BIXAFEN (262)

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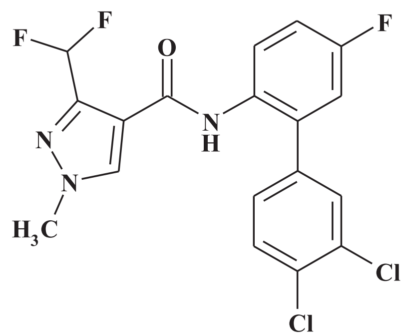
EXPLANATION

Bixafen (ISO common name) is a pyrazole-carboxamide fungicide used to control diseases on rape plants and cereals. Bixafen inhibits fungal respiration by binding to mitochondrial respiratory complex II. It was considered for the first time by the 2013 JMPR for toxicology and residues.

IDENTITY

ISO common name	Bixafen
Chemical name	
IUPAC	N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
CA	1H-pyrazole-4-carboxamide, N-(3',4'-dichloro-5-fluoro[1,1'-biphenyl]-2-yl)-3-(difluoromethyl)-1-methyl-
CIPAC No.	819
CAS No.	581809-46-3
Structural formula	
Molecular formula	$C_{18}H_{12}Cl_2F_3N_3O$
Molecular mass	414.21 g/mol

Specifications

Specifications for bixafen are not yet developed by FAO.

PHYSICAL AND CHEMICAL PROPERTIES

Property	Results	Method (test material)	Reference
Melting point	The melting point of pure bixafen (purity 98.9%) at atmospheric pressure is 146.6 °C The melting point of bixafen technical (purity 95.8%) at atmospheric pressure is 142.9 °C	EC A.1, OECD 102 batch 1 + batch 2	Smeykal, H 2007, BIXAFEN_001 & Smeykal, H 2007, BIXAFEN_002

Property	Results	Method (test material)	Reference
Boiling point & temperature of decomposition	No boiling before decomposition at approx. 250 °C observed	EC A.2, OECD 103 + OECD 113 batch 1 + batch 2	Smeykal, H 2007, BIXAFEN_001 & Smeykal, H 2007, BIXAFEN_002
Relative density	Bixafen, pure (purity 98.9%): $D_4^{20} = 1.43$ Bixafen, technical (purity 95.8%): $D_4^{20} = 1.51$	EC A.3, OECD 109 batch 1 + batch 2	Bogdoll, B and Strunk, B 2007, BIXAFEN_003
Vapour pressure	Extrapolated: $4.6 \cdot \times 10^{-8}$ Pa at 20 °C 1.1×10^{-7} Pa at 25 °C 5.9×10^{-6} Pa at 50 °C	EC A.4, OECD 104 batch 3 (purity 98.8%)	Smeykal, H 2006, BIXAFEN_004
Henry´s Law Coefficient	Henry's law constant at 20 °C (calculated): 3.89×10^{-5} Pa m^3 mol^{-1}	Calculation	Bogdoll, B and Lemke, G 2007, BIXAFEN_005
Physical state, colour	Active substance, pure: no noticeable odour Active substance as manufactured: no noticeable odour	OPPTS 830.6302 OPPTS 830.6303 batch 1 (purity 98.9%) + batch 2 (purity 95.8%)	Bogdoll, B and Strunk, B 2007, BIXAFEN_006
Odour	Active substance, pure: white powder Active substance as manufactured: light brown powder		
Solubility in water including effect of pH	0.49 mg/L at 20 °C Investigation on different pH is not necessary, because bixafen has no acidic or basic properties in the range of pH 1 to pH 12.	EC A.6, OECD 105 batch 4 (purity 99.2%)	Jungheim, R, 2005, BIXAFEN_007 & Bogdoll, B 2008, BIXAFEN_008
Solubility in organic solvents	[g/L at 20 °C] n-heptane 0.056 dichloromethane 102 methanol 32 toluene 16 acetone > 250 ethylacetate 82 dimethylsulfoxide > 250	CIPAC MT 157 based on EC A.6, OECD 105 batch 1 (purity 98.9%) + batch 3 (purity 98.8%)	Bogdoll, B and Eyrich, U, 2007, BIXAFEN_009
Dissociation constant	No dissociation constant of bixafen was found in the pH range of pH 1–pH 12. This result is in line with the chemical structure of bixafen which does not contain an acidic or a basic group.	OECD 112 batch 3 (purity 98.8%)	Bogdoll, B and Wiche, A 2007, BIXAFEN_010
Partition coefficient n-octanol / water	Determination of the partition coefficient of Bixafen in 1-octanol / water (HPLC column 40 °C) P_{ow} 2046	EC A.8, OECD 117 (HPLC-method)	Bogdoll, B and Lemke, G 2005, BIXAFEN_011

Property	Results	Method (test material)	Reference
	log P_{ow} 3.3	batch 3 (purity 98.8%)	
Hydrolysis rate	Pyrazole-[^{14}C] Bixafen had shown no hydrolytic breakdown at pH 4, 7 and 9 over 5 days (120 hours) at 50 °C.	EPA 161-1, OECD 111, EC C.7 batch 5 (purity 99.2%)	Oddy, AM 2005, BIXAFEN_012
Photochemical degradation	In sterile aqueous buffer solution at 25 °C under artificial sunlight exposure (λ > 290 nm) the calculated experimental half-life was 82 days of intensive continuous irradiation (791 W/m2 in the wavelength range 300–800 nm).	EPA 161-2 batch 6	Muehmel, T and Fliege, R 2006, BIXAFEN_013

Hydrolysis of bixafen

A hydrolysis study was carried out by Oddy, AM (2005, BIXAFEN_012). Pyrazole-^{14}C-bixafen at concentrations of 0.25 mg/L was investigated in aqueous buffer solutions at three pH values at 50 °C for up to 120 h.

