28 mg/kg).

In the samples of the depuration group (dose of 150 mg/kg feed) residues declined rapidly to levels near the LOQ after a depuration of 14 days (muscle, liver 0.02 mg/kg, kidney 0.01 mg/kg, fat <0.01 mg/kg). Residues in other dose groups were lower. Group mean residue levels for groups B-E indicate a linear dose dependency, particularly for muscle, liver and fat.

For TA, quantifiable residue levels for groups B, and C, as well as D for fat are comparable to untreated control group A, suggesting that this is a background level coming from ingestion of TA residues present in feedstuff, although no analysis of the feedstuff has been undertaken to confirm this. Dose group E shows a treatment related increase of TA level (in muscle E: 0.178 mg/kg compared to A: 0.03 mg/kg, in liver E: 0.65 mg/kg compared to A: 0.14 mg/kg, in kidney E: 0.19 mg/kg compared to A: 0.04 mg/kg, in perirenal fat E: 0.06 mg/kg compared to A: 0.01 mg/kg), in mesenterial fat E: 0.02 mg/kg compared to A: 0.01 mg/kg), in subcutaneous fat E: 0.06 mg/kg compared to A: 0.02 mg/kg). Upon withdrawal, TA levels rapidly declined within 3 days, yet remaining at levels >LOQ in muscle, liver and kidney for the rest of the depuration phase.

Residues of TLA were < LOQ for all samples. Residues of TAA also were below the LOQ in all samples except kidney where the mean residues were 0.011 mg/kg in the control group and 0.011-0.027 mg/kg in the treated groups, indicating these residues are largely treatment unrelated.

Table 7.4.2-9: Residues of 1,2,4-T in tissues

Tissue	1,2,4-T : residues in mg/kg							
	Group A *(0 mg/kg)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F1**[1)] *(150 mg/kg)*	**Group F1**[2)] *(150 mg/kg)*	**Group F1**[3)] *(150 mg/kg)*
muscle	0.010 0.013 0.015 **(0.013)**	<0.01 <0.01 0.012 **(0.011)**	0.023 0.030 0.030 **(0.028)**	0.098 0.108 0.108 **(0.105)**	0.232 0.288 0.328 **(0.283)**	**0.141** [1]	**0.047** [1]	**0.016** [1]
liver	<0.01 0.012 0.013 **(0.011)**	0.013 0.013 0.016 **(0.014)**	0.027 0.031 0.034 **(0.031)**	0.115 0.122 0.014 **(0.121)**	0.187 0.282 0.301 **(0.257)**	**0.166** [1]	**0.048** [1]	**0.016** [1]
kidney	<0.01 <0.01 0.015 **(0.012)**	<0.01 0.011 0.017 **(0.013)**	0.025 0.027 0.032 **(0.028)**	0.032 0.108 0.117 **(0.086)**	0.193 0.258 0.386 **(0.279)**	**0.139** [1]	**0.044** [1]	**0.014** [1]
fat (perir.)	<0.01 <0.01 0.011 **(0.010)**	**<0.01** [3]	0.011 0.016 0.017 **(0.014)**	<0.01 <0.01 0.028 **(0.016)**	0.065 0.087 0.191 **(0.114)**	**<0.01** [1]	**<0.01** [1]	**<0.01** [1]
fat (mesen.)	<0.01 <0.01 0.052 **(0.024)**	**<0.01** [3]	ND <0.01 0.011 **(0.011)**	<0.01 <0.01 0.035 **(0.018)**	0.011 0.049 0.0254 **(0.105)**	**0.085** [1]	**<0.01** [1]	**<0.01** [1]
fat (subcut.)	**<0.01** [3]	**<0.01** [3]	<0.01 0.020 0.021 **(0.017)**	0.010 0.018 0.065 **(0.031)**	0.088 0.148 0.277 **(0.171)**	**0.040** [1]	**0.010** [1]	**<0.01** [1]

Note, <0.01 denotes residues below LOQ, ND denotes non-detection, "-" denotes non-analysis. [1)] *3 days withdrawal,* [2)] *7 days withdrawal,* [3)] *14 days withdrawal*

Table 7.4.2-10: Residues of TA in tissues

Tissue	TA : residues in mg/kg							
	Group A *(0 mg/kg)*	**Group B** *(1.5 mg/kg)*	**Group C** *(7.5 mg/kg)*	**Group D** *(50 mg/kg)*	**Group E** *(150 mg/kg)*	**Group F1**[1)] *(150 mg/kg)*	**Group F1**[2)] *(150 mg/kg)*	**Group F1**[3)] *(150 mg/kg)*
muscle	0.022 0.023 0.034 **(0.026)**	0.026 0.032 0.058 **(0.038)**	0.031 0.056 0.063 **(0.050)**	0.047 0.055 0.066 **(0.056)**	0.139 0.141 0.255 **(0.178)**	**0.051** [1]	**0.037** [1]	**0.080** [1]
liver	0.097 0.147 0.179 **(0.141)**	0.108 0.154 0.168 **(0.143)**	0.138 0.148 0.216 **(0.167)**	0.201 0.219 0.229 **(0.216)**	0.487 0.691 0.777 **(0.652)**	**0.231** [1]	**0.221** [1]	**0.404** [1]
kidney	0.029 0.034 0.052 **(0.038)**	0.032 0.048 0.050 **(0.043)**	0.030 0.047 0.065 **(0.048)**	0.024 0.050 0.068 **(0.047)**	0.149 0.184 0.231 **(0.188)**	**0.064** [1]	**0.083** [1]	**0.120** [1]
fat (perir.)	<0.01 <0.01 0.018 **(0.013)**	**<0.01** [3]	<0.01 0.011 0.023 **(0.015)**	<0.01 <0.01 0.013 **(0.011)**	0.034 0.049 0.083 **(0.055)**	**<0.01** [1]	**<0.01** [1]	**<0.01** [1]
fat (mesen.)	<0.01 <0.01 0.019 **(0.013)**	**<0.01** [3]	<0.01 0.014 0.020 **(0.015)**	<0.01 <0.01 0.015 **(0.012)**	<0.01 0.015 0.034 **(0.020)**	**0.016** [1]	**<0.01** [1]	**<0.01** [1]
fat (subcut.)	<0.02 0.011 0.030 **(0.017)**	<0.01 <0.01 0.015 **(0.012)**	0.012 0.018 0.037 **(0.022)**	<0.01 0.011 0.023 **(0.015)**	0.051 0.056 0.085 **(0.064)**	**<0.01** [1]	**<0.01** [1]	**0.012** [1]

Note, <0.01 denotes residues below LOQ, ND denotes non-detection, "-" denotes non-analysis.
[1)] 3 days withdrawal, [2)] 7 days withdrawal, [3)] 14 days withdrawal

Conclusion

Milk and tissue samples from cows that had been dosed with BAS 750 F for 28 days were analysed for triazole derived metabolites, for BAS 750 F and for M750F022.

Residues in milk reached a plateau level within 3 days for both BAS 750 F and 1,2,4-triazole. The plateau levels found for the highest dose group (given as group mean, highest individual results in parenthesis) were for BAS 750 F at 0.21 mg/kg (0.35 mg/kg) and 1,2,4-triazole at 0.27 mg/kg (0.33 mg/kg). M750F022 measured at one sampling date representative of the plateau phase of the highest dose (group F, day 21) was quantified at 0.011 mg/kg. Upon withdrawal residues declined rapidly, levels <LOQ (BAS 750 F) and background levels (1,2,4-triazole) were reached in 4 days demonstrating that residues do not accumulate in milk.

In milk of the highest dose group, residues were localized mainly in the cream fraction for BAS 750 F (1.23 mg/kg) and M750F022 (0.099 mg/kg) while for 1,2,4-triazole residues were slightly higher in skim milk (0.24 mg/kg) than for cream (0.23 mg/kg). TAA and TLA were not detected >LOQ , TA where detected, was not detected at a higher level than in the control samples.

At a realistic dose level (group B) residues of BAS 750 F and M750F022 in milk and cream were <0.01 mg/kg. Levels of 1,2.4-T were <0.02 mg/kg in samples dosed at this level, which is not significantly higher than the levels in the control samples. TA, TAA and TLA were not detected >LOQ at this dose level.

Residues in tissues were highest for BAS 750 F in liver, lower in kidney and fat and much lower in muscle. For the highest dose group (E), group mean levels (highest individual) in liver were 3.03 mg/kg (3.58 mg/kg), in kidney 1.29 mg/kg (1.88 mg/kg), in different fat types 0.60-1.71 mg/kg (1.20-

2.29 mg/kg) while in muscle only 0.16 mg/kg (0.22 mg/kg). The same pattern is seen at realistic dose levels (group B) were residues in muscle were not detected >LOQ.

For M750F022 residues were higher in fat compared to liver, kidney and muscle. For the highest dose group (150 mg/kg feed), group mean levels (highest individual) in different fat types were 0.07 - 0.16 mg/kg (0.13 -0.21 mg/kg), in liver and kidney 0.04 mg/kg (0.04 mg/kg), while in muscle only 0.02 mg/kg (0.02 mg/kg). Generally, much higher levels were found for parent BAS 750 F than for its metabolite M750F022. At 5x realistic dose levels (group C) residues above >LOQ were not generally detected, therefore analysis at lower dose levels was not undertaken.

For 1,2,4-triazole, residues were higher in muscle, liver, kidney compared to different fat types. For the highest dose group (150 mg/kg feed), group mean levels (highest individual) in muscle were 0.28 mg/kg (0.33 mg/kg), in kidney 0.28 mg/kg (0.39 mg/kg), in liver 0.26 mg/kg (0.30 mg/kg) while in different fat types level were 0.11 - 0.17 mg/kg (0.19 - 0.28 mg/kg). The same pattern is seen at realistic dose levels (group B) were residues in fat were not detected >LOQ, and residues in muscle, liver and kidney were <0.015 mg/kg.

For TA, residues were highest in liver, lower in muscle and kidney and lowest in different fat types. For the highest dose group (150 mg/kg feed), group mean levels (highest individual) in muscle were 0.178 mg/kg (0.255 mg/kg), in kidney 0.188 mg/kg (0.231 mg/kg), in liver 0.652 mg/kg (0.777 mg/kg) while in different fat types level were 0.02 - 0.064 mg/kg (0.034 - 0.085 mg/kg). The same pattern is seen at realistic dose levels (group B), as well as in the control group (A), where mean residues in fat were >LOQ, mean residues in muscle and kidney were <0.05 mg/kg and mean residues in liver were <0.15 mg/kg. TAA and TLA were not detected >LOQ in any sample (except TAA in kidney where dose unrelated levels of <0.02 mg/kg were determined in all samples).

Upon withdrawal, residues declined to levels below or near the LOQ demonstrating absence of accumulation for BAS 750 F (after 7 days for muscle, kidney and after 14 days for liver, and perirenal/mesenterial fat) as well as for M750F022 (after 3 days for muscle and kidney, after 7 days for liver, and after 14 days for perirenal/mesenterial fat). In subcutaneous fat residues also declined, with levels near the LOQ for both BAS 750 F and M750F022 (0.023 mg/kg) reached after 14 days of withdrawal.

The relative amounts of BAS 750 F and its metabolites (M750F022 and TDM) are seen most clearly in the highest dose group, although comparable relative amounts are seen in the lower dose groups as a result of the observed linear dose-dependency for all three analytes (with the exception of TA where largely dose independent residues were observed). Table 7.4.2-11 shows the mean residues of BAS 7450 F, M750F022, 1,2,4-T and TA in both the highest dose group (E) and the group dosed at realistic levels (B).

Table 7.4.2-11: Overview tissue distribution for BAS 750 F, M750F022, 1,2,4-triazole and TA in groups B (1.5 mg/kg feed) and E (150 mg/kg feed) (mean values)

Commodity	BAS 750 F		M750F022		1,2,4-T		TA	
	Group B *(mg/kg)*	Group E *(mg/kg)*	Group B *(mg/kg)*	Group E *(mg/kg)*	Group B *(mg/kg)*	Group E *(mg/kg)*	Group B *(mg/kg)*	Group E *(mg/kg)*
milk [1)]	<0.01	0.21	-	0.02	0.015	0.27	<0.01	0.011
cream [2)]	<0.01	1.23	<0.01	0.09	0.011	0.23	<0.01	<0.01
skimmilk[2)]	<0.01	0.07	-	<LOQ	0.016	0.24	<0.01	0.022
muscle	<0.01	0.16	<0.01[3)]	0.02	0.011	0.28	0.038	0.178
liver	0.031	3.03	<0.01[3)]	0.04	0.014	0.26	0.143	0.652
kidney	0.012	1.29	<0.01[3)]	0.04	0.013	0.28	0.043	0.188
fat peri.	0.017	1.71	<0.01[3)]	0.16	<0.01	0.11	<0.01	0.055
fat mesen.	0.018	1.16	0.01[3)]	0.13	<0.01	0.11	<0.01	0.020
fat subcut.	0.015	0.59	<0.01[3)]	0.07	<0.01	0.17	0.012	0.064

[1)] plateau level [2)] taken during plateau phase [3)] Determined for group C (7.5 mg/kg feed) as no determinations made for group B

In conclusion, BAS 750 F residues present in feed items are transferred to ruminant commodities showing a linear dose-residue-dependency. Residues in milk reach plateau level indicating absence of accumulation for parent BAS 750 F, its metabolite M750F022 and 1,2,4-triazole. Residues in milk and tissues decline rapidly after dose withdrawal.

B.7.4.3. Pigs

No study is required for pig. Estimations on magnitude of residues in commodities of pig can be based on the feeding study in ruminants.

B.7.4.4. Fish

A fish feeding study is not required. Currently no test method or guidance document is available. As a consequence waiving of this particular data requirement is considered acceptable according to the "Guidance document for applicants on preparing dossiers for the approval of a chemical new active substance and the renewal of the chemical active substance according to regulation (EU) No. 283/2013 and regulation (EU) No. 284/2013" (SANCO/10181/2013-rev.2 of 2-May-2013).

B.7.4.5. Conclusions on the magnitude of residues in animals

The maximum animal burden is greater than >0.004 mg/kg bw/day for all animals (except breeding pig), therefore feeding studies were undertaken on poultry and ruminants.

Laying hens were dosed with BAS 750 F at nominal doses of 0.15, 1.5, 4.5 and 15.0 mg/kg feed (predicted dietary burden in laying hens 2.2 mg/kg DM) over a period of 34 days. Eggs and tissue samples were analysed for BAS 750 F, M750F022 and TDMs. The study demonstrated that BAS 750 F residues present in feed items were transferred to poultry commodities showing a linear dose-residue-dependency. Residues in eggs reached a plateau level within 10 days indicating absence of accumulation for parent BAS 750 F, its metabolite M750F022 and 1,2,4-triazole. Residues in eggs and tissues declined rapidly within a few days after withdrawal of the dose.

Lactating cows were dosed with BAS 750 F at nominal doses of 1.5, 7.5, 50 and 150 mg/kg feed (predicted dietary burden in cattle 6.2 mg/kg DM) over a period of 28 days. Milk and tissue samples were analysed for BAS 750 F, M750F022 and TDMs. The study demonstrated that BAS 750 F residues present in feed items were transferred to ruminant commodities showing a linear dose-residue-dependency. Residues in milk reached a plateau level indicating absence of accumulation for parent BAS 750 F, its metabolite M750F022 and 1,2,4-triazole. Residues in milk and tissues declined rapidly after dose withdrawal.

The residue levels determined in the feeding studies have been used to determine the STMR, HR and MRL for animal commodities using the Excel calculator proposed by EFSA.

Calculations for poultry must account for both the different residue definitions for risk assessment and monitoring, as well as the matrix specific conversion factors for fatty acid conjugates of M750F022.

Residues of BAS 750 F and M750F022 at the four feeding levels were taken from the feeding study. The values for BAS 750 F are used directly for the calculations based on the residue definition monitoring (parent BAS 750 F only). For calculation of the values for the residue definition for risk assessment (sum of parent BAS 750 F, metabolite M750F022 and fatty acid conjugates of M750F022, expressed in parent equivalents) the following process was used:

- Conversion M750F022 residue data to BAS 750 F equivalents based on molecular weight (x1.15).
- Multiplication of M750F022 residue data (as mg/kg BAS 750 F equivalents) with a matrix-specific correction factor to account for the amounts of fatty acid-conjugates of M750F022 (4X (fat), 1.5X (muscle, egg), and 1X (liver))
- Summation of calculated M750F022 residue data with BAS 750 F residue data
- Derivation of conversion factor (CF) using Excel calculator based on RD-MO and RD-RA

The STMR and HR estimations and MRL proposals are given in Table 7.4.3-1. The conversion factors calculated are 6.2 for muscle, 16.3 for fat, and 4.9 for liver and egg.

Table 7.4.3-1: Poultry commodities: MRL proposal and conversion factor

Poultry									
Closest level 0.096 mg/kg bw [1)] 0.6 N Layer 12.9 N Broiler		Residues at closest level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)	CF	**STMR (mg/kg)**	**HR (mg/kg)**
		Mean	Highest	$STMR_{Mo}$ (mg/kg)	HR_{Mo} (mg/kg)				
	Meat [2)]	-	-	0.01	0.01	-	-	**0.07**	**0.10**
	Muscle	0.01	0.01	0.01	0.011	0.015	6.2	**0.06**	**0.07**
	Fat	0.01	0.01	0.01	0.022	0.03	16.3	**0.16**	**0.36**
	Liver [3)]	0.01	0.017	0.011	0.026	0.03	4.9	**0.05**	**0.13**
	Egg	0.01	0.01	0.01	0.011	0.015	4.9	**0.05**	**0.05**

[1)]1.5 mg/kg DM (dose group C) [2)] *STMR and HR for meat calculated based on composition of10% fat and 90 % muscle as BAS 750 F and M750F022 are fat soluble,* [3)] *Also used for poultry kidney*

Calculations for ruminants (and swine) are more straightforward than for poultry as the proposed residue definition for both monitoring and risk assessment is parent BAS 750 F only. The STMR and HR estimations and MRL proposals are given in Table 7.4.3-2. Data for all three fat types was entered separately as significantly different residue data was observed for the three fat types, the 'fat' STMR, HR and MRL in the table below is taken from the fat type which gave the highest levels.

Table 7.4.3-2: Ruminant and swine commodities: MRL proposals

Closest level	Residues at the closest feeding level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)
	Mean	Highest	STMR (mg/kg)	HR (mg/kg)	
Bovine					
0.192 mg/kg bw [2)]					
0.8 N Dairy C.					
1.3 N Beef C.					
Meat[1)]	-	-	0.024	0.065	-
Muscle	0.010	0.010	0.014	0.032	**0.04**
Fat (perineal)	0.049	0.059	0.062	0.197	**0.2**
Liver	0.150	0.182	0.088	0.337	**0.4**
Kidney	0.048	0.074	0.021	0.105	**0.1**
Milk	0.010	0.010	0.010	0.017	**0.02**
Fat per	0.049	0.059	0.062	0.197	0.2
Fat Sub.	0.023	0.041	0.026	0.080	0.08
Fat Mes.	0.053	0.077	0.063	0.184	0.2
Sheep					
0.192 mg/kg bw [2)]					
0.4 N Lamb					
0.5 N Ewe					
Meat[1)]	-	-	0.032	0.118	-
Muscle	0.010	0.010	0.017	0.052	**0.06**
Fat (perineal)	0.049	0.059	0.092	0.384	**0.4**
Liver	0.150	0.182	0.141	0.652	**0.7**
Kidney	0.048	0.074	0.033	0.248	**0.3**
Milk	0.010	0.010	0.010	0.026	**0.03**
Fat per	0.049	0.059	0.092	0.384	0.4
Fat Sub.	0.023	0.041	0.019	0.328	0.4
Fat Mes.	0.053	0.077	0.083	0.350	0.4
Swine					
0.034 mg/kg bw [3)]					
7.9 N Finishing					
10.3N Breeding					
Meat[1)]	-	-	0.002	0.026	-
Muscle	0.010	0.010	0.000	0.000	**0.01***
Fat (perineal)	0.015	0.017	0.002	0.002	**0.01***
Liver	0.031	0.034	0.004	0.004	**0.01***
Kidney	0.012	0.014	0.002	0.002	**0.01***
Milk	0.017	0.018	0.002	0.002	**0.01***
Fat per	0.015	0.017	0.002	0.002	0.01*
Fat Sub.	0.018	0.018	0.002	0.002	0.01*
Fat Mes.	0.010	0.010	0.010	0.000	0.01*

[1)] *STMR and HR for meat calculated based on composition of 20% fat and 80 % muscle as BAS 750 F and M750F022 are fat soluble,* [2)]7.5 mg/kg DM (dose group C), [3)]1.5 mg/kg DM (dose group B)

B.7.5. EFFECTS OF PROCESSING

B.7.5.1. Nature of the residue

Report: CA 6.5.1/1
Hassink J.,Bartmann S., 2014 a
BAS 750 F: Hydrolysis at 90°C, 100°C and 120°C
2014/1170665

Guidelines: EEC 7035/VI/95 rev. 5, OECD 507 - Nature of the residues in processed commodities - High temperature hydrolysis

GLP: yes

Materials and methods

A standard hydrolysis study was performed with ^{14}C-BAS 750 F labelled either at the chlorophenyl ring (C-label 99.3% purity) or at the triazole ring (T-label 97.7% purity). Different processes (pasteurisation, baking, brewing, boiling, and sterilisation) were simulated in order to investigate any potential degradation of BAS 750 F during industrial processing or household preparation.

The ^{14}C-labeled test substance was dissolved in aqueous buffer solutions of different pH values and heated according to the parameters given in Table 7.5.1-1. Samples were prepared by dissolving 0.25 mg of ^{14}C-labelled BAS 750 F in 250 mLs (1 mg/L) of pH 4, 5 or 6 buffer solution. In each case the pH was checked before and after processing and found to be constant.

Table 7.5.1-1: Conditions tested in the standard hydrolysis study

Temperature (°C)	pH	Time (min)	Simulated processing procedure
90	4	20	pasteurisation
100	5	60	baking, brewing, boiling
120	6	20	sterilisation (in the dark)

Total radioactivity was determined by liquid scintillation counting (LSC), composition of the radioactive residue was analysed directly by HPLC (radio detection) without any prior work-up. Two HPLC systems were used:

System 1 was used for chromatographic confirmation of radiochemical purity prior to incubation as well as for analysis of chromatographic profile subsequent to incubation (investigation of potential degradation products).

System 2 was used for the chiral separation of the two enantiomers of BAS 750 F. Samples were cleaned-up by solid phase extraction. The C18 SPE cartridges were loaded with 5 ml of each sample, washed with 1 ml water and eluted with 5 mL ethanol. 1 ml of the eluate was evaporated under nitrogen to dryness and dissolved in 1 ml n-Heptane/ethanol/2-propanol (500 + 25 + 25) for HPLC injection.

Results and Discussion

Total radioactivity before and after incubation were similar, indicating absence of major loss of radioactivity (see Table 7.5.1-2). Before and after incubation, BAS 750 F accounted for almost all of the radioactivity, indicating absence of any degradation product at levels of 2% TAR or higher.

Table 7.5.1-2: BAS 750 F levels before and after incubation

	T-label			C-label		
	pH 4, 90°C (20 min)	**pH 5, 100°C (60 min)**	**pH 6, 120°C (20 min)**	**pH 4, 90°C (20 min)**	**pH 5, 100°C (60 min)**	**pH 6, 120°C (20 min)**
	Total Radioactivity (TAR)					
	% TAR	**% TAR**	**% TAR**	**% TAR**	**% TAR**	**% TAR**
total (prior to treatment) [1)]	100.0	100.0	100.0	100.0	100.0	100.0
total (post treatment)	110.3	110.2	105.2	110.2	108.3	110.1
BAS 750 F	110.3	110.2	103.8	107.9	107.1	107.3
unknown [2)]	n.d.	n.d.	1.4	2.3	1.2	2.8

[1)] actual concentration of each sample was determined by LSC before each incubation to use as value for 100% TAR.
[2)] sum of unknown peaks, each < 2% TAR

The relative amounts of S-enantiomer and R-enantiomer before and after incubation were similar, indicating absence of any significant change of the enantiomer ratio upon incubation at hydrolytic conditions (see Table 7.5.1-3).

Table 7.5.1-3: Enantiomer ratio of BAS 750 F before and after treatment

	T-label						C-label					
	BAS 750 F *%TAR*				**others** *%TAR*	**sum** [1)] *%TAR*	**BAS 750 F** *%TAR*				**others** *%TAR*	**sum** [1)] *%TAR*
	R [2)]	**S**[2)]	**sum**	**R:S**	-	-	**R**[2)]	**S**[2)]	**sum**	**R:S**	-	-
before treatment												
pH 4, 90°C	51.2	48.8	100.0	1.05	-	100.0	49.1	50.1	99.2	0.98	0.8	100.0
pH 5, 100°C	49.6	50.4	100.0	0.98	-	100.0	50.1	48.7	98.8	1.03	1.2	100.0
pH 6, 120°C	50.1	49.9	100.0	1.00	-	100.0	48.4	50.2	98.5	0.96	1.5	100.0
after treatment												
pH 4, 90°C	54.4	55.1	109.5	0.99	0.7	110.2	55.0	54.5	109.5	1.01	0.7	110.2
pH 5, 100°C	55.7	54.5	110.2	1.02	-	110.2	52.9	55.4	108.3	0.95	-	108.3
pH 6, 120°C	53.3	51.9	105.2	1.03	-	105.2	55.4	54.0	109.4	1.03	0.7	110.1

[1)] The actual concentration of each sample was determined by LSC before each test and set to 100 % TAR.
[2)] R denotes R enantiomer (no. 5934591), S denotes S-enantiomer (no. 5434588)

Conclusion

Under conditions representative of pasteurisation (pH 4, 90 °C, 20 min), baking, boiling, brewing (pH 5, 100 °C, 60 min) and sterilisation (pH 6, 120 °C, 20 min) BAS 750 F is stable. Notably, no degradation product exceeding 2% of total radioactivity was detected. Also, no change in the isomer ratio was observed. In conclusion, as BAS 750 F can be regarded as stable to hydrolysis, the nature of the residue is not affected by processing operations.

Stability of TDMs under high temperature hydrolysis has not been considered in this dossier; however it was investigated in the TDM review (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015), whereupon they were found to be stable, see section 7.5.4 for further discussion.

B.7.5.2. Distribution of the residue in peel and pulp

The representative uses to be evaluated in this dossier (cereals) are crops with no peel or edible peel only. Therefore, studies on the distribution between peel and pulp are not required.

B.7.5.3. Magnitude of residues in processed commodities

Report:	CA 6.5.3/1 Plier S.,Elze M., 2015 a Determination of residues of BAS 750 F (Reg.No. 5834378) in wheat and its processed products after two applications of BAS 750 01 F in Germany, 2014 2014/1315283
Guidelines:	OECD 508 Magnitude of the Pesticide Residues in Processed Commodities (2008), OECD 509 Crop Field Trial (2009), OECD Series on Testing and Assessment No. 96 (2008) - Magnitude of Pesticide Residues in Processed Commodities, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7035/VI/95 rev. 5, BBA IV 3-3, BBA IV 3-4, IVA Guideline IA-III (1992)
GLP:	yes

Materials and methods

A processing study on wheat (variety *Triticum aestivum*) was conducted outdoors at three locations in Germany during the 2014 growing season. Normal agricultural practices were followed, and no unusual weather events were recorded. Each field trial included one treated plot. In addition, an application-free control plot was also included in one of the three field trials. Two foliar applications of BAS 750 F (10% EC formulation) were made to each of the treated plots at a target rate of 0.45 kg ai/ha to obtain 3x the maximum per season rate in the GAP. The applications were made at crop growth stages BBCH 49 and BBCH 69 using a spray volume of 200 L/ha. Samples were taken at the following time points:

- DALA 0 (plants at BBCH 69, application control)

- DALA 7-9 (plants at BBCH 71, sub-samples processed into silage)

- DALA 45-60 (grain at BBCH 89)

The processing of wheat was conducted using sub-samples taken from grain (BBCH 89, DALA 45-60) as well as plants (BBCH 71, DALA 7-9). The following fractions of wheat were generated following industrial processing procedures at a laboratory scale: wet silage, wilted silage, bran, flour, germ, middlings, shorts, gluten, gluten feed meal, starch, whole meal flour, whole grain bread, milled by products and aspirated grain fraction. The processing was carried out in the following stages.

Silage - For wilted silage production the fresh harvested whole plants of the wheat were dried in the field (sunny weather) or in a dry oven at 35 °C until the dry matter content of the wheat reached 35-55 %. The fresh wheat for wet silage production and the dried wheat for wilted silage production were placed in silage glass containers (under pressure). The glass containers were closed and stored at 20-25 °C for approx. 6 weeks. *Wet silage* and *wilted silage* were sampled.

Cleaning - Each of the grain samples for processing was cleaned. *Aspirated grain fraction* was sampled. Therefore, the cleaned grain samples for processing which had no optimal moisture content were moistened to 15-16% by addition of tap water if necessary.

Milling to flour - The grain was milled to straight flour, bran and middlings in a closed system with different pairs of smooth rollers and sifter passages. Samples of *bran* and *middlings* were taken. In a further processing step bran and middlings were mixed together and low grade meal was separated using a centrifuge/scouring machine. This process resulted in shorts and low grade meal. Samples of *shorts* were taken. The mineral content of straight flour was in the range of 510-630 g/100 kg. Therefore the flour was not mixed with low grade meal. Samples of *flour* were taken.

Milling to wholemeal flour and baking - For the generation of whole-meal flour and whole-meal bread the same milling procedure as used for the production of flour was used. After milling the shorts were cracked with an impact mill to smaller pieces. All milling products of the process were used completely for the whole-meal and mixed homogeneously in a special flour mixer. Samples of the *whole-meal flour* were taken.

For baking an 1.0 kg whole-meal bread, whole-meal (approx. 1.3 kg), yeast (approx. 52 g), salt (approx. 26 g) and water (approx. 0.9 L) were mixed. Subsequently, the resulting dough was kneaded for 7 min. After kneading the dough fermented for 20 min. It was then reprocessed for 5 min and a second rest for fermentation followed (40 min in a baking tin). The baking process was conducted at 210 to 230°C for 50 min. Sample of *whole-grain bread* were taken.

Wheat germ - The grain was broken to bruised grain in a special mill using 0.2-0.5 mm roller distances). The fraction 400-1000 µm was collected and the fraction above 1000 µm was broken again. This milling/sieving process was performed in total three times. The fractions obtained <400 µm and > 1000 µm were excluded from further processing.

The fraction 400-1000 µm, a mixture of bran, middlings and germs, was separated by weight to give a middlings/germ mixture and the bran. The bran was retained for mixing to the milled by-products. The middlings/germ mixture was milled to flour, bran and small wheat germ discs. This product was then sieved to give a flour and a bran/germ fraction. The flour was retained for mixing to the specimen milled by-products. The bran/germ fraction was sieved once again to fine bran/germ fraction and coarse bran/germ fraction. Samples of *germ* were taken. The bran was mixed with the other fractions which were retained for the specimen milled by-products. *Milled by-products* were sampled.

