ANNEX B

Pyraflufen-ethyl

B.4 Methods of analysis

B.4.1 Analytical methods for formulation analysis

B.4.1.1 Analytical methods for the determination of pure active substance in the active substance as manufactured (Annex IIA 4.1.1)

Two methods were presented for the determination of pure ET-751 in ET-751 technical :
- a GC-method, for which two different sets of validation data were provided (Kudo, 1993 and Kudo, 1997)
- a HPLC method (Gladdines, 1995)

GC-method

- Validation of the analytical method of ET-751 technical (Kudo, 1993) = validation set 1)
- Analytical profile of batches of ET-751 technical (Kudo, 1997) = validation set 2)

GLP :
GLP-compliance is stated for the batch analysis study (Kudo, 1997).
Principle of the method :
ET-751 technical (50 mg) is dissolved in internal standard solution (Di-(2-ethylhexyl)-phthalate in acetone), after which the solution is further diluted with acetone.
ET-751 content is determined by GC (15 m x 0.53 mm i.d.; TC-1 (1.5 µm) (Kudo, 1993) or Nutra Bond-1 (2.0 µm) (Kudo, 1997)) using FID detection.Quantification by internal standard method.
Findings :
Specificity - interferences : According to the representative chromatograms of ET-751 technical and analytical standard, the method is suitable to determine ET-751 in ET-751 technical; no interferences are observed.
Linearity : the response of the GC-FID system to ET-751 (= peak area ratio of ET-751 to IS) was found to be linear
1) r = 0.999965; y = 0.0193 x - 0.0123 (n = 6; range from 11 to 100 mg)
2) calibration 1 : r = 0.999904; y = 0.0201 x - 0.0220 (n = 5; range from 29 to 70 mg)
calibration 2 : r = 0.999984; y = 0.0193 x - 0.0039 (n = 5; range from 30 to 71 mg)
Accuracy : determined by analysis of 3 independent weighings of analytical standard (50 mg), resp. 3 consecutive injections of the same standard solution, using the calibration curve
1) mean recovery = 101.5% (RSD = 1.5%)
2) mean recovery = 99.8% (RSD = 0.05%)
Repeatability : determined by 6 consecutive injections of the same standard solution
1) RSD = 0.4%
2) RSD = 0.08%
Conclusions :
The GC-method is suitable for the determination of ET-751 in ET-751 technical.

HPLC-method

Development and validation of an analytical method for ET-751 (Gladdines, 1995)
GLP :
GLP-compliance stated.
Principle of the method :
ET-751 technical is dissolved in acetonitrile and the ET-751 content is determined by RP-HPLC (LiChrospher 100 RP-18; 125 mm x 4 mm i.d., 5 µm; isocratic elution) using UV-detection at 243 nm.Quantification by external standardization.
Findings :
Specificity - interferences : According to the representative chromatograms of ET-751 technical and solvent blank, the method is able to separate the a.s. from the impurities in ET-751 technical, while the solvent causes no interference.
Linearity : the response of the HPLC-UV system to ET-751 (= peak height) was found to be linear over a concentration range from 1.19 to 24.8 mg/L
r = 0.9999; y = 1076 x + 125 (n = 6, analyzed in duplicate)
Accuracy : recovery was not addressed

Repeatability : determined by 10 consecutive injections of standard solutions
at 1.19 mg/L : RSD = 1.1%
at 24.8 mg/L : RSD = 1.3%

Limit of detection (LOD) : determined as the absolute amount of ET-751 at which the signal to noise ratio equals 3
LOD = 1.4 ng (i.e. 0.142 mg/L at an injection volume of 10 µL)

Conclusions :
The HPLC-method appears suitable for the determination of ET-751 in ET-751 technical, although accuracy was not addressed.

B.4.1.2 Analytical methods for the determination of significant and/or relevant impurities and additives in the active substance as manufactured (Annex IIA 4.1.2)

Analytical profile of batches of ET-751 technical (Kudo, 1997)
GLP :
GLP-compliance stated.
Principle of the method :
1) Determination of impurities 1-9 : ET-751 technical is dissolved in acetonitrile, after which the impurities are determined by HPLC (Inertsil ODS-2; 250 mm x 4.6 mm i.d., 5 µm; gradient elution) with UV detection at 254 nm. Quantification by external standardization.
2) Determination of impurities 11-12 : ET-751 technical is dissolved in internal standard solution (anisole in dioxane) and subsequently diluted to the mark with dioxane. Impurities 11 and 12 are determined by GC (25 m x 0.53 mm i.d.; OV-1 Bonded (2.0 µm)) with FID; quantification by internal standard method.
3) Determination of sulfate (impurity 10) : ET-751 technical is dissolved in 25 mL of ethyl acetate in a separatory funnel, after which 25 mL of distilled water is added and the funnel is vigorously shaken by a mechanical shaker. The aqueous layer is transferred to a volumetric flask, 1 mL of 10% hydrochloric acid is added and the mixture is diluted to the volume. After filtration by membrane filter, the test solution is transferred to a Nessler tube, 2 mL of barium chloride solution is added and the mixture is thoroughly mixed. After standing for 10 minutes, impurity 10 is quantified by comparing the turbidity of the test solution to that of an authentic solution of known content.
4) Determination of moisture (impurity 13) : ET-751 technical is dissolved in ethyl acetate, after which impurity 13 is determined by injecting test solution and ethyl acetate into coulometric moisture meter.
Findings :
Validation data were presented for methods 1) and 2) (see Table B.4.1.2-1).