Duplicate samples were analysed after 2.4, 48 and 120 hours by HPLC-LSC and TLC for confirmation purposes.

A summary of the results is presented in the following table:

Table 1 Summary of radioactive residues in sterile buffer solutions fortified with [^{14}C] pyrazole labelled bixafen at a concentration of 0.25 mg/L

Time (hours)	pH 4		pH 7		pH 9	
	% TRR recovered	% recovered as bixafen in mg eq/kg	% TRR recovered	TRR recovered as bixafen in mg eq/kg	% TRR recovered	TRR recovered as bixafen in mg eq/kg
0	97 101	100 100	101.49	100 100	103 103	100 100
2.4	96 93	100 100	97 96	100 100	93 96	100 100
48	100 95	100 100	99 90	100 100	99 101	100 100
120	97 94	100 100	99 93	100 100	95 92	100 100

The hydrolysis of bixafen under processing conditions is described under fate of residues in storage and processing.

Formulations

Bixafen is registered as the following EC formulations:

Table 2 Formulations registered containing bixafen as active ingredient

Formulation	Content of active ingredients
EC 125	125 g/L bixafen
EC 190	40 g/L bixafen 50 g/L fluoxastrobin 100 g/L prothioconazole

Formulation	Content of active ingredients
EC 216	50 g/L bixafen 166 g/L tebuconazole
EC 225	75 g/L bixafen 150 g/L prothioconazole
EC 235	75 g/L bixafen 160 g/L prothioconazole
EC 260	60 g/L bixafen 200 g/L prothioconazole
EC 275	75 g/L bixafen 100 g/L prothioconazole 100 g/L tebuconazole
EC 275	75 g/L bixafen 110 g/L prothioconazole 90 g/L tebuconazole
EC 285	75 g/L bixafen 110 g/L prothioconazole 100 g/L tebuconazole
EC 400	50 g/L bixafen 250 g/L spiroxamine 100 g/L prothioconazole

METABOLISM AND ENVIRONMENTAL FATE

Metabolism studies were conducted using [^{14}C-5-pyrazole]-bixafen (pyrazole-label) and [^{14}C-dichlorophenyl-UL]-bixafen (dichlorophenyl-label). The position of the label for both substances is presented in the following figures:

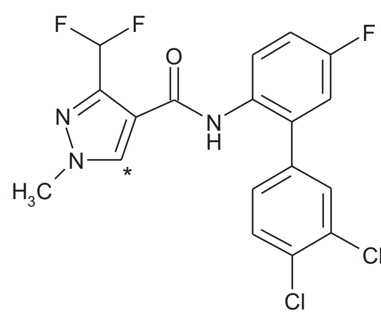


Figure 1 [pyrazole-5-^{14}C]-bixafen

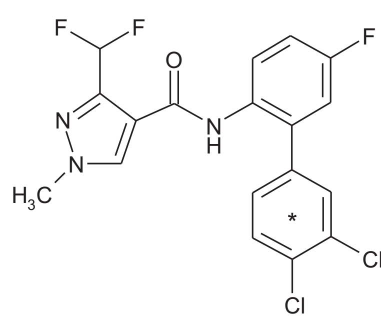
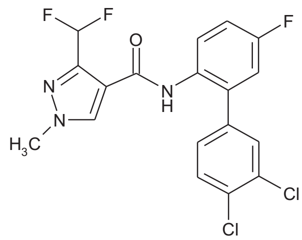
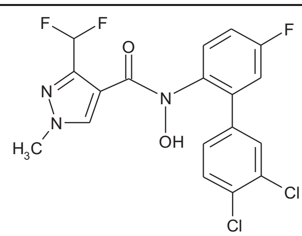
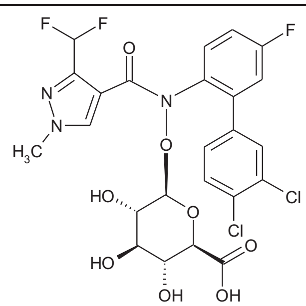
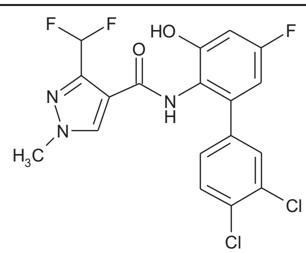
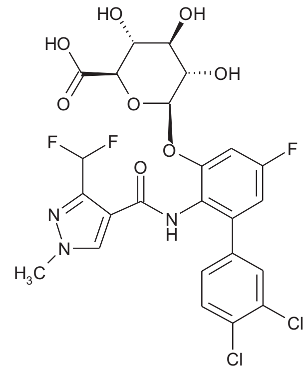
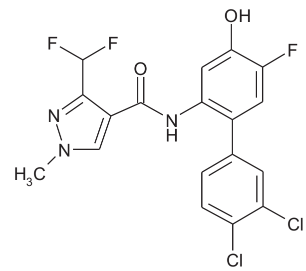
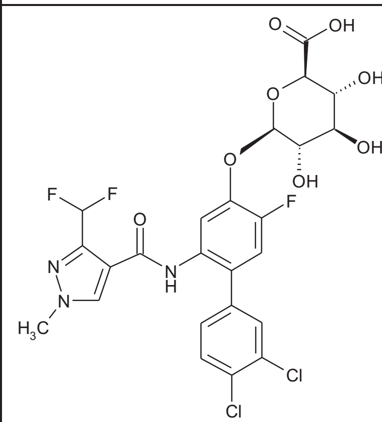
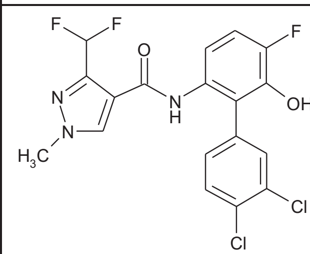
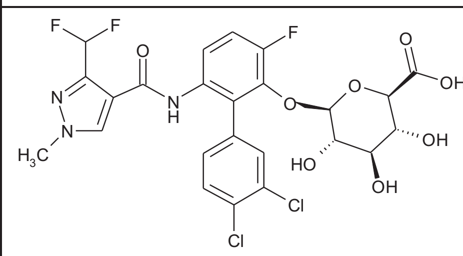