Starch and gluten - The first step of the production of starch and gluten was milling the grain to straight flour, bran and middlings. straight flour and water were mixed to obtain a hydrated dough. The dough was separated by centrifugation into wet starch, water and gluten (containing starch). Subsequently, the starch was washed out with water and separated by centrifugation into starch , water and gluten. This process was repeated.

The gluten (containing starch) was washed out to produce gluten and water (containing starch and fibre). This process was repeated. The water was separated by centrifugation to give starch, fibre (containing starch) and water. Subsequently fibre was washed out to give starch, fibre and water. Fibre and wet starch were dried at 60 °C and wet gluten was dried by freeze drying. After the drying process the dried products were milled. *Starch* and *gluten* samples were collected. The dried and milled fibre, starch and gluten were mixed to produce gluten feed meal. Samples of *gluten feed meal* were taken.

Details of how each processed fraction are produced are given in Figures 7.5.3-1 to 7.5.3-4. The mass of each processed fraction is given in Table 7.5.3-1.

The RAC (raw agricultural commodity) samples were stored frozen at ≤-18°C with 12 hours of harvest. Samples for processing were stored at ambient temperature prior to processing (1 day for whole plant samples/up to 4 months for grain samples), and then frozen at ≤-18°C immediately afterwards. Samples were stored frozen for up to 344 days. Sufficient storage stability is available (see section 7.1.1) to support frozen storage for this period.

Figure 7.5.3-1 Processing of wheat plant to silage

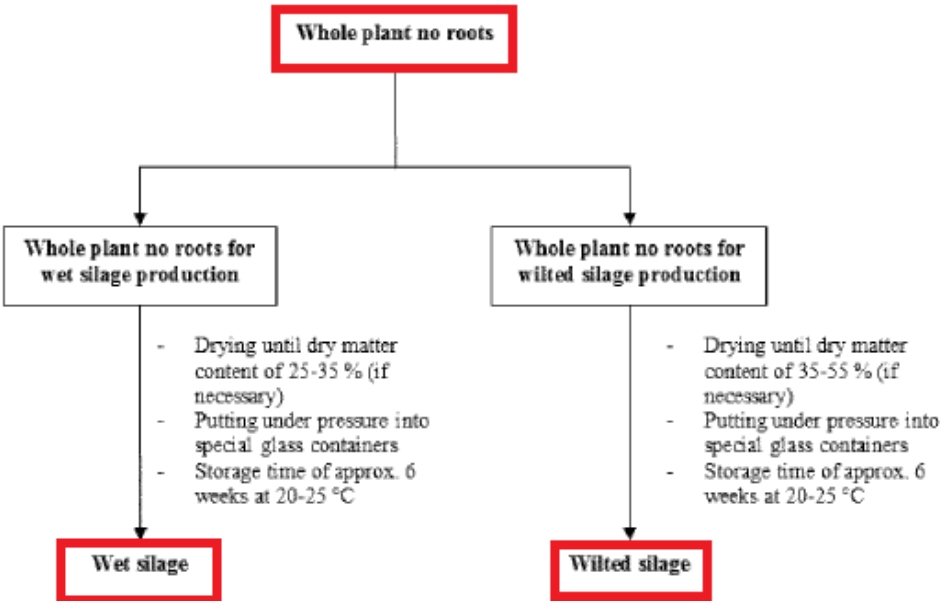


Figure 7.5.3-2 Processing of wheat grain (part 1)

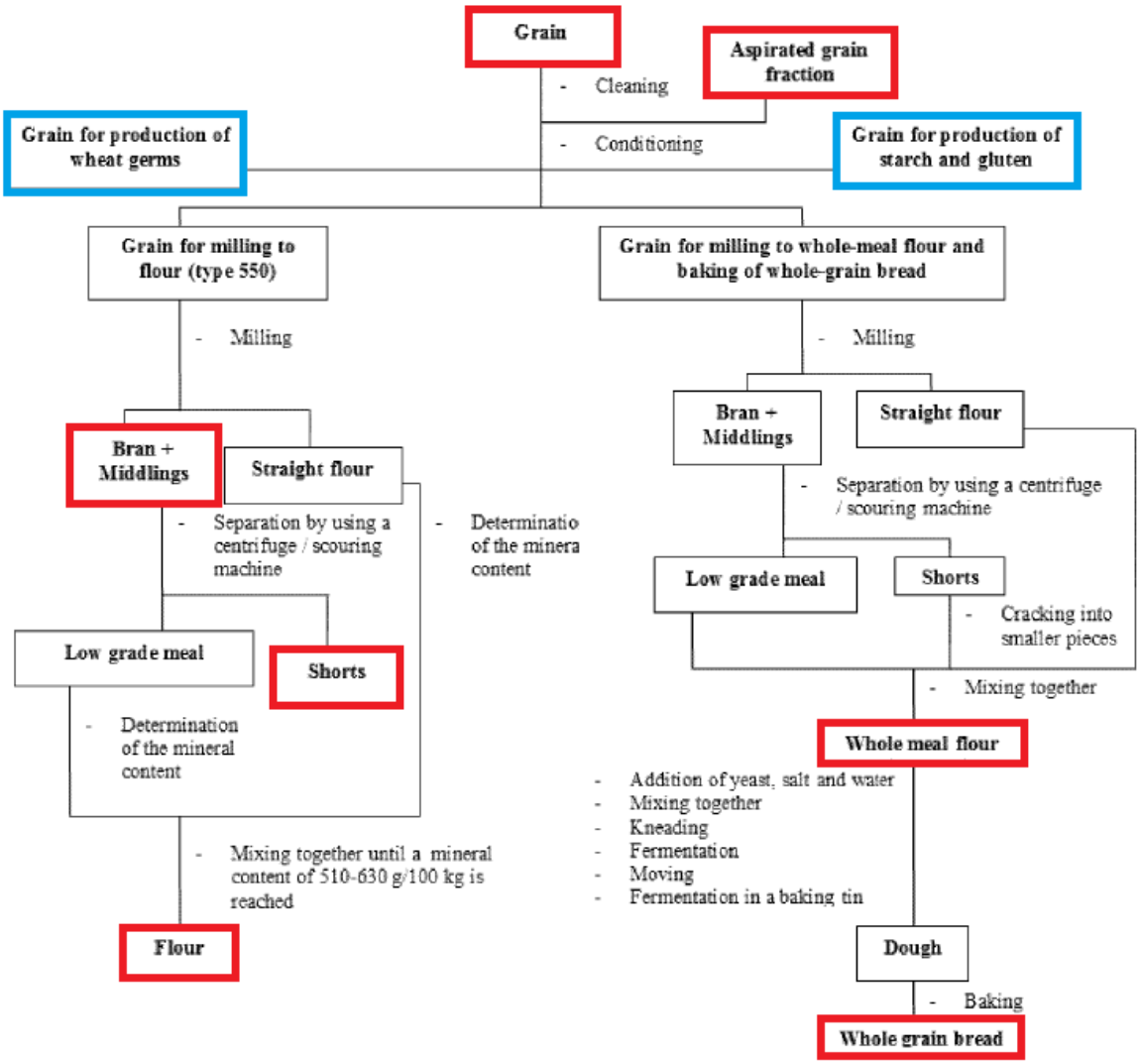


Figure 7.5.3-3 Processing of wheat grain (part 2)

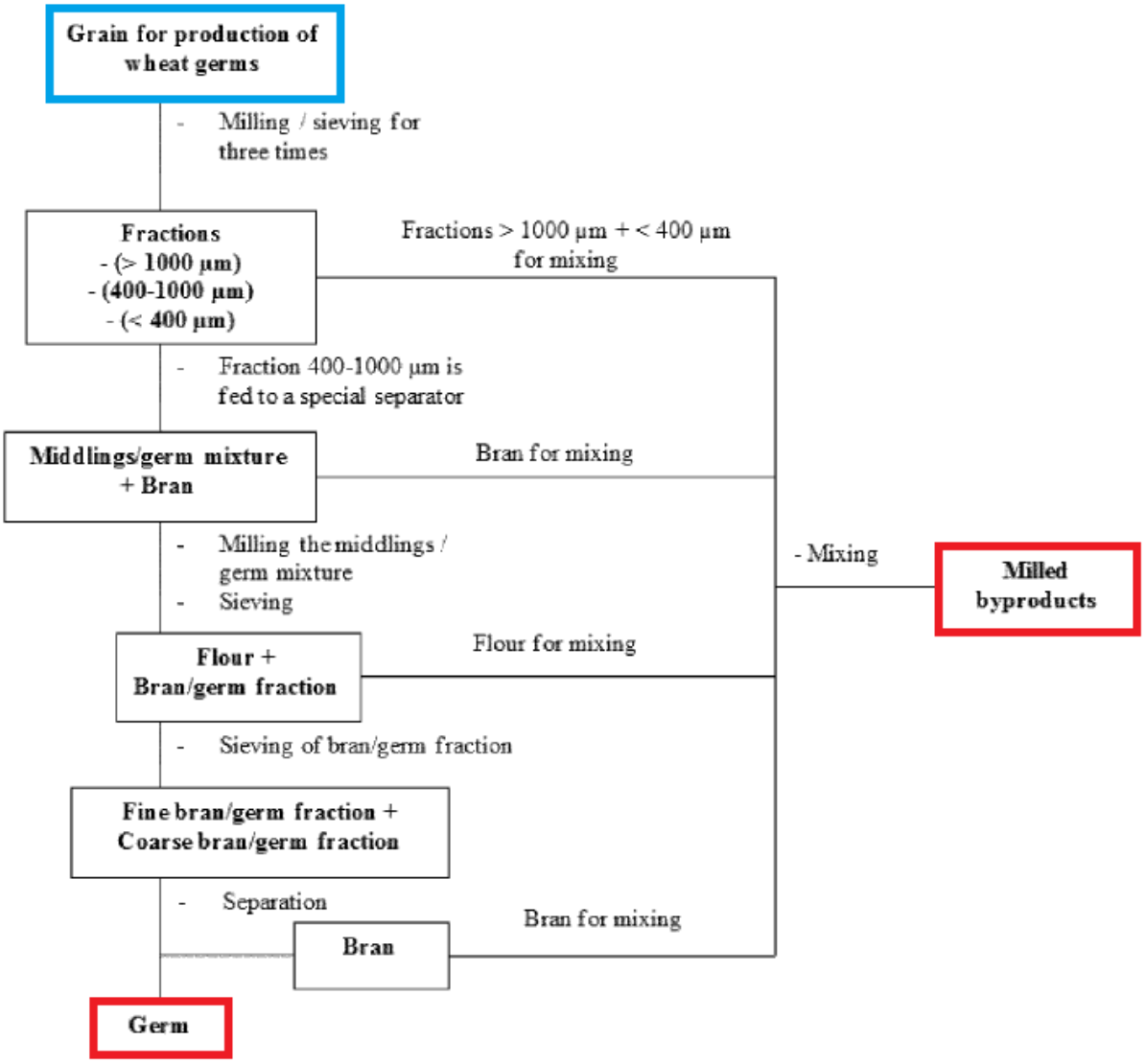


Figure 7.5.3-4 Processing of wheat grain (part 3)

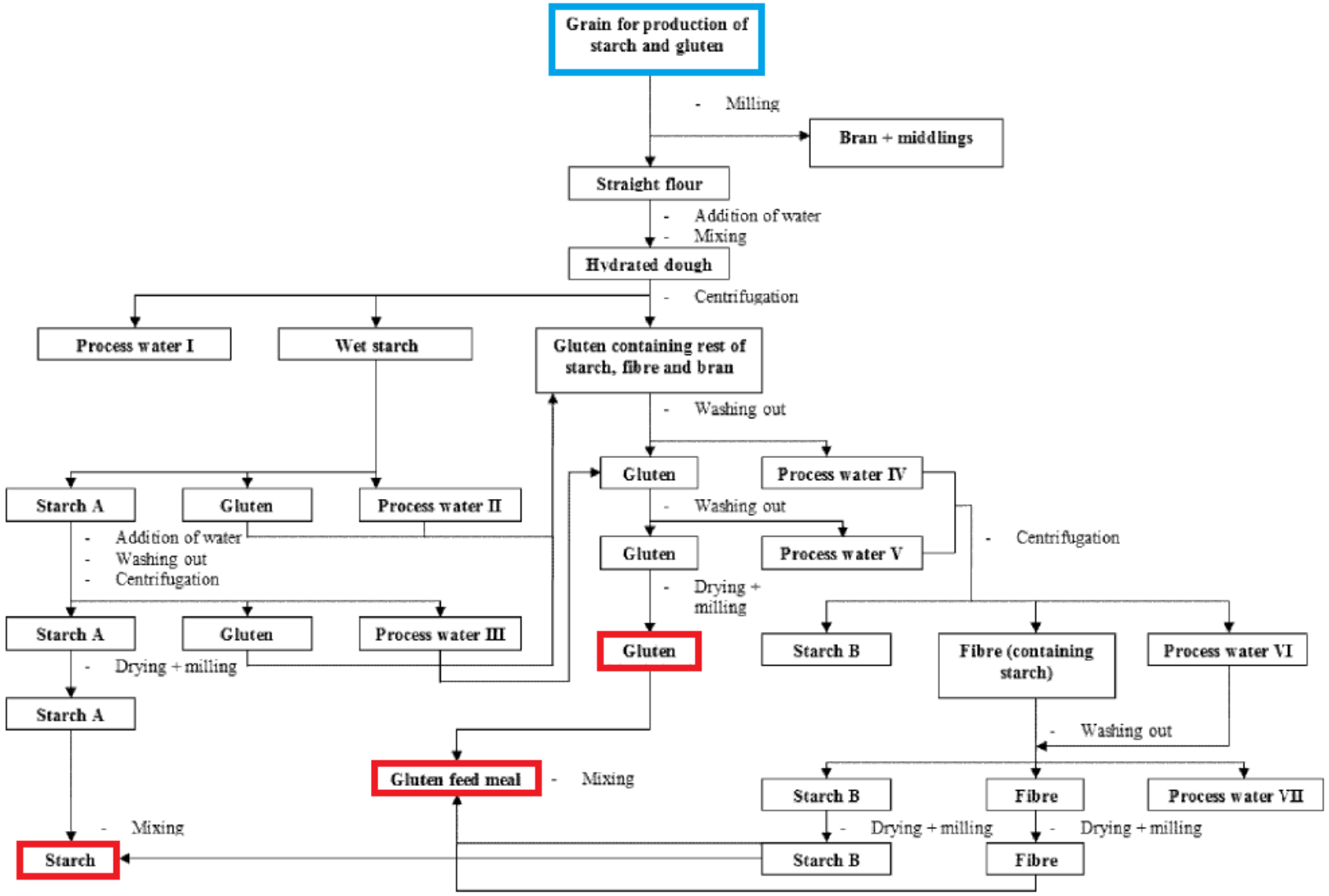


Table 7.5.3-1 Weight of processed fractions sampled for each trial

Processed fraction	Sample weight (kg)			
	Trial 1 (L140181)	**Trial 2 (L140182)**	**Trial 3 (L140183)**	**Mean**
whole plant (no roots)	0.53	0.53	0.53	0.53
wet silage	1.95	1.78	2.11	1.95
wilted silage	1.71	1.54	1.68	1.64
grain	0.54	0.56	0.67	0.59
bran	0.21	0.22	0.22	0.22
flour	1.02	1.02	1.01	1.02
germ	0.01	0.01	0.01	0.01
middlings	0.2	0.2	0.2	0.20
shorts	0.21	0.18	0.13	0.17
gluten	0.05	0.05	0.05	0.05
gluten feed meal	0.1	0.1	0.1	0.10
starch	0.1	0.1	0.1	0.10
whole meal flour	0.41	0.21	0.24	0.29
whole grain bread	1.39	1.35	1.32	1.35
milled by products	0.28	0.27	0.26	0.27
aspirated grain fraction	0.12	0.13	0.11	0.12

The level of residues in each processed fraction was determined for BAS 750 F (BASF method L0076/09, LOQ of 0.01 mg/kg) and for the triazole derivative metabolites (TDM) 1,2,4-T, TA, TAA, TLA (BASF method L0170/02, LOQ of 0.01 mg/kg for each of the four analytes). Full details of sample preparation and validation data for these methods is given in section CA B.5.1.2.5. A brief summary of the methods is outlined below. Details of the procedural recoveries are given in Table 7.5.3-2 and 7.5.3-3.

Principle of the method L0076/09: The analyte is extracted with methanol/water/HCl (70/25/5, v/v/v). After extraction with cyclohexane, an aliquot is concentrated, dissolved in methanol/water (50/50, v/v) and transferred into an autosampler vial for LC-MS/MS analysis (method validated for BAS 750 F analysis in plant matrices).

Principle of the method L0170/02: The analytes are extracted with methanol/water (4/1, v/v), an aliquot is filtered, concentrated and cleaned-up by a simple dispersive C18-SPE-step. The analytes are determined by LC-DMS/MS/MS (method validated for TDM analysis in plant matrices, i.e. 1,2,4-T, TA, TAA, TLA).

Table 7.5.3-2: Recoveries for BAS 750 F in wheat processed fractions

Processed fraction	**Fortification level (mg/kg) /recovery (%)**				**Precision/recovery**			
	0.01	**1**	**5**	**50**	**Mean**	**SD**	**RSD**	**n**
whole plant	85.8	-	-	77.8	81.8	-	-	2
wet silage	76.0	-	-	88.3	82.1	-	-	2
wilted silage	85.8, 89.0, 92.5	-	-	90.0, 94.5, 94.5	91.0	3.4	3.8	6
grain	91.5	86.8	-	-	89.1	-	-	2
bran	75.3	-	84.3	-	79.8	-	-	2
flour	79.3	91.0	-	-	85.1	-	-	2
germ	97.0	-	85.0	-	91.0	-	-	2
middlings	82.8	89.5	-	-	86.1	-	-	2

Processed fraction	Fortification level (mg/kg) /recovery (%)				Precision/recovery			
	0.01	1	5	50	Mean	SD	RSD	n
shorts	96.0	93.3	-	-	94.6	-	-	2
gluten	87.8	89.5	-	-	88.6	-	-	2
gluten feed meal	76.0, 84.3, 89.5	84.5, 84.5, 90.0	-	-	84.8	5	5.9	6
starch	89.3	95.3	-	-	92.3	-	-	2
whole meal flour	89.3	81.5	-	-	85.4	-	-	2
whole grain bread	69.8, 70.0, 72.5	82.3, 92.5, 94.5	-	-	80.3	11	14	6
milled by products	88.5	-	86.0	-	87.3	-	-	2
aspirated grain	85.8	-	83.3	-	84.5	-	-	2

Table 7.5.3-3: Recoveries for TDMs in wheat processed fractions

Processed fraction	Fortification level (mg/kg)				Precision/recovery			
	0.01	1	2	4	Mean	SD	RSD	n
1,2,4-Triazole								
whole plant	90.0, 94.2	95.6, 103			95.7	5.4	5.7	4
wet silage	103, 108	88.5, 88.9			97.1	9.9	10	4
wilted silage	78.3, 97.9, 103, 115	79.2, 92.1, 95.9, 98.5			95.0	12	13	8
grain	85.3, 116	88.8, 105			98.8	14	15	4
bran	116	106	109		110	5.1	4.7	3
flour	80.5, 85.5	78.0, 94.5			84.6	7.3	8.6	4
germ	96.5		65.5		81.0			2
middlings	109, 114	94.8	92.1		102	11	10	4
shorts	119			103	111			2
gluten	69.6	90.4, 91.6			83.9	12	15	3
gluten feed meal	79.8, 81.5, 87.1, 90.4	78.3, 79.5, 82.5, 84.2			82.9	4.1	5.0	8
starch	83.0, 103	85.9, 101			93.2	10	11	4
whole meal flour	96.7, 108	97.3, 108			103	6.4	6.2	4
whole grain bread	110 111, 113, 124	103, 105, 106, 119			111	7.2	6.5	8
milled by products	103, 104	109			105	3.1	2.9	3
aspirated grain	97.5, 110	97.0			102	7.4	7.3	3
TAA								
whole plant	89.0, 105	110, 115			105	11	11	4
wet silage	80.0, 80.0	112, 115			96.8	19	20	4
wilted silage	70.0, 80.0, 90.0	90.0, 105, 108, 109			93.1	15	16	7
grain	100	76.6			88.3			2
bran	90		70.1		80.0			2
flour	90.0, 100	79.7, 81.4			87.8	9.3	10.6	4
germ	110		73.1		91.5			2
middlings	90.0	64.2	88.6		80.9	14	18	3
shorts	90.0			68.6	79.3			2
gluten	90.0, 110	99.2, 103			101	8.4	8.3	4
gluten feed meal	80.0, 90.0, 100, 110	86.2, 88.8, 90.0, 93..0			92.3	9.1	9.9	8
starch	76.4, 78.1	62.6			72.4	8.5	12	3
whole meal flour	80.0, 110	87.3, 87.3			91.2	13	14	4
whole grain bread	80.0, 80.0, 80.0, 90.0	66.5, 68.6, 71.0, 74.3			76.3	7.7	10	8

Processed fraction	Fortification level (mg/kg)				Precision/recovery			
	0.01	1	2	4	Mean	SD	RSD	n
milled by products	100, 100	66.9, 74.4			85.3	17	20	4
aspirated grain	80.0, 110	78.3			89.4	18	20	3
TLA								
whole plant	101	87.9, 105			98.0	8.9	9.1	3
wet silage	120	99.0, 109			109	11	9.6	3
wilted silage	80.0, 90.0, 100	92.6, 109, 114, 114			99.8	13	13	7
grain	70.2, 95.9	70.6, 71.6			77.1	13	16	4
bran	104	91.3	78.1		91.1	13	14	3
flour	82.5, 88.5	65.2, 67.2			75.9	11	15	4
germ	116		78.4		97.2			2
middlings	89.9, 111	79.4	85.0		91.3	14	15	4
shorts	112			71.3	91.7			2
gluten	88.0, 98.4	81.6, 87.3			88.8	7.0	7.9	4
gluten feed meal	63.0 76.0, 78.0, 79.0	71.5, 75.0, 77.1, 79.9			74.9	5.5	7.3	8
starch	108, 115	88.7, 90.5			101	13	13	4
whole meal flour	72.0, 91.7	72.2, 93.0			82.2	12	14	4
whole grain bread	86.0, 93.1, 108	86.4, 86.9, 92.9, 97.0			92.9	7.9	8.5	7
milled by products	83.2, 106	74.2, 87.1			87.6	13	15	4
aspirated grain	89.0, 114	90.9			98.0	14	14	3
TA								
whole plant	110	87.8, 112			103	13	13	3
wet silage	70.0, 90.0	106, 114			94.7	19	20	4
wilted silage	90.0, 100, 110	89.7, 102, 103			99.0	7.9	8.0	6
grain	110	79.5			94.8			2
bran	80.0	60.8			70.4			2
flour	100, 111	98.5, 106			104	5.8	5.5	4
germ	80.0		66.5		73.2			2
middlings	90.0		56.0		73.0			2
shorts	100			64.5	82.7			2
gluten	81.0, 90.0	64.7, 77.7			78.4	10	13	4
gluten feed meal	72.0, 75.0, 101, 105	63.1, 69.4, 77.1, 90.8			81.7	15	19	8
starch	115	117, 123			118	4.2	3.5	3
whole meal flour	70, 90	69.7, 77.3			76.7	9.5	12	4
whole grain bread	70.0, 80.0 100, 110	72.7, 80.2, 82.2, 83.2			84.8	14	16	8
milled by products	80.0, 100	80.6, 86.7			86.8	9.3	11	4
aspirated grain	90.0, 100	73.5			87.8	13	15	3

Results and discussion

The transfer of BAS 750 F and triazole derivative metabolites (TDM) from wheat grain (sampled at BBCH 89) and whole plant (sampled at BBCH 71) to processed fractions was determined for three trials. TDMs were not formed on processing (see section 7.5.1); however as they are present in the RAC, a consideration of their levels in processed commodities is required.In each case a processing factor was calculated, this is defined as the residue in the processed product divided by the residue in the RAC. The median processing factor was then determined for each component in each processed commodity.

BAS 750 F

BAS 750 F residue levels in processed grain fractions were compared with residue levels in grain (prior to processing 0.13 or 0.017 or 0.034 mg/kg) in order to calculate processing factors for each trial (PF1, PF2, PF3), as well as a median processing factor (PF). Overall, comparable processing factors were obtained. Results are summarized in Table 7.5.3-4. No residues of BAS 750F were detected above the respective LOQs in any of the untreated samples.

Processing factors indicating reduction of residues during processing (PF < 1.00), were obtained for flour, gluten, gluten feed meal as well as starch, whole meal flour, whole grain bread and milled by-products. Increased residue levels are determined for germ (PF=1.12), middlings (PF=2.26), shorts (PF=3.53), bran (PF=2.94), and aspirated grain fractions (PF=38.46).

BAS 750 F residue levels in silage compared with residue levels in whole plant (prior to processing 6.9 or 2.5 or 3.2 mg/kg) were used to calculate processing factors for each trial (PF1, PF2, PF3) as well as an average processing factor (PF). Overall, comparable processing factors were obtained. The processing factor indicates a slight increase of residues in silage (1.19 for wet silage and 1.88 for wilted silage).

Table 7.5.3-4: Processing factors for BAS 750 F in wheat processed fractions

Matrix	BAS 750 F [mg/kg]			Processing factor [5]			
	trial 1[4]	trial 2[4]	trial 3[4]	PF 1	PF 2	PF 3	PF median
RAC GRAIN [1]	***0.13***	***0.017***	***0.034***	**1.00**	**1.00**	**1.00**	**1.00**
bran	*0.31*	*0.063*	*0.10*	2.38	3.71	2.94	**2.94**
flour	*< 0.01*	*< 0.01*	*< 0.01*	< 0.08	< 0.59	< 0.29	**<0.29**
germ	*0.11*	*0.031*	*0.038*	0.85	1.82	1.12	**1.12**
middlings	*0.25*	*0.066*	*0.077*	1.92	3.88	2.26	**2.26**
shorts	*0.34*	*0.077*	*0.12*	2.62	4.53	3.53	**3.53**
gluten	*0.072*	*< 0.01*	*0.015*	0.55	< 0.59	0.44	**0.55**
gluten feed meal	*0.038*	*< 0.01*	*< 0.01*	0.29	< 0.59	< 0.29	**<0.29**
starch	*< 0.01*	*< 0.01*	*< 0.01*	< 0.08	< 0.59	< 0.29	**<0.29**
whole meal flour	*0.10*	*0.017*	*0.027*	0.77	1.00	0.79	**0.79**
whole grain bread	*0.070*	*< 0.01*	*0.019*	0.54	< 0.59	0.56	**0.56**
milled by-products	*0.081*	*0.019*	*0.014*	0.62	1.12	0.41	**0.62**
aspirated grain fraction	*5.0*	*0.37*	*1.5*	38.46	21.76	44.12	**38.46**
RAC PLANT [2]	***6.9***	***2.5***	***3.2***	**1.00**	**1.00**	**1.00**	**1.00**
silage, wet	*7.6*	*3.6*	*3.8*	1.10	1.44	1.19	**1.19**
silage, wilted	*8.0*	*4.7*	*6.5*	1.16	1.88	2.03	**1.88**
plant (DALA 0) [3]	*7.0*	*7.6*	*5.9*	-	-	-	-
plant (DALA 7-9) [3]	*3.1*	*3.0*	*4.4*	-	-	-	-
grain (DALA 45-60) [3]	*0.14*	*0.024*	*0.028*	-	-	-	-

[1] RAC, sub-sample of grain used for processing
[2] RAC, whole plant without roots, sub-sample of plant used for processing
[3] samples taken on field (purpose: application control)
[4] trial numbers are L140181 (trial 1), L140182 (trial 2), L140183 (trial 3)
[5] for calculation purposes residue level of "< 0.01" are set to a value of "0.01"

TA

TA residue levels in processed grain fractions were compared with residue levels in grain (prior to processing 0.27 – 0.48 mg/kg) in order to calculate processing factor for each trial (PF1, PF2, PF3), as well as a median processing factor (PF). Overall, comparable processing factors were obtained. Results for TA are summarized in Table 7.5.3-5.

Processing factors below or around 1.00 were obtained for flour, gluten, gluten feed meal as well as starch, whole meal flour, whole grain bread, milled by-products, germ and aspirated grain fractions. Increased residue levels are indicated by the processing factors for middlings (PF=2.74), for bran (PF=2.86) and for shorts (PF=3.54).

TA levels in plant samples prior to processing were 0.13 - 0.31 mg/kg. For silage production, average processing factors indicate a slight increase of residues (wet silage PF=1.31, wilted silage PF=1.46).

Table 7.5.3-5: Processing factors for TA in wheat processed fractions

Matrix	TA [mg/kg]			Processing factor [5]			
	trial 1[4]	trial 2[4]	trial 3[5]	PF1	PF2	PF3	PF median
RAC GRAIN [1]	***0.48***	***0.27***	***0.35***	**1.00**	**1.00**	**1.00**	**1.00**
bran	*1.0*	*0.95*	*1.0*	2.08	3.52	2.86	**2.86**
flour	*0.20*	*0.19*	*0.18*	0.42	0.70	0.51	**0.51**
germ	*0.18*	*0.71*	*0.34*	0.38	2.63	0.97	**0.97**
middlings	*1.2*	*0.74*	*0.96*	2.50	2.74	2.74	**2.74**
shorts	*1.7*	*1.6*	*1.1*	3.54	5.93	3.14	**3.54**
gluten	*0.067*	*0.14*	*0.18*	0.14	0.52	0.51	**0.51**
gluten Feed Meal	*0.090*	*0.052*	*0.094*	0.19	0.19	0.27	**0.19**
starch	*< 0.01*	*< 0.01*	*< 0.01*	< 0.02	< 0.04	< 0.03	**<0.03**
whole meal flour	*0.44*	*0.41*	*0.35*	0.92	1.52	1.00	**1**
whole grain bread	*0.35*	*0.37*	*0.30*	0.73	1.37	0.86	**0.86**
milled by-products	*0.28*	*0.36*	*0.039*	0.58	1.33	0.11	**0.58**
aspirated grain fraction	*0.33*	*0.26*	*0.22*	0.69	0.96	0.63	**0.69**
RAC PLANT [2]	***0.31***	***0.13***	***0.19***	**1.00**	**1.00**	**1.00**	**1.00**
wet silage	*0.28*	*0.17*	*0.31*	0.90	1.31	1.63	**1.31**
wilted silage	*0.18*	*0.23*	*0.27*	0.58	1.77	1.42	**1.42**
plant (DALA 0) [3]	*0.26*	*0.090*	*0.12*	-	-	-	-
plant (DALA 7-9) [3]	*<0.01*	*0.14*	*0.29*	-	-	-	-
grain (DALA 45-60) [3]	*0.44*	*0.43*	*0.36*	-	-	-	-

[1] *RAC, sub-sample of grain used for processing*
[2] *RAC, whole plant without roots, sub-sample of plant used for processing*
[3] *samples taken on field (purpose: application control)*
[4] *trial numbers are L140181 (trial 1), L140182 (trial 2), L140183 (trial 3)*
[5] *for calculation purposes residue level of "< 0.01" are set to a value of "0.01"*

TAA

TAA residue levels in processed grain fractions were compared with residue levels in grain (prior to processing 0.12 – 0.32 mg/kg) in order to calculate processing factor for each trial (PF1, PF2, PF3), as well as a median processing factor (PF). Overall, comparable processing factors were obtained. Results for TAA are summarized in Table 7.5.3-6.