Specificity - interferences : According to the representative chromatograms of ET-751 technical and impurity analytical standard mixtures, the HPLC-method is able to separate impurities 1 to 9 from one another and from the a.s., while the GC-method is able to separate impurities 11 and 12 from one another and from the internal standard. No interferences were observed.

Linearity : The response of the HPLC-UV system (= peak area) to each of the impurities 1-9 was found to be linear. The same also goes for the response of the GC-FID system (= peak area ratio of impurity to IS) to impurities 11 and 12.

Accuracy : Determined by analysis of 3 consecutive injections of the same impurity standard solution (10 mg/L of each impurity), using the calibration curves

Repeatability : Determined by 6 consecutive injections of 3 standard solutions (resp. 1, 4 and 10 mg/L of each impurity)

Limit of detection (LOD) : Determined as the amount which gave a detectable peak on the chromatograph
Conclusions :
The HPLC-method is suitable for determination of the structurally related impurities in ET-751 technical, while the GC-method is suitable for the determination of residual solvents.
No actual validation data were presented with regard to the methods for sulfate and moisture analysis.

Table B.4.1.2-1 : Validation of methods for determination of impurities in ET-751 technical (Kudo, 1997)

Impurity	Level (%)*	Repeatability (% RSD)	Recovery (%)***	Linearity**	LOD (%)*
				Concentration range (mg/100 mL), resp. (%)*	
1	0.01 0.04 0.10	4.7 0.6 3.8	101.7	y = 258142 x - 27978 r = 0.999952 0.1 - 20 (0.01 - 2.0)	0.01
2	0.01 0.04 0.10	1.3 0.7 0.6	102.7	y = 260590 x - 4573 r = 0.999981 0.1 - 20 (0.01 - 2.0)	0.01
3	0.01 0.04 0.10	1.6 0.7 0.7	102.5	y = 208999 x - 6616 r = 0.999982 0.1 - 20 (0.01 - 2.0)	0.01
4	0.01 0.04 0.10	1.5 0.6 0.5	103.4	y = 249420 x - 4980 r = 0.999980 0.1 - 20 (0.01 - 2.0)	0.01
5	0.01 0.04 0.10	2.4 0.4 0.8	102.8	y = 214380 x - 4879 r = 0.999978 0.1 - 20 (0.01 - 2.0)	0.01
6	0.01 0.04 0.10	5.8 1.9 1.7	102.9	y = 141613 x - 6933 r = 0.999985 0.1 - 20 (0.01 - 2.0)	0.01
7	0.01 0.04 0.10	3.2 0.7 0.7	101.5	y = 362474 x - 8835 r = 0.999984 0.1 - 20 (0.01 - 2.0)	0.01
8	0.01 0.04 0.10	1.6 1.0 0.8	102.8	y = 219302 x - 3895 r = 0.999981 0.1 - 20 (0.01 - 2.0)	0.01
9	0.01 0.04 0.10	- 4.9 2.5	105.2	y = 215323 x - 37774 r = 0.999971 0.4 - 20 (0.04 - 2.0)	0.04
11	0.01 0.04 0.10	4.3 1.2 0.3	98.2	y = 0.37895 x - 0.00583 r = 0.999981 0.1 - 10 (0.01 - 1.0)	0.01
12	0.01 0.04 0.10	- 2.7 9.3	95.5	y = 0.25992 x - 0.00817 r = 0.999465 0.4 - 10 (0.04 - 1.0)	0.04

Notes

* relative to the amount of technical ET-751 (250 mg in 25 mL for impurities 1-9, resp. 1000 mg in 100 mL for impurities 11 and 12)

** for impurities 1-8 : n = 7
for impurities 9, 11 : n = 6
for impurity 12 : n = 5

*** recalculated by RMS (results in report were found to be incorrect)

B.4.1.3 Analytical methods for the determination of pure active substance in plant protection products (Annex IIIA 5.1.1)

Method B-887-11-96(E) : Bifenox/ET-751 determination by HPLC-analysis in formulation EXP31279A (SC) (Sciolla and Uceda, 1996)

GLP :

GLP-compliance stated.

Principle of the method :

EXP31279A is extracted with methanol by sonication, after which a portion of the solution is filtered for analysis. ET-751 and bifenox are determined simultaneously by RP-HPLC (Nucleosil 100C18; 125 mm x 4 mm i.d.; isocratic elution) with UV detection at 254 nm; quantification by external standardization.