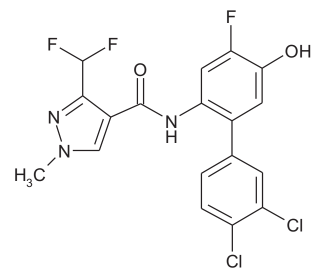
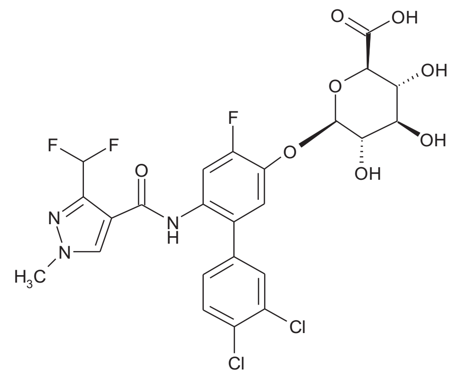
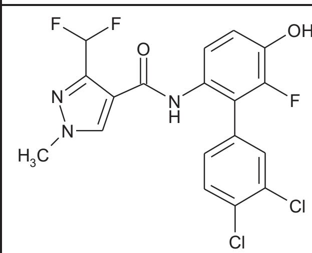
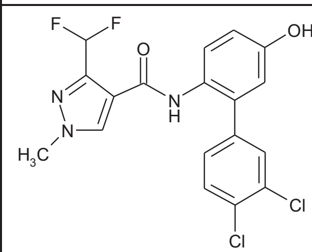
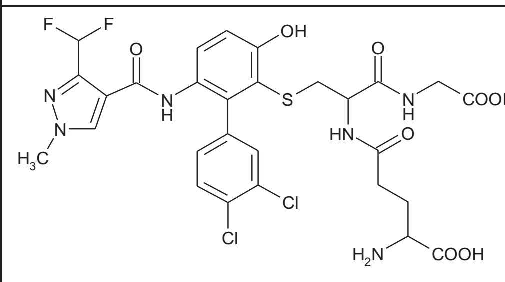
Figure 2 [dichlorophenyl-UL-^{14}C] bixafen

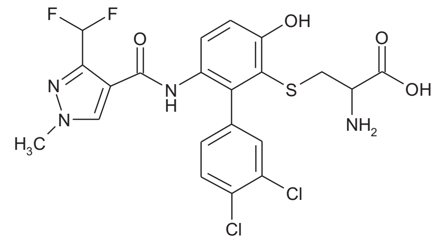
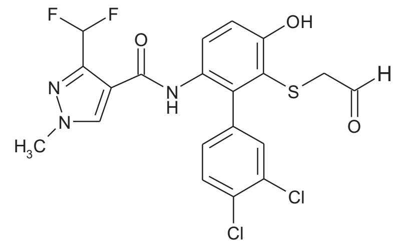
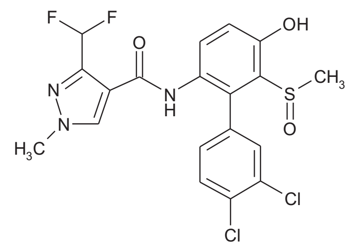
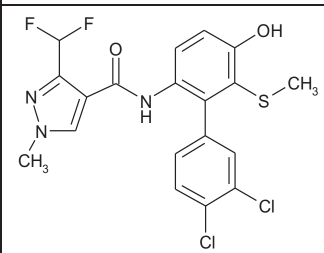
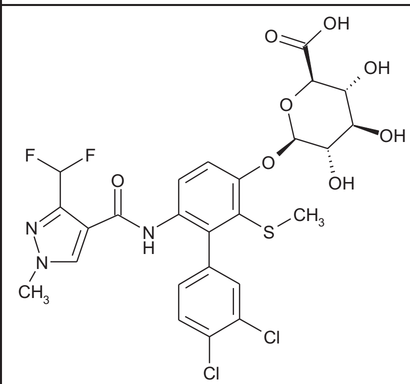
Chemical names, structures and code names of metabolites and degradation products of bixafen are shown in Table 3.