Processing factors below or around 1.00 were obtained for flour, germ, gluten feed meal as well as starch, whole meal flour, whole grain bread, milled by-products and aspirated grain fractions. Increased residue levels are indicated by the processing factors for middlings (PF=1.42), gluten (PF=1.15), bran (PF=1.35), whole grain bread (PF=1.19) and shorts (PF=2.00).

TAA levels in plant samples prior to processing were 0.06 - 0.1 mg/kg. For silage production, average processing factors indicate increase of residues (wet silage PF=1.96, wilted silage PF=1.79).

Table 7.5.3-6: Processing factors for TAA in wheat processed fractions

Matrix	TAA [mg/kg]			Processing factor [5]			
	trial 1[4]	trial 2[4]	trial 3[4]	PF1	PF2	PF3	PFmedian
RAC GRAIN[1]	***0.32***	***0.20***	***0.12***	**1.00**	**1.00**	**1.00**	**1.00**
bran	*0.41*	*0.27*	*0.30*	1.28	1.35	2.50	**1.35**
flour	*0.26*	*0.16*	*0.18*	0.81	0.80	1.50	**0.81**
germ	*0.15*	*0.14*	*0.14*	0.47	0.70	1.17	**0.70**
middlings	*0.48*	*0.22*	*0.17*	1.50	1.10	1.42	**1.42**
shorts	*0.73*	*0.34*	*0.24*	2.28	1.70	2.00	**2.00**
gluten	*0.28*	*0.23*	*0.24*	0.88	1.15	2.00	**1.15**
gluten feed meal	*0.19*	*0.19*	*0.14*	0.59	0.95	1.17	**0.95**
starch	*0.012*	*< 0.01*	*< 0.01*	0.04	< 0.05	< 0.08	**<0.05**
whole meal flour	*0.25*	*0.18*	*0.17*	0.78	0.90	1.42	**0.90**
whole grain bread	*0.38*	*0.15*	*0.15*	1.19	0.75	1.25	**1.19**
milled by-products	*0.20*	*0.13*	*0.19*	0.63	0.65	1.58	**0.65**
aspirated grain fraction	*0.20*	*0.083*	*0.14*	0.63	0.42	1.17	**0.63**
RAC PLANT [2]	***0.099***	***0.056***	***0.10***	**1.00**	**1.00**	**1.00**	**1.00**
wet silage	*0.25*	*0.082*	*0.19*	2.53	1.46	1.90	**1.90**
wilted silage	*0.13*	*0.11*	*0.21*	1.31	1.96	2.10	**1.96**
plant (DALA 0) [3]	*0.14*	*0.049*	*0.15*	-	-	-	-
plant (DALA 7-9) [3]	*0.090*	*0.058*	*0.10*	-	-	-	-
grain (DALA 45-60) [3]	*0.16*	*0.093*	*0.14*	-	-	-	-

[1] *RAC, sub-sample of grain used for processing*
[2] *RAC, whole plant without roots, sub-sample of plant used for processing*
[3] *samples taken on field (purpose: application control)*
[4] *trial numbers are L140181 (trial 1), L140182 (trial 2), L140183 (trial 3)*
[5] *for calculation purposes residue level of "< 0.01" are set to a value of "0.01"*

TLA

TLA residue levels in grain prior to processing were below the LOQ of 0.01 mg/kg. Processing factors were therefore not determined. Residue data from wheat plant and silage produced thereof indicates an increase of residues upon silaging (PF=2.62 for wet silage and PF= 2.86 for wilted silage). Results for TAA are summarized in Table 7.5.3-7.

Table 7.5.3-7: Processing factors for TLA in wheat processed fractions

Matrix	TLA [mg/kg]			Processing factor			
	trial 1[3]	trial 2[3]	trial 3[3]	PF1	PF2	PF3	median PF
RAC PLANT [1]	***0.10***	***0.042***	***0.040***	**1.00**	**1.00**	**1.00**	**1.00**
wet silage	*0.20*	*0.11*	*0.17*	2.00	2.62	4.25	**2.62**
wilted silage	*0.22*	*0.12*	*0.18*	2.20	2.86	4.50	**2.86**
plant (DALA 0) [2]	*0.11*	*0.050*	*0.083*	-	-	-	-
plant (DALA 7-9) [2]	*0.047*	*0.046*	*0.052*	-	-	-	-
grain (DALA 45-60) [2]	*< 0.01*	*< 0.01*	*< 0.01*	-	-	-	-

[1] *RAC, whole plant without roots, sub-sample of plant used for processing*
[2] *samples taken on field (purpose: application control)*
[3] *trial numbers are L140181 (trial 1), L140182 (trial 2), L140183 (trial 3)*

1,2,4-triazole

No residues above the limit of quantitation were found in any field sample or processed fraction. Processing factors were therefore not determined.

Residues above the LOQ (0.01 mg/kg) were determined in control samples for TA, TAA and TLA (not for 1,2.4-T). The residue levels determined are summarized in Table 7.5.3-8. TDMs are common metabolites from a range of pesticides, and hence are often present in soil. These results are not considered to have an adverse impact on the study, as the processing factors for these metabolites are not impacted by the source of the metabolites, although they demonstrate why higher levels of TDMs were observed in this study than expected based on the nature of the residues study in section 7.5.1.

Table 7.5.3-8: Summary of residues in untreated samples

Matrix	Residues [mg/kg]		
	TA	**TAA**	**TLA**
wet silage	*0.14*	*0.11*	*0.15*
wilted silage	*0.11*	*0.11*	*0.14*
bran	*0.83*	*0.29*	*< 0.01*
flour	*0.072*	*0.16*	*< 0.01*
germ	*0.28*	*0.12*	*< 0.01*
middlings	*0.37*	*0.19*	*< 0.01*
shorts	*0.37*	*0.28*	*< 0.01*
gluten	*0.067*	*0.21*	*< 0.01*
gluten feed meal	*0.046*	*0.10*	*< 0.01*
starch	*< 0.01*	*0.014*	*< 0.01*
whole meal flour	*0.20*	*0.19*	*< 0.01*
whole grain bread	*0.24*	*0.12*	*< 0.01*
milled by-products	*0.18*	*0.12*	*< 0.01*
aspirated grain fraction	*0.20*	*0.11*	*0.021*
plant (DALA 0)	*0.10*	*0.081*	*0.087*
plant (DALA 7-9)	*0.13*	*0.081*	*0.074*
grain (DALA 45-60)	*0.42*	*0.17*	*< 0.01*

Conclusion

Average processing factors for BAS 750 F and TDMs were calculated based on residue data from three trials. For 1,2,4-triazole and triazole lactic acid in grain, processing factors could not be calculated due to residue level <LOQ. For BAS 750F, TA and TAA processing factors demonstrate an increase of residues upon silaging (PF=1.19-1.96 for wet silage and PF= 1.46-1.88 for wilted silage).

For BAS 750 F the median processing factors for middlings (PF=2.26), shorts (PF=3.53), bran (PF=2.94), and aspirated grain fractions (PF=38.46) indicated an increase of residues upon processing, while PF below 1.00 were obtained for flour, gluten, gluten feed meal, starch, whole meal flour, whole grain bread and milled by-products.

For TA the median processing factors for germ (PF=1.33), middlings (PF=2.74), bran (PF=2.86), and shorts (PF=3.54) indicate an increase of residues upon processing, while PF below 1.00 were obtained for flour, gluten, gluten feed meal as well as starch, whole meal flour, whole grain bread, milled by-products and aspirated grain fractions.

For TAA the median processing factors for middlings (PF=1.42), gluten (PF=1.15), bran (PF=1.35), whole grain bread (PF=1.19) and shorts (PF=2.00) indicate an increase of residues upon processing, while PF below 1.00 were obtained for flour, germ, gluten feed meal as well as starch, whole meal flour, whole grain bread, milled by-products and aspirated grain fractions.

Report:	CA 6.5.3/2 Plier S.,Elze M., 2015 b Determination of residues of BAS 750 F (Reg.No. 5834378) in barley and its processed products after two applications of BAS 750 01 F in Germany, 2014 2014/1315282
Guidelines:	IVA Guideline IA-III (1992), BBA IV 3-3, BBA IV 3-4, OECD 508 Magnitude of the Pesticide Residues in Processed Commodities (2008), OECD 509 Crop Field Trial (2009), OECD-ENV/JM/MONO(2008)23, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7035/VI/95 rev. 5
GLP:	yes

Materials and methods

A processing study on barley (variety *Quench, Grace*) was conducted outdoors at three locations in Germany during the 2014 growing season. Normal agricultural practices were followed, and no unusual weather events were recorded. Each field trial included one treated plot. In addition, an application-free control plot was also included in one of the three field trials. Two foliar applications of BAS 750 F (10% EC formulation) were made to each of the treated plots at a target rate of 0.45 kg ai/ha to obtain 3x the maximum per season rate in the GAP. The applications were made at crop growth stages BBCH 49 and BBCH 69 using a spray volume of 200 L/ha. Samples were taken at the following time points:

- DALA 0 (plants at BBCH 69, application control)

- DALA 43-56 (grain at BBCH 89)

The processing of barley was conducted using sub-samples taken from grain (BBCH 89, DALA 45-60). The following fractions of barley were generated following industrial processing procedures at a laboratory scale: pearled barley (pot barley), flour, bran, brewing malt, malt sprouts, beer, brewers grain (dried) and brewers yeast. The processing was carried out in the following stages.

Malting - The grain specimens were cleaned and sieved (sieve mesh 2.5 mm) before malting was started. After sieving, a combined wet and dry steeping was conducted. After steeping, a germination procedure followed ("still" germination). Kiln-drying was conducted in a dry chamber. After kiln-drying the germs were removed mechanically by a trimmer. *Brewing malt* and *malt sprouts* were sampled immediately after malting. Until brewing the malt was stored at room temperature (malt rest).

Brewing – Before mashing (homogeneous mixing), the brewing malt was dried milled in a special malt mill. The crushed malt was mixed with brew water. The mashing of ground malt and water was undertaken according to a definite temperature time regime (mash program). After mash boiling, the wort was separated from the insoluble malt components (brewer's grain).The extract remaining in the brewer's grain was extracted by washing with hot water. After separation, brewers grain was dried at 50 °C until a dry matter content of < 10 % was reached and sampled as *brewers grain* (dried). After addition of hop pellets, the separated wort was boiled (about 90 min at normal pressure). After boiling, the flocs (hops draff) were separated in a whirlpool. For the wort was cooled and ventilated and the conditions for the start of the fermentation were prepared by adding oxygen.

In the pilot plant the primary fermentation was carried out in bottom fermentation containers at approx. 9 °C. As soon as the extract content of the fermented young beer was 2 % higher than the final attenuation, the storing time began.. During the main fermentation the yeast deposited on the tank bottom and was sampled as *brewers yeast*. At the beginning of maturation the young beer was stored at room temperature in casks. Then the young beer was stored under pressure (approx. 0.7 - 1.0 bar) at approx. 2 °C (cold maturation) for about 3 - 4 weeks. In this time the remaining extract was

fermented. The rack beer was filtered using a special filter combination to remove yeast, bacteria and sludge. The final product *beer* was sampled.

Pot barley - The grain was cleaned and an optimal moisture content of barley grain of approx. 14 % achieved (via drying or damping). Each sample was hulled until the stipulated abrasion for pot barley (20 - 25 %) was reached. Abrasion was sieved to bran and flour. *Pot barley*, *bran* and *flour* were sampled.

Details of how each processed fraction are produced are given in Figures 7.5.3-5 and 7.5.3-6. The mass of each processed fraction is given in Table 7.5.3-9.

The RAC (raw agricultural commodity) samples were stored frozen at ≤-18°C with 12 hours of harvest. Samples for processing were stored at ambient temperature prior to processing (for up to 6 months), and then frozen at ≤-18°C immediately afterwards. Samples were stored frozen for up to 304 days. Sufficient storage stability is available to support frozen storage for this period (see section 7.1.1).

Figure B.7.5.3-5 Processing of barley grain (1)

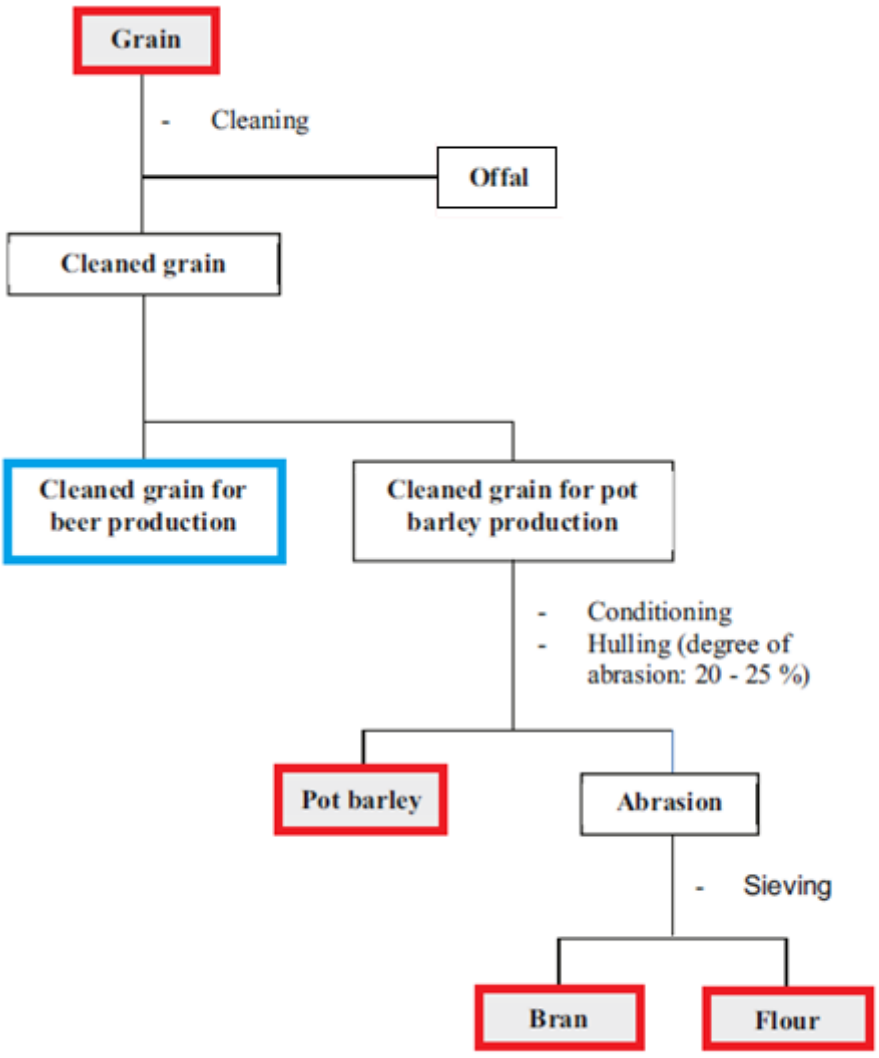


Figure B.7.5.3-6 Processing of barley grain (2)

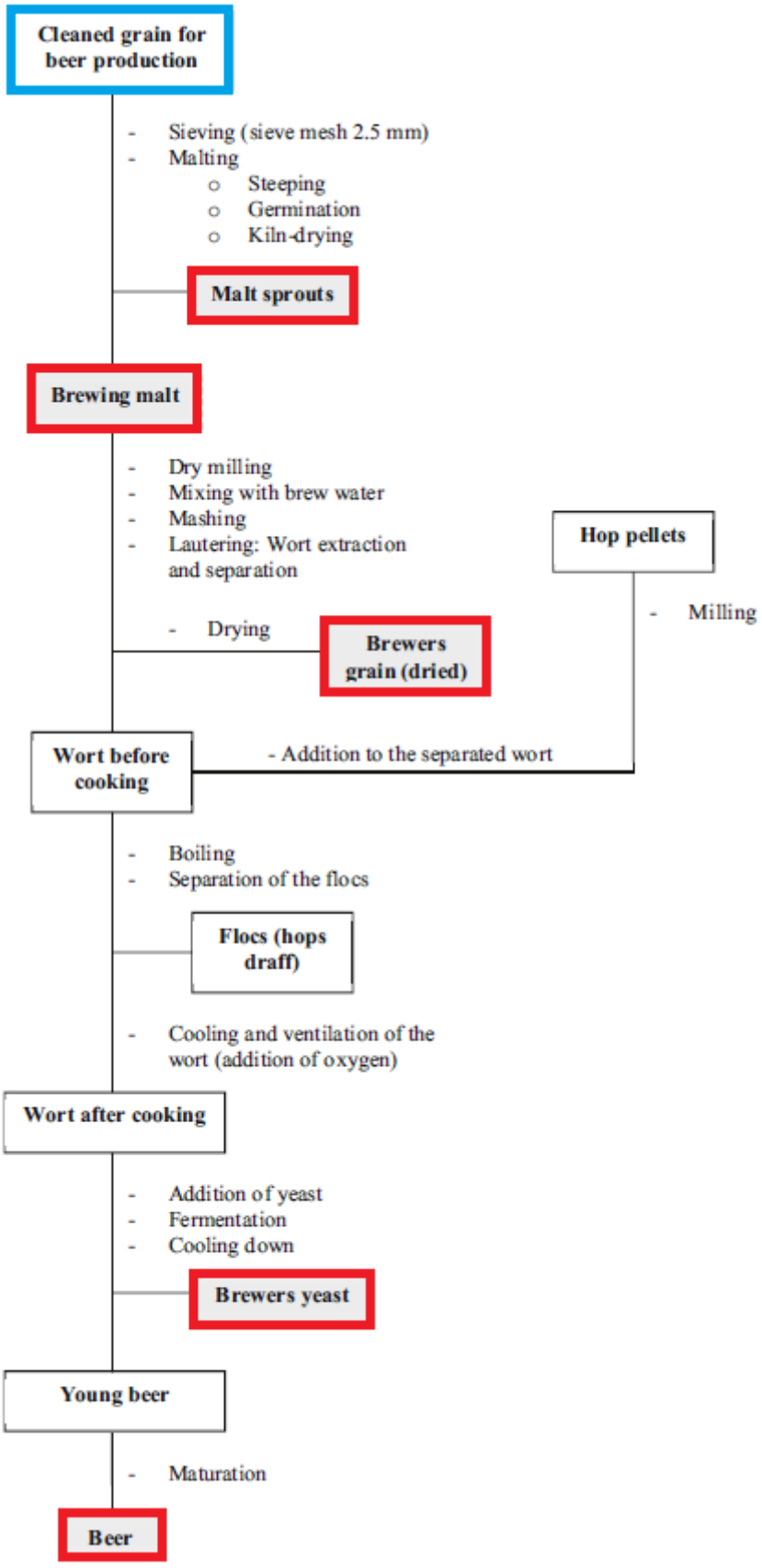


Table 7.5.3-9 Weight of processed fractions sampled for each trial

Processed fraction	Sample weight (kg)			
	Trial 1 (L140178)	**Trial 2 (L140179)**	**Trial 3 (L140180)**	**Mean**
Grain	1.05	0.96	0.89	0.97
Pot barley	0.54	0.58	0.61	0.58
Bran	0.11	0.13	0.15	0.13
Flour	0.11	0.13	0.15	0.13
Malt sprouts	0.61	0.62	0.69	0.64
Brewing malt	0.62	0.57	0.57	0.59
Brewers grain	0.60	0.60	0.60	0.60
Brewer's yeast	0.30	0.30	0.30	0.30
Beer	0.75	1.10	1.10	0.98

The level of residues in each processed fraction was determined for BAS 750 F (BASF method L0076/09, LOQ of 0.01 mg/kg) and for the triazole derivative metabolites (TDM) 1,2,4-T, TA, TAA, TLA (BASF method L0170/02, LOQ of 0.01 mg/kg for each of the four analytes). Full details of sample preparation and validation data for these methods is given in section CA B.5.1.2.5. A brief summary of the methods is outlined below. Details of the procedural recoveries are given in Table 7.5.3-10 and 7.5.3-11.

Principle of the method L0076/09: The analyte is extracted with methanol/water/HCl (70/25/5, v/v/v). After extraction with cyclohexane, an aliquot is concentrated, dissolved in methanol/water (50/50, v/v) and transferred into an autosampler vial for LC-MS/MS analysis (method validated for BAS 750 F analysis in plant matrices).

Principle of the method L0170/02: The analytes are extracted with methanol/water (4/1, v/v), an aliquot is filtered, concentrated and cleaned-up by a simple dispersive C18-SPE-step. The analytes are determined by LC-DMS/MS/MS (method validated for TDM analysis in plant matrices, i.e. 1,2,4-T, TA, TAA, TLA).

Table 7.5.3-10: Recoveries for BAS 750 F in barley processed fractions

Processed fraction	**Fortification level (mg/kg)/recovery (%)**				**Precision/recovery**			
	0.01	**1**	**5**	**50**	**Mean**	**SD**	**RSD**	**n**
Whole plant	80.5	83.8	-	85.5	83.3	2.5	3.0	3
Grain	92.3	97.2	-	-	94.7	-	-	2
Pot barley	90.3	94.0	-	-	92.1	-	-	2
Flour	87.0, 87.5 89.5	92.5, 92.5, 92.5	79.8	-	88.8	4.6	5.2	7
Bran	80.3	90.8	81.5	-	84.2	5.7	6.8	3
Brewing malt	85.5	93.0	-	-	89.3	-	-	2
Malt sprouts	78.8	85.8	-	-	82.3	-	-	2
Brewers grain	83.5	86.8	-	-	85.1	-	-	2
Brewers yeast	82.0	78.8	-	-	80.4	-	-	2
Beer	91.5, 93.0, 97.8	90.5, 92.8, 100	-	-	94.3	3.8	4.0	6

Table 7.5.3-11 Recoveries for TDMs in barley processed fractions

Processed fraction	Fortification level (mg/kg)/recovery (%)		Precision/recovery			
	0.01	1	Mean	SD	RSD	n
Triazole						
Whole plant	93.2, 104	76.9, 89.3	90.9	11	12	4
Grain	91.3, 97.3	80.1, 89.9	89.7	7.1	8	4
Pot barley	90.7, 103	85.9, 93.0	93.2	7.2	7.7	4
Flour	96.2, 100	91.5, 91.7, 93.9, 99.9	95.6	3.9	4.1	6
Bran	87.8, 96.8	82.8, 91.8	89.8	5.9	6.6	4
Brewing malt	110, 121	92.5, 105	107	12	11	4
Malt sprouts	81.0, 98.3	80.8, 95.3	88.9	9.3	10	4
Brewers grain	87.1, 97.1	85.1, 91.9	90.3	5.4	5.9	4
Brewers yeast	103, 106	98.1, 102	102	3.3	3.2	4
Beer	92.1, 99.1, 113, 117	78.9, 86.7, 96.9, 99.0	97.8	13	13	8
TAA						
Whole plant	80.0, 106	109, 114	102	15	15	4
Grain	90.0, 90.0	84.3, 89.7	88.5	2.8	3.2	4
Pot barley	83.0, 111	79.8, 87.2	90.3	14	16	4
Flour	80.0, 90.0	67.6, 75.7, 78.8, 84.2	79.4	7.6	9.6	6
Bran	80.0, 110	89.6, 95.6	98.3	13	13	4
Brewing malt	80.0, 110	93.3, 93.3	94.1	12	13	4
Malt sprouts	90.0, 90.0	63.2, 70.2	78.4	14	18	4
Brewers grain	74.0, 94.0	104, 109	95.3	15	16	4
Brewers yeast	79.0, 95.0	107, 111	98.0	14	15	4
Beer	74.0, 74.0, 78.0, 87.0	75.8, 82.1, 86.7, 107	83.1	11	13	8
TLA						
Whole plant	99.0, 109	107, 109	106	4.8	4.5	4
Grain	93.0, 94.0	74.2, 94.7	89.0	9.9	11	4
Pot barley	72.0, 94.0	66.1, 84.1	79.1	12	16	4
Flour	71.0, 103	74.7, 85.7, 89.4, 95.6	86.6	12	14	6
Bran	79.0, 85.0	79.7, 108	87.9	14	16	4
Brewing malt	99.9, 109	86.5, 88.8	96.1	10	11	4
Malt sprouts	72.5, 92.5	105, 115	96.3	18	19	4
Brewers grain	80.6, 88.6	65.5, 82.1	79.2	9.8	12	4
Brewers yeast	96.5, 97.9	80.2, 91.3	91.5	8.0	8.8	4
Beer	90.0, 90.0, 90.0, 110	93.5, 93.5, 94.5, 97.5	94.9	6.7	7.0	8
TA						
Whole plant	84.0, 99.0	86.7, 96.2	91.5	7.2	7.9	4
Grain	100	81.9, 91.6	91.2	9.1	9.9	3
Pot barley	95.0, 113	72.1, 115	98.8	20	20	4
Flour	90.0	66.5, 87.3, 95.3, 97.3	87.3	12	14	5
Bran	75.0, 84.0	80.9	80.0	4.6	5.7	3
Brewing malt	85.0, 88.0	82.0, 93.9	87.2	5.1	5.8	4
Malt sprouts	80.0	69.8, 69.8	73.2	5.9	8.0	3
Brewers grain	67.0, 73.0	94.8, 94.9	82.4	15	18	4
Brewers yeast	96.0, 103	89.5, 97.9	96.6	5.6	5.8	4
Beer	78.6, 104, 105, 109	86.2, 96.6, 97.1, 122	99.8	13	14	8

Results and discussion

The processing of BAS 750 F and triazole derivative metabolites (TDM) from barley grain (sampled at BBCH 89) to processed fractions was determined for three trials. TDMs were not formed on processing (see section 7.5.1); however as they are present in the RAC, a consideration of their levels

in processed commodities is required. In each case a processing factor was calculated, this is defined as the residue in the processed product divided by the residue in the RAC. A median processing factor was then determined for each component in each processed commodity.

BAS 750 F

BAS 750 F residue levels in processed grain fractions compared with residue levels in grain (prior to processing 0.40 or 0.24 or 0.22 mg/kg) were used to calculate processing factors for each trial (PF1, PF2, PF3)), as well as a median processing factor (PF). Overall, comparable processing factors were obtained. Results are summarized in Table 7.5.3-12. No residues of BAS 750 F were detected above the respective LOQs in any of the untreated samples.

Processing factors indicating reduction of residues during processing (PF < 1.00), were obtained for pot barley, brewing malt, beer, and brewers yeast. Increase of residue levels are determined for flour (PF=3.67), bran (PF=5.00), malt sprouts (PF=1.09), and dried brewers grain (PF=2.38).

Table 7.5.3-12: Processing factors for BAS 750 F in barley processed fractions

Matrix	BAS 750 F [mg/kg]			Processing factor [5]			
	trial 1[4]	trial 2[4]	trial 3[4]	PF1	PF2	PF3	PF median
RAC GRAIN [1]	***0.40***	***0.24***	***0.22***	**1.00**	**1.00**	**1.00**	**1.00**
pearled barley (pot b.)	*0.065*	*0.029*	*0.018*	0.16	0.12	0.08	**0.12**
flour	*1.8*	*0.88*	*0.70*	4.50	3.67	3.18	**3.67**
bran	*1.7*	*1.2*	*1.2*	4.25	5.00	5.45	**5.00**
brewing malt	*0.20*	*0.12*	*0.067*	0.50	0.50	0.30	**0.5**
malt sprouts	*0.67*	*0.23*	*0.24*	1.68	0.96	1.09	**1.09**
beer	*< 0.01*	*< 0.01*	*< 0.01*	< 0.03	< 0.04	< 0.05	**<0.04**
brewers grain (dried)	*0.95*	*0.58*	*0.47*	2.38	2.42	2.14	**2.38**
brewers yeast	*0.076*	*0.064*	*0.042*	0.19	0.27	0.19	**0.19**
plant (DALA 0)	*12*	*9.3*	*15*	-	-	-	-
grain (DALA 43-56) [2]	*0.35*	*0.21*	*0.23*	-	-	-	-

[1] *RAC, sub-sample of grain used for processing*
[2] *RAC, whole plant without roots, sub-sample of plant used for processing*
[3] *samples taken on field (purpose: application control)*
[4] *trial numbers are L140178 (trial 1), L140179 (trial 2), L140180 (trial 3).*
[5] *for calculation purposes residue level of "< 0.01" are set to a value of "0.01".*

1,2,4-triazole

No residues above the limit of quantitation were found in any field sample or processed fraction. Processing factors were therefore not determined.

TA

TA residue levels in processed grain fractions were compared with residue levels in grain (prior to processing 0.20 – 0.53 mg/kg) in order to calculate processing factors for each trial (PF1, PF2, PF3), as well as a median processing factor (PF). Overall, comparable processing factors were obtained. Results for TA are summarized in Table 7.5.3-13.

Processing factors below or around 1.00 were obtained for pot barley, brewing malt, beer, and dried brewers grain. Increase of residue levels are indicated by the average processing factors for flour (PF=1.20), bran (PF=2.08) and malt sprouts (PF=1.72).

Table 7.5.3-13: Processing factors for TA in barley processed fractions

Matrix	TA [mg/kg]			Processing factor[5)]			
	trial 1[4)]	trial 2[4)]	trial 3[4)]	PF1	PF2	PF3	PFmedian
RAC GRAIN [1)]	***0.25***	***0.20***	***0.53***	**1.00**	**1.00**	**1.00**	**1.00**
pearled barley (pot b.)	*0.21*	*0.27*	*0.15*	0.84	1.35	0.28	**0.84**
flour	*0.27*	*0.24*	*0.71*	1.08	1.20	1.34	**1.20**
bran	*0.52*	*0.47*	*0.26*	2.08	2.35	0.49	**2.08**
brewing malt	*0.045*	*0.29*	*0.27*	0.18	1.45	0.51	**0.51**
malt sprouts	*0.33*	*1.1*	*0.91*	1.32	5.50	1.72	**1.72**
beer	*< 0.01*	*< 0.01*	*< 0.01*	< 0.04	< 0.05	< 0.02	**< 0.04**
brewers grain (dried)	*< 0.01*	*< 0.01*	*< 0.01*	< 0.04	< 0.05	< 0.02	**< 0.04**
brewers yeast	*0.15*	*0.56*	*0.069*	0.60	2.80	0.13	**0.60**
plant (DALA 0)	*0.076*	*0.055*	*0.049*	-	-	-	-
grain (DALA 43-56) [2)]	*0.16*	*0.55*	*0.54*	-	-	-	-

[1)] RAC, sub-sample of grain used for processing
[2)] RAC, whole plant without roots, sub-sample of plant used for processing
[3)] samples taken on field (purpose: application control)
[4)] trial numbers are L140178 (trial 1), L140179 (trial 2), L140180 (trial 3).
[5)] for calculation purposes residue level of "< 0.01" are set to a value of "0.01".