Findings :

Specificity - interferences : Method is capable to separate both active substances from one another and from the co-formulants. Examination of a formulation blank revealed that there was no interference likely to affect the peaks of the active substances.

Linearity : the response of the HPLC-UV system to ET-751 and bifenox was found to be linear over a concentration range from 0.004 to 0.040 g/L for ET-751 and over a range from 0.2 to 2.0 g/L for bifenox.

1) for ET-751 : $R^2 = 1.000$; $y = 13480 x + 0.2881$ ($n = 10$)
2) for bifenox : $R^2 = 1.000$; $y = 7079 x + 26.12$ ($n = 10$)

Accuracy : determined by analysis of 6 independent spiked formulation blanks

1) for ET-751 : mean recovery = 100.6% (RSD = 2.7%)
2) for bifenox : mean recovery = 99.4% (RSD = 1.4%)

Repeatability : determined by analysis of 6 independent preparations of the formulation

1) for ET-751 : RSD = 0.7%
2) for bifenox : RSD = 0.2%

Conclusions :

The HPLC-method is suitable for determination of ET-751 and bifenox in formulation EXP31279A.

B.4.1.4 Analytical methods for the determination of relevant impurities, additives and formulants in plant protection products (Annex IIIA 5.1.2)

No methods were submitted (applicant refers to the methods that were provided in Annex II).

Conclusion :

No methods required since none of the impurities and formulants are considered to be of toxicological, ecotoxicological or environmental concern.

B.4.2 Analytical methods (residue) for food and feed (Annex IIA 4.2.1; Annex IIIA 5.2.1)

B.4.2.1 Analytical methods (residue) for target crops

Analytical method of ET-751 and its metabolite (E-1) in wheat grains (Ikemoto, 1995)
Analytical method validation of ET-751 and its metabolite E-1 in wheat (grain, straw and shoot) (Anding, 1997a)
GLP :
GLP-compliance stated for the independent laboratory validation study (Anding, 1997a).
Principle of the method :
1) Original method (Ikemoto, 1995) : Milled wheat grain samples are extracted by shaking with acidic acetonitrile. The extract is cleaned up by solvent partitioning with hexane/ethyl acetate (9/1), followed by three different types of column chromatography (Bond Elut LRC SCX, Bond Elut LRC C18 and Bond Elut LRC SI). After the first chromatographic step, the sample is divided in two fractions for separate analysis of ET-751 and E-1. While the sample for ET-751 analysis is immediately further cleaned up, the sample for E-1 analysis is first methylated using 10% trimethylsilyl diazomethane in hexane.
ET-751 and E-15 (= methylated derivative of E-1) are determined by GC (15 m x 0.53 mm i.d.; DB-17 (1 µm)) with nitrogen-phosphorus detection (GC-NPD); quantification by external standardization.
2) Adapted method (Anding, 1997a) : Wheat samples (grain, straw, shoot) are extracted by blending with acidic acetonitrile. The extract is cleaned up by solvent partitioning with hexane/ethyl acetate (9/1) and is then methylated using diazomethane. After derivatisation, the extract is further cleaned up by column chromatography, first on C18 Mega Bond Elut cartridges and finally on Florisil Mega Bond Elut cartridges.
ET-751 and E-15 are determined by GC (30 m x 0.53 mm i.d.; DB-608 (0.83 µm)) with nitrogen-phosphorus detection (GC-NPD); quantification by external standardization.
Findings :
Only the validation data regarding simultaneous fortification with ET-751 and E-1 are discussed here.
Specificity - interferences : According to the representative chromatograms of standard solutions, controls and spiked samples, the method is able to determine ET-751 and its metabolite E-1 (= E-15) through a one step analysis.
Untreated control samples exhibit no significant interfering peaks at the retention time of ET-751 or E-15.
Linearity : The response of the GC-NPD system to ET-751 and E-1 (i.e. E-15) (= peak height) was stated to be linear for each compound over a concentration range from 0.025 to 0.50 mg/L (Anding, 1997a).
Recovery - precision : see Table B.4.2.1-1
Validation by an independent laboratory : the original analytical method, validated by Nihon Nohyaku, was partially modified and subsequently validated by Defitraces, an independent laboratory.
Limit of determination (LOQ) : LOQ was stated to be 0.005 mg/kg by Ikemoto (1995), but this value was not substantiated with recovery trials at the corresponding concentration level.
Anding (1997a) stated the LOQ to be 0.01 mg/kg for wheat grain and 0.02 mg/kg for straw and shoot.
Conclusions :
The method is suitable for residue analysis of ET-751 and metabolite E-1 in wheat matrices with a LOQ of 0.01 mg/kg for grain and 0.02 mg/kg for straw and shoot.
According to the validation recovery rates, the method doesn't seem to meet all the requirements for ET-751 analysis in wheat grain (overall RSD 30.7%). However, taking into account the limited number of recovery samples tested during that particular study, as well as the acceptable results obtained at LOQ in the same matrix on 2 other occasions (control recovery rates from 95 and 96), this deviation is considered not to detract from the suitability of the method. The same conclusion is also valid with respect to the determination of ET-751 in wheat straw, where on one occasion a RSD of 23.9% was observed at LOQ.