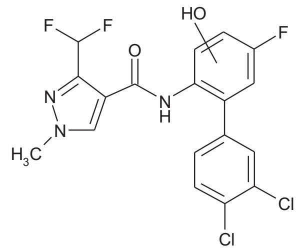
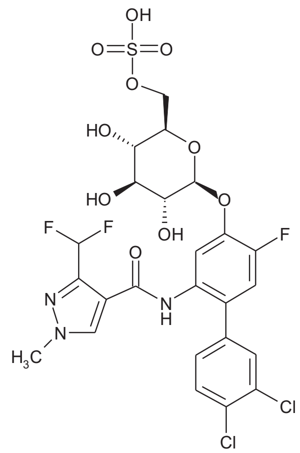
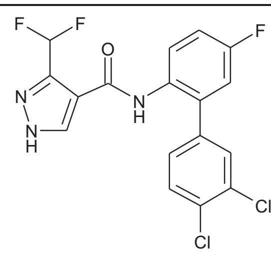
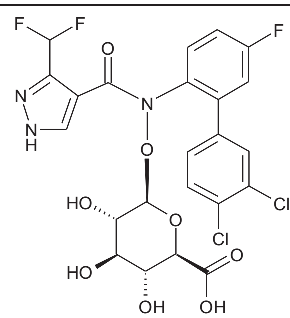
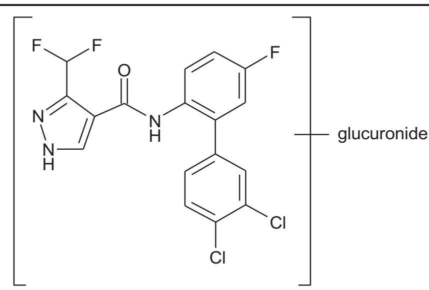
Table 3 Known metabolites of bixafen

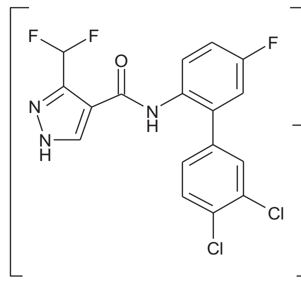
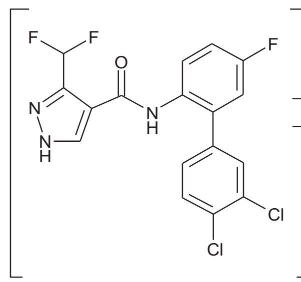
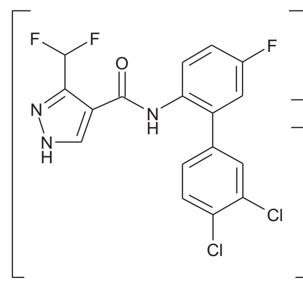
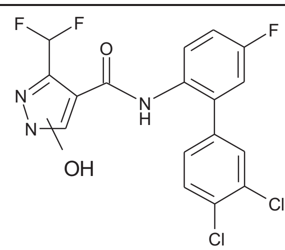
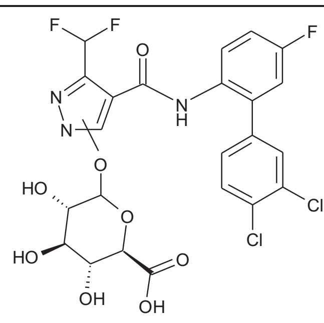
Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
bixafen (parent substance) BYF 00587 (“ai”)	N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) 1H-Pyrazole-4-carboxamide, N-(3',4'-dichloro-5-fluoro[1,1'-biphenyl]-2-yl)-3-(difluoromethyl)-1-methyl- (CAS) [CAS No.: 581809-46-3] $C_{18}H_{12}Cl_2F_3N_3O$ 414.2 g/mol		All matrices
M01 BYF 00587-N-hydroxy	N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-N-hydroxy-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{18}H_{12}Cl_2F_3N_3O_2$ 430.2 g/mol		Rat
M02 BYF 00587-N-O-glucuronide	tbd1-O-((3',4'-dichloro-5-fluorobiphenyl-2-yl){[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-beta-L-glucopyranuronic acid (IUPAC) $C_{24}H_{20}Cl_2F_3N_3O_8$ 606.3 g/mol		Rat
M03 BYF 00587-3-hydroxyphenyl	N-(3',4'-dichloro-5-fluoro-3-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{18}H_{12}Cl_2F_3N_3O_2$ 430.2 g/mol		Rat