TAA

TAA residue levels in processed grain fractions were compared with residue levels in grain (prior to processing 0.17 – 0.19 mg/kg) in order to calculate processing factors for each trial (PF1, PF2, PF3), as well as a median processing factor (PF). Overall, comparable processing factors were obtained. Results for TAA are summarized in Table 7.5.3-14.

Processing factors below or around 1.00 were obtained for pot barley, brewing malt, beer, dried brewers grain and brewers yeast. Increase of residue levels are indicated by the average processing factors for flour (PF=2.11), bran (PF=1.33), and malt sprouts (PF=2.71).

Table 7.5.3-14: Processing factors for TAA in barley processed fractions

Matrix	TAA [mg/kg]			Processing factor [5)]			
	trial 1[4)]	trial 2[4)]	trial 3[4)]	PF1	PF2	PF3	PFmedian
RAC GRAIN [1)]	***0.19***	***0.18***	***0.17***	**1.00**	**1.00**	**1.00**	**1.00**
pearled barley (pot b.)	*0.16*	*0.11*	*0.12*	0.84	0.61	0.71	**0.71**
flour	*0.36*	*0.38*	*0.38*	1.89	2.11	2.24	**2.11**
bran	*0.22*	*0.24*	*0.34*	1.16	1.33	2.00	**1.33**
brewing malt	*0.17*	*0.20*	*0.14*	0.89	1.11	0.82	**0.89**
malt sprouts	*0.40*	*0.57*	*0.46*	2.11	3.17	2.71	**2.71**
beer	*0.028*	*< 0.01*	*0.025*	0.15	< 0.06	0.15	**0.15**
brewers grain (dried)	*0.022*	*0.011*	*0.014*	0.12	0.06	0.08	**0.08**
brewers yeast	*0.042*	*0.044*	*0.038*	0.22	0.24	0.22	**0.22**
plant (DALA 0)	*0.074*	*0.059*	*0.076*	-	-	-	-
grain (DALA 43-56) [2)]	*0.17*	*0.18*	*0.19*	-	-	-	-

[1)] RAC, sub-sample of grain used for processing
[2)] RAC, whole plant without roots, sub-sample of plant used for processing
[3)] samples taken on field (purpose: application control)
[4)] trial numbers are L140178 (trial 1), L140179 (trial 2), L140180 (trial 3)
[5)] for calculation purposes residue level of "< 0.01" are set to a value of "0.01"

TLA

TA residue levels in processed grain fractions compared with residue levels in grain (prior to processing 0.055 – 0.14 mg/kg) were used to calculate processing factor for each trial (PF1, PF2, PF3), as well as a median processing factor (PF). Overall, comparable processing factors were obtained and used to calculate an average processing factor (PF). Results for TLA are summarized in Table 7.5.3-15..

Processing factors below or around 1.00 were obtained for pot barley, bran, brewing malt, malt sprouts, dried brewers grain and brewers yeast. Increase of residue levels are indicated by the average processing factors for flour (PF=3.86), and beer (PF=1.71).

Table 7.5.3-15: Processing factors for TLA in barley processed fractions

Matrix	TLA [mg/kg]			Processing factor [5]			
	trial 1[4]	trial 2[4]	trial 3[4]	PF1	PF2	PF3	PFmedian
RAC GRAIN [1]	***0.055***	***0.14***	***0.14***	**1.00**	**1.00**	**1.00**	**1.00**
pearled barley (pot b.)	*0.028*	*0.077*	*0.073*	0.51	0.55	0.52	**0.52**
flour	*0.36*	*0.54*	*0.045*	6.55	3.86	0.32	**3.86**
bran	*0.035*	*0.11*	*0.055*	0.64	0.79	0.39	**0.64**
brewing malt	*0.021*	*0.032*	*0.011*	0.38	0.23	0.08	**0.23**
malt sprouts	*< 0.01*	*< 0.01*	*< 0.01*	< 0.18	< 0.07	< 0.07	**< 0.07**
beer	*0.25*	*0.15*	*0.24*	4.55	1.07	1.71	**1.71**
brewers grain (dried)	*< 0.01*	*< 0.01*	*< 0.01*	< 0.18	< 0.07	< 0.07	**< 0.07**
brewers yeast	*0.040*	*0.042*	*0.021*	0.73	0.30	0.15	**0.30**
plant (DALA 0)	*0.087*	*0.12*	*0.062*	-	-	-	-
grain (DALA 43-56) [3]	*0.049*	*0.17*	*0.13*	-	-	-	-

[1] *RAC, sub-sample of grain used for processing*
[2] *RAC, whole plant without roots, sub-sample of plant used for processing*
[3] *samples taken on field (purpose: application control)*
[4] *trial numbers are L140178 (trial 1), L140179 (trial 2), L140180 (trial 3)*
[5] *for calculation purposes residue level of "< 0.01" are set to a value of "0.01"*

Residues above the LOQ (0.01 mg/kg) were determined in control samples for TA, TAA and TLA (not for 1,2.4-T). The residue levels determined are summarized in Table 7.5.3-16. TDMs are common metabolites from a range of pesticides, and hence are often present in soil. These results are not considered to have an adverse impact on the study, as the processing factors for these metabolites are not impacted by their source, although they demonstrate why higher levels of TDMs were observed in this study than indicated based on the nature of the residues study.

Table 7.5.3-16: Summary of residues in the untreated samples

Matrix	Residues [mg/kg]		
	TA	TLA	TAA
pearled barley (pot barley)	*0.026*	*0.087*	*0.010*
flour	*0.14*	*0.18*	*0.033*
bran	*0.079*	*0.13*	*0.046*
brewing malt	*0.075*	*0.14*	*< 0.01*
malt sprouts	*0.31*	*0.32*	*< 0.01*
beer	*< 0.01*	*0.023*	*0.14*
brewers grain (dried)	*0.029*	*0.011*	*< 0.01*
brewers yeast	*0.038*	*0.036*	*< 0.01*
plant (DALA 0)	*0.023*	*0.073*	*0.067*
grain (DALA 52)	*0.096*	*0.11*	*0.033*

Conclusion

Average processing factors for BAS 750 F and TDMs were calculated based on residue data from three trials. For 1,2,4-triazole, processing factors could not be calculated due to residue level <LOQ.

For BAS 750 F the average transfer factors for flour (PF=3.67), bran (PF=5.00), malt sprouts (PF=1.09), and dried brewers grain (PF=2.38) indicated an increase of residues upon processing, while a PF below 1.00 was obtained for pot barley, brewing malt, beer, and brewers yeast.

For TA the average transfer factors flour (PF=1.20), bran (PF=2.08) and malt sprouts (PF=1.72) indicate an increase of residues upon processing, while a PF below 1.00 was obtained for pot barley, brewing malt, brewers yeast, beer, and dried brewers grain.

For TAA the average transfer factors for flour (PF=2.11), bran (PF=1.33), and malt sprouts (PF=2.71) indicate an increase of residues upon processing, while a PF below 1.00 was obtained for pot barley, brewing malt, beer, and brewers yeast.

For TLA the average transfer factors for flour (PF=3.87), and beer (PF=1.71) indicate an increase of residues upon processing, while a PF below 1.00 was obtained for pot barley, bran, brewing malt, malt sprouts, dried brewers grain and brewers yeast.

B.7.5.4. Conclusion on the effects of processing

In the nature of the residues processing study, under conditions representative of pasteurisation (pH 4, 90 °C, 20 min), baking, boiling, brewing (pH 5, 100 °C, 60 min) and sterilisation (pH 6, 120 °C, 20 min) BAS 750 F was stable. No degradation product exceeding 2% of total radioactivity was detected and no change in the isomer ratio was observed. BAS 750 F can be regarded as stable to hydrolysis and the nature of the residue is not affected by processing operations. Stability of TDMs under high temperature hydrolysis has not been considered in this dossier; however it was investigated in the TDM review (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015), whereupon they were found to be stable. The following summary is taken from this review:

"The test compounds triazole alanine, triazole acetic acid, triazole lactic acid and 1,2,4-triazole were stable under three sets of hydrolytic conditions representative of the main food processing procedures (pasteurization, baking, brewing, boiling and sterilization). No significant amounts of hydrolysis products of these triazole derived metabolites could be detected after the high temperature hydrolysis mimicking industrial and domestic food processing. The mass balance was complete in each test; no radioactivity has been lost. Consequently, it can be concluded that food processing does not change the nature of the triazole derived metabolites (triazole alanine, triazole acetic acid, triazole lactic acid or 1,2,4-triazole)."

The representative uses are on wheat and barley, therefore a magnitude of the residues study on each of these crops was presented. A summary of the processing factors determined for BAS 750 F is given in Table 7.5.4-1.

For wheat, BAS 750 F was concentrated on processing (PF>1) to germ, middlings, shorts, bran and aspirated grain fractions. For barley, BAS 750 F was concentrated on processing (PF>1) to flour, bran, malt sprouts and brewers grain. For other processed wheat and barley commodities the processing factor was less than 1.

In both studies, control samples of RAC and processed commodities contained the TDMs TA, TLA and TAA. TDMs are common metabolites from a range of pesticides, and hence are often present in soil. These results are not considered to have an adverse impact on the study, as the processing factors for these metabolites are not impacted by their source. TDMs were not formed on processing (see section 7.5.1); however as they are present in the RAC (either through treatment with BAS 750 F or due to their presence in the soil) a consideration of the effect of processing on their levels has been made.

For 1,2,4-triazole in wheat and barley and TLA in wheat grain, processing factors could not be calculated due to residue level <LOQ. For barley, TLA was concentrated on processing (PF>1) to flour and beer, but for other processed commodities the processing factor was less than 1.

For both TA and TAA in processed wheat commodities, these metabolites were concentrated on processing to middlings, bran and shorts. TA was also concentrated on processing to germ, and TAA on processing to gluten and whole grain bread. In barley both metabolites were concentration on processing to flour, bran and malt sprouts. For other processed wheat and barley commodities the processing factor was less than 1.

Levels of TDMs in wheat and barley were also considered in the TDM review, and the results obtained were broadly in agreement with the results obtained in this evaluation. The following summary is taken from this review:

"For wheat, the data clearly show that the triazole derived metabolite TA does not concentrate in flour (straight, type 550 or wholemeal) or aspirated grain fractions, but concentrates in bran (fine and coarse) and germ. The results for TA in shorts and meal were more variable and overall residues levels were similar to the raw agricultural commodity. The results for TAA in all commodities were variable but showed a concentration in bran though overall residues levels in all other processed commodities were similar to the raw agricultural commodity. Limited data in flour or bran indicated that T does not concentrate whereas TLA does concentrate in these commodities. In most studies,

residues of T were below the LOQ of 0.01 mg/kg in the raw agricultural commodity and all the processed commodities.

In barley, the data show that the triazole derived metabolites TA and TAA do not concentrate in brewer's malt, brewer's grain brewer's yeast or beer. For most commodities TLA was not found but the results showed that this metabolite concentrates in brewer's malt. Residues of 1,2,4-T were below the LOQ of 0.01 mg/kg in the raw agricultural commodity and all the processed commodities."

Table 7.5.4-1: Median processing factors for wheat and barley commodities

Crop	Matrix	Median Processing Factor			
		BAS 750 F	**TA**	**TAA**	**TLA**
Wheat	bran	**2.94**	*2.86*	*1.35*	-
	flour	**<0.29**	*0.51*	*0.81*	-
	germ	**1.12**	*0.97*	*0.70*	-
	middlings	**2.26**	*2.74*	*1.42*	-
	shorts	**3.53**	*3.54*	*2.00*	-
	gluten	**0.55**	*0.51*	*1.15*	-
	gluten feed meal	**<0.29**	*0.19*	*0.95*	-
	starch	**<0.29**	*<0.03*	*<0.05*	-
	whole meal flour	**0.79**	*1*	*0.90*	-
	whole grain bread	**0.56**	*0.86*	*1.19*	-
	milled by-products	**0.62**	*0.58*	*0.65*	-
	aspirated grain fraction	**38.46**	*0.69*	*0.63*	-
Barley	pearled barley (pot b.)	**0.12**	*0.84*	*0.71*	*0.52*
	flour	**3.67**	*1.20*	*2.11*	*3.86*
	bran	**5.00**	*2.08*	*1.33*	*0.64*
	brewing malt	**0.5**	*0.51*	*0.89*	*0.23*
	malt sprouts	**1.09**	*1.72*	*2.71*	*< 0.07*
	beer	**<0.04**	*< 0.04*	*0.15*	*1.71*
	brewers grain (dried)	**2.38**	*< 0.04*	*0.08*	*< 0.07*
	brewers yeast	**0.19**	*0.60*	*0.22*	*0.30*

B.7.6. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS

B.7.6.1. Metabolism in rotational crops

Report:	CA 6.6.1/1 Rabe U.,Glaessgen W., 2015 a Confined rotational crop study with 14C LS 5834378 2015/1001871
Guidelines:	OECD 502 Metabolism in Rotational Crops (January 2007), EPA 860.1850: Confined Accumulation in Rotational Crops, EPA 860.1000, PMRA Residue Chemistry Guidelines Section 97.13 Confined Accumulation in Rotational Crops (Canada)
GLP:	yes

Materials and methods

Materials

1. C-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Chlorophenyl-U-C14 (spec. activity 7.88 MBq/mg) was added in a 1:1 (w:w) mixture of Chlorophenyl-1-C13-labelled test item	
Lot/Batch #:	Chlorophenyl-U-C14:	CFQ41561
	Chlorophenyl-1-C13:	RS4-2012-173A2
Purity:	Chlorophenyl-U-C14:	99.1% (radiochem 98.9%)
	Chlorophenyl-1-C13:	97.7%

2. T-label BAS 750 F (CAS No. 1417782-03-6)

Description:	Triazole-3(5)-C14 (spec. activity 5.46 MBq/mg) was added in a 2:1 (w:w) mix with Triazole-3(5)-C13-labelled test item	
Lot/Batch #:	Triazole-3(5)-C14:	1062-1101
	Triazole-3(5)-C13:	1077-1001
Purity:	Triazole-3(5)-C14:	98.9% (radiochem 98.2%)
	Triazole-3(5)-C13:	97.1%

Methods

A metabolism study on the rotational crops spinach, white radish and spring wheat in Limburgerhof, Germany was carried out in 2013-2015. 23 plastic containers (0.20 m^2) filled with a sandy loam soil (Table 7.6.1-1) were used in the study. Twelve containers were treated with the triazole label (2.45 m^2 in total) and eleven with the chlorophenyl label (2.25 m^2 in total). Containers were located in a vegetation hall/greenhouse for application of the BAS 750F.

One foliar spray application of either triazole or chlorophenyl labelled BAS 750F was made to the bare soil in each container using an automatic spray track system. BAS 750 F was prepared as outlined in the materials section and combined with a blank EC formulation (EXP 5834378F-AW) and water, and applied at 300 g a.s./ha.

After aging for 30-31 days the top layer of soil (20 cm) in each container was mixed before returning to the container to simulate ploughing. Directly after this, the crops were planted into the containers. After harvest the soil was mixed again and the crops were replanted (at plant back intervals of 120-122 and 364 days). In each case the plants were cultivated using normal growing practices. A summary of the study details are given in Table 7.6.1-2.

Table 7.6.1-1: Soil Physicochemical Properties

soil series	soil type	pH	OM %	sand %	silt%	slay %	maximal water holding capacity	CEC cmol/kg
Bruch West	sandy loam [1]	7.4 [2]	2.54 [3]	72.1	16.6	11.2	26.6 g / 100 g dry soil	11.2

[1] USDA scheme, [2] ($CaCl_2$) , [3] organic matter, corresponds to the total organic carbon (TOC)

Table 7.6.1-2: Study design

Label	Spring wheat		White radish		Spinach	
Application rate [g a.s./ha]	300		300		300	
Plant back interval (PBI)	30/31 (C/T), 120/122 (C/T) 365/364 (C/T)		30/31 (C/T), 120/122 (C/T) 365/364 (C/T)		30/31 (C/T), 120/122 (C/T) 365/364 (C/T)	
sampled matrices	Immature: forage (hay) (BBCH 37-39) Mature: grain and straw (BBCH 89)		Mature: root and top (BBCH 49)		Immature: leaf (BBCH 15-19) Mature: leaf (BBCH 49)	
sampling [DAP] 1)	forage	49-55	Root	57-70	Imm. leaf	25-33
	grain	105-144	Top	57-70	Mat. leaf	40-46
	straw	105-144				

1) days after planting – crop sampled harvested at these timings for each PBI

Analysis and Identification

The same growth stages were sampled for each PBI. For spinach immature leaf and mature leaf were sampled with the root remaining in the soil. Radish was sampled when mature, and separated into edible part (radish root) and the remaining green part (radish top). Wheat was harvested both as immature green plant (wheat forage) and mature straw and ears. A subsample of wheat forage was dried to obtain wheat hay. Mature ears were separated by threshing into wheat grain and chaff. Chaff and straw were combined to obtain the sample wheat straw.

Soil samples were taken immediately after "ploughing" (i.e. at the end of soil aging intervals) as well as after harvest (i.e. crop maturation). Plant and soil samples were stored frozen at ≤-20°C during the course of the study. The maximum time of frozen storage between sampling and analysis was 242 days for the triazole label and 231 days for the chlorophenyl label. The maximum interval between extraction and analysis was 298 days (T-label) and 351 days (C-label). Stability data to support this duration of storage is presented below.

Aliquots of homogenised plant or soil samples were subjected to oxidative combustion (liquid scintillation measurement) to determine total radioactive residue (TRR combusted). In addition, aliquots were subjected to repetitive solvent extraction (methanol three times, water two times). Where necessary SPE clean up was used to remove chlorophyll from the methanol extracts. ERR was calculated as the sum of radioactivity in the extracts. Residual radioactive residues (RRR) was determined by combustion of the residue after solvent extraction. TRR calculated was obtained as the sum of ERR and RRR.

The nature of the residue in extracts was investigated using two different HPLC methods (radiodetection). Assignment of chromatographic peaks and identification of components of the residue was based on co-chromatography with ^{14}C-labelled reference items as well as on comparison of retention times and elution pattern (metabolic profile). Further characterization of the RRR was done by sequential solubilization including treatments with aqueous ammonia, amylases/amyloglucosidase, macerozyme/cellulase, and tyrosinase/laccase. During each treatment the sample was incubated for 1-2 day at 37 °C, and post treatment acetonitrile was added prior to centrifuging and filtering.

Results and Discussion

Total radioactive residue

TRR in soil was determined directly after "ploughing" as well as after harvest of a mature crop (see Table 7.6.1-3). For both labels (C-label/T-label), residue levels were similar and only slightly decreased from the start of crop cultivation until the date of harvesting (30 DAT: 0.094/0.094 mg/kg, 120 DAT: 0.080/0.095 mg/kg, 365 DAT: 0.064/0.072 mg/kg). No significant change of the TRR was observed during crop cultivation.

Table 7.6.1-3: TRR of soil treated with ^{14}C-BAS 750 F

Soil samples	C-label		T-label	
	DAT [1)]	**TRR** [2)] *[mg/kg]*	**DAT** [1)]	**TRR** [2)] *[mg/kg]*
plant back interval:	**30**		**31**	
soil after ploughing	30	0.094	31	0.094
after harvest of mature crops				
soil post cultivation of spinach	71	0.083	75	0.109
soil post cultivation of radish	98	0.108	101	0.072
soil post cultivation of wheat	135	0.087	136	0.081
plant back interval:	**120**		**122**	
soil after ploughing	120	0.080	122	0.095
after harvest of mature crops				
soil post cultivation of spinach	161	0.086	165	0.094
soil post cultivation of radish	177	0.065	181	0.075
soil post cultivation of wheat	264	0.085	270 (148 DAP)	0.072
plant back interval:	**365**		**364**	
soil after ploughing	365	0.064	364	0.072
after harvest of mature crops				
soil post cultivation of spinach	405	0.070	410	0.075
soil post cultivation of radish	426	0.063	425	0.067
soil post cultivation of wheat	502	0.054	502	0.068

[1)] days after treatment (bare soil application of ^{14}C-BAS 750 F), [2)] TRR=total radioactive residue determined by combustion

TRR for rotational crops of different replant intervals was determined by combustion ("TRR combusted"). For samples extracted, comparable values were obtained for the "TRR calculated" (sum of ERR and RRR) and were therefore used as "100% TRR" for all further calculations (for non-extracted samples "TRR combusted" was used) (see Table 7.6.1-4). In contrast to soil samples, significantly different results were obtained with the two labels.

Table 7.6.1-4: TRR in rotational crops cultivated on ^{14}C-BAS 750 F- treated soil

Crop parts	C-label			T-label		
	DAP [1]	**TRR** [2] *combusted [mg/kg]*	**TRR** [2] *calculated [mg/kg]*	**DAP**	**TRR** *combusted [mg/kg]*	**TRR** *calculated [mg/kg]*
plant back interval 30/31 DAT [1]						
spinach (immature)	28	*0.016*	*0.013*	25	*0.055*	*0.052*
spinach (mature)	41	*0.014*	*0.009*	44	*0.063*	*0.057*
radish (top)	68	*0.013*	*0.011*	70	*0.194*	*0.186*
radish (root)	68	*0.010*	*0.009*	70	*0.281*	*0.267*
wheat (forage)	49	*0.027*	*0.021*	53	*0.318*	*0.288*
wheat (hay)	49	*0.085*	*0.076*	53	*0.761*	*0.681*
wheat (straw) [4]	105	*0.240*	*0.239*	105	*1.058*	*1.039*
wheat (grain)	105	*0.015*	*0.014*	105	*2.400*	*2.311*
plant back interval 120/122 DAT [1]						
spinach (immature)	33	*0.011*	*0.009*	32	*0.114*	*0.116*
spinach (mature)	41	*0.016*	*0.014*	43	*0.171*	*0.150*
radish (top)	57	*0.006*	*0.006*	59	*0.209*	*0.197*
radish (root)	57	*0.009*	*0.008*	59	*0.206*	*0.198*
wheat (forage)	50	*0.030*	*0.024*	52	*0.417*	*0.387*
wheat (hay)	50	*0.181*	*0.155*	52	*2.561*	*2.260*
wheat (straw) [4]	144	*0.105*	*0.094*	148	*1.102*	*1.008*
wheat (grain)	144	*0.039*	*0.039*	148	*3.389*	*3.252*
plant back interval 365/364 DAT [1]						
spinach (immature)	27	*0.007*	- [3]	33	*0.096*	*0.094*
spinach (mature)	40	*0.007*	- [3]	46	*0.108*	*0.097*
radish (top)	61	*0.005*	- [3]	61	*0.100*	*0.100*
radish (root)	61	*0.005*	- [3]	61	*0.093*	*0.098*
wheat (forage)	55	*0.012*	*0.010*	54	*0.189*	*0.193*
wheat (hay)	55	*0.035*	*0.033*	54	*0.873*	*0.860*
wheat (straw) [4]	137	*0.078*	*0.076*	138	*0.947*	*0.916*
wheat (grain)	137	*0.032*	*0.033*	138	*2.258*	*2.221*

[1] *DAT=days after soil treatment (soil aging interval), DAP=days after planting/sowing (cultivation interval),*
[2] *TRR=sum of ERR and RRR (ERR: methanol extract and water extract, RRR: residues after solvent extraction),*
[3] *no extraction performed,*
[4] *straw samples including chaff fraction*

With the C-label, low levels of TRR (maximal 0.02 mg/kg, decreasing further at longer replant intervals) was seen for spinach (mature and immature leaf), for radish (root and top) and for wheat forage. Higher residues in wheat hay may reflect the loss of water during its production from forage (0.08, 0.16, 0.03 mg/kg for the three replant intervals). In straw (including chaff), levels were higher at short replant interval (30 DAT: 0.24 mg/kg) and decreased to 0.10 mg/kg (120 DAT) and 0.08 mg/kg (365 DAT). The TRR in grain was low at 30 DAT (0.014 mg/kg) increasing towards longer replant intervals (120 DAT: 0.039 mg/kg, 365 DAT: 0.033 mg/kg).

With the T-label, throughout replant intervals DAT31/122/364, significantly higher TRR levels than for the C-label were found in spinach (mature leaf: 0.06/0.15/0.10 mg/kg, similar in immature leaf), in radish (root: 0.27/0.20/0.10 mg/kg, similar in top), in forage (0.29/0.39/0.19 mg/kg) and straw

(1.0/1.0/0.9 mg/kg). In grain the largest TRR difference between T-label (2.3/3.3/2.2 mg/kg) and C-label (<0.04 mg/kg) was seen. The decline in residues generally observed with increasing PBI in the C-label is not observed for the T-label.

Taken together, the TRR values obtained indicate uptake of radioactive residue from the soil for all representative crops. In most crop parts, residues remained similar or decreased at longer replant intervals (except for C-labelled grain, potentially attributable to an analytical anomaly). For all crop parts, several fold higher residues were seen with the T-label compared with the C-label indicating the presence (and plant uptake) of T-label-specific cleavage products (as defined by absence of C-ring). In crop metabolism studies similar label-specific differences were seen (see section 7.2.1).The largest difference was determined for grain (DAT30/31) with TRR of 2.3 mg/kg (T-label) and only 0.014 mg/kg (C-label).

It should be noted that plants were cultivated under plastic with limited drainage. Limited drainage is assumed to enhance the accessibility of mobile compounds to uptake by plant roots. As BAS 750 F demonstrates limited absorbance at wavelengths >290 nm, there is some minor potential for photolytic degradation (see section CA B.2.4). However, the large majority of absorbance is at wavelengths <290 nm, therefore it is not considered that photolytic degradation will have any significant effect on the behaviour of BAS 750 F.

Extractability

The extractabilities of ^{14}C residues from wheat, radish and spinach are summarized in Table 7.6.1-5 and Table 7.6.1-6.

With the C-label, solvent extraction of spinach and radish resulted in low RRR (maximum 0.005 mg/kg). Given the low TRR in these samples, the RRR represented up to 17-60% TRR while the corresponding ERR was 40-83% TRR (ERR was extracted mainly by methanol, while water extracted only an additional 2-6% TRR with the exception of radish top where water extraction amounted to 8-12% TRR). Similar extractability was seen for wheat forage. RRR was low (max 0.014 mg/kg, representing up to 61% of the TRR). Hay containing several fold higher residues than forage (water loss during production from forage) showed similar extractabilities resulting in RRR up to 58% TRR (representing 0.09 mg/kg). For both forage and hay, methanol extraction (35-70% TRR) was more effective than water (only additional 2-7% TRR).

In straw/chaff, the proportion of the residue not extracted by solvent was high (35%, 53% and 66% TRR for DAT 30, 120, 365). With longer replant intervals, the methanol extractable residue decreased from 54% to 26% TRR, while the water extractable residue decreased from 12 to 8% TRR.

In grain, the predominant proportion of the residue was not extracted by solvent resulting in RRR of 79 to 92% TRR. Extraction with methanol and water did retrieve similar amounts of residue (7-11% TRR), with the exception of one grain sample (30 DAT, where methanol did not extract any detectable residue).

Table 7.6.1-5: Extractability of radioactive residues in rotational crops (C-Label)

Crop part	Distribution of radioactive residues								
	TRR [1]	methanol extract		water extract		ERR [1]		RRR [1]	
	[mg/kg]	%TRR	*[mg/kg]*	%TRR	*[mg/kg]*	%TRR	*[mg/kg]*	%TRR	*[mg/kg]*
plant back interval 30 DAT [1]									
spinach, imm.	*0.013*	**80.5**	*0.010*	**2.2**	*0.0003*	**82.7**	*0.011*	**17.3**	*0.002*
spinach, mature	*0.009*	**78.0**	*0.007*	**1.8**	*0.0002*	**79.8**	*0.007*	**20.2**	*0.002*
radish, top	*0.011*	**67.3**	*0.007*	**12.0**	*0.0013*	**79.3**	*0.008*	**20.7**	*0.002*
radish, root	*0.009*	**53.2**	*0.005*	**2.4**	*0.0002*	**55.6**	*0.005*	**44.4**	*0.004*
wheat, forage	*0.021*	**68.7**	*0.015*	**2.1**	*0.0005*	**70.8**	*0.015*	**29.2**	*0.006*
wheat, hay	*0.076*	**70.0**	*0.053*	**5.0**	*0.0038*	**74.9**	*0.057*	**25.1**	*0.019*
wheat, straw [4]	*0.239*	**53.7**	*0.128*	**11.6**	*0.0278*	**65.3**	*0.156*	**34.7**	*0.083*
wheat, grain	*0.014*	**0.0**	*0.000*	**7.8**	*0.0011*	**7.8**	*0.001*	**92.2**	*0.013*
plant back interval 120 DAT [1]									
spinach, imm	*0.009*	**63.9**	*0.006*	**5.0**	*0.0005*	**68.9**	*0.006*	**31.1**	*0.003*
spinach, mature	*0.014*	**53.8**	*0.007*	**6.0**	*0.0008*	**59.8**	*0.008*	**40.2**	*0.005*
radish, top	*0.006*	**31.7**	*0.002*	**8.3**	*0.0005*	**39.9**	*0.002*	**60.1**	*0.004*
radish, root	*0.008*	**54.2**	*0.005*	**4.1**	*0.0003*	**58.2**	*0.005*	**41.8**	*0.004*
wheat, forage	*0.024*	**40.0**	*0.010*	**3.5**	*0.0009*	**43.5**	*0.011*	**56.5**	*0.014*
wheat, hay	*0.155*	**35.0**	*0.054*	**6.8**	*0.0106*	**41.8**	*0.065*	**58.2**	*0.090*
wheat, straw [4]	*0.094*	**38.7**	*0.036*	**8.7**	*0.0082*	**47.4**	*0.045*	**52.6**	*0.050*
wheat, grain	*0.039*	**11.4**	*0.004*	**9.9**	*0.0039*	**21.2**	*0.008*	**78.8**	*0.031*
plant back interval 365 DAT [1]									
wheat, forage	*0.010*	**34.8**	*0.004*	**4.6**	*0.0005*	**39.4**	*0.004*	**60.6**	*0.006*
wheat, hay	*0.033*	**44.5**	*0.014*	**6.1**	*0.0020*	**50.6**	*0.016*	**49.4**	*0.016*
wheat, straw [4]	*0.076*	**25.5**	*0.019*	**8.3**	*0.0063*	**33.8**	*0.026*	**66.2**	*0.050*
wheat, grain	*0.033*	**7.3**	*0.002*	**7.8**	*0.0025*	**15.1**	*0.005*	**84.9**	*0.028*

[1] *DAT=days after soil treatment, TRR=total radioactive residue calculated as sum of ERR and RRR, ERR=extractable radioactive residue calculated as sum of the methanol extract and water extract, RRR=radioactive residue after solvent extraction,* [4] *straw samples including chaff fraction*

With the T-label, extractabilities were higher, thus correlating with the higher TRR when compared to the C-label. In addition, no replant-interval-dependency was observed.