Table B.4.2.1-1 : Validation of method for determination of ET-751 and E-1 in wheat (grain, straw, shoot)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation by Nihon Nohyaku (Ikemoto, 1995)						
wheat grain	ET-751	0.1	3	83 - 92	87	5.2
	E-1	0.1	3	102 - 105	103	1.5
Validation by independent laboratory Defitraces (Anding, 1997a)						
Validation recovery rates						
wheat grain	ET-751	0.01 - 0.2	4	71 - 123	84	30.7*
	E-1	0.01 - 0.2	4	74 - 97	86	11.9
wheat straw	ET-751	0.02 - 0.4	4	79 - 110	98	14.8
	E-1	0.02 - 0.4	4	73 - 106	86	17.5
wheat shoot	ET-751	0.02 - 0.4	4	70 - 87	79	10.5
	E-1	0.02 - 0.4	4	69 - 90	79	14.3
Control recovery rates (studies from 1995)						
wheat grain	ET-751	0.01	10	73 - 120	99	15.7
	E-1	0.01	10	89 - 120	107	9.6
wheat straw	ET-751	0.02 0.04	6 4	77 - 126 85 - 126	97 101	23.9* 18.6
		0.02 - 0.04	10	77 - 126	99	20.8
	E-1	0.02 0.04	6 4	69 - 111 76 - 119	90 100	20.4 20.3
		0.02 - 0.04	10	69 - 119	94	19.8
wheat shoot	E-751	0.02 0.1 - 1.0	5 5	78 - 97 68 - 76	86 73	9.9 4.7
		0.02 - 1.0	10	68 - 97	80	11.3
	E-1	0.02 0.1 - 0.5	5 5	77 - 110 69 - 101	98 84	12.8 15.9
		0.02 - 0.5	10	69 - 110	91	15.7
Control recovery rates (studies from 1996)						
wheat grain	ET-751	0.01	9	73 - 127	92	17.2
	E-1	0.01	9	69 - 112	95	16.6
wheat straw	ET-751	0.02 0.04	8 1	69 - 118 116	94 -	17.3 -
		0.02 - 0.04	9	69 - 118	96	17.5
	E-1	0.02 0.04	8 1	70 - 120 95	92 -	19.9 -
		0.02 - 0.04	9	70 - 120	92	18.6
wheat shoot	ET-751	0.02 0.05 - 0.25	6 5	71 - 97 71 - 121	85 97	11.5 18.3
		0.02 - 0.25	11	71 - 121	90	16.3
	E-1	0.02 0.1 - 1.5	6 5	71 - 109 76 - 108	84 88	18.2 14.5
		0.02 - 1.5	11	71 - 109	86	15.9

Determination of pyraflufen-ethyl (ET-751) in cereal by the modified mulri-residue enforcement method DFG-S19 : Results of method try-out experiments (Bacher, 1998)

GLP :

GLP-compliance stated.

Principle of the method :

Cereal samples (grain, straw, shoot) are extracted using a neutral water/acetone (1/2) mixture. Saturation by NaCl and addition of ethyl acetate/cyclohexane (1/1) is used for phase separation and partition of pesticides into the organic phase, which is subsequently dried and concentrated, filtered and applied to gel permeation chromatography (GPC) on Bio-Beads S-X3.

For determination of residues of the acidic metabolite E-1, the concentrated GPC eluate is methylated using diazomethane in dichloromethane to form the methyl ester E-15. After complete elimination of excess reagent, the extract is redissolved in isooctane and fractionated on silicagel (SiO_2, 1.5% water). Pyraflufen-ethyl and the methyl derivative E-15 elute mainly with toluene/5% acetone (3^{rd} fraction) and toluene/20% acetone (4^{th} fraction). The combined fractions are analysed using GC/MS or GC/MS/MS with external calibration.

Findings :

Method recoveries of less than 50% were observed for puraflufen-ethyl. Also, a portion of approximately 5% of the pyraflufen-ethyl was hydrolysed to E-1 and analysed as E-15 after methylation, indicating a partial hydrolysis or degradation of the parent compound. The extraction/partition step is most likely the critical step in the sample preparation procedure.

Conclusions :

The modified multi-residue enforcement method DFG-S19 was found to be not applicable to the analysis of pyraflufen-ethyl and its acidic metabolite E-1 in cereals.

B.4.2.2 Analytical methods (residue) for food of animal origin

no methods were submitted

Conclusions :

Methods for the determination of residues in food matrices of animal origin are not required since residues of pyraflufen-ethyl are not expected in animal products for human consumption.