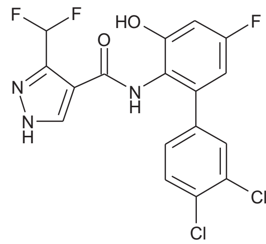
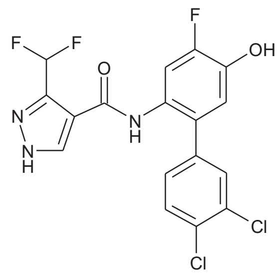
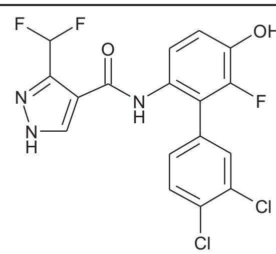
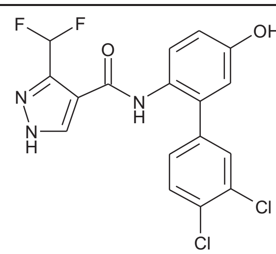
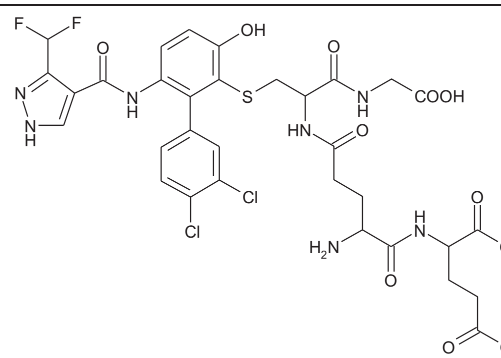
Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M04 BYF 00587-3-hydroxyphenyl-glucuronide	3',4'-dichloro-2-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-5-fluorobiphenyl-3-yl beta-L-glucopyranosiduronic acid (IUPAC) $C_{24} H_{20} Cl_2 F_3 N_3 O_8$ 606.3 g/mol		Rat
M05 BYF 00587-4-hydroxyphenyl	N-(3',4'-dichloro-5-fluoro-4-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{18} H_{12} Cl_2 F_3 N_3 O_2$ 430.2 g/mol		Rat
M06 BYF 00587-4-hydroxyphenyl-glucuronide	3',4'-dichloro-2-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-5-fluorobiphenyl-4-yl beta-L-glucopyranosiduronic acid (IUPAC) $C_{24} H_{20} Cl_2 F_3 N_3 O_8$ 606.3 g/mol		Rat
M07 BYF 00587-6-hydroxyphenyl (&BYF 00587-5-hydroxyphenyl due to F/OH substitution)	N-(3',4'-dichloro-5-fluoro-6-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{18} H_{12} Cl_2 F_3 N_3 O_2$ 430.2 g/mol		Rat (postulated intermediate
M08 BYF 00587-6-hydroxyphenyl-glucuronide	3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-3-fluorobiphenyl-2-yl beta-L-glucopyranosiduronic acid (IUPAC) $C_{24} H_{20} Cl_2 F_3 N_3 O_8$ 606.3 g/mol		Rat

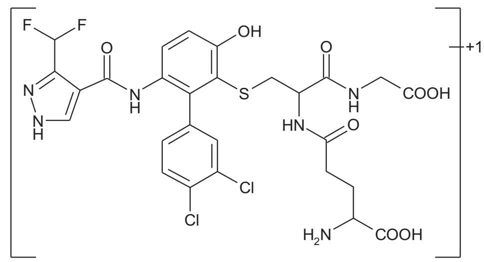
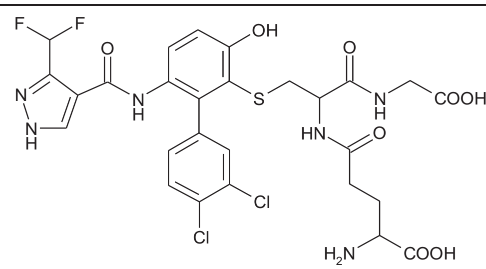
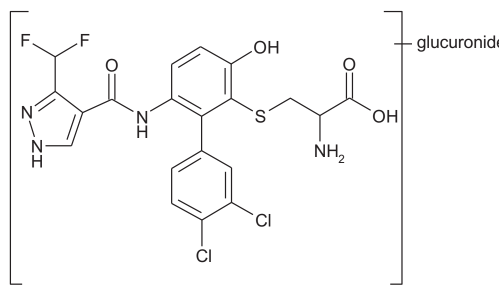
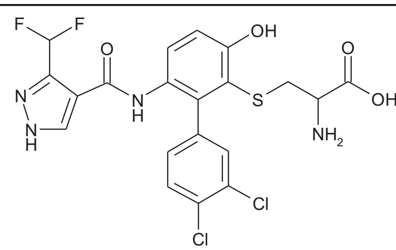
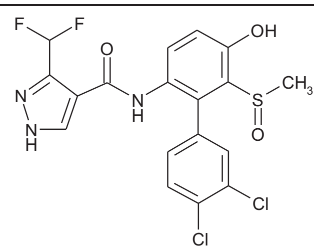
Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M09 BYF 00587-4-fluoro-5-hydroxyphenyl	N-(3',4'-dichloro-4-fluoro-5-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{18}\ H_{12}\ Cl_2\ F_3\ N_3\ O_2$ 430.2 g/mol		Rat
M10 BYF 00587-4-fluoro-5-hydroxyphenyl-glucuronide	3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-4-fluorobiphenyl-3-yl beta-L-glucopyranosiduronic acid (IUPAC) $C_{24}\ H2_0\ Cl_2\ F_3\ N_3\ O_8$ 606.3 g/mol		Rat
M11 BYF 00587-6-fluoro-5-hydroxyphenyl	N-(3',4'-dichloro-6-fluoro-5-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{18}\ H_{12}\ Cl_2\ F_3\ N_3\ O_2$ 430.2 g/mol		Rat
M12 BYF 00587-5-hydroxyphenyl	N-(3',4'-dichloro-5-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{18}\ H_{13}\ Cl_2\ F_2\ N_3\ O_2$ 412.2 g/mol		Rat
M13 BYF 00587-5-hydroxyphenyl-6-glutathionyl	gamma-glutamyl-S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteinylglycine (IUPAC) $C_{28}\ H_{28}\ Cl_2\ F_2\ N_6\ O_8\ S$ 717.5 g/mol		Goat, hen (postulated intermediate)