Solvent extraction of spinach and radish samples resulted in RRR <5% TRR (maximum 0.009 mg/kg, thus similar to RRR with the C-label). Extraction was most efficient with methanol (81% TRR or higher) while water extracted <5% TRR (except for radish tops 8-14% TRR).

For forage and hay similar extractability patterns were observed with RRR at 6-13% TRR, the ERR was mostly extracted with methanol (forage 85-87% TRR, hay 57-83% TRR), to lesser extent with water (forage 6% TRR, hay 11-30%).

In straw/chaff, extractability was >82% TRR (methanol extraction with 49-68% TRR exceeding water extraction with 20-34% TRR). Given TRR at 1.0 mg/kg, the RRR of <18% represented residue levels of up to 0.19 mg/kg.

In grain, extractability was very high (93% TRR or higher) with similar amounts obtained by methanol and water extraction steps (41-53% TRR). The RRR (< 7% TRR) represented up to 0.15 mg/kg.

Table 7.6.1-6: Extractability of radioactive residues in rotational crops (T-Label)

Crop part	Distribution of radioactive residues								
	TRR [1]	methanol extract		water extract		ERR [1]		RRR [1]	
	[mg/kg]	% TRR	*[mg/kg]*	% TRR	*[mg/kg]*	% TRR	*[mg/kg]*	% TRR	*[mg/kg]*
plant back interval 31 DAT [1]									
spinach,	*0.052*	**93.4**	*0.049*	**2.4**	*0.0012*	**95.7**	*0.050*	**4.3**	*0.002*
spinach, mature	*0.057*	**95.2**	*0.054*	**1.3**	*0.0008*	**96.6**	*0.055*	**3.4**	*0.002*
radish, top	*0.186*	**87.3**	*0.162*	**7.7**	*0.0144*	**95.1**	*0.177*	**4.9**	*0.009*
radish, root	*0.267*	**92.7**	*0.248*	**4.2**	*0.0112*	**96.9**	*0.259*	**3.1**	*0.008*
wheat, forage	*0.288*	**87.0**	*0.251*	**5.8**	*0.0167*	**92.8**	*0.267*	**7.2**	*0.021*
wheat, hay	*0.681*	**78.9**	*0.537*	**13.5**	*0.0921*	**92.4**	*0.630*	**7.6**	*0.052*
wheat, straw [4]	*1.039*	**57.6**	*0.598*	**24.6**	*0.2559*	**82.2**	*0.854*	**17.8**	*0.185*
wheat, grain	*2.311*	**47.5**	*1.097*	**46.0**	*1.0634*	**93.5**	*2.161*	**6.5**	*0.150*
plant back interval 122 DAT [1]									
spinach,	*0.116*	**93.1**	*0.108*	**2.3**	*0.0026*	**95.4**	*0.110*	**4.6**	*0.005*
spinach, mature	*0.150*	**92.2**	*0.139*	**2.7**	*0.0040*	**94.9**	*0.143*	**5.1**	*0.008*
radish, top	*0.197*	**81.4**	*0.160*	**14.1**	*0.0278*	**95.5**	*0.188*	**4.5**	*0.009*
radish, root	*0.198*	**91.8**	*0.182*	**4.3**	*0.0086*	**96.2**	*0.190*	**3.8**	*0.008*
wheat, forage	*0.387*	**84.9**	*0.328*	**6.4**	*0.0247*	**91.3**	*0.353*	**8.7**	*0.034*
wheat, hay	*2.260*	**56.7**	*1.282*	**30.0**	*0.6772*	**86.7**	*1.959*	**13.3**	*0.302*
wheat, straw [4]	*1.008*	**68.2**	*0.687*	**20.0**	*0.2018*	**88.2**	*0.889*	**11.8**	*0.119*
wheat, grain	*3.252*	**49.3**	*1.603*	**46.4**	*1.5087*	**95.7**	*3.111*	**4.3**	*0.141*
plant back interval 364 DAT [1]									
spinach,	*0.094*	**94.6**	*0.089*	**2.1**	*0.0020*	**96.7**	*0.091*	**3.3**	*0.003*
spinach, mature	*0.097*	**94.2**	*0.091*	**2.3**	*0.0022*	**96.4**	*0.093*	**3.6**	*0.003*
radish, top	*0.100*	**83.6**	*0.083*	**12.9**	*0.0128*	**96.5**	*0.096*	**3.5**	*0.004*
radish, root	*0.098*	**96.4**	*0.094*	**2.3**	*0.0023*	**98.7**	*0.097*	**1.3**	*0.001*
wheat, forage	*0.193*	**85.6**	*0.165*	**6.2**	*0.0120*	**91.8**	*0.177*	**8.2**	*0.016*
wheat, hay	*0.860*	**83.0**	*0.715*	**10.8**	*0.0928*	**93.8**	*0.807*	**6.2**	*0.053*
wheat, straw [4]	*0.916*	**48.9**	*0.448*	**33.8**	*0.3095*	**82.7**	*0.757*	**17.3**	*0.159*
wheat, grain	*2.221*	**40.6**	*0.901*	**52.9**	*1.1742*	**93.4**	*2.075*	**6.6**	*0.146*

[1] *TRR=total radioactive residue calculated as sum of ERR and RRR, ERR=extractable radioactive residue calculated as sum of the methanol extract and water extract, RRR=radioactive residue after solvent extraction, for precise values see* Table 6.6.1-11 (further characterization of RRR), [4] straw samples including the chaff fraction

The extractability data indicates that the T-label specific components of the residue are not only present at high levels but also to a large extent solvent-extractable (and thus accessible to further analysis).

Characterisation and Identification

The results of the ammonia incubations and enzyme solubilisations of the residue after solvent extraction are summarized in Tables 7.6.1-7 to 7.6.1-9.

With the C-label, RRR of spinach (mature leaf, DAT30, 0.002 mg/kg), radish root (30 DAT, 0.004 mg/kg) and radish top (30 DAT, 0.002 mg/kg) were subjected to treatment with ammonia (AM) followed by enzyme digestion with cell wall degrading enzymes macerozyme/cellulase (M/C) obtaining similar results. For spinach, treatment of the RRR (20% TRR) resulted in characterization of 10% TRR (3% AM, 7% M/C, leaving a final unextractable residue of 6% TRR (0.0005 mg/kg). For radish top, treatment of the RRR (21% TRR) resulted in characterization of 10% TRR (4% AM, 6% M/C, leaving a final unextractable residue of 10% TRR (0.0010 mg/kg). For radish root, treatment of the RRR (44% TRR) resulted in characterization of 14% TRR (4% AM, 10% M/C, leaving a final unextractable residue of 31% TRR (0.0029 mg/kg).

No analysis of immature spinach was undertaken due to the lower residue levels observed than for mature spinach. C-labelled spinach (mature leaf, DAT120, RRR 0.0055 mg/kg) was subjected to AM, which resulted in characterization of 5% TRR, leaving a final unextractable residue of 32% TRR (0.004 mg/kg). No further analysis of spinach or radish matrices at DAT 120 or 365 was made, due to the low level of residues determined. As lower levels of RRR were present for the T-labelled samples, no further characterization of spinach or radish was undertaken.

RRR of C-labelled wheat forage from replant intervals DAT30 (0.006 mg/kg), DAT120 (0.014 mg/kg), and DAT365 (0.006 mg/kg) was subjected to AM followed by enzyme digestion (M/C, and polyphenol-oxidases tyrosinase/laccase (T/L) for the DAT365 samples). Treatment of RRRs (representing 29%, 57% and 61% TRR) resulted in characterization of 12%, 38% and 20% TRR leaving final unextractable residues of <0.004 mg/kg (<13-32% TRR). Similar characterization was obtained for RRR of hay (0.019 mg/kg, 0.091 mg/kg, 0.016 mg/kg). Treatments resulted in characterization of 12%, 34% and 15% TRR leaving final unextractable residues of <0.04 mg/kg.

RRR of straw from replant intervals DAT30 (0.083 mg/kg), DAT120 (0.050 mg/kg), and 365 DAT (0.050 mg/kg) was subjected to AM followed by enzyme digestion (M/C, T/L). Treatment of RRRs (representing 35%, 53% and 66% TRR) resulted in characterization of 11%, 20% and 14% TRR leaving final unextractable residues of up to 0.05 mg/kg (21-51%% TRR). Most solubilization was obtained with AM and M/C while T/L added only 1.9% TRR or less.

RRR of grain from replant intervals DAT30 (0.01 mg/kg), DAT120 (0.03 mg/kg), and 365 DAT (0.03 mg/kg) was subjected to AM followed by two enzyme digestion steps (M/C, then starch-degrading enzymes amylase/amyloglucosidase (A/G)). Treatment of RRRs (representing 92%, 79% and 85% TRR) resulted in characterization of the major proportion of the RRR (representing 72%, 62% and 50% TRR) leaving final residues of <0.008 mg/kg. The three treatments released similar amounts of the residue (15-30% TRR).

With the T-label, RRR of forage from replant intervals DAT30 (0.021 mg/kg), DAT120 (0.034 mg/kg), and 365 DAT (0.016 mg/kg) was subjected to solubilization including treatment with AM, with M/C, and with T/L). Treatment of RRRs (representing 7-8% TRR) resulted in characterization of 3.5-5.5% TRR leaving final unextractable residues of <0.01 mg/kg (<3% TRR).

Similar characterization was obtained for RRR of hay (0.05 mg/kg, 0.30 mg/kg, 0.05 mg/kg representing 6-13% TRR). Treatments resulted in characterization of 4-10% TRR leaving final unextractable residues of <3% TRR (up to 0.06 mg/kg).

RRR of straw from replant intervals DAT30 (0.19 mg/kg), DAT120 (0.12 mg/kg), and DAT365 (0.16 mg/kg) was subjected to AM followed by enzyme digestion (M/C, and T/L). Treatment of RRRs (representing 12-18% TRR) resulted in characterization of 6-12% TRR leaving final unextractable residues of up to 0.05 mg/kg (<6% TRR). Most solubilization was obtained with AM (6-10%), less with M/C (<2% TRR) and T/L (<1% TRR).

RRR of grain from replant intervals DAT30 (0.15 mg/kg), DAT120 (0.14 mg/kg), and DAT365 (0.15 mg/kg) was subjected to AM followed by two enzyme digestion steps (A/G, M/C). Treatment of RRRs (representing 4-7% TRR) resulted in characterization of the major proportion of the RRR (representing 3.6-5.4% TRR) leaving final unextractable residues of <0.7 % TRR (maximum 0.017 mg/kg). AM and A/G together released higher amounts than M/C indicating preferential association with starch compounds.

For wheat, a similar pattern of solubilisation was observed upon sequential treatment with ammonia (AM), starch-degrading enzymes amylase/amyloglucosidase (A/G), cell wall degrading enzymes macerozyme/cellulase (M/C) and polyphenol-oxidases tyrosinase/laccase (T/L).

Following ammonia incubations and enzyme solubilisations in some cases residues were >0.01 mg/kg; however no further efforts were made to characterise these residues as the contribution to the TRR is < 10%, and a significant proportion of the residue was available for characterisation/identification, therefore further characterisation was not considered to impact significantly on the study results.

Table 7.6.1-7: C-label: characterization of RRR of spinach and radish matrices

Solubilization fraction[1)]	**Matrices**							
	spinach (imm)		**spinach (mature)**		**radish top**		**radish root**	
	% TRR	*[mg/kg]*	*% TRR*	*[mg/kg]*	*% TRR*	*[mg/kg]*	*% TRR*	*[mg/kg]*
Plant back interval 30 DAT								
RRR	**17.3**	*0.0022*	**20.2**	*0.0018*	**20.7**	*0.0022*	**44.4**	*0.0041*
AM solubilizate [2)]	- [3)]	- [3)]	**2.9**	*0.0003*	**4.0**	*0.0004*	**4.2**	*0.0004*
AM residue	- [3)]	- [3)]	**14.3**	*0.0013*	**15.5**	*0.0016*	**40.7**	*0.0038*
A/G solubilizate	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
A/G residue	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
M/C solubilizate	- [3)]	- [3)]	**7.3**	*0.0007*	**5.7**	*0.0006*	**10.1**	*0.0009*
M/C residue	- [3)]	- [3)]	**6.1**	*0.0005*	**10.3**	*0.0011*	**31.0**	*0.0029*
T/L solubilizate	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
T/L residue	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
Sum Solubilized	- [3)]	- [3)]	**10.2**	*0.0009*	**9.7**	*0.0010*	**14.2**	*0.0013*
Unextractable Residue	- [3)]	- [3)]	**6.1**	*0.0005*	**10.3**	*0.0011*	**31.0**	*0.0029*
Plant back interval 120 DAT								
RRR	**31.1**	*0.0029-*[3]	**40.2**	*0.0055*	**60.1**	*0.0037*	**41.8**	*0.0035*
AM solubilizate [2)]	- [3)]	- [3)]	**5.2**	*0.0007*	- [3)]	- [3)]	- [3)]	- [3)]
AM residue	- [3)]	- [3)]	**31.7**	*0.0043*	- [3)]	- [3)]	- [3)]	- [3)]
A/G solubilizate	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
A/G residue	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
M/C solubilizate	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
M/C residue	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
T/L solubilizate	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
T/L residue	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]
Sum Solubilized	-[3]	-[3]	**5.2**	*0.0007*	-[3]	-[3]	-[3]	-[3]
Unextractable Residue	-[3]	-[3]	**31.7**	*0.0043*	-[3]	-[3]	-[3]	-[3]

[1)] AM, ammonia, A/G amylase/amyluglucosidase, M/C macerozyme/cellulase, T/L tyrosinase/laccase, [2)] combined ammonia solubilizate from two solubilisation steps in the cases of spring wheat hay, straw and grain; only one solubilisation step was performed in the cases of mature spinach, white radish top and root and spring wheat forage, 3) not analysed

Table 7.6.1-8: C-label: characterization of RRR of in wheat matrices

Solubilization fraction[1)]	wheat matrices							
	forage		hay		straw [4)]		grain	
	% TRR	*[mg/kg]*	*% TRR*	*[mg/kg]*	*% TRR*	*[mg/kg]*	*% TRR*	*[mg/kg]*
Plant back interval 30 DAT								
RRR	**29.2**	*0.0062*	**25.1**	*0.0189*	**34.7**	*0.0828*	**92.2**	*0.0132*
AM solubilizate [2)]	**6.1**	*0.0013*	**8.5**	*0.0064*	**7.7**	*0.0183*	**23.2**	*0.0033*
AM residue	**21.6**	*0.0046*	**17.1**	*0.0129*	**25.9**	*0.0618*	**63.5**	*0.0091*
A/G solubilizate	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**28.4**	*0.0041*
A/G residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**31.3**	*0.0045*
M/C solubilizate	**5.4**	*0.0012*	**3.9**	*0.0030*	**2.2**	*0.0053*	**20.4**	*0.0029*
M/C residue	**13.3**	*0.0029*	**9.6**	*0.0072*	**22.8**	*0.0544*	**10.5**	*0.0015*
T/L solubilizate	-[3]	-[3]	-[3]	-[3]	**0.9**	*0.0020*	-[3]	-[3]
T/L residue	-[3]	-[3]	-[3]	-[3]	**21.6**	*0.0516*	-[3]	-[3]
Sum Solubilized	**11.5**	*0.0025*	**12.4**	*0.0094*	**10.8**	*0.0257*	**71.9** [2)]	*0.0103*
Unextractable Residue	**13.3**	*0.0029*	**9.6**	*0.0072*	**21.6**	*0.0516*	**10.5**	*0.0015*
Plant back interval 122 DAT								
RRR	**56.5**	*0.0138*	**58.2**	*0.0901*	**52.6**	*0.0495*	**78.8**	*0.0309*
AM solubilizate [2)]	**20.0**	*0.0049*	**5.4**	*0.0084*	**8.5**	*0.0081*	**17.5**	*0.0069*
AM residue	**35.1**	*0.0086*	**48.8**	*0.0756*	**43.2**	*0.0407*	**59.5**	*0.0234*
A/G solubilizate	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**29.6**	*0.0116*
A/G residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**26.5**	*0.0104*
M/C solubilizate	**18.0**	*0.0044*	**28.9**	*0.0447*	**9.1**	*0.0085*	**14.9**	*0.0059*
M/C residue	**14.9**	*0.0036*	**21.9**	*0.0339*	**33.8**	*0.0318*	**8.5**	*0.0034*
T/L solubilizate	-[3]	-[3]	-[3]	-[3]	**1.9**	*0.0018*	-[3]	-[3]
T/L residue	-[3]	-[3]	-[3]	-[3]	**29.3**	*0.0275*	-[3]	-[3]
Sum Solubilized	**38.0**	*0.0093*	**34.3**	*0.0530*	**19.5**	*0.0184*	**62.0**	*0.0244*
Unextractable Residue	**14.9**	*0.0036*	**21.9**	*0.0339*	**29.3**	*0.0275*	**8.5**	*0.0034*
Plant back interval 365 DAT								
RRR	**60.6**	*0.0063*	**49.4**	*0.0161*	**66.2**	*0.0500*	**84.9**	*0.0276*
AM solubilizate [2)]	**8.7**	*0.0009*	**8.0**	*0.0026*	**8.6**	*0.0065*	**14.7**	*0.0048*
AM residue	**44.0**	*0.0046*	**34.8**	*0.0113*	**54.9**	*0.0415*	**65.6**	*0.0213*
A/G solubilizate	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**18.3**	*0.0060*
A/G residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]
M/C solubilizate	**8.6**	*0.0009*	**5.8**	*0.0019*	**3.9**	*0.0029*	**17.2**	*0.0056*
M/C residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**25.2**	*0.0082*
T/L solubilizate	**3.0**	*0.0003*	**1.6**	*0.0005*	**1.7**	*0.0013*	-[3]	-[3]
T/L residue	**32.2**	*0.0033*	**32.2**	*0.0105*	**50.8**	*0.0384*	-[3]	-[3]
Sum Solubilized	**20.4**	*0.0021*	**15.4**	*0.0050*	**14.3**	*0.0108*	**50.2**	*0.0163*
Unextractable Residue	**32.2**	*0.0033*	**32.2**	*0.0105*	**50.8**	*0.0384*	**25.2**	*0.0082*

[1)] AM, ammonia, A/G amylase/amyluglucosidase, M/C macerozyme/cellulase, T/L tyrosinase/laccase, [2)] combined ammonia solubilizate from two solubilisation steps in the cases of spring wheat hay, straw and grain; only one solubilisation step was performed in the cases of mature spinach, white radish top and root and spring wheat forage, 3) not analysed, [4)] straw samples include chaff

Table 7.6.1-9: T-label: characterization of RRR of in wheat matrices

Solubilization fraction[1)]	wheat forage		wheat hay		wheat straw [4]		wheat grain	
	% TRR	*[mg/kg]*	*% TRR*	*[mg/kg]*	*% TRR*	*[mg/kg]*	*% TRR*	*[mg/kg]*
Plant back interval 31 DAT								
RRR	**7.2**	*0.0208*	**7.6**	*0.0519*	**17.8**	*0.1851*	**6.5**	*0.1499*
AM solubilizate [2)]	**3.5**	*0.0100*	**3.7**	*0.0249*	**9.5**	*0.0990*	**1.9**	*0.0436*
AM residue	**2.7**	*0.0079*	**3.0**	*0.0208*	**6.6**	*0.0687*	**4.4**	*0.1020*
A/G solubilizate	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**3.1**	*0.0712*
A/G residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**0.9**	*0.0216*
M/C solubilizate	-[3]	-[3]	**0.7**	*0.0044*	**1.5**	*0.0155*	**0.5**	*0.0109*
M/C residue	-[3]	-[3]	**2.1**	*0.0144*	**4.9**	*0.0504*	**0.4**	*0.0097*
T/L solubilizate	-[3]	-[3]	-[3]	-[3]	**0.5**	*0.0055*	-[3]	-[3]
T/L residue	-[3]	-[3]	-[3]	-[3]	**4.3**	*0.0450*	-[3]	-[3]
Sum Solubilized	**3.5**	*0.0100*	**4.3**	*0.0294*	**11.6**	*0.1201*	**5.4**	*0.1257*
Unextractable Residue	**2.7**	*0.0079*	**2.1**	*0.0144*	**4.3**	*0.0450*	**0.4**	*0.0097*
Plant back interval 365 DAT								
RRR	**8.7**	*0.0338*	**13.3**	*0.3017*	**11.8**	*0.1188*	**4.3**	*0.1410*
AM solubilizate [2)]	**3.0**	*0.0116*	**7.3**	*0.1652*	**4.3**	*0.0430*	**1.6**	*0.0505*
AM residue	**5.0**	*0.0193*	**5.4**	*0.1219*	**6.3**	*0.0637*	**2.6**	*0.0830*
A/G solubilizate	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**1.7**	*0.0558*
A/G residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**0.7**	*0.0215*
M/C solubilizate	**2.4**	*0.0094*	**2.6**	*0.0586*	**1.7**	*0.0167*	**0.3**	*0.0107*
M/C residue	**2.4**	*0.0091*	**2.6**	*0.0597*	**4.6**	*0.0465*	**0.2**	*0.0078*
T/L solubilizate	-[3]	-[3]	-[3]	-[3]	**0.5**	*0.0049*	-[3]	-[3]
T/L residue	-[3]	-[3]	-[3]	-[3]	**3.9**	*0.0395*	-[3]	-[3]
Sum Solubilized	**5.4**	*0.0210*	**9.9**	*0.2238*	**6.4**	*0.0646*	**3.6**	*0.1170*
Unextractable Residue	**2.4**	*0.0091*	**2.6**	*0.0597*	**3.9**	*0.0395*	**0.2**	*0.0078*
Plant back interval 365 DAT								
RRR	**8.2**	*0.0159*	**6.2**	*0.0532*	**17.3**	*0.1587*	**6.6**	*0.1460*
AM solubilizate [2)]	**4.5**	*0.0088*	**3.1**	*0.0266*	**7.2**	*0.0657*	**4.1**	*0.0902*
AM residue	**3.4**	*0.0065*	**2.6**	*0.0227*	**8.6**	*0.0788*	**2.8**	*0.0616*
A/G solubilizate	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**1.3**	*0.0285*
A/G residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]
M/C solubilizate	**0.8**	*0.0015*	**0.5**	*0.0041*	**1.6**	*0.0145*	**0.3**	*0.0068*
M/C residue	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	**0.7**	*0.0165*
T/L solubilizate	**0.2**	*0.0004*	**0.2**	*0.0014*	**0.7**	*0.0061*	-[3]	-[3]
T/L residue	**2.3**	*0.0045*	**1.9**	*0.0165*	**5.9**	*0.0540*	-[3]	-[3]
Sum Solubilized	**5.5**	*0.0107*	**3.7**	*0.0320*	**9.4**	*0.0863*	**5.7**	*0.1256*
Unextractable Residue	**2.3**	*0.0045*	**1.9**	*0.0165*	**5.9**	*0.0540*	**0.7**	*0.0165*

[1)] AM, ammonia, A/G amylase/amyluglucosidase, M/C macerozyme/cellulase, T/L tyrosinase/laccase, [2)] combined ammonia solubilizate from two solubilisation steps in the cases of spring wheat hay, straw and grain; only one solubilisation step was performed in the cases of mature spinach, white radish top and root and spring wheat forage, 3) not analysed, [4)] straw samples include chaff

Data on identification and characterization of radioactive components of the residue (ERR and RRR) is summarized in Table 7.6.1-10 (C-label) and Table 7.6.1-11 (T-label). Detail of the characterization of components in the RRR is given above. Identification and characterization of the ERR was via HPLC-MS.

Chiral analysis of spinach, radish and wheat forage and hay samples (C-label), and spinach (T-label) confirmed that the racemic mixture (1:1 ratio of S-enantiomer and R-enantiomer) of the application formulation is essentially maintained, and hence that there is no significant change in BAS 750 F enantiomers applied to bare soil. The unchanged enantiomer ratio indicates absence of preferential metabolism and /or translocation of one of the two enantiomers in rotational crops. Details of the isomer ratio are given in Table 7.6.1-12.

Table 7.6.1-12: Determination of isomer ratio of BAS 750 F in rotational crop matrices

Matrix	S-enantiomer [%]	R-enantiomer [%]
C-label		
application formulation	51.65	48.35
application formulation	48.97	51.03
spinach, mature	56.44	43.56
radish, root	49.57	50.43
wheat, forage	50.94	49.06
wheat, hay	57.84	42.16
T-label		
application formulation	50.51	48.87
spinach, immature	52.96	47.04

For the C-label, representative data on identity of residue components could be obtained for all crop parts (except grain). For the T-label, data on identity of residue components could be obtained for all crop parts/replant intervals including grain. Correlating with TRR and extractability data, significantly different results were obtained with the two labels.

For the C-label, the identification rate reflects the amounts of residue retrieved by extraction and solubilization, e.g. high for spinach at DAT30 (>85% TRR) and moderate for radish at DAT30 (55 and 62% TRR). The only identified component of the radioactive residue in any commodity is unchanged parent BAS 750 F. No residues of parent BAS 750 F (or other metabolites) were found in grain. In spinach (mature leaf), parent BAS 750 F amounted to 85% TRR at DAT30 (0.008 mg/kg) and 52% of TRR at DAT120 (0.007 mg/kg). Similar values were obtained for immature leaf (91% and 61% TRR). In radish top, parent BAS 750 F amounted to 55% TRR at DAT30 (0.006 mg/kg) and 62% (0.006 mg/kg) at DAT30 in radish root. In wheat forage, parent BAS 750 F amounted to 70%, 44%, 18% TRR (DAT30, 120, 365), in hay 62%, 36% and 41% TRR and in straw 43%, 36%, 24% indicating a decrease of BAS 750 F from DAT30 to DAT365 (forage: from 0.015 to 0.002 mg/kg, hay: from 0.046 to 0.013 mg/kg, for straw: from 0.101 to 0.018 mg/kg).

For the T-label, identification rate was generally high with >88% TRR in spinach, >78% TRR in radish, > 89% TRR in grain, >83% TRR in forage/hay, and >73% TRR in straw at all plant back intervals.

AT 30DAT unchanged parent BAS 750 F was detected at levels of 0.01 mg/kg in spinach (mature, immature leaf) and radish top, at level of <0.015 mg/kg in forage and <0.043 mg/kg in straw, which are similar absolute amounts to those seen in C-labelled samples, but representing much smaller proportions of the residue (spinach up to 25% TRR, radish top 6 % TRR, forage/hay/straw at maximum 5 % TRR). Parent BAS 750 F was not present in detectable amounts in spinach leaf, radish

top and wheat forage of DAT122/365, or in radish roots and wheat grain at any DAT. At DAT 112 and 365BAS 750 F was detected in wheat straw at 1.3 % TRR (0.014 mg/kg) and 0.8 % TRR (0.008 mg/kg). In line with the C-labelled samples a decrease in BAS 750 F from DAT30 to DAT365 is observed.

In T-labelled samples, the predominant components of the residue were the triazole derived metabolites 1,2,4-triazole, triazole alanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA). Unlike BAS 750 F, similar levels for all plant back intervals were observed.

In mature spinach of all replant intervals, TA was the predominant component with 53-56% of TRR (corresponding to 0.03 – 0.08 mg/kg). The second most abundant component was TLA with 18-34% TRR (0.01 – 0.05 mg/kg). Lower amounts were seen for 1,2,4-triazole (8-12% TRR) and TAA (0-6% TRR). Similar results were found for immature spinach with TA amounting up to 71% TRR (0.07 mg/kg). In these samples at DAT30, BAS 750 F was present in significant amounts (14% and 25% in mature and immature spinach), which was not detected at longer PBIs.

TA was also the predominant component in radish root (62-79% TRR, 0.08-0.17 mg/kg) and radish top (45-94% TRR, 0.08-0.18 mg/kg), with TLA as the second most abundant component (17-31% TRR/0.017-0.083 mg/kg root, 0-22 % TRR/0-0.042 mg/kg top). TAA and 1,2,4-triazole were not detected in radish root at any PBI. In radish top levels above 4.5 % TRR (0.004 mg/kg) were not detected for these metabolites.

In forage and hay, TA and TLA were present as a similar proportion of the residue (forage: TA 38-44% TRR, TLA 34-38% TRR, hay: TA 32-46% TRR, TLA 24-38% TRR); however the absolute amounts in hay were significantly higher in each case (*ca* 4x higher in hay) with residues between 0.073-0.171 mg/kg for TA and 0.072-0.141 mg/kg for TLA in forage, and 0.311-0.717 mg/kg for TA and 0.162-0.827 for TLA in hay. Lower amounts of TAA and 1,2,4-triazole were present in forage and hay (up to 0.101 mg/kg 1,2,4-triazole and 0.266 mg/kg TAA), with the same pattern of increased absolute amounts observed in hay. BAS 750 F was present only in minor amounts at DAT30 (at maximum at 5% TRR) and was not detected at longer PBIs.

In straw, TLA was the component present in highest amounts (34-39% TRR, 0.344-0.366 mg/kg), followed by TA (13-33%, 0.137-0.333 mg/kg), and TAA (15-19% TRR, 0.156-0.182 mg/kg) with 1,2,4-triazole present at the lowest level 3-6 % TRR, 0.026-0.065 mg/kg). BAS 750 F was present only in minor amounts (<5% TRR) at all PBIs.

In grain, a different metabolite pattern was seen: the predominant component was TA (43-73 % TRR, 0.982-2.361 mg/kg), the second most abundant was TAA (20-24% TRR, 0.462-0.593 mg/kg), together amounting to 63-94% TRR. 1,2,4-triazole was only seen in lower amounts (15% TRR/0.339 mg/kg at 30 DAT, 1% TRR/0.023 mg/kg at 365 DAT), as was TLA (14% TRR/0.319 mg/kg) at 30 DAT). BAS 750 F was not detected in grain.

For the TDMs, TA, TAA and TLA were present overall at higher levels than 1,2,4-triazole which was present at the lowest level of these metabolites in all commodities and plant back intervals.

Characterised components of the ERR were a total of 21.4 % TRR (0.051 mg/kg) in C-label in wheat straw at 30DAT. In all other commodities/PHIs characterised components were significantly lower.