B.4.3 Analytical methods (residue) in soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2.2 to 5.2.4)

B.4.3.1 Analytical methods for soil (Annex IIA 4.2.2; Annex IIIA 5.2.2)

Analytical procedure CHE 608/26-02R : Determination of ET-751 and its metabolites E-1, E-2 and E-3 residues in soil
in : ET-751 SC (containing 20 g ET-751/L) - Dissipation from four field soils following spring application (Analytical method validation) (Wright and Burden, 1997)
GLP :
GLP-compliance stated.
Principle of the method :
Soil samples are extracted by shaking with acidic acetonitrile. The extract is centrifuged, after which an aliquot is diluted with water and cleaned-up on a Bond Elut C18 solid phase extraction cartridge. Samples are eluted from the cartridge with acetonitrile and diluted with water for analysis.
Residues of ET-751, E-1, E-2 and E-3 are determined by HPLC (Inert Pack Phenyl, 25 cm x 4.6 mm i.d., 5µm; isocratic elution) with multiple reaction monitoring tandem mass spectrometry using electrospray ionisation (LC/MS-MS); quantification by external standardization.
Findings :
Specificity - interferences : According to the representative chromatograms of standard solutions, control and recovery samples, the method is suitable to determine ET-751 and its metabolites E-1, E-2 and E-3.
Untreated control samples were found not to contain significant concentrations of any analyte or other co-extracted material which interfered with analysis (< 0.01 mg/kg).
Linearity : The response of the LC/MS-MS system to ET-751, E-1, E-2 and E-3 (= peak area) was demonstrated to be linear for each compound over a concentration range from 2.5 to 75 µg/L.
Calibration solutions were prepared in control matrix (= cleaned-up control soil extract).
Recovery - precision : see Table B.4.3.1-1
Table B.4.3.1-1 : Validation of method CHE 608/26-02R (Wright and Burden, 1997)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil (silty clay loam, clay loam, silt loam, sandy clay loam)	ET-751	0.01	6	94 - 108	102	6.1
		0.05	6	95 - 101	98	2.2
		0.50	6	99 - 104	101	1.8
		0.01 - 0.50	18	94 - 108	100	4.2
	E-1	0.01	6	89 - 109	103	7.2
		0.05	6	94 - 106	100	4.0
		0.50	6	95 - 106	101	4.0
		0.01 - 0.50	18	89 - 109	101	5.1
	E-2	0.01	6	93 - 108	100	5.6
		0.05	6	88 - 105	97	6.7
		0.50	6	93 - 108	99	5.9
		0.01 - 0.50	18	88 - 108	99	5.8
	E-3	0.01	6	96 - 106	101	3.7
		0.05	6	91 - 105	99	6.2
		0.50	6	98 - 102	100	1.6
		0.01 - 0.50	18	91 - 106	100	4.0

Limit of determination (LOQ): 0.01 mg/kg

Conclusions :
The method is suitable for residue analysis of ET-751 and metabolites E-1, E-2, E-3 in soil with a LOQ of 0.01 mg/kg.

B.4.3.2 Analytical methods for water (Annex IIA 4.2.3; Annex IIIA 5.2.3)

Analytical method validation of ET-751 and its metabolite E-1 in water (Anding, 1997b)
GLP :
GLP-compliance stated.
Principle of the method :
Water samples are acidified and extracted with ethyl acetate, after which the concentrated organic extract is methylated with diazomethane.
ET-751 and E-15 (= the methylated E-1) are determined by GC (15 m x 0.53 mm i.d. or 30 m x 0.53 mm i.d.; DB-608 (0.83 µm)) with electron capture detection (GC-ECD); quantification by external standardization.
Findings :
Specificity - interferences : According to the representative chromatograms of standard solutions, control and recovery samples, the method is suitable to determine ET-751 and its metabolite E-1 (as E-15).
Untreated control samples exhibit no significant interfering peaks at the retention time of ET-751 and E-15 (control values are stated to be < LOQ)
Linearity : The response of the GC-ECD system to ET-751 and E-15 (= peak height) was stated to be linear for each compound over a concentration range from 0.005 to 0.10 mg/L.
Recovery - precision : see Table B.4.3.2-1
Limit of determination (LOQ) : - 0.1 µg/L for mineral water and tap water
- 1.0 µg/L for surface water
Conclusions :
In terms of interferences, accuracy (mean recoveries between 70 and 110%) and precision (overall RSD's lower than 20%), the method is generally suitable for residue analysis of ET-751 and metabolite E-1 in water with a LOQ of 0.1 µg/L for mineral and tap water and 1.0 µg/L for surface water.
At the lower fortification level of 0.1 µg/L, the method doesn't seem to meet all requirements for determination of E-1 in mineral water (RSD slightly exceeds 20%). However, taking into account the acceptable results obtained for tap water, as well as the fact that the corresponding overall RSD falls within limits, this small deviation is considered not to detract from the suitability of the method.