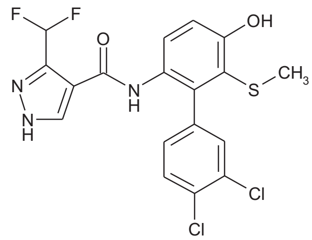
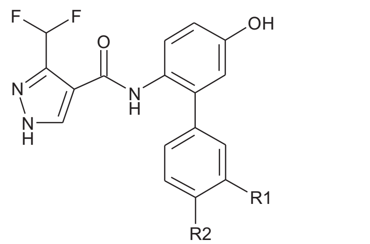
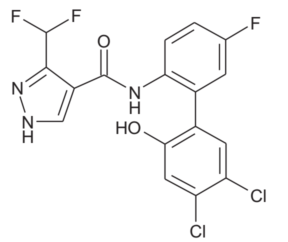
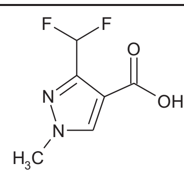
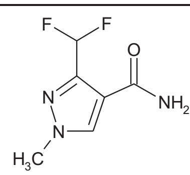
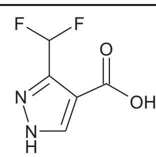
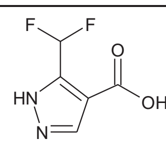
Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M14 BYF 00587-5-hydroxyphenyl-6-cysteinyl	S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteine (IUPAC) $C_{21} H_{18} Cl_2 F_2 N_4 O_4 S$ 531.4 g/mol		Goat, hen, rat
M15 BYF 00587-5-hydroxyphenyl-6-thiol-acetaldehyde	N-{3',4'-dichloro-5-hydroxy-6-[(2-oxoethyl)thio]biphenyl-2-yl}-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{20} H_{15} Cl_2 F_2 N_3 O_3 S$ 486.3 g/mol		Rat
M16 BYF 00587-5-hydroxyphenyl-6-(methylsulfinyl)	N-[3',4'-dichloro-5-hydroxy-6-(methylsulfinyl)biphenyl-2-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{19} H1_5 Cl_2 F_2 N_3 O_3 S$ 474.3 g/mol		Rat
M17 BYF 00587-5-hydroxyphenyl-6-(methylthio)	N-[3',4'-dichloro-5-hydroxy-6-(methylthio)biphenyl-2-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_{19} H_{15} Cl_2 F_2 N_3 O_2 S$ 458.3 g/mol		Rat
M18 BYF 00587-5-hydroxyphenyl-6-(methylthio)-glucuronide	3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-2-(methylthio)biphenyl-3-yl beta-L-glucopyranosiduronic acid (IUPAC) $C_{25} H_{23} Cl_2 F_2 N_3 O_8 S$ 634.4 g/mol		Hen

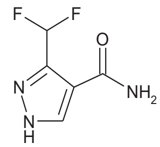
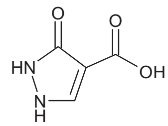
Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M19 BYF 00587-hydroxy	not possible—position of hydroxy group not specified $C_{18} H1_2 Cl_2 F_3 N_3 O_2$ 430.2 g/mol		Rotational crops (postulated intermediate)
M20 BYF 00587-hydroxy-glycoside-sulfate	not possible—position of hydroxy group not specified $C_{24} H_{22} Cl_2 F_3 N_3 O_{10} S$ 672.4 g/mol	 or isomer	Rotational crops (Swiss chard)
M21 Bixafen-desmethyl BYF 00587-desmethyl	N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{17} H_{10} Cl_2 F_3 N_3 O$ 400.2 g/mol		Goat, hen, rat, rotational crops, soya bean, wheat, soil
M22 BYF 00587-desmethyl-N-O-glucuronide	1-O-((3',4'-dichloro-5-fluorobiphenyl-2-yl){[3-(difluoromethyl)-1H-pyrazol-4-yl]carbonyl}amino)-beta-L-glucopyranuronic acid (IUPAC) $C_{23} H_{18} Cl_2 F3 N_3 O_8$ 592.3 g/mol		Rat
M23 BYF 00587-desmethyl-N-glucuronide (isomer 1 and isomer 2)	structure not specified $C_{23} H_{18} Cl_2 F_3 N_3 O_7$ 576.3 g/mol		Goat, rat

Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M24 BYF 00587-desmethyl-glycoside	structure not specified C_{23} $H2_0$ Cl2 F_3 N_3 O_6 562.3 g/mol		Hen
M25 BYF 00587-desmethyl-hydroxy-glycoside	structure not specified C_{23} $H2_0$ Cl_2 F_3 N_3 O_7 578.3 g/mol		Hen
M26 BYF 00587-desmethyl-hydroxy-pentoside	structure not specified C_{22} H_{18} Cl_2 F_3 N_3 O_6 548.3 g/mol		Hen
M27 BYF 00587-desmethyl-hydroxypyrazole	structure not specified C_{17} H_{10} Cl_2 F_3 N_3 O_2 416.2 g/mol		Hen, rat
M28 BYF 00587-desmethyl-hydroxypyrazole-glucuronide	structure not specified C_{23} H_{18} Cl_2 F_3 N_3 O_8 592.3 g/mol		Rat

Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M29 BYF 00587-desmethyl-3-hydroxyphenyl	N-(3',4'-dichloro-5-fluoro-3-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{17} H_{10} Cl_2 F_3 N_3 O_2$ 416.2 g/mol		Rat
M30 BYF 00587-desmethyl-4-fluoro-5-hydroxyphenyl	N-(3',4'-dichloro-4-fluoro-5-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{17} H_{10} Cl_2 F_3 N_3 O_2$ 416.2 g/mol		Rat
M31 BYF 00587-desmethyl-6-fluoro-5-hydroxyphenyl	N-(3',4'-dichloro-6-fluoro-5-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{17} H1_0 Cl_2 F_3 N_3 O_2$ 416.2 g/mol		Rat
M32 BYF 00587-desmethyl-5-hydroxyphenyl	N-(3',4'-dichloro-5-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{17} H_{11} Cl_2 F_2 N_3 O_2$ 398.2 g/mol		Rat
M33 BYF 00587-desmethyl-5-hydroxyphenyl-6-(glutathionyl-glutamic acid)	N-(1,3-dicarboxypropyl)-alpha-glutaminyl-S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteinylglycine (IUPAC) $C_{32} H_{33} Cl_2 F_2 N_7 O_{11} S$ 832.6 g/mol		Rat

Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M34 BYF 00587-desmethyl-hydroxy-5-hydroxyphenyl-6-glutathionyl	structure not specified $C_{27} H_{26} Cl_2 F_2 N_6 O_9 S$ 719.5 g/mol		Rat
M35 BYF 00587-desmethyl-5-hydroxyphenyl-6-glutathionyl	$C_{27} H2_{6} Cl_2 F_2 N_6 O_8 S$ 703.5 g/mol		Rat
M36 BYF 00587-desmethyl-5-hydroxyphenyl-6-cysteinyl-glucuronide	structure not specified $C_{26} H2_{4} Cl_2 F_2 N_4 O_{10} S$ 693.5 g/mol		Rat
M37 BYF 00587-desmethyl-5-hydroxyphenyl-6-cysteinyl	S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteine (IUPAC) $C_{20} H_{16} Cl_2 F_2 N_4 O_4 S$ 517.3 g/mol		Hen, rat
M38 BYF 00587-desmethyl-5-hydroxyphenyl-6-(methylsulfinyl)	N-[3',4'-dichloro-5-hydroxy-6-(methylsulfinyl)biphenyl-2-yl]-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{18} H_{13} Cl_2 F_2 N_3 O_3 S$ 460.3 g/mol		Rat

Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M39 BYF 00587-desmethyl-5-hydroxyphenyl-6-(methylthio)	N-[3',4'-dichloro-5-hydroxy-6-(methylthio)biphenyl-2-yl]-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{18}H_{13}Cl_2F_2N_3O_2S$ 444.3 g/mol		Rat
M40 BYF 00587-desmethyl-5-hydroxyphenyl-deschloro-(methylthio)	structure not specified $C_{18}H_{14}ClF_2N_3O_2S$ 409.8 g/mol	 R1, R2 = -Cl,-SCH3 or -SCH3, -Cl	Rat
M41 BYF 00587-desmethyl-6'-hydroxy	N-(4',5'-dichloro-5-fluoro-2'-hydroxybiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_{17}H_{10}Cl_2F_3N_3O_2$ 416.2 g/mol		Rat
M42 BYF 00587-pyrazole-4-carboxylic acid AE 1954999	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid (IUPAC) $C_6H_6F_2N_2O_2$ 176.1 g/mol		Goat, rat, rotational crops, soil
M43 BYF 00587-pyrazole-4-carboxamide	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC) $C_6H_7F_2N_3O$ 175.1 g/mol		Rat, rotational crops
M44 BYF 00587-desmethyl-pyrazole-4-carboxylic acid (tautomer 1)	3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid (IUPAC) $C_5H_4F_2N_2O_2$ 162.1 g/mol	 proposal for tautomer 1	Rotational crops, soya bean, soil
M45 BYF 00587-desmethyl-pyrazole-4-carboxylic acid (tautomer 2)	5-(difluoromethyl)-1H-pyrazole-4-carboxylic acid (IUPAC) $C_5H_4F_2N_2O_2$ 162.1 g/mol	 proposal for tautomer 2	Rotational crops, soya bean,

Code Names	Chemical Abstracts Name (IUPAC Name), molecular formula, molar mass	Structure	Where found
M46 BYF 00587-desmethyl-pyrazole-4-carboxamide	3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC) $C_5 H_5 F_2 N_3 O$ 161.1 g/mol		Rat
M47 BYF 00587-pyrazolone-4-carboxylic acid	3-hydroxy-1H-pyrazole-4-carboxylic acid (IUPAC) $C_4 H_4 N_2 O_3$ 128.1 g/mol		Rotational crops, soya bean

Animal metabolism

The Meeting received metabolism studies on laboratory animals, poultry and lactating goats using the pyrazole- and the dichlorophenyl-label of bixafen.