Table 7.6.1-10: C-label: summary of identified and characterized components of ERR and RRR in rotational crop matrices [1)]

Component		Matrices															
		spinach (imm)		spinach (mature)		radish top		radish root		wheat forage		wheat hay		wheat straw[3]		wheat grain	
		%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]
Plant back interval 30 DAT																	
TRR		**100**	*0.013*	**100**	*0.009*	**100**	*0.011*	**100**	*0.009*	**100**	*0.021*	**100**	*0.076*	**100**	*0.239*	**100**	*0.014*
ERR	BAS 750 F	**91.2**	*0.012*	**85.2**	*0.008*	**54.8**	*0.006*	**61.5**	*0.006*	**70.4**	*0.015*	**61.6**	*0.046*	**42.5**	*0.101*	- [3)]	- [3)]
	Total ID	**91.2**	*0.012*	**85.2**	*0.008*	**54.8**	*0.006*	**61.5**	*0.006*	**70.4**	*0.015*	**61.6**	*0.046*	**42.5**	*0.101*	-	-
	Total CHA	**2.2**	*<0.001*	**1.8**	*<0.001*	**25.0**	*0.003*	**2.4**	*<0.001*	**2.1**	*<0.001*	**11.6**	*0.009*	**21.4**	*0.051*	-	-
	Sum ID/CHA	**93.4**	*0.012*	**87.0**	*0.008*	**79.8**	*0.008*	**63.9**	*0.006*	**72.5**	*0.016*	**73.1**	*0.055*	**63.9**	*0.153*	-	-
RRR	***Total CHA***	**-**	-	**10.2**	*<0.001*	**9.1**	*0.001*	**14.2**	*0.001*	**11.5**	*0.0025*	**12.4**	*0.009*	**10.8**	*0.026*	**71.9**	*0.010*
	Final residue[4]	**17.3**	*0.002*	**6.1**	*<0.001*	**10.3**	*0.001*	**31.0**	*0.003*	**13.3**	*0.003*	**9.6**	*0.007*	**21.6**	*0.052*	**10.5**	*0.0015*
Total		**110.7**	*0.014*	**103.3**	*0.009*	**99.7**	*0.011*	**109.2**	*0.010*	**97.4**	*0.021*	**95.1**	*0.072*	**96.3**	*0.230*	**81.9**	*0.0115*
Plant back interval 120 DAT																	
TRR		**100**	*0.009*	**100**	*0.014*	**100**	*0.006*	**100**	*0.008*	**100**	*0.024*	**100**	*0.155*	**100**	*0.094*	**100**	*0.039*
ERR	BAS 750 F	**60.8**	*0.006*	**51.7**	*0.007*	- [3)]	- [3)]	- [3)]	- [3)]	**43.7**	*0.011*	**35.7**	*0.055*	**35.7**	*0.034*	- [3)]	- [3)]
	Total ID	**60.8**	*0.006*	**51.7**	*0.007*	-	-	-	-	**43.7**	*0.011*	**35.7**	*0.055*	**35.7**	*0.034*	-	-
	Total CHA	**5.0**	*<0.001*	**6.0**	*<0.001*	-	-	-	-	**3.5**	*0.001*	**6.8**	*0.011*	**8.7**	*0.008*	**21.2**	*0.008*
	SUM ID/CHA	**65.8**	*0.006*	**57.7**	*0.008*	-	-	-	-	**47.2**	*0.012*	**42.5**	*0.066*	**44.4**	*0.042*	**21.2**	*0.008*
RRR	***Total CHA***	**-**	-	**5.2**	*<0.001*	-	-	-	-	**38.0**	*0.010*	**34.3**	*0.053*	**19.5**	*0.018*	**62.0**	*0.024*
	Final residue[4]	**31.1**	*0.003*	**31.7**	*0.004*	**60.1**	*0.004*	**41.8**	*0.0035*	**14.9**	*0.004*	**21.9**	*0.034*	**29.3**	*0.028*	**8.5**	*0.003*
Total		**96.9**	*0.009*	**94.6**	*0.013*	**60.1**	*0.004*	**41.8**	*0.0035*	**100.1**	*0.026*	**98.7**	*0.153*	**93.2**	*0.088*	**91.7**	*0.035*
Plant back interval 365 DAT																	
TRR		**100**	*0.007*	**100**	*0.007*	**100**	*0.005*	**100**	*0.005*	**100**	*0.010*	**100**	*0.033*	**100**	*0.076*	**100**	0.033
ERR	BAS 750 F	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	**17.7**	*0.002*	**41.0**	*0.013*	**23.9**	*0.018*	- [3)]	- [3)]
	Total ID	-	-	-	-	-	-	-	-	**17.7**	*0.002*	**41.0**	*0.013*	**23.9**	*0.018*	-	-
	Total CHA	-	-	-	-	-	-	-	-	**22.9**	*0.002*	**6.1**	*0.002*	**8.3**	*0.006*	**15.1**	*0.005*
	SUM ID/CHA	-	-	-	-	-	-	-	-	**40.6**	*0.004*	**47.1**	*0.015*	**32.2**	*0.024*	**15.1**	*0.005*
RRR	***Total CHA***	-	-	-	-	-	-	-	-	**20.4**	*0.002*	**15.4**	*0.005*	**14.3**	*0.011*	**50.2**	*0.016*
	Final residue[4]	-	-	-	-	-	-	-	-	**32.2**	*0.003*	**32.2**	*0.01*	**50.8**	*0.038*	**25.2**	*0.008*

Table 7.6.1-10: C-label: summary of identified and characterized components of ERR and RRR in rotational crop matrices [1)]

Component	Matrices															
	spinach (imm)		spinach (mature)		radish top		radish root		wheat forage		wheat hay		wheat straw[3]		wheat grain	
	%TRR	*[mg/kg]*	*%TRR*	*[mg/kg]*	*%TRR*	*[mg/kg]*	*%TRR*	*[mg/kg]*	*%TRR*	*[mg/kg]*	*%TRR*	*[mg/kg]*	*%TRR*	*[mg/kg]*	*%TRR*	*[mg/kg]*
Total	-	-	-	-	-	-	-	-	**93.2**	*0.010*	**94.8**	*0.031*	**97.3**	*0.074*	**90.5**	*0.029*

1) ERR=extractable radioactive residue, RRR=radioactive residue after solvent extraction, 2) straw including chaff, 3) not detected 4) Unextracted residue

Table 7.6.1-11: T-label: summary of identified and characterized components of ERR and RRR in rotational crop matrices [1)]

Component		Matrices															
		spinach (imm)		spinach (mature)		radish top		radish root		wheat forage		wheat hay		wheat straw [2)]		wheat grain	
		%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]
Plant back interval 31 DAT																	
TRR		**100**	*0.052*	**100**	*0.057*	**100**	*0.186*	**100**	*0.267*	**100**	*0.288*	**100**	*0.681*	**100**	*1.039*	**100**	*2.311*
ERR	1,2,4-triazole	**10.5**	*0.005*	**7.7**	*0.004*	- [3)]	- [3)]	- [3)]	- [3)]	**4.0**	*0.012*	**5.0**	*0.034*	**6.3**	*0.065*	**14.7**	*0.339*
	TA	**42.9**	*0.022*	**56.0**	*0.032*	**45.4**	*0.084*	**61.8**	*0.165*	**43.1**	*0.124*	**45.6**	*0.311*	**13.2**	*0.137*	**42.5**	*0.982*
	TAA	**2.5**	*0.001*	**5.5**	*0.003*	**4.2**	*0.008*	- [3)]	- [3)]	**10.9**	*0.031*	**20.8**	*0.142*	**15.0**	*0.156*	**20.0**	*0.462*
	TLA	**8.8**	*0.005*	**18.3**	*0.010*	**22.4**	*0.042*	**30.9**	*0.083*	**33.7**	*0.097*	**23.7**	*0.162*	**35.3**	*0.366*	**13.8**	*0.319*
	BAS 750 F	**25.2**	*0.013*	**13.9**	*0.008*	**5.6**	*0.010*	- [3)]	- [3)]	**5.0**	*0.015*	**4.5**	*0.031*	**4.1**	*0.043*	- [5]	- [5]
	Total ID	**89.9**	*0.047*	**101.5**	*0.058*	**77.6**	*0.144*	**92.8**	*0.248*	**96.7**	*0.279*	**99.7**	*0.679*	**73.8**	*0.767*	**91.0**	*2.103*
	Total CHA	**2.4**	*0.001*	**1.3**	*0.001*	**10.5**	*0.020*	**0.7**	*0.002*	**0.8**	*0.002*	**1.8**	*0.012*	**1.3**	*0.014*	-	-
	Sum ID/CHA	**92.3**	*0.048*	**102.8**	*0.059*	**88.1**	*0.164*	**93.5**	*0.250*	**97.5**	*0.281*	**101.5**	*0.692*	**75.1**	*0.781*	**91.0**	*2.103*
RRR	***Total CHA***	-	-	-	-	-	-	-	-	**3.5**	*0.010*	**4.3**	*0.029*	**11.6**	*0.120*	**5.4**	*0.126*
	Final residue [4]	**4.3**	*0.002*	**3.4**	*0.002*	**4.9**	*0.009*	**3.1**	*0.008*	**2.7**	*0.008*	**2.1**	*0.014*	**4.3**	*0.045*	**0.4**	*0.010*
Total		**96.6**	*0.050*	**106.2**	*0.061*	**93.0**	*0.173*	**96.6**	*0.258*	**103.7**	*0.299*	**107.9**	*0.735*	**91.0**	*0.946*	**96.8**	*2.238*
Plant back interval 122 DAT																	
TRR		**100**	*0.116*	**100**	*0.150*	**100**	*0.197*	**100**	*0.198*	**100**	*0.387*	**100**	*2.260*	**100**	*1.008*	**100**	*3.252*
ERR	1,2,4-triazole	- [3)]	- [3)]	**12.4**	*0.019*	- [3)]	- [3)]	- [3)]	- [3)]	**3.9**	*0.015*	**4.5**	*0.101*	**2.6**	*0.026*	- [3)]	- [3)]
	TA	**60.1**	*0.070*	**52.9**	*0.080*	**93.5**	*0.184*	**62.6**	*0.124*	**44.3**	*0.171*	**31.7**	*0.717*	**33.1**	*0.333*	**72.6**	*2.361*
	TAA	**3.2**	*0.004*	- [5]	- [5]	- [5]	- [5]	- [5]	- [5]	**7.9**	*0.030*	**10.0**	*0.226*	**18.0**	*0.182*	**21.2**	*0.689*
	TLA	**25.0**	*0.029*	**34.2**	*0.051*	- [5]	- [5]	**23.0**	*0.046*	**36.4**	*0.141*	**36.6**	*0.827*	**34.2**	*0.344*	- [3)]	- [3)]
	BAS 750 F	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [5]	- [5]	**1.3**	*0.030*	**1.3**	*0.014*	- [3)]	- [3)]
	Total ID	**88.4**	*0.102*	**99.5**	*0.150*	**93.5**	*0.184*	**85.6**	*0.169*	**92.4**	*0.357*	**84.1**	*1.901*	**89.2**	*0.899*	**93.8**	*3.050*
	Total CHA	**2.3**	*0.003*	**2.7**	*0.004*	-	-	**4.3**	*0.009*	-	-	-	-	**3.9**	*0.039*	-	-
	Sum ID/CHA	**90.7**	*0.105*	**102.2**	*0.154*	**93.5**	*0.184*	**89.9**	*0.178*	**92.4**	*0.357*	**84.1**	*1.901*	**93.1**	*0.939*	**93.8**	*3.050*
RRR	***Total CHA***	-	-	-	-	-	-	-	-	**5.4**	*0.021*	**9.9**	*0.224*	**6.4**	*0.065*	**3.6**	*0.117*
	Final residue [4]	**4.6**	*0.005*	**5.1**	*0.008*	**4.5**	*0.009*	**3.8**	*0.008*	**2.4**	*0.009*	**2.6**	*0.060*	**3.9**	*0.039*	**0.2**	*0.008*

Table 7.6.1-11: T-label: summary of identified and characterized components of ERR and RRR in rotational crop matrices [1)]

Component		Matrices															
		spinach (imm)		spinach (mature)		radish top		radish root		wheat forage		wheat hay		wheat straw [2)]		wheat grain	
		%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]	%TRR	[mg/kg]
Total		**95.3**	*0.110*	**107.3**	*0.162*	**98.0**	*0.193*	**93.7**	*0.186*	**100.2**	*0.387*	**96.6**	*2.185*	**103.4**	*1.043*	**97.6**	*3.175*
Plant back interval 364 DAT																	
TRR		**100**	*0.094*	**100**	*0.097*	**100**	*0.100*	**100**	*0.098*	**100**	*0.193*	**100**	*0.860*	**100**	*0.916*	**100**	*2.221*
ERR	1,2,4-triazole	**-** [5]	- [5]	**7.9**	*0.008*	**2.6**	*0.003*	- [3)]	- [3)]	- [3)]	- [3)]	**4.2**	*0.036*	**4.0**	*0.036*	**1.1**	*0.023*
	TA	**71.1**	*0.067*	**56.2**	*0.054*	**77.5**	*0.077*	**79.1**	*0.077*	**37.7**	*0.073*	**38.3**	*0.330*	**16.6**	*0.152*	**64.2**	*1.425*
	TAA	**-** [5]	- [5]	**3.7**	*0.004*	**4.5**	*0.004*	**-** [5]	- [5]	**8.7**	*0.017*	**11.7**	*0.101*	**18.9**	*0.173*	**24.3**	*0.539*
	TLA	**25.0**	*0.023*	**33.6**	*0.032*	**10.7**	*0.011*	**17.5**	*0.017*	**37.5**	*0.072*	**37.5**	*0.323*	**38.6**	*0.354*	- [3)]	- [3)]
	BAS 750 F	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	- [3)]	**0.8**	*0.008*	- [3)]	- [3)]
	Total ID	**96.1**	*0.090*	**101.4**	*0.098*	**95.2**	*0.095*	**96.6**	*0.095*	**83.8**	*0.162*	**91.7**	*0.789*	**78.9**	*0.722*	**89.5**	*1.987*
	Total CHA	**2.1**	*0.002*	**2.3**	*0.002*	**-**	-	**2.3**	*0.002*	**0.6**	*0.001*	**-**	-	**1.7**	*0.016*	**-**	-
	Sum ID/CHA	**98.2**	*0.092*	**103.7**	*0.100*	**95.2**	*0.095*	**98.9**	*0.097*	**84.4**	*0.163*	**91.7**	*0.789*	**80.6**	*0.738*	**89.5**	*1.987*
RRR	***Total CHA***	**-**	-	**-**	-	**-**	-	**-**	-	**5.5**	*0.011*	**3.7**	*0.032*	**9.4**	*0.086*	**5.7**	*0.126*
	Final residue [4]	**3.3**	*0.003*	**3.6**	*0.003*	**3.5**	*0.004*	**1.3**	*0.001*	**2.3**	*0.005*	**1.9**	*0.017*	**5.9**	*0.054*	**0.7**	*0.017*
Total		**101.5**	*0.095*	**107.3**	*0.103*	**98.7**	*0.099*	**100.2**	*0.098*	**92.2**	*0.179*	**97.3**	*0.838*	**95.9**	*0.878*	**95.9**	*2.129*

1) *ERR=extractable radioactive residue, RRR=radioactive residue after solvent extraction, SUM includes all identified compounds,* [2)] *straw including chaff,* [3)] *not detected* [4)] *Unextracted residue*

Storage stability

Analysis of the storage stability confirmed the stability of radioactive residues over the period of the study, both in the frozen matrix (prior to extraction) and in extracts.

Stability during storage of matrix at ≤-18°C was demonstrated by comparison of the metabolic HPLC profiles for T-labelled wheat straw extracted and analysed within 6 months, with samples stored for longer time periods, and no change in profile was observed. No other matrices were investigated; however, this finding is supported by the storage stability studies in section B7.1.1 which demonstrate stability of BAS 750 F and M750F022 (non-radiolabelled) for up to 2 years and TDMs for up to 48 months, in plant commodities from all commodity groups. Therefore it is considered there is sufficient information available to support the storage of frozen samples in this study.

Stability during storage of extract was investigated by comparing the metabolic HPLC profiles after extended storage of the extract. Methanol extracts were analysed. The periods of stability for the extracts are given in Table 7.6.1-13.

For the storage of both matrix samples and extract samples, comparison of metabolic HPLC profiles confirmed absence of significant changes. For the triazole label, the residues were stable for a period of at least 239 days in methanol extract. For the chlorophenyl label, the residues were stable for a period of at least 234 days in methanol extract.

Table 7.6.1-13: Storage intervals of plant samples and extract samples (spring wheat)

Matrix	Storage of extract		
	storage interval (analysis 1) [2)]	*storage interval (analysis 2)* [2)]	*Storage period*
	[days]	*[days]*	*[days]*
C-label			
spinach, imm. (30 DAT)	72	350	278
spinach, mature (30 DAT)	127	380	253
spinach, mature (120 DAT)	59	378	319
radish, top (30 DAT)	115	349	234
radish, root (30 DAT)	115	512	397
wheat, forage (30 DAT)	75	351	276
wheat, hay (30 DAT)	71	379	308
wheat, straw (30 DAT)	60	459	399
T-label			
spinach, imm. (31 DAT)	43	512	469
spinach, mature (31 DAT)	44	365	321
radish, top (31 DAT)	16	286	270
radish, root (31 DAT)	16	450	434
wheat, forage (31 DAT)	47	286	239
wheat, hay (31 DAT)	46	452	406
wheat, straw (31 DAT)	14	284	270
wheat, grain (31 DAT)	14	448	434

1) sampling to extraction, 2) extraction to analysis, 3

Translocation and proposed metabolic pathway

In rotational crops, the metabolic profile of an active substance is potentially influenced by soil metabolites and their plant uptake and translocation. When the results from both labels are considered

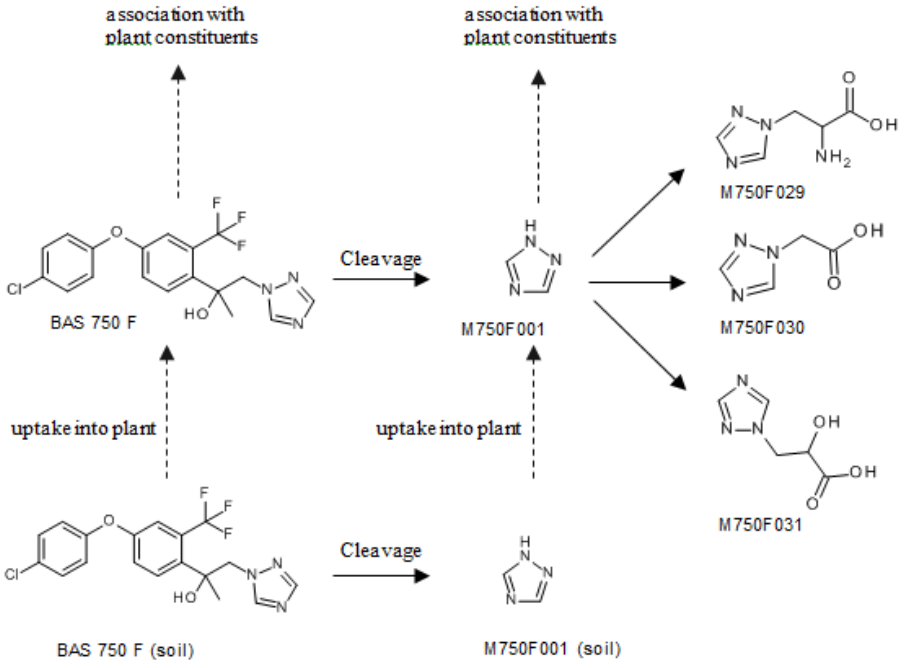
together the data demonstrate consistent metabolic pathways in radish, spinach and wheat forage, straw and grain. The proposed metabolic pathway is outlined in Figure 7.6.1-1.

Determination of total radioactive residue (TRR) indicates label-specific uptake and translocation. The C-label enables radiodetection specifically of parent BAS 750 F (due to non-detectability of TDM). In contrast, the T-label captures both the parent molecule as well as the TDM. Due to the fact that TDM are the predominant component of the T-labelled radioactive residue, the T-label largely represents the TDM. Taking results obtained with both labels together, different uptake and translocation properties can be deduced for parent and TDMs.

The data shows that the unchanged parent BAS 750 F is taken up into the plant to a certain extent followed by association with plant constituents. It is possible that parent BAS 750 F is cleaved within the plant, at the triazole bridge generating 1,2,4-triazole and the other triazole derivative metabolites TA, TAA, and TLA, although its abundance and the absence of any breakdown products with a C-label indicates that breakdown in this way is very limited. Instead, the main proportion of TDMs in rotational crops appear to result from degradation of BAS 750 F in the soil followed by preferential uptake of T-labelled cleavage products into the plant where further transformation (TA, TAA, TLA) and association with plant constituents can occur.

Comparison of metabolic profiles in different rotational crops representing leafy vegetables, root vegetable, and cereals provides a consistent picture. Subsequent to uptake from soil, parent BAS 750 F and TDMs are translocated within the plant, namely the leaf (spinach), top (radish), and plant, (cereal forage/hay/straw). While BAS 750 F is not translocated into the grain, TDMs show high concentration into grain. In conclusion, uptake from soil and translocation within plant is high for TDM, and significantly lower for parent BAS 750 F, and no components specific to rotational crops were identified.

Figure 7.6.1-1: Proposed metabolic pathway of ^{14}C BAS 750 F in rotational crops



Conclusion

Metabolism of BAS 750 F was investigated in three representative succeeding crops spinach (leafy vegetable), radish (root and tuber) and wheat (cereal) using test substance either labelled in the chlorophenyl ring (C-label) or labelled in the triazole ring (T-label). The study included soil aging intervals of 30, 120 and 365 days following bare soil application of the maximal annual use rate of 300 g as/ha, corresponding to a soil concentration of 0.1 mg/kg (depth 20 cm).

The residue in rotational crops was shown to consist of two components, the unchanged parent BAS 750 F (carrying C- and T-label) as well as the TDMs (carrying only the T-label, the group of 1,2,4-triazole, TA, TAA and TLA). No further components were identified.

The metabolic pathway consists of cleavage of the parent backbone structure at the triazole bridge, releasing 1,2,4-triazole, which is further transformed to the derivative metabolites TA, TAA, and TLA. The data obtained shows that parent and cleavage products are taken up by rotational crops from the soil followed by translocation within the plant.

The amount of parent BAS 750 F in rotational crops is generally lower than the amount of TDMs as seen in the metabolic profile of T-labelled samples. Major amounts are only seen in spinach leaf (up to 25% TRR in immature leaf, with TDMs amounting to 65% TRR). In other T-labelled samples, BAS 750 F was present at < 6% TRR and BAS 750 F was not detected in grain. This was confirmed in C-labelled samples, where BAS 750 F was determined at similar absolute amounts.

The TDM amounts, generally exceeded the BAS 750 F amounts. Particularly high levels were determined in T-labelled grain (up to 3.2 mg/kg, while levels in C-labelled grain were < 0.04 mg/kg).

Comparison of metabolic profiles in different rotational crops representing leafy vegetables, root vegetable, and cereals reveals that, subsequent to uptake from soil, parent BAS 750 F and TDMs both are translocated within the “green plant parts” (namely the leaf of spinach, top of radish, and plant of cereal, thus forage/hay/straw). However, only TDM are translocated into the grain, BAS 750 F is not detected.

Comparison of metabolic profiles obtained at different replant intervals reveals that BAS 750 F residues tend to decrease during longer soil aging intervals, whereas TDM levels remained largely unchanged over the intervals investigated.

In summary, the metabolism of BAS 750 F in rotational crops has been comprehensively studied and is thus well understood. Parent BAS 750 F and the TDMs were identified as the major components of the residue. No components specific to rotational crops were detected.

B.7.6.2. Magnitude of residues in rotational crops

Report:	CA 6.6.2/1 Martin T., 2015 a Study on the residue behaviour of BAS 750 F on the rotational crops: wheat, carrots or radish, broccoli or cauliflower and spinach or lettuce after one application of BAS 750 01 F to bare soil under field conditions, 2014-2015 2015/1106682
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011), OECD 504
GLP:	yes

Materials and Methods

During the growing season of 2014-2015, field trials on four rotational crops (wheat, radish/carrot, cauliflower/broccoli and lettuce/spinach) using the formulated product 'BAS 750 01 F', an EC formulation containing 100 g/L BAS 750 F were conducted in Germany, the Netherlands, Italy and Spain. Each trial consisted of three control and three treated plots, corresponding to different replant intervals. The formulation was applied once as a foliar spray at a rate of 300 g as/ha (0.1 mg/kg soil assuming soil depth: 20 cm, soil density 1.5 g/cm^3) in a spray volume of 200 L/ha. This application rate is equivalent to the maximum proposed per season rate for BAS 750 F. The spray was applied to bare soil in each treated plot at either 29-31, 117-121 or 363-365 days before planting/seeding.

Soil samples were collected directly after treatment, at planting and at harvest. In each case 10 soil cores (0-20 cm) weighing ≥1kg were taken from each sub plot. Crop samples were collected as outlined in Table 7.6.2-1. Control specimens were collected prior to collection of the treated specimens to avoid contamination.

Table 7.6.2-1: Target sampling parameters of the rotational crop study

		Sampling event no.1	*Sampling event no.2*	*Sampling event no.3*
Rotational crop (sampling timing, sample size)	wheat	BBCH 30-33 whole plant no root (1 kg)	BBCH 65 whole plant (no root, 1kg)	BBCH 89 grain (1 kg) straw (0.5 kg)
	carrot / radish	BBCH 41 whole plant (with root, 1 kg)	-	BBCH 49 root (1 kg, 12 pieces) top (1 kg, 12 pieces)
	cauliflower / broccoli	BBCH 41 whole plant (no root, 0.5 kg)	-	BBCH 49 inflorescence (1 kg, 12 pieces)
	lettuce / spinach	BBCH 41 head/leaf (0.5 kg)	-	BBCH 49 head/leaf (1 kg)

Weather data were reported for the trials and no exceptional events were noted. Normal agricultural practice was followed during the field stage. Samples weighing at least 1 kg were collected for mature plants, and samples weighing at least 0.5 kg were collected for immature plants. Samples were stored frozen at ≤-18 oC within 6 hours of harvest, and maintained at this temperature until extraction.

The maximum storage interval from sampling until extraction was 305 days for BAS 750 F and 347 days for triazole metabolites.

The level of residues in each crop was determined for BAS 750 F (BASF method L0076/09, LOQ of 0.01 mg/kg) and for the triazole derivative metabolites (TDM) 1,2,4-T, TA, TAA, TLA (BASF method L0170/02, LOQ of 0.01 mg/kg for each of the four analytes). Full details of sample preparation and validation data for these methods are given in section CA B.5.1.2.5. Details of the procedural recoveries are given in Table 7.6.2-2 and 7.6.2-3.

Table 7.6.2-2: Summary of recoveries for BAS 750 F

Matrix		Fortification level (mg/kg)	Summary Recoveries			
			n	mean (%)	SD (+/-)	RSD (%)
Method L0076/09			BAS 750 F			
Broccoli	Whole plant[1]	0.01, 0.10, 1.0	4	87.1	11	13
	Inflorescences	0.01, 0.10, 1.0	4	89.9	9.8	11
Carrots	Whole plant[2]	0.01, 0.10, 1.0	4	83.5	9.3	11
	Tops	0.01, 0.10, 1.0	4	85.0	10	12
	Roots	0.01, 0.10, 1.0	4	83.3	7.7	9.3
Cauliflower	Whole plant[1]	0.01, 0.10, 1.0	4	91.6	7.6	8.3
	Inflorescences	0.01, 0.10, 1.0	6	85.3	6.1	7.2
Lettuce	Leaves	0.01, 0.10, 1.0	4	87.0	3.8	4.4
Radish	Whole plant[2]	0.01, 0.10, 1.0	6	83.4	8.6	10
	Tops (leaves/stem)	0.01, 0.10, 1.0	6	86.8	9.1	11
	Roots	0.01, 0.10, 1.0	6	84.0	5.3	6.3
Spinach	Leaves	0.01, 0.10, 1.0	6	81.7	8.3	10
Wheat	Whole plant[1]	0.01, 0.10, 1.0	6	85.4	6.7	7.8
	Grain	0.01, 0.10, 1.0	8	86.7	5.8	6.7
	straw	0.01, 0.10, 1.0	8	77.1	6.7	8.7
Overall			80	84.7	7.7	9.1

[1] no roots, [2] with roots

Table 7.6.2-3: Summary of recoveries for T, TA, TAA and TLA

Matrix		**Fortification level (mg/kg)**	**Summary Recoveries**			
			n	**mean (%)**	**SD (+/-)**	**RSD (%)**
Method L0170/02			**1,2,4-triazole (T)**			
Broccoli	Whole plant[1]	0.01, 0.10, 1.0	4	97.6	9.8	10
	Inflorescences	0.01, 0.10, 1.0	4	91.3	8.8	9.7
Carrots	Whole plant[2]	0.01, 0.10, 1.0	4	97.4	10	10
	Tops	0.01, 0.10, 1.0	4	93.0	6.6	6.6
	Roots	0.01, 0.10, 1.0	4	102	5.6	5.6
Cauliflower	Whole plant[1]	0.01, 0.10, 1.0	4	94.2	6.1	6.1
	Inflorescences	0.01, 0.10, 1.0	6	89.4	10	10
Lettuce	Leaves	0.01, 0.10, 1.0	3	95.7	6.1	6.1
Radish	Whole plant[2]	0.01, 0.10, 1.0	6	97.7	7.4	7.4
	Tops (leaves/stem)	0.01, 0.10, 1.0	6	102	5.0	5.0
	Roots	0.01, 0.10, 1.0	6	97.5	10	10
Spinach	Leaves	0.01, 0.10, 1.0	8	99.9	6.2	6.2
Wheat	Whole plant[1]	0.01, 0.10, 1.0	6	98.9	8.1	8.1
	Grain	0.01, 0.10, 1.0	8	99.1	7.9	7.9
	straw	0.01, 0.10, 1.0	8	94.2	9.4	10
Overall			**81**	**96.9**	**8.0**	**8.3**
Method L0170/02			**triazole alanine (TA)**			
Broccoli	Whole plant[1]	0.01, 0.10, 1.0	4	101	6.1	6.0
	Inflorescences	0.01, 0.10, 1.0	4	96.0	13	13
Carrots	Whole plant[2]	0.01, 0.10, 1.0	4	95.0	7.2	7.6
	Tops	0.01, 0.10, 1.0	4	96.6	2.5	2.6
	Roots	0.01, 0.10, 1.0	4	101	5.9	5.8
Cauliflower	Whole plant[1]	0.01, 0.10, 1.0	4	101	7.9	6.9
	Inflorescences	0.01, 0.10, 1.0	6	101	6.1	6.1
Lettuce	Leaves	0.01, 0.10, 1.0	3	107	2.9	2.7
Radish	Whole plant[2]	0.01, 0.10, 1.0	6	101	6.7	6.6
	Tops (leaves/stem)	0.01, 0.10, 1.0	6	105	3.7	3.6

Matrix		Fortification level (mg/kg)	Summary Recoveries			
			n	mean (%)	SD (+/-)	RSD (%)
	Roots	0.01, 0.10, 1.0	6	102	5.5	5.4
Spinach	Leaves	0.01, 0.10, 1.0	8	103	5.4	5.2
Wheat	Whole plant[1]	0.01, 0.10, 1.0	6	96.8	7.1	7.4
	Grain	0.01, 0.10, 1.0	8	94.8	11	12
	straw	0.01, 0.10, 1.0	8	83.8	3.6	4.3
Overall			**81**	**98.6**	**8.7**	**8.7**
Method L0170/02			**triazole acetic acid (TAA)**			
Broccoli	Whole plant[1]	0.01, 0.10, 1.0	4	103	4.0	3.9
	Inflorescences	0.01, 0.10, 1.0	4	97.0	3.3	3.4
Carrots	Whole plant[2]	0.01, 0.10, 1.0	4	94.4	7.7	8.2
	Tops	0.01, 0.10, 1.0	4	104	2.9	2.8
	Roots	0.01, 0.10, 1.0	4	101	4.1	4.1
Cauliflower	Whole plant[1]	0.01, 0.10, 1.0	4	100	3.5	3.5
	Inflorescences	0.01, 0.10, 1.0	6	96.3	3.7	3.8
Lettuce	Leaves	0.01, 0.10, 1.0	4	99.3	6.4	6.5
Radish	Whole plant[2]	0.01, 0.10, 1.0	6	99.3	2.7	2.7
	Tops (leaves/stem)	0.01, 0.10, 1.0	6	99.3	7.5	7.6
	Roots	0.01, 0.10, 1.0	6	103	5.2	5.1
Spinach	Leaves	0.01, 0.10, 1.0	8	101	6.6	6.6
Wheat	Whole plant[1]	0.01, 0.10, 1.0	6	94.6	7.4	7.8
	Grain	0.01, 0.10, 1.0	8	97.2	8.2	8.5
	straw	0.01, 0.10, 1.0	8	92.7	8.7	9.4
Overall			**82**	**98.4**	**6.6**	**6.7**
Method L0170/02			**triazole lactic acid (TLA)**			
Broccoli	Whole plant[1]	0.01, 0.10, 1.0	4	100	2.5	2.5
	Inflorescences	0.01, 0.10, 1.0	4	105	3.0	2.9
Carrots	Whole plant[2]	0.01, 0.10, 1.0	4	94.0	8.5	9.0
	Tops	0.01, 0.10, 1.0	4	88.1	12	13
	Roots	0.01, 0.10, 1.0	4	96.0	13	14
Cauliflower	Whole plant[1]	0.01, 0.10, 1.0	4	93.1	5.2	5.7
	Inflorescences	0.01, 0.10, 1.0	6	95.5	6.1	6.4
Lettuce	Leaves	0.01, 0.10, 1.0	4	103	12	12
Radish	Whole plant[2]	0.01, 0.10, 1.0	6	99.4	1.8	1.9
	Tops (leaves/stem)	0.01, 0.10, 1.0	6	94.7	3.7	3.9
	Roots	0.01, 0.10, 1.0	6	98.8	2.9	2.9
Spinach	Leaves	0.01, 0.10, 1.0	8	97.1	6.1	6.3
Wheat	Whole plant[1]	0.01, 0.10, 1.0	6	91.4	7.8	8.6
	Grain	0.01, 0.10, 1.0	8	88.0	3.7	4.2
	straw	0.01, 0.10, 1.0	8	94.1	2.7	2.9
Overall			**82**	**95.4**	**7.4**	**7.8**

Results and Discussion

Field trials were conducted on wheat, radish/carrot, cauliflower/broccoli and lettuce/spinach planted at 30, 120 or 365 days after foliar spray to soil with 300 g ai/ha BAS 750 F.