Table B.4.3.2-1 : Validation of method for determination of ET-751 and E-1 in water (Anding, 1997b)

Matrix	Analyte	Fortification level (µg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
mineral water	ET-751	0.1	4	85 - 123	102	15.7
		0.1 - 10	8	85 - 123	99	13.8
	E-1	0.1	4	68 - 113	89	21.1*
		0.1 - 10	8	68 - 120	92	19.8
tap water	ET-751	0.1	4	85 - 124	105	16.9
		0.1 - 10	8	85 - 124	105	13.4
	E-1	0.1	4	79 - 119	96	18.1
		0.1 - 10	8	79 - 119	104	13.9
surface water	ET-751	1.0	4	101 - 119	110	6.8
		1.0 - 100	8	89 - 119	101	10.8
	E-1	1.0	4	77 - 98	87	10.3
		1.0 - 100	8	77 - 111	97	13.1

B.4.3.3 Analytical methods for air (Annex IIA 4.2.4; Annex IIIA 5.2.4)

ET-751 : Analytical method for the determination in air (Mörtl and Class, 1996)

GLP :

GLP-compliance was stated.

Principle of the method :

A defined volume of air (200 to 350 mL/min for 6 h, 0.1 m³) is sucked through an ORBO 44 (XAD-2 equivalent) air sampling cartridge; particles and aerosols are trapped by filtration or impact onto the adsorbent material. The adsorbent portions are extracted twice with toluene and the combined extract is adjusted to a final volume of 10 mL with toluene.

ET-751 is determined by capillary GC (15 m x 0.32 mm i.d.; DB1 (0.25 µm)) with ECD; quantification by external calibration. GC-NPD or GC-MS is proposed as confirmatory method.

Findings :

Specificity - interferences : According to the representative chromatograms of standard solutions, control and recovery samples, the method is suitable to determine ET-751.

Untreated control samples exhibit no significant interfering peaks at the retention time of ET-751 (analyte signals in control samples are stated to be < 10% of LOQ). Other volatile pesticides may interfere in GC-ECD analysis, but a different temperature program, capillary column or detector may solve this problem.

Linearity : The response of the GC-ECD system to ET-751 (= peak area) was stated to be quadratic over a concentration range from 1 to 1000 µg/L. However, it is advised to use a more narrow range for evaluation of sample extracts, to prevent memory effect caused by injecting high concentrations.

Recovery - precision : - Dynamic retention efficiency : see Table B.4.3.3-1 (breakthrough was always < 0.5% of spiked).

- Extraction efficiency : 101% (n = 6; RSD = 22%)

Storage stability : at least 7 d at room temperature, refrigerated and frozen (mean recovery : 96%; n = 8; RSD = 6%)

Limit of determination (LOQ) : 6 µg/m³

Table B.4.3.3-1 : Validation of method for determination of ET-751 in air (Mörtl and Class, 1996)

Matrix	Analyte	Fortification level (µg/m³ commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
ambient air (22 C, 42% RH)	ET-751	6 600	4 4	105.0 - 134.2 86.6 - 104.4	121.9* 91.9	10.1 9.1
		6 - 600	8	86.6 - 134.2	106.9	17.5
warm, humid air (31 C, 83% RH)	ET-751	6 600	4 4	83.3 - 117.5 86.2 - 93.9	98.8 89.5	14.6 4.0
		6 - 600	8	83.3 - 117.5	94.1	11.6

Conclusions :
In terms of interferences, accuracy (overall mean recoveries between 70 and 110%) and precision (RSD's lower than 20%), the method is generally suitable for ET-751 residue analysis in air with a LOQ of 6 µg/m³.
At the lower fortification level of 6 µg/m³ in ambient air, the method doesn't seem to meet all requirements (mean recovery > 110%), which may have been caused by improper dilution or memory effect in the injector. However, taking into account the acceptable results obtained in warm humid air, as well as the fact that the overall mean recovery in ambient air falls within limits, this deviation is considered not to detract from the suitability of the method.

B.4.4 Analytical methods (residue) wildlife and for use in support of diagnostic and therapeutic regimes (Annex IIA 4.2.5; Annex IIIA 5.2.5)