Samples were taken at two time points after the last application; immature (BBCH 30-33 for wheat and BBCH 41 for other crops) and mature (BBCH 89 for wheat and BBCH 49 for other crops). Residues trials data are presented in Table 7.6.2-8 – 7.6.2-10.

Samples were stored frozen for up to 305 days prior to analysis for BAS 750 F and for up to 347 days for triazole and related analytes. Storage stability data is available to support storage of BAS 750 F for up to 550 days, where no significant degradation is observed during this time frame (see section X). This is sufficient to support the storage times in this study. Storage stability data is available to support storage of 1,2,4-triazole and TA for 54 months, TTA for 26 months (TDM review) and TLA for 49 months (section X). This is sufficient to support the storage times in this study.

The methods of analysis for determination of BAS 750 F and TDMs are considered to be satisfactorily validated in accordance with SANCO 3029/99 rev.4. Acceptable procedural recovery data, using an appropriate number of samples were presented.

BAS 750 F

In untreated samples no residues of BAS 750 F exceeding the LOQ (0.01 mg/kg) were detected. A summary of the results from the treated samples is presented in Table 7.6.2-4.

For all four crop groups, cereals, root/tuber, brassica vegetables and leafy vegetables, BAS 750 F residue levels in samples from treated plots were below the LOQ of 0.01 mg/kg.

Table 7.6.2-4: Summary of BAS 750 F residues in representative succeeding crops

Crop	Portion analysed	Growth stage	30±1 days			120±3 days			365±5 days		
			Sampling	n	BAS[2]	Sampling	n	BAS[2]	Sampling	n	BAS[2]
		BBCH	*DALA*		*mg/kg*	*DALA*		*mg/kg*	*DALA*		*mg/kg*
wheat	whole plant[1]	31 - 33	69 - 195	4	<0.01	196 - 321	4	<0.01	403 - 441	4	<0.01
	whole plant[1]	65	89 - 211	4	<0.01	238 - 370	4	<0.01	423 - 455	4	<0.01
	grain	89	135 - 257	4	<0.01	301 - 413	4	<0.01	467 - 497	4	<0.01
	straw	89	135 - 257	4	<0.01	301 - 413	4	<0.01	467 - 497	4	<0.01
carrots / radish	whole plant	41	52 - 101	4	<0.01	142 - 188	4	<0.01	396 - 445	4	<0.01
	top	49	65 - 138	4	<0.01	155 - 228	4	<0.01	410 - 482	4	<0.01
	root	49	65 - 138	4	<0.01	155 - 228	4	<0.01	410 - 482	4	<0.01
broccoli / cauliflower	whole plant	41	66 - 87	4	<0.01	153 - 177	4	<0.01	404 - 447	4	<0.01
	inflorescence	49	83 - 135	4	<0.01	184 - 225	4	<0.01	427 - 470	4	<0.01
spinach / lettuce	leaf	41	58 - 74	4	<0.01	148 - 180	4	<0.01	393 - 426	4	<0.01
	leaf	49	69 - 88	4	<0.01	162 - 190	4	<0.01	407 - 434	4	<0.01

[1] no roots [2] BAS 750 F

TDM

For 1,2,4-triazole, no residues exceeding the LOQ were detected in any sample at any plant back interval, both for treated samples and untreated samples. For TA, TAA, and TLA, residue levels above LOQ were determined, both for treated samples and untreated samples. Details of the previous treatments to the plots are not available in the study reports; however it is considered that the most likely explanation as to the source of the TDMs in untreated crops, is residual TDMs in the soil from previous applications of a triazole containing pesticide. A comparison of the results is presented in Table 7.6.2-5 -7.6.2-7.

For TA, residues were determined for all treated commodities at all plant back intervals, with the exception of wheat straw, where residues >0.01 mg/kg were not determined. Residues in treated commodities ranged between <0.01-0.23 mg/kg at the 30 day replant interval, compared with <0.01-0.084 mg/kg in untreated commodities. At the 120 day replant interval residues in treated commodities ranged between <0.01-0.5 mg/kg, compared with <0.01-0.11 mg/kg in untreated commodities. At the 365 day replant interval residues in treated commodities ranged between <0.01-0.52 mg/kg, compared with <0.01-0.13 mg/kg in untreated commodities. Residues were highest in wheat commodities. Residues of TA are therefore considered to be to an extent treatment unrelated as

residues are also present in control samples, although residues in treated samples are higher. Based on the results it appears that levels of TA are constant, and not affected by increasing PBI.

For TAA, residues >0.01 mg/kg were only detected in wheat (both control and treated samples). No residues above LOQ were detected in control or treated samples on the other commodities. At the 30 day replant interview residues of TAA in untreated grain and straw were <0.01-0.034 mg/kg and <0.01-0.13 mg/kg in the treated commodities. A similar pattern was observed at longer PBIs, with residues up to 0.072 mg/kg in untreated wheat samples at 120 days, compared to 0.21 mg/kg for treated samples, and at 365 days residues up to 0.055 mg/kg were observed in untreated samples and up to 0.35 mg/kg in treated samples. Residues were highest in grain and lowest in immature whole plant. As for TA, residues are considered to be to an extent treatment unrelated as residues are also present in control samples, although residues in treated samples are higher. Based on the results it appears that levels of TAA are largely constant, and not significantly affected by increasing PBI.

For TLA, residues >0.01 mg/kg were detected in all commodities (both control and treated samples) with the exception of broccoli and cauliflower in which no residues were detected for either control and treated samples. At the 30 day replant interview residues of TLA in untreated commodities were <0.01-0.037 mg/kg and <0.01-0.084 mg/kg in the treated commodities. Residues up to 0.032 mg/kg were detected in untreated samples at 120 days, compared to 0.29 mg/kg for treated samples, and at 365 days residues up to 0.06 mg/kg were observed in untreated samples and up to 0.11 mg/kg in treated samples. Residues were highest in wheat commodities. As for TA and TAA, residues are considered to be to an extent treatment unrelated as residues are also present in control samples, although residues in treated samples are higher. Based on the results it appears that levels of TLA are constant, and not affected by increasing PBI.

Overall residues of TA were highest for all commodities; relatively high levels of TAA in wheat were also detected, although these were not present in other commodities. Somewhat lower levels of TLA were detected in all commodities (except brassicas), and no residues of 1,2,4-triazole were detected in any sample. Where residues were detected in treated samples, they were in almost every case also detected in control samples albeit at a lower level. As such, it is considered that residues of TDMs are likely to be present in the soil where triazole containing pesticides have been used previously, and hence in these cases the entirety of the residue cannot be attributed to the BAS 750 F application. For further discussion see section 7.3.3. Overall it does not appear that levels of TDMs are affected by differing PBIs, the exception of TAA in wheat grain and straw, where a moderate increase is observed with increasing PBI.

Table 7.6.2-5: Summary of TDM residues in representative succeeding crops (replant interval 30±1 days, plot 1 untreated, plot 2 treated)

Plot	Crop	Portion analysed	GS [3] *[mg/kg]*	n	Residues *[mg/kg]*			
					T	TA	TAA	TLA
1	wheat	whole plant[1]	31-33	4	< 0.01	< 0.01 - 0.021	< 0.01	< 0.01 – 0.037
		whole plant[1]	65	4	< 0.01	< 0.01 - 0.014	< 0.01	< 0.01 – 0.015
		grain	89	4	< 0.01	0.041 - 0.069	< 0.01 - 0.034	< 0.01
		straw	89	4	< 0.01	< 0.01	< 0.01 – 0.023	< 0.01 – 0.020
2		whole plant[1]	31-33	4	< 0.01	0.010 - 0.017	< 0.01 – 0.026	< 0.01 - 0.023
		whole plant[1]	65	4	< 0.01	< 0.01 - 0.044	< 0.01 - 0.030	< 0.01 – 0.066
		grain	89	4	< 0.01	0.042 - 0.23	0.019 - 0.13	< 0.01
		straw	89	4	< 0.01	< 0.01 - 0.011	< 0.01 - 0.041	0.012 – 0.037
1	carrot / radish	whole plant[2]	41	4	< 0.01	< 0.01 - 0.049	< 0.01	< 0.01 - 0.011
		root	49	4	< 0.01	< 0.01 - 0.014	< 0.01	< 0.01
		top	49	4	< 0.01	< 0.01 - 0.014	< 0.01	< 0.01 – 0.025
2		whole plant[2]	41	4	< 0.01	< 0.01 - 0.077	< 0.01	< 0.01 - 0.029

Plot	Crop	Portion analysed	GS [3] *[mg/kg]*	n	Residues *[mg/kg]*			
					T	TA	TAA	TLA
		root	49	4	< 0.01	0.014 - 0.017	< 0.01	< 0.01
		top	49	4	< 0.01	< 0.01 - 0.061	< 0.01	< 0.01 – 0.023
1	broccoli / cauliflower	whole plant[1]	41	4	< 0.01	0.012 - 0.026	< 0.01	< 0.01
		inflorescence	49	4	< 0.01	0.020 - 0.084	< 0.01	< 0.01
2		whole plant[1]	41	4	< 0.01	0.014 - 0.076	< 0.01	< 0.01
		inflorescence	49	4	< 0.01	0.060 - 0.12	< 0.01	< 0.01
1	spinach / lettuce	leaf	41	4	< 0.01	< 0.01 - 0.024	< 0.01	< 0.01 - 0.031
			49	4	< 0.01	< 0.01	< 0.01	< 0.01 - 0.032
2		leaf	41	4	< 0.01	< 0.01 - 0.048	< 0.01	< 0.01 - 0.092
			49	4	< 0.01	< 0.01 - 0.026	< 0.01	< 0.01 - 0.084

[1] roots removed, [2] roots included, [3] growth stage (BBCH)

Table 7.6.2-6: Summary of TDM residues in representative succeeding crops (replant interval 120±3 days, plot 3 untreated, plot 4 treated)

Plot	Crop	Portion analysed	GS [3] *[mg/kg]*	n	Residues *[mg/kg]*			
					T	TA	TAA	TLA
3	wheat	whole plant[1]	31-33	4	< 0.01	< 0.01 - 0.031	< 0.01	< 0.01 – 0.027
		whole plant[1]	65	4	< 0.01	< 0.01 - 0.016	< 0.01 - 0.020	< 0.01 – 0.016
		grain	89	4	< 0.01	0.034 - 0.11	0.018 - 0.072	< 0.01
		straw	89	4	< 0.01	< 0.01	< 0.01 – 0.027	< 0.01 – 0.032
4		whole plant[1]	31-33	4	< 0.01	0.026 - 0.12	< 0.01 – 0.036	0.010 - 0.29
		whole plant[1]	65	4	< 0.01	0.017 - 0.093	0.020 – 0.070	0.019 - 0.10
		grain	89	4	< 0.01	0.093 - 0.50	0.066 - 0.21	< 0.01 – 0.012
		straw	89	4	< 0.01	< 0.01 - 0.035	0.020 – 0.13	0.022 – 0.16
3	carrot / radish	whole plant[2]	41	4	< 0.01	< 0.01 - 0.014	< 0.01	< 0.01 - 0.017
		root	49	4	< 0.01	< 0.01 - 0.013	< 0.01	< 0.01
		top	49	4	< 0.01	< 0.01 - 0.015	< 0.01	< 0.01 – 0.017
4		whole plant[2]	41	4	< 0.01	0.011 - 0.090	< 0.01	< 0.01 - 0.018
		root	49	4	< 0.01	< 0.01 - 0.039	< 0.01	< 0.01
		top	49	4	< 0.01	< 0.01 - 0.056	< 0.01	< 0.01 – 0.038
3	broccoli / cauliflower	whole plant[1]	41	4	< 0.01	0.021 - 0.034	< 0.01	< 0.01
		inflorescence	49	4	< 0.01	0.029 - 0.10	< 0.01	< 0.01
4		whole plant[1]	41	4	< 0.01	0.034 - 0.18	< 0.01	< 0.01
		inflorescence	49	4	< 0.01	0.064 - 0.35	< 0.01	< 0.01
3	spinach / lettuce	leaf	41	4	< 0.01	< 0.01	< 0.01	< 0.01 - 0.012
			49	4	< 0.01	< 0.01	< 0.01	< 0.01 - 0.016
4		leaf	41	4	< 0.01	< 0.01 - 0.048	< 0.01	0.014 - 0.042
			49	4	< 0.01	< 0.01 - 0.043	< 0.01	0.017 - 0.050

[1] roots removed, [2] roots included, [3] growth stage (BBCH)

Table 7.6.2-7: Summary of TDM residues in representative succeeding crops (replant interval 365±1 days, plot 5 untreated, plot 6 treated)

Plot	Crop	Portion analysed	GS [3] *[mg/kg]*	n	Residues *[mg/kg]*			
					T	TA	TAA	TLA
5	wheat	whole plant[1]	31-33	4	< 0.01	< 0.01 - 0.02	< 0.01	< 0.01
		whole plant[1]	65	4	< 0.01	< 0.01 - 0.024	< 0.01 - 0.01	< 0.01 – 0.014
		grain	89	4	< 0.01	0.040 - 0.13	0.019 - 0.055	< 0.01
		straw	89	4	< 0.01	< 0.01	< 0.01 - 0.022	< 0.01 – 0.060
6		whole plant[1]	31-33	4	< 0.01	0.026 - 0.13	< 0.01 - 0.010	< 0.01 – 0.11
		whole plant[1]	65	4	< 0.01	0.010 - 0.10	< 0.01 - 0.068	< 0.01 – 0.017
		grain	89	4	< 0.01	0.048 - 0.52	0.021 - 0.35	< 0.01 - 0.015
		straw	89	4	< 0.01	< 0.01 - 0.037	< 0.01 - 0.15	< 0.01 – 0.090
5	carrot / radish	whole plant[2]	41	4	< 0.01	< 0.01	< 0.01	< 0.01 - 0.015
		root	49-55	4	< 0.01	< 0.01	< 0.01	< 0.01
		top	49-55	4	< 0.01	< 0.01 - 0.024	< 0.01	< 0.01 – 0.015
6		whole plant[2]	41	4	< 0.01	< 0.01 - 0.040	< 0.01	< 0.01 - 0.014
		root	49-55	4	< 0.01	< 0.01 - 0.020	< 0.01	< 0.01
		top	49-55	4	< 0.01	< 0.01 - 0.047	< 0.01	< 0.01 – 0.043
5	broccoli / cauliflower	whole plant[1]	41	4	< 0.01	< 0.01 - 0.022	< 0.01	< 0.01
		inflorescence	49	4	< 0.01	< 0.01	< 0.01	< 0.01
6		whole plant[1]	41	4	< 0.01	0.019 - 0.12	< 0.01	< 0.01
		inflorescence	49	4	< 0.01	0.054 - 0.17	< 0.01	< 0.01
5	spinach / lettuce	leaf	41	4	< 0.01	< 0.01 - 0.011	< 0.01	< 0.01 - 0.014
			49-55	4	< 0.01	< 0.01 - 0.012	< 0.01	< 0.01 - 0.024
6		leaf	41	4	< 0.01	< 0.01 - 0.015	< 0.01	< 0.01 - 0.017
			49-55	4	< 0.01	< 0.01 - 0.025	< 0.01	< 0.01 - 0.045

[1] roots removed, [2] roots included, [3] growth stage (BBCH)

Conclusion

Residue data was obtained for 8 independent field trials in wheat, radish/carrot, broccoli/cauliflower, and spinach/lettuce, planted at 30, 120 and 365 days after soil treatment with 300 g/ha of BAS 750 F. BAS 750 F residues do not exceed the LOQ of 0.01 mg/kg for different representative succeeding crops at any plant back interval, therefore demonstrating that no residues of BAS 750 F are expected in rotational crops, after treatment of the primary crop at the maximum per season application rate (0.1 mg/kg soil).

Application to bare soil can be considered a worst case scenario in respect of BAS 750 F as crop interception would usually occur. Considering 80 % crop interception, BAS 750 F concentration in soil during the first year of application of the maximal yearly rate of BAS 750 F, is calculated at maximum 0.08 mg/kg (PECsoil, max, see section CA B.8). For a multi-year application of the maximal yearly rate, the plateau is calculated to be 0.308 mg/kg (PECsoil, plateau), demonstrating accumulation in soil. This level is significantly higher than the soil concentration of 0.1 mg/kg calculated in this study.

However the PECsoil, plateau calculation is based on a soil depth of 5 cm, whereas the calculation of concentration in the soil uses a depth of 20 cm (Lundehn Appendix C 7524/VI/95 rev.2). If the PECsoil, max and PECsoil, plateau were calculated using a 20 cm soil depth, they would be 0.02 mg/kg and 0.077 mg/kg respectively. Hence the dose in the trial of 0.1 mg/kg would cover these, (as

well as the sum of the maximum and plateau PECsoil (0.097 mg/kg) and hence it is considered that the trials are dosed at a level which is worst case with respect to the expected plateau level of BAS 750 F in soil.

For the TDMs, residues of 1,2,4-triazole were not detected in any crop samples (treated or control) at any plant back interval. For TA, and TLA, residue levels above LOQ were determined in all crop samples (except TLA in brassicas) and all PBIs, both for treated samples and untreated samples. Residues were highest in wheat commodities. Overall higher residues of these metabolites in treated crops were detected than untreated crops, which were not affected by varying PBI. For TAA, residue levels above LOQ were only detected in wheat samples (at all PBIs) for both treated and control samples. Overall higher residues of these metabolites in treated crops were detected than untreated crops, which were not significantly affected by varying PBI.

The residue data obtained for the TDMs, is comparable to the data package considered in the TDM review (Triazole Derivative Metabolites Addendum – Confirmatory Data, November 2015). As for primary crop trials (see B.7.3.3), slight variations in the levels of TDMs in rotational crops are not considered to have any significant impact on the risk assessment, and hence no further consideration is required.

Table 7.6.2-8: Residues in succeeding crops (30 ± 1 DAA replanting interval, treated samples)

Study details	Formulation, Appl. rate (kg as/ha)	Crop	Country	GS 1)	Matrix	Treated Sample Residues (mg/kg)					Control Sample Residues (mg/kg)				
						BAS 750 F	T	TA	TAA	TLA	BAS 750 F	T	TA	TAA	TLA
PLOT 1/2: 30 ± 1 DAA replanting interval															
Study code: 727902 **Doc ID:** 2015/1106682 **GLP:** Yes **Year:** 2014-2015	BAS 750 01 F 1 x 3.0 L/ha to bare soil	Wheat	Germany	31-33	whole plant without root	<0.01	<0.01	0.017	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.010	<0.01	<0.01	<0.01	<0.01	0.021	<0.01	0.011
			Italy			<0.01	<0.01	0.105	0.026	0.23	<0.01	<0.01	0.012	<0.01	0.037
			Spain			<0.01	<0.01	0.016	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Germany	65		<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01
			Italy			<0.01	<0.01	0.044	0.0301	0.066	<0.01	<0.01	0.011	<0.01	0.015
			Spain			<0.01	<0.01	0.017	0.013	0.012	<0.01	<0.01	<0.01	<0.01	<0.01
			Germany	89	grain	<0.01	<0.01	0.0901	0.028	<0.01	<0.01	<0.01	0.041	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.042	0.019	<0.01	<0.01	<0.01	0.069	0.034	<0.01
			Italy			<0.01	<0.01	0.226	0.13	<0.01	<0.01	<0.01	0.053	0.021	<0.01
			Spain			<0.01	<0.01	0.089	0.054	<0.01	<0.01	<0.01	0.050	0.022	<0.01
			Germany	89	straw	<0.01	<0.01	<0.01	0.016	0.012	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	0.023	0.020
			Italy			<0.01	<0.01	0.011	0.041	0.037	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	<0.01	0.026	0.033	<0.01	<0.01	<0.01	0.016	0.017
		Carrots	Germany	41	whole plant with roots	<0.01	<0.01	<0.01	<0.01	0.029	<0.01	<0.01	<0.01	<0.01	0.010
			Netherlands			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.011	<0.01	0.011
		Radish	Italy			<0.01	<0.01	0.077	<0.01	0.028	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.049	<0.01	<0.01	<0.01	<0.01	0.049	<0.01	<0.01
		Carrots	Germany	49	tops	<0.01	<0.01	<0.01	<0.01	0.023	<0.01	<0.01	<0.01	<0.01	0.0112
			Netherlands			<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	0.025
		Radish	Italy			<0.01	<0.01	0.045	<0.01	0.017	<0.01	<0.01	0.014	<0.01	<0.01
			Spain			<0.01	<0.01	0.061	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Carrots	Germany	49	roots	<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01
		Radish	Italy			<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.017	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Broccoli	Germany	41	whole plant without root	<0.01	<0.01	0.035	<0.01	<0.01	<0.01	<0.01	0.012	<0.01	<0.01
		Cauliflower	Netherlands			<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	0.026	<0.01	<0.01
			Italy			<0.01	<0.01	0.064	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01
			Spain			<0.01	<0.01	0.076	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01

Table 7.6.2-8: Residues in succeeding crops (30 ± 1 DAA replanting interval, treated samples)

Study details	Formulation, Appl. rate (kg as/ha)	Crop	Country	GS [1)]	Matrix	Treated Sample Residues (mg/kg)					Control Sample Residues (mg/kg)				
						BAS 750 F	T	TA	TAA	TLA	BAS 750 F	T	TA	TAA	TLA
		Broccoli	Germany	49	inflorescences	<0.01	<0.01	0.085	<0.01	<0.01	<0.01	<0.01	0.0457	<0.01	<0.01
		Cauliflower	Netherlands			<0.01	<0.01	0.060	<0.01	<0.01	<0.01	<0.01	0.084	<0.01	<0.01
			Italy			<0.01	<0.01	0.087	<0.01	<0.01	<0.01	<0.01	0.030	<0.01	<0.01
			Spain			<0.01	<0.01	0.12	<0.01	<0.01	<0.01	<0.01	0.019	<0.01	<0.01
		Lettuce	Germany	41	leaves	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01	<0.01	<0.01	<0.01
		Spinach	Netherlands			<0.01	<0.01	0.016	<0.01	0.014	<0.01	<0.01	0.024	<0.01	0.016
			Italy			<0.01	<0.01	0.048	<0.01	0.092	<0.01	<0.01	0.015	<0.01	0.031
			Spain			<0.01	<0.01	0.040	<0.01	0.030	<0.01	<0.01	<0.01	<0.01	<0.01
		Lettuce	Germany	49	leaves	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01	<0.01	<0.01	<0.01
		Spinach	Netherlands			<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	0.020
			Italy			<0.01	<0.01	0.020	<0.01	0.084	<0.01	<0.01	<0.01	<0.01	0.032
			Spain			<0.01	<0.01	0.026	<0.01	0.017	<0.01	<0.01	<0.01	<0.01	<0.01

[1)] Growth stage as planned (BBCH)

Table 7.6.2-9: Residues in succeeding crops (120 ± 3 DAA replanting interval, treated samples)

Study details	Formulation, Appl. rate (kg as/ha)	Crop	Country	GS [1)]	Matrix	Treated Sample Residues (mg/kg)					Control Sample Residues (mg/kg)				
						BAS 750 F	T	TA	TAA	TLA	BAS 750 F	T	TA	TAA	TLA
PLOT3/4 120 ± 3 DAA replanting interval															
Study code: 727902	BAS 750 01 F	Wheat	Germany	31-33	whole plant without root	<0.01	<0.01	0.026	<0.01	0.010	<0.01	<0.01	<0.01	<0.01	<0.01
Doc ID: 2015/1106682	1 x 3.0 L/ha		Netherlands			<0.01	<0.01	0.044	<0.01	0.017	<0.01	<0.01	0.031	<0.01	0.0146
GLP: Yes	to bare soil		Italy			<0.01	<0.01	0.12	0.036	0.29	<0.01	<0.01	0.012	<0.01	0.0273
Year: 2014-2015			Spain			<0.01	<0.01	0.038	<0.01	0.010	<0.01	<0.01	0.011	<0.01	<0.01
			Germany	65		<0.01	<0.01	0.017	0.0203	0.019	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.024	0.024	0.024	<0.01	<0.01	0.017	0.020	0.016
			Italy			<0.01	<0.01	0.093	0.0703	0.100	<0.01	<0.01	0.011	<0.01	0.0122
			Spain			<0.01	<0.01	0.026	0.025	0.029	<0.01	<0.01	<0.01	<0.01	<0.01
			Germany	89	grain	<0.01	<0.01	0.093	0.066	<0.01	<0.01	<0.01	0.034	0.018	<0.01
			Netherlands			<0.01	<0.01	0.19	0.094	<0.01	<0.01	<0.01	0.109	0.072	<0.01
			Italy			<0.01	<0.01	0.503	0.21	0.012	<0.01	<0.01	0.068	0.028	<0.01
			Spain			<0.01	<0.01	0.13	0.0930	<0.01	<0.01	<0.01	0.084	0.040	<0.01
			Germany	89	straw	<0.01	<0.01	<0.01	0.020	0.022	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.014	0.034	0.039	<0.01	<0.01	<0.01	0.027	0.032
			Italy			<0.01	<0.01	0.035	0.13	0.16	<0.01	<0.01	<0.01	0.0121	0.011
			Spain			<0.01	<0.01	0.011	0.048	0.052	<0.01	<0.01	<0.01	0.022	0.027
		Carrots	Germany	41	whole plant with roots	<0.01	<0.01	0.014	<0.01	0.016	<0.01	<0.01	<0.01	<0.01	0.011
			Netherlands			<0.01	<0.01	0.011	<0.01	0.015	<0.01	<0.01	<0.01	<0.01	0.017
		Radish	Italy			<0.01	<0.01	0.0601	<0.01	0.018	<0.01	<0.01	0.013	<0.01	<0.01
			Spain			<0.01	<0.01	0.0902	<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01
		Carrots	Germany	49	tops	<0.01	<0.01	<0.01	<0.01	0.021	<0.01	<0.01	<0.01	<0.01	0.010
			Netherlands			<0.01	<0.01	<0.01	<0.01	0.026	<0.01	<0.01	<0.01	<0.01	0.0174
		Radish	Italy			<0.01	<0.01	0.056	<0.01	0.038	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.031	<0.01	<0.01	<0.01	<0.01	0.015	<0.01	<0.01
		Carrots	Germany	49	roots	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.016	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01
		Radish	Italy			<0.01	<0.01	0.037	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.039	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Broccoli	Germany	41	whole plant without root	<0.01	<0.01	0.034	<0.01	<0.01	<0.01	<0.01	0.021	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.059	<0.01	<0.01	<0.01	<0.01	0.034	<0.01	<0.01
		Cauliflower	Italy			<0.01	<0.01	0.057	<0.01	<0.01	<0.01	<0.01	0.026	<0.01	<0.01
			Spain			<0.01	<0.01	0.18	<0.01	<0.01	<0.01	<0.01	0.024	<0.01	<0.01

Table 7.6.2-9: Residues in succeeding crops (120 ± 3 DAA replanting interval, treated samples)

Study details	Formulation, Appl. rate (kg as/ha)	Crop	Country	GS [1)]	Matrix	Treated Sample Residues (mg/kg)					Control Sample Residues (mg/kg)				
						BAS 750 F	T	TA	TAA	TLA	BAS 750 F	T	TA	TAA	TLA
		Broccoli	Germany	49	inflorescences	<0.01	<0.01	0.064	<0.01	<0.01	<0.01	<0.01	0.043	<0.01	<0.01
		Cauliflower	Netherlands			<0.01	<0.01	0.26	<0.01	<0.01	<0.01	<0.01	0.101	<0.01	<0.01
			Italy			<0.01	<0.01	0.089	<0.01	<0.01	<0.01	<0.01	0.0305	<0.01	<0.01
			Spain			<0.01	<0.01	0.35	<0.01	<0.01	<0.01	<0.01	0.029	<0.01	<0.01
		Lettuce	Germany	41	leaves	<0.01	<0.01	0.012	<0.01	0.022	<0.01	<0.01	<0.01	<0.01	<0.01
		Spinach	Netherlands			<0.01	<0.01	<0.01	<0.01	0.015	<0.01	<0.01	<0.01	<0.01	0.0119
			Italy			<0.01	<0.01	0.027	<0.01	0.042	<0.01	<0.01	<0.01	<0.01	0.0102
			Spain			<0.01	<0.01	0.048	<0.01	0.040	<0.01	<0.01	<0.01	<0.01	<0.01
		Lettuce	Germany	49	leaves	<0.01	<0.01	<0.01	<0.01	0.017	<0.01	<0.01	<0.01	<0.01	<0.01
		Spinach	Netherlands			<0.01	<0.01	<0.01	<0.01	0.019	<0.01	<0.01	<0.01	<0.01	<0.01
			Italy			<0.01	<0.01	0.018	<0.01	0.050	<0.01	<0.01	<0.01	<0.01	0.016
			Spain			<0.01	<0.01	0.043	<0.01	0.030	<0.01	<0.01	<0.01	<0.01	<0.01

[1)] Growth stage as planned (BBCH)

Table 7.6.2-10: Residues in succeeding crops (365 ± 5 DAA replanting interval, treated samples)

Study details	Formulation, Appl. rate (kg as/ha)	Crop	Country	GS [1)]	Matrix	Treated Sample Residues (mg/kg)					Control Sample Residues (mg/kg)				
						BAS 750 F	T	TA	TAA	TLA	BAS 750 F	T	TA	TAA	TLA
PLOT 5/6: 365 ± 5 DAA replanting interval															
Study code: 727902	BAS 750 01 F	Wheat	Germany	31-33	whole plant without root	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Doc ID: 2015/1106682	1 x 3.0 L/ha		Netherlands			<0.01	<0.01	0.026	<0.01	0.014	<0.01	<0.01	0.024	<0.01	<0.01
GLP: Yes	to bare soil		Italy			<0.01	<0.01	0.1301	0.0104	0.11	<0.01	<0.01	0.014	<0.01	<0.01
Year: 2014-2015			Spain			<0.01	<0.01	0.031	<0.01	0.013	<0.01	<0.01	0.0120	<0.01	<0.01
			Germany	65		<0.01	<0.01	0.0101	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.030	0.019	0.017	<0.01	<0.01	0.024	0.015	0.013
			Italy			<0.01	<0.01	0.100	0.068	0.15	<0.01	<0.01	0.011	<0.01	0.014
			Spain			<0.01	<0.01	0.0203	0.017	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Germany	89	grain	<0.01	<0.01	0.065	0.021	<0.01	<0.01	<0.01	0.0402	0.018	<0.01
			Netherlands			<0.01	<0.01	0.048	0.024	<0.01	<0.01	<0.01	0.10	0.039	<0.01
			Italy			<0.01	<0.01	0.52	0.35	0.015	<0.01	<0.01	0.065	0.039	<0.01
			Spain			<0.01	<0.01	0.14	0.062	<0.01	<0.01	<0.01	0.13	0.055	<0.01
			Germany	89	straw	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	<0.01	<0.01	0.021	<0.01	<0.01	<0.01	0.017	0.0103
			Italy			<0.01	<0.01	0.037	0.15	0.090	<0.01	<0.01	<0.01	0.016	<0.01
			Spain			<0.01	<0.01	<0.01	0.027	0.015	<0.01	<0.01	<0.01	0.022	0.060
		Carrots	Germany	41	whole plant with roots	<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	0.015
			Netherlands			<0.01	<0.01	<0.01	<0.01	0.014	<0.01	<0.01	<0.01	<0.01	0.015
		Radish	Italy			<0.01	<0.01	0.040	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.021	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Carrots	Germany	49	tops	<0.01	<0.01	<0.01	<0.01	0.0432	<0.01	<0.01	<0.01	<0.01	0.014
			Netherlands			<0.01	<0.01	<0.01	<0.01	0.0417	<0.01	<0.01	<0.01	<0.01	0.015
		Radish	Italy			<0.01	<0.01	0.035	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.047	<0.01	0.0102	<0.01	<0.01	0.024	<0.01	<0.01
		Carrots	Germany	49	roots	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Netherlands			<0.01	<0.01	0.020	<0.01	<0.01	<0.01	<0.01	0.020	<0.01	<0.01
		Radish	Italy			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Broccoli	Germany	41	whole plant without root	<0.01	<0.01	0.019	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Cauliflower	Netherlands			<0.01	<0.01	0.057	<0.01	<0.01	<0.01	<0.01	0.022	<0.01	<0.01
			Italy			<0.01	<0.01	0.12	<0.01	<0.01	<0.01	<0.01	0.018	<0.01	<0.01
			Spain			<0.01	<0.01	0.033	<0.01	<0.01	<0.01	<0.01	0.012	<0.01	<0.01

Table 7.6.2-10: Residues in succeeding crops (365 ± 5 DAA replanting interval, treated samples)

Study details	Formulation, Appl. rate (kg as/ha)	Crop	Country	GS [1)]	Matrix	Treated Sample Residues (mg/kg)					Control Sample Residues (mg/kg)				
						BAS 750 F	T	TA	TAA	TLA	BAS 750 F	T	TA	TAA	TLA
		Broccoli	Germany	49	inflorescences	<0.01	<0.01	0.054	<0.01	<0.01	<0.01	<0.01	0.022	<0.01	<0.01
		Cauliflower	Netherlands			<0.01	<0.01	0.11	<0.01	<0.01	<0.01	<0.01	0.061	<0.01	<0.01
			Italy			<0.01	<0.01	0.17	<0.01	<0.01	<0.01	<0.01	0.061	<0.01	<0.01
			Spain			<0.01	<0.01	0.087	<0.01	<0.01	<0.01	<0.01	0.027	<0.01	<0.01
		Lettuce	Germany	41	leaves	<0.01	<0.01	<0.01	<0.01	0.010	<0.01	<0.01	<0.01	<0.01	<0.01
		Spinach	Netherlands			<0.01	<0.01	0.013	<0.01	0.017	<0.01	<0.01	<0.01	<0.01	0.014
			Italy			<0.01	<0.01	0.014	<0.01	0.065	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.015	<0.01	0.017	<0.01	<0.01	0.011	<0.01	<0.01
		Lettuce	Germany	49	leaves	<0.01	<0.01	<0.01	<0.01	0.011	<0.01	<0.01	<0.01	<0.01	<0.01
		Spinach	Netherlands			<0.01	<0.01	<0.01	<0.01	0.036	<0.01	<0.01	<0.01	<0.01	0.024
			Italy			<0.01	<0.01	0.025	<0.01	0.045	<0.01	<0.01	<0.01	<0.01	<0.01
			Spain			<0.01	<0.01	0.015	<0.01	0.012	<0.01	<0.01	0.012	<0.01	<0.01

[1)] Growth stage as planned (BBCH)

B.7.6.3. Conclusion on rotational crops

To investigate residues in rotational crops, a nature of the residue study and a magnitude of the residue study have been conducted in different crops representing three different crop categories, namely leafy vegetables, root and tuber vegetables and cereals. BAS 750 F was applied at 300 g ai/ha to bare soil, corresponding to a BAS 750 F concentration in soil of 0.1 mg/kg (soil depth 20 cm, soil density 1.5 g/cm^3). The rotational crops were cultivated after soil aging intervals of 30d, 120d and 365 days, samples were taken at both mature and immature growth stages. The trials are dosed at a level which is worst case with respect to the expected plateau level of BAS 750 F in soil.

Based on results obtained in the nature of the residue study conducted with two labels (C-label, T-label), the residue in rotational crops is identified as unchanged parent BAS 750 F as well as the triazole derivative metabolites (TDM). The ratio of R- and S-enantiomers of BAS 750 F residue in plant remained unchanged compared with the test substance, indicating absence of preferential metabolism or uptake. Overall, the metabolism in rotational crops is similar to metabolism in primary crops (see section 7.2.1) with no rotational crop specific metabolites. The magnitude of both BAS 750 F and TDM was investigated under field conditions. Based on the results obtained in the magnitude of the residue study, absence of residues of BAS 750 F are expected for the use of BAS 750 F supported in the present dossier. The residue data obtained for the TDMs, is comparable to the data package considered in the TDM review. As for primary crop trials (see B.7.3.3), slight variations in the levels of TDMs in rotational crops are not considered to have any significant impact on the risk assessment, and hence no further consideration is required.

In conclusion, for the use of BAS 750 F supported in the present dossier, no replant restrictions are required. As no significant residues of BAS 750 F are expected, the default MRL of 0.01 mg/kg is appropriate for rotational crops.

B.7.7. OTHER STUDIES

B.7.7.1. Effect on the residue level in pollen and bee products

At present there are no agreed EU guidance documents or test methods to address these data requirements. At the SCoPAFF (PPP legislation) meeting in October 2014 the COM emphasised, as laid down in document SANCO/10181/2013 Rev 2.1, that these data requirements can be waived until test methods or guidance documents are made available. SANCO/10181/2013 Rev 2.1 states:

In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02. Applicants should follow on a routine basis the current developments, e.g. activities of the European Food Safety Authority for guidance documents and in particular publications in the Official Journal.

B.7.8. REFERENCES RELIED ON

Literature Search
BAS 750 F
The following databases were searched:

BIOSIS		1926 – to present
CABA	- CAB Abstracts	1973 – to present
CAPLUS	- Chemical Abstracts Plus	1907 – to present

Search criteria:
- BAS 750 F, synonyms and CAS numbers were used
- Appropriate metabolites, synonyms and CAS numbers were used
- Suitable terms relating to the assessment of residues were used.
- 1,2,4-triazole, synonyms and CAS numbers were used

A two-step process for selection of relevant scientific peer-reviewed open literature was undertaken:

First selection step for relevance based on summary records
- Obviously irrelevant records were tagged as “Ballast” and not further processed.
- Records which appeared to be relevant and those of unclear relevance were tagged for further evaluation (“Hits”)

Second detailed assessment for records requiring further information.
“Hits” were reviewed based on the title and the abstract with regard to relevance for the regulatory endpoints. Those records which were clearly not assignable to any regulatory endpoint were categorised as "no relevant endpoint". All remaining records were assessed in detail based on the complete report and separated into relevant and non-relevant reports.

Criteria to assign records as "evaluated - not-relevant" were:
- Records which did not provide any new relevant data or information
- Records which were not assignable to the substance of interest
- Secondary literature linking to primary literature already discussed under relevant records
- Records with limited reliability of grade 3 or 4 based on the 'Klimisch' scoring system.

Any remaining records were assigned to the category "used for dossier".

8 records relating to BAS 750 F and metabolites, and 55 records relating to 1,2,4-triazole were identified under a consideration of metabolism and residues in animals. Of these 10 records (3 BAS 750 F/7 1,2,4-T) were considered to be “hits”.

8 records relating to BAS 750 F and metabolites, and 65 records relating to 1,2,4-triazole were identified under a consideration of metabolism and residues in plants. Of these 28 records (4 BAS 750 F/24 1,2,4-T) were considered to be “hits”.

The 7 records relating to BAS 750 F were assessed further and considered not to be relevant to the residues and consumer safety assessment, and have therefore not been included in the dossier.

The methodology used in the search, and determination of records as non-relevant is considered acceptable.

A further assessment of the records relating to 1,2,4-triazole was not made, a more in-depth assessment of literature relating to1,2,4-triazole and related metabolites (TDMs) were considered by the ‘Triazole Metabolite Derivatives Group’ (TMDG), and the results of this review were used instead.

TDMs

The following databases were searched:

Agricola		1979 – to present
ANABSTR	- Analytical Abstracts	1980 – to present
BIOSIS		1926 – to present
CABA	- CAB Abstracts	1973 – to present
CAPLUS	- Chemical Abstracts Plus	1907 – to present
EMBASE		1947 – to present
ESBIOBASE		1994 – to present
FSTA		1969 – to present
FROSTI		1972 – to present
GEOREF		1669 – to present
MEDLINE		1946 – to present
PQSCITECH (CSA databases)		1962 – to present
PASCAL		1977 – to present
SCISEARCH		1974 – to present
TOXCENTER		1907 – to present

Search criteria :

- 1,2,4-triazole, triazole lactic acid, triazole acetic acid and triazole alanine, synonyms and CAS numbers were used
- Suitable terms relating to the assessment of residues were used.

The process for selection of relevant scientific peer-reviewed open literature was undertaken as outlined below:

1. A very broad search was conducted for each section of the dossier
2. Duplicate titles were automatically removed
3. A rapid assessment of the titles was conducted to remove any obviously irrelevant titles
4. A further rapid assessment was conducted using abstracts and any clearly irrelevant titles were removed
5. A detailed assessment of the full-text documents for the remaining titles was conducted using the criteria (based on the relevant annex points in the dossier) developed for study relevance.
6. Any relevant papers were highlighted and assessed for reliability.

856 records relating to TDMs were identified under a consideration of metabolism and residues in animals. Of these, one was considered for detailed assessment. Upon further assessment it was considered not to be relevant to the residues and consumer safety assessment, and has therefore not been included in the dossier.

The methodology used in the search, and determination of records as non-relevant is considered acceptable.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6.1/1	Guedez-Orozco A.-A. Eilers B.	2016 a	Storage stability of BAS 750 F in plant matrices 2016/1112644 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A.
KCA 6.1/2	Heger N. Taraschewski I.	2015 a	Storage stability of Reg.No. 6011210 in animal matrices 2015/1106710 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.1/3	Heger N. Guedez-Orozco A.-A.	2015 b	Storage stability of BAS 750 F in animal matrices 2015/1106711 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.1/4	Perez R.	2015 a	Freezer storage stability of Triazolyl lactic acid in plant samples 2015/7005764 ADPEN Laboratories Inc., Jacksonville FL, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.2.1/1	Rabe U. Bogen C.	2015 a	Metabolism of 14C LS 5834378 in wheat 2015/1001872 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.2.1/2	Thiaener J. Bogen C.	2015 a	Metabolism of 14C-BAS 750 F in soybean 2014/1224012 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.2.1/3	Birk B.,Bogen C.	2015 a	Metabolism of 14C-BAS 750 F in grape 2015/1073822 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6.2.1/4	Birk B. et al.	2015 b	Investigation of the extractability of BAS 750 F in samples from 14C plant metabolism studies 2014/1261057 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.2.2/1	[redacted]	2015 a	The metabolism of 14C-Reg. No 5834378 (BAS 750 F) in laying hens 2015/1001001 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	
KCA 6.2.2/2	Thiaener J. Glaessgen W.E.	2015 b	Investigation of the extractability of BAS 750 F and M750F022 in samples from 14C animal metabolism studies 2015/1161960 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.2.3/1	[redacted]	2015 a	The metabolism of 14C-Reg. No. 5834378 (BAS 750 F) in lactating goats 2015/1078841 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	
KCA 6.2.3/2	Thiaener J. Glaessgen W.E.	2015 b	Investigation of the extractability of BAS 750 F and M750F022 in samples from 14C animal metabolism studies 2015/1161960 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	

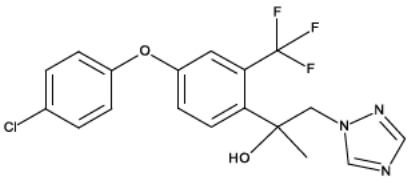
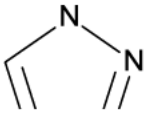
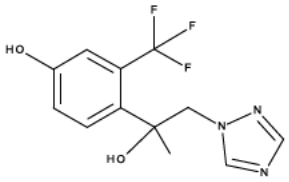
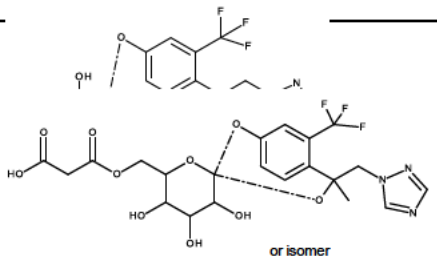
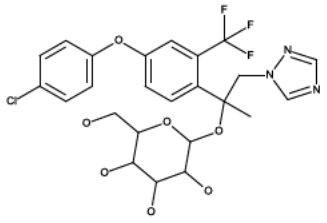
Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6.3.1/1	Erdmann H.-P.	2015 a	Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in wheat after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013 2014/1010809 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.3.1/2	Ale E.	2015 a	Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to wheat in Northern and Southern Europe in 2014 2015/1099704 Envigo CRS Limited Sucursal en Espana, Valencia, Spain yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.3.2/1	Erdmann H.-P.	2015 b	Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in barley after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013 2014/1010808 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.3.2/2	Ale E.	2015 b	Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to barley in Northern and Southern Europe in 2014 2015/1099703 Envigo CRS Limited Sucursal en Espana, Valencia, Spain yes Unpublished	No	Yes	Data for first Approval	BASF	

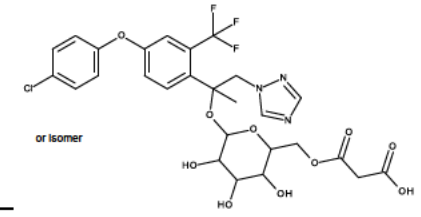
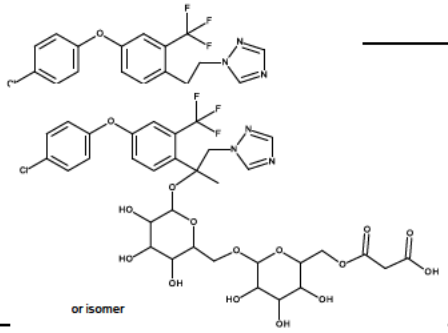
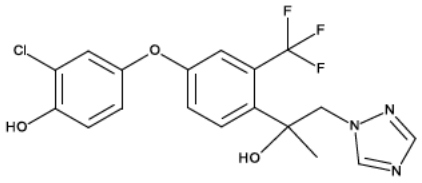
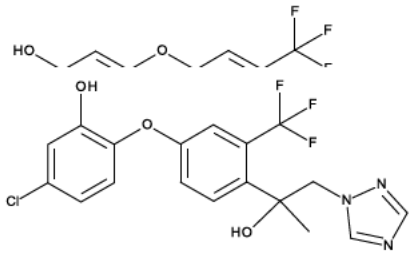
Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6.4.1/1	[redacted]	2015 a	Magnitude of residues in tissues and eggs of laying hens following multiple oral administrations of BAS 750 F 2015/1106667 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	
KCA 6.4.2/1	[redacted]	2015 a	Magnitude of residues in milk and tissues of dairy cows following multiple oral administration of BAS 750 F 2015/1107649 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	
KCA 6.4.2/2	Guedez Orozco A.A. Heger N.	2016 a	Determination of the fatty conjugates metabolites of M750F022 (Reg. No. 6011210) in animal matrices 2016/1001326 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.5.1/1	Hassink J. Bartmann S.	2014 a	BAS 750 F: Hydrolysis at 90°C, 100°C and 120°C 2014/1170665 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.5.3/1	Plier S.,Elze M.	2015 a	Determination of residues of BAS 750 F (Reg.No. 5834378) in wheat and its processed products after two applications of BAS 750 01 F in Germany, 2014 2014/1315283 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	

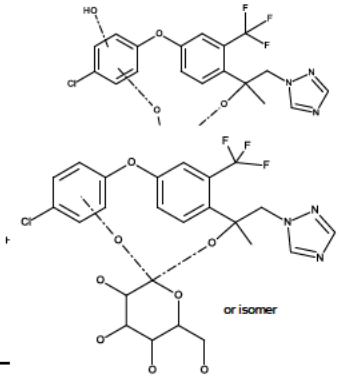
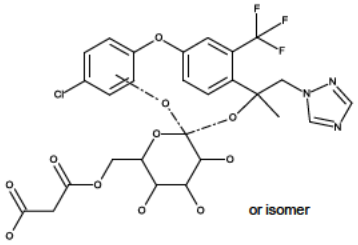
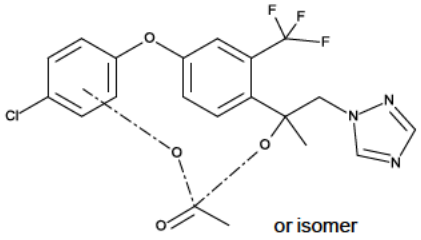
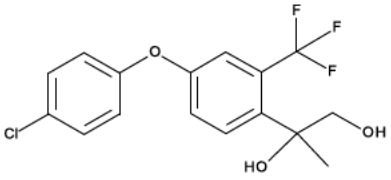
Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6.5.3/2	Plier S.,Elze M.	2015 b	Determination of residues of BAS 750 F (Reg.No. 5834378) in barley and its processed products after two applications of BAS 750 01 F in Germany, 2014 2014/1315282 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.6.1/1	Rabe U. Glaessgen W.	2015 a	Confined rotational crop study with 14C LS 5834378 2015/1001871 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	
KCA 6.6.2/1	Martin T.	2015 a	Study on the residue behavior of BAS 750 F on the rotational crops: wheat, carrots or radish, broccoli or cauliflower and spinach or lettuce after one application of BAS 750 01 F to bare soil under field conditions, 2014-2015 2015/1106682 Agrologia SLU, Utrera, Spain yes Unpublished	No	Yes	Data for first Approval	BASF	

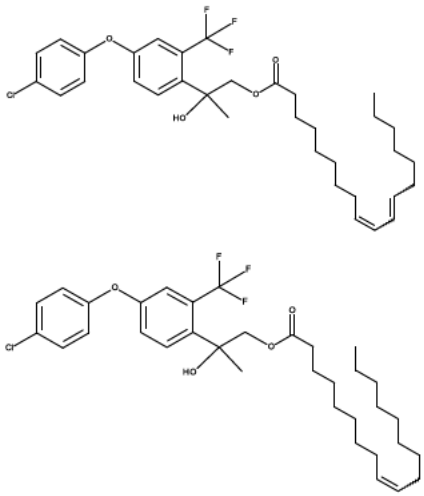
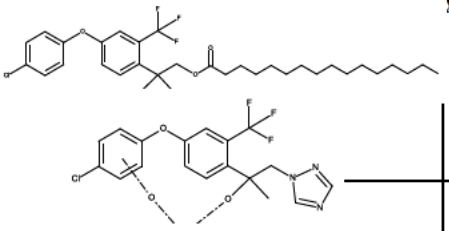
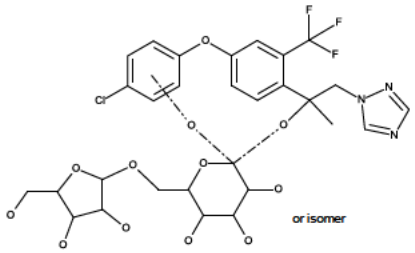
B.7.9. APPENDICES

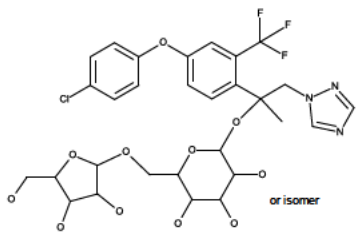
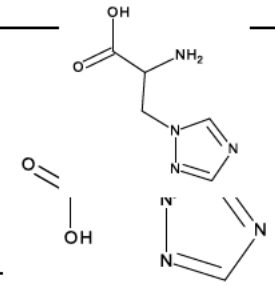
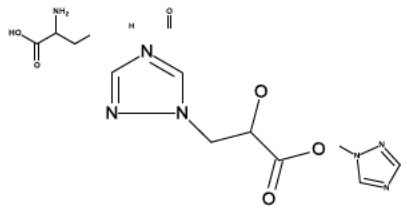
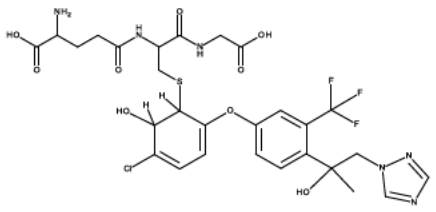
Appendix 1: Structure and identity of metabolites

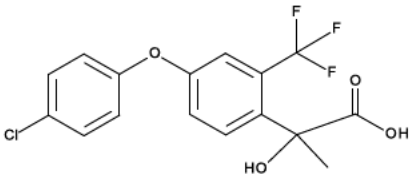
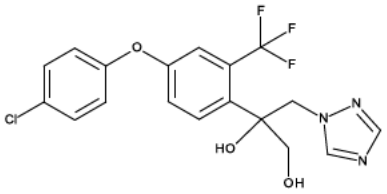
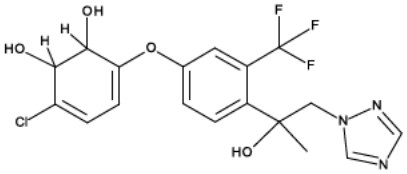
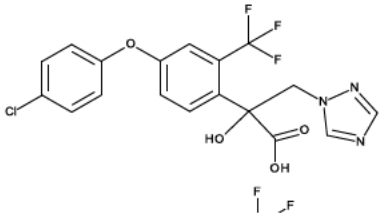
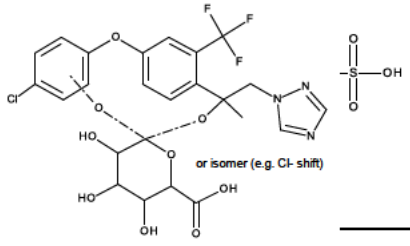
Code Number (Reg. Number)	Chemical Name	Molecular Structure	Compound found in		
			Livestock (Hen & Goat, Fish)	Crop (Wheat, Grape & Soy)	Rot Crop
BAS 750F (5834378)	(2RS)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol		x	x	
M750F001 (87084)	1,2,4-(1H)-triazole		x	x	x
M750F003 (5924326)	4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol		x		
M750F009		 or isomer		x	
M750F010				x	
M750F011	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-yl hexopyranoside			x	

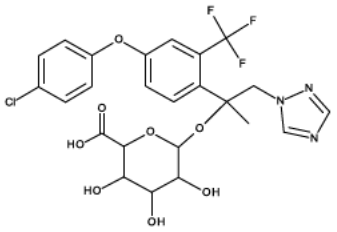
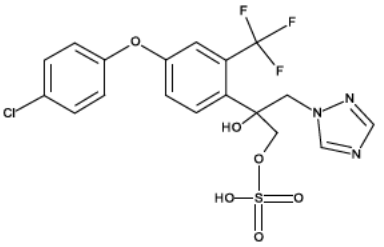
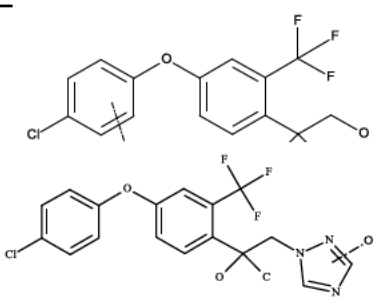
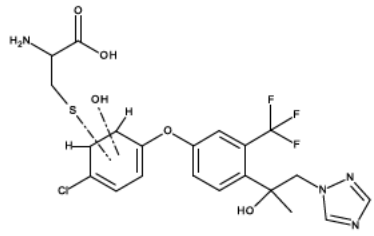
Code Number (Reg. Number)	Chemical Name		Compound found in		
			Livestock (Hen & Goat, Fish)	Crop (Wheat, Grape & Soy)	Rot Crop
M750F012	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-yl 6-O-(carboxyacetyl)hexopyranoside			x	
M750F013	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-yl 6-O-hexopyranosylhexopyranoside			x	
M750F014	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-yl 6-O-[6-O-(carboxyacetyl)hexopyranosyl]hexopyranoside			x	
M750F015 (6011549)	2-chloro-4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol		x		
M750F016 (6010140)	2-chloro-5-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol		x		
M750F017 (6010139)	5-chloro-2-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol		x		

Code Number (Reg. Number)	Chemical Name	Molecular Structure	Compound found in		
			Livestock (Hen & Goat, Fish)	Crop (Wheat, Grape & Soy)	Rot Crop
M750F018				X	
M750F019				X	
M750F020				X	
M750F021				X	
M750F022 (6011210)	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]propane-1,2-diol		X		

Code Number (Reg. Number)	Chemical Name	Molecular Structure	Compound found in		
			Livestock (Hen & Goat, Fish)	Crop (Wheat, Grape & Soy)	Rot Crop
M750F023	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-2-hydroxypropyl (9Z,11E)-octadeca-9,11-dienoate		x		
M750F024	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-2-hydroxypropyl (9Z)-octadec-9-enoate		x		
M750F025 (6056452)	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-2-hydroxypropyl hexadecanoate		x		
M750F026				x	
M750F027				x	

Code Number (Reg. Number)	Chemical Name	Molecular Structure	Compound found in		
			Livestock (Hen & Goat, Fish)	Crop (Wheat, Grape & Soy)	Rot Crop
M750F028	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-yl 6-O-pentofuranosylhexopyranoside			x	
M750F029 (270412)	2-amino-3-(1H-1,2,4-triazol-1-yl)propionic acid			x	
M750F030 (137281)	(1H-1,2,4-triazol-1-yl)acetic acid			x	
M750F031 (5050862)	2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propanoic acid			x	
M750F033	L-gamma-glutamyl-S-(2-chloro-5-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenyl)-L-cysteinylglycine				
M750F034	gamma-glutamyl-S-(5-chloro-6-hydroxy-2-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}cyclohexa-2,4-dien-1-yl)cysteinylglycine		x		

Code Number (Reg. Number)	Chemical Name	Molecular Structure	Compound found in		
			Livestock (Hen & Goat, Fish)	Crop (Wheat, Grape & Soy)	Rot Crop
M750F038	(2R)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-2-hydroxypropanoic acid		x		
M750F039	(2S)-2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-3-(1H-1,2,4-triazol-1-yl)propane-1,2-diol		x		
M750F041	3-chloro-6-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}cyclohexa-3,5-diene-1,2-diol		x		
M750F042	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propanoic acid		x		
M750F043	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-2-hydroxypropyl hydrogen sulfate		x		
M750F063			x		

Code Number (Reg. Number)	Chemical Name	:e	Compound found in		
			Livestock (Hen & Goat, Fish)	Crop (Wheat, Grape & Soy)	Rot Crop
M750F064			x		
M750F068	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-yl hexopyranosiduronic acid		x		
M750F072	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl hydrogen sulfate		x		
M750F078			x		
M750F086			x		
M750F091			x		