Validation of an analytical method for the quantitative estimation of ET-751 and metabolites E-1 and E-9 in dog plasma (Oldfield, 1996)
GLP :
GLP-compliance stated.
Principle of the method :
Plasma samples are acidified with phosphate/citrate buffer (pH 3) and subsequently extracted using dichloromethane, after which the organic layer is evaporated and reconstituted in mobile phase.
ET-751, E-1 and E-9 are determined by HPLC (Inertsil Ph; 150 x 4.6 mm, 5 µm; isocratic elution) with UV detection at 248 nm; quantification by external standardization.
Findings :
Specificity - interferences : According to the representative chromatograms of control plasma and spiked samples, the method allows good separation of ET-751, E-1 and E-9 from one another and from endogenous compounds.
Untreated control sample (control dog plasma with lithium heparin anticoagulant) exhibited no significant interfering peaks at the retention time of ET-751, E-1 or E-9.
Linearity : The response of the HPLC-UV system to ET-751, E-1 and E-9 (=peak height) was found to be linear over a concentration range from 0.3 to 12 mg/L for ET-751 and E-1 and over a range from 0.3 to 2.4 mg/L for E-9.
Calibration solutions were prepared in control plasma and taken through the extraction procedures, thus eliminating the effect of the extraction efficiency. The overall extraction efficiency was determined to be 60.9% and 87.7% for ET-751 and E-1 resp. (over the range 0.3 to 12 mg/L) and 34.3% for E-9 (over the range 0.3 to 2.4 mg/L).
Recovery - precision : see Table B.4.4-1
Limit of determination (LOQ) : 0.3 mg/L

Table B.4.4-1 : Validation of method for determination of ET-751, E-1 and E-9 in dog plasma (Oldfield, 1996)

Matrix	Analyte	Fortification level (mg/L commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
dog plasma	ET-751	0.3 0.6 2.4 12	5 6 6 6	81.2 - 108.1 81.7 - 112.3 100.7 - 128.1 87.7 - 114.7	96.9 101.5 108.6 104.6	13.9 10.6 9.4 11.3
		0.3 - 12	23	81.2 - 128.1	103.2	11.2
	E-1	0.3 0.6 2.4 12	5 6 6 6	77.6 - 119.5 78.1 - 116.4 99.6 - 113.5 85.8 - 109.0	101.5 104.7 107.6 99.4	18.0 13.2 4.7 9.4
		0.3 - 12	23	77.6 - 119.5	103.4	11.5
	E-9	0.3 0.6 2.4	5 6 6	86.5 - 138.9 84.2 - 127.2 96.0 - 119.9	113.7* 108.1 109.2	17.3 13.2 8.1
		0.3 - 2.4	17	84.2 - 138.9	110.1	12.5

Conclusions :

In terms of interferences, accuracy (overall mean recoveries between 70 and 110%) and precision (RSD's lower than 20%), the method is generally suitable for residue analysis of ET-751, E-1 and E-9 in dog plasma with a LOQ of 0.3 mg/L.

At the lower fortification level of 0.3 mg/L, the method doesn't seem to meet all requirements for E-9 determination (mean recovery slightly exceeds 110%). However, taking into account that the overall mean recovery for E-9 falls within limits, this small deviation is considered not to detract from the suitability of the method.

B.4.5 Evaluation and assessment

B.4.5.1 Evaluation and assessment of analytical methods for technical active substance and formulation analysis

Table B.4.5.1-1 : Summary of analytical methods for technical active substance and formulation analysis

Matrix	Analyte	Type of method	Validation	References
technical active substance	ET-751	GC with FID	full	Kudo, 1993 Kudo, 1997
		HPLC with UV detection	full, except for accuracy	Gladdines, 1995
technical active substance	impurities 1-9	HPLC with UV detection	full	Kudo, 1997
	impurities 11-12	GC with FID	full	
	impurity 10	turbidimetry	no data	
	impurity 13	coulometric moisture tester	no data	
formulation (SC) EXP31279A	ET-751 Bifenox	HPLC with UV	full	Sciolla and Uceda, 1996

Evaluation :
The methods submitted allow to determine the purity and the impurities of the technical a.s., as well as the a.s. content of formulation EXP31279A (SC). However, with respect to the determination of pure ET-751 in ET-751 technical, accuracy of the HPLC-method remains to be addressed. No actual validation data were provided with regard to the methods for sulfate (turbidimetry) and moisture (coulometric moisture tester) analysis.

B.4.5.2 Evaluation and assessment of the analytical methods (residue) for food and feed

Table B.4.5.2-1 : Summary of analytical methods (residue) for target crops

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation	References
wheat grain	ET-751 E-1 (as E-15)	GC with NPD after derivatisation	0.01*-0.2	full	Ikemoto, 1995 Anding, 1997a
wheat straw	ET-751 E-1 (as E-15)		0.02*-0.4		Anding, 1997a
wheat shoot	ET-751 E-1 (as E-15)		0.02* - 1.0 0.02* - 1.5		
cereal (grain, straw, shoot)	ET-751 E-1 (as E-15)	GC/MS or GC/MS/MS after derivatisation	-	recoveries < 50% + partial hydrolysis of parent compound	Bacher, 1998 (DFG-S19)

* LOQ = limit of determination

Evaluation :
The GC-method submitted allows determination of parent ET-751 and its main metabolite E-1 (as E-15) in *food matrices of plant origin* with a LOQ of 0.01 mg/kg for wheat grain and 0.02 mg/kg for wheat straw and shoot.

The modified multi-residue enforcement method DFG-S19 was found to be not applicable to the analysis of ET-751 and E-1 in cereals.

Methods for the determination of residues in *food matrices of animal origin* are not required since residues of pyraflufen-ethyl are not expected in animal products for human consumption.

B.4.5.3 Evaluation and assessment of the analytical methods (residue) in soil, water and air

Table B.4.5.3-1 : Summary of analytical methods (residue) for soil, water and air

Matrix	Analyte	Type of method	Method range	Validation	References
soil	ET-751 E-1 E-2 E-3	LC/MS-MS	0.01* - 0.5 mg/kg	full	Wright and Burden, 1997
mineral water, tap water	ET-751 E-1 (as E-15)	GC with ECD after derivatisation	0.10* - 10 µg/L	full	Anding, 1997b
surface water			1.0* - 100 µg/L		
air	ET-751	GC with ECD	6* - 600 µg/m³	full	Mörtl and Class, 1996

* LOQ = limit of determination

Evaluation :
The LC/MS-MS method submitted for *soil analysis* allows determination of parent ET-751 and its main metabolites E-1, E-2 and E-3 in different soil types with a LOQ of 0.01 mg/kg.
Although LC/MS-MS is currently not considered to be a commonly available technique, the method can be accepted taking into account the justification stated by the notifier. According to Nihon Nohyaku there was a degree of uncertainty as to the GC/NPD method supplied (cfr. B.4.2.1) being able to achieve the required LOQ for all soil types.

The GC-ECD method provided for *water analysis* allows determination of parent ET-751 and its metabolite E-1 (as E-15) in drinking water and surface water with a LOQ of resp. 0.1 µg/L and 1.0 µg/L.

The GC-ECD method submitted for *air analysis* allows to determine parent ET-751 in ambient and warm, humid air with a LOQ of 6 µg/m³.

B.4.5.4 Evaluation and assessment of analytical methods (residue) wildlife and for use in support of diagnostic and therapeutic regimes

Table B.4.5.4-1 : Summary of analytical methods (residue) for body fluids

Matrix	Analyte	Type of method	Method range (mg/L commodity)	Validation	References
dog plasma	ET-751 E-1 E-9	HPLC with UV detection	0.3* - 12 0.3* - 2.4	full	Oldfield, 1996

*LOQ = limit of determination

Evaluation :
The HPLC method submitted allows determination of parent ET-751 and its main metabolites E-1 and E-9 in dog *plasma* with a LOQ of 0.3 mg/L.
Further analytical methodology for residue analysis in *tissues* is not required as pyraflufen-ethyl is not classified as toxic or highly toxic.

A summarized description of analytical methods for the determination of bifenox residues (in crops, soil, water, air and milk) was provided by the notifier.

B.4.6 References relied on

Methods of analysis for the active substance (Annex IIA 4)

Author(s)	Year	Annex IIA Point Title Company, Report No.	GLP GEP Y/N	Published or not Y/N	Owner
Anding, C.	1997a	IIA, 4.2.1/02 Analytical method validation of ET-751 and its metabolite E-1 in wheat (grain, straw and shoot). Nihon Nohyaku, Report No.: A-5018	Y	N	NN
Anding, C.	1997b	IIA, 4.2.3 Analytical method validation of ET-751 and its metabolite E-1 in water. Nihon Nohyaku, Report No.: A-5027	Y	N	NN
Gladdines, M.	1995	IIA, 4.1/02 Development and validation of an analytical method for ET-751. Nihon Nohyaku, Report No.: A-500?	Y	N	NN
Ikemoto, Y.	1995	IIA, 4.2.1/01 Analytical method of ET-751 and its metabolite (E-1) in wheat grains. Nihon Nohyaku, Report No.: A-5	N	N	NN
Kudo, M.	1993	IIA, 4.1/01 Validation of the analytical method of ET-751 technical. Nihon Nohyaku, Report No.: A-5002	N	N	NN
Mörtl, S. Class, T.	1996	IIA, 4.2.4 Analytical method for determination in air Nihon Nohyaku, Report No.:	Y	N	NN
Oldfield, P. R.	1996	IIA, 4.2.5 Validation of an analytical method for the quantitative estimation of ET-751 and metabolite E1 and E9 in dog plasma Nihon Nohyaku, Report No.: A-5023	Y	N	NN
Wright, D. R. Burden, A. N.	1997	IIA, 4.2.2 ET-751 SC (Containing 20g ET-751/L): Dissipation from four field soils following spring application (Analytical method validation). Nihon Nohyaku, Report No.:	Y	N	NN

Methods of analysis for the formulation MILAN (Annex IIIA 5)

Author(s)	Year	Annex IIA Point Title Company, Report No.	GLP GEP Y/N	Published or not Y/N	Owner
Sciolla, C. and Uceda, L.	1996	Annex IIIA, 5.1 BIFENOX/ET-751 Determination by HPLC analysis in formulation EXP31279A (SC) Report n : B-887-11-96 (E), 20 December 1996 Rhône-Poulenc Agro, Lyon, France	Y	N	RPA