The metabolism of bixafen in livestock animals was limited, showing unchanged parent bixafen as main residue in all matrices except liver. Besides the unchanged parent substance the metabolite M21 (bixafen-desmethyl) was found as major residue in all tissues, and represented the main residue in liver following exhaustive extraction involving hydrolysis. The sum of parent and M21 represented more than 50% of the total residue in all samples of animal origin, often more than 80% TRR. The only other major metabolites present at > 10% of the TRR were the two isomers of M23 in goat livers and kidneys.

The most important metabolic reaction was the demethylation of the pyrazole ring resulting in bixafen-desmethyl. Further biochemical reactions included substitution of the fluorine atom by a hydroxy group and an adjacent glutathione conjugation, an unspecified hydroxylation of an aromatic ring and conjugation with various sugar molecules, and hydroxylation of the pyrazole ring.

In general the metabolic pathway of bixafen was comparable between ruminants and hens. All metabolites identified in goats were also found in rats. The metabolites M26, M25 and M18 found in eggs and poultry liver were not directly identified in the rat.

Laboratory animals

Rats

In rats given (dichlorophenyl-U-^{14}C)-labelled bixafen orally by gavage, absorption was rapid and accounted for at least 83% of the total administered radioactivity after a single low dose (2 mg/kg bw). The maximum plasma concentrations of radioactivity were reached approximately 2–4 and 8 hours after administration of the low and high doses (2 and 50 mg/kg bw), respectively. Radioactivity was widely distributed throughout the body. Elimination of the radioactivity was mainly via faeces (≥ 91%), whereas elimination via urine accounted for 1–3% of the administered dose. In bile duct-cannulated rats, extensive biliary excretion (up to 83%) was demonstrated. Elimination of the radioactivity from the body was rapid, with a half-life in plasma of 8–9 hours and a mean residence time of 13–19 hours (for the low dose). Residues in tissues at 72 hours after a single oral dose as well as after repeated oral dosing accounted for 0.1–3% of the administered radioactivity, with liver and kidneys containing the highest concentrations of residues.

Metabolism of bixafen in rats was extensive, and more than 30 metabolites were identified. The main metabolic routes included demethylation, hydroxylation of the parent and bixafen-desmethyl, and conjugation with glucuronic acid or glutathione. A minor metabolic reaction was the cleavage of the amide bridge of bixafen.

Lactating goats

The kinetic behaviour and the metabolism of [pyrazole-5-^{14}C]-bixafen was investigated in the lactating goat by Spiegel, K and Koester, J (2007, BIXAFEN_014). One goat (41 kg bw) was dosed orally five times in 24 h intervals with 2.0 mg radiolabelled bixafen per kg body weight per day (equivalent to 34.7 ppm in the diet). The goat was sacrificed about 24 hours after the last dose. Milk, plasma, urine and faeces were collected during the whole dosing period. After sacrifice liver, kidney, muscle and fat were sampled.

Analysis of the total radioactive residues (TRR) was carried out using combustion and liquid scintillation counting (LSC).

Milk pools, muscle and kidney were extracted with acetonitrile/water mixtures. The resulting extracts were degreased by a clean-up step using a C_{18}-SPE cartridge. Milk sugar was removed in a second clean-up step using an XAD-4 cartridge.

Liver was extracted analogously to muscle and kidney but the solids after conventional extraction were additionally subjected to four exhaustive extraction steps at increased temperature with microwave assistance. In the third and fourth step formic acid and hydrochloric acid, respectively were added to the acetonitrile/water mixtures. These extracts were also degreased by a clean-up step using a C18-SPE cartridge in the same way as the conventional extracts.

An aliquot of the fat composite was extracted subsequently with hexane and acetonitrile. The hexane phases were partitioned with acetonitrile and vice versa. The acetonitrile phases were combined, concentrated and analysed.

The radioactivity in all extracts was determined by LSC. Aliquots were concentrated and analysed by radio-HPLC and UV detection (254 nm) using a reversed phase column and an acidic acetonitrile/water gradient. The identification of metabolites was conducted either by comparison with reference substances and/or confirmation by LC-MS/MS.

At the end of the dosing period most of the administered radioactivity was excreted via the urine (5.42% AR) and the faeces (82.088% AR).

The total radioactive residues (TRR) found were highest in liver with 1.178 mg eq/kg, followed by fat (perirenal: 0.318 mg eq/kg, omental: 0.544 mg eq/kg), kidney (0.203 mg eq/kg) and muscle (round muscle: 0.057 mg eq/kg, loin muscle: 0.063 mg eq/kg). In milk the TRR increased from 0.003 mg eq/kg directly after the first administration up to a plateau of 0.153 mg eq/kg after three days. The TRR levels found are summarized in Table 5. The radioactivity in milk and plasma are presented in Tables 5 and 6.

Table 4 TRR in goats milk and tissues after administration of pyrazole-^{14}C-bixafen at 2.0 mg/kg bw and day (34.7 ppm in the diet)

Matrix	% of total dose administered	TRR [mg eq/kg]
Liver	0.278	1.18
Kidney	0.007	0.203
Round muscle	–	0.057
Loin muscle	–	0.063
Total body muscle [a]	0.17	0.057
Perirenal fat	–	0.318
Omental fat	–	0.544
Total body fat [a]	0.55	0.466
Total in organs and tissues	1.01	–
Milk, 0–120 h	0.28	0.092
Urine, 0–120 h	5.42	2.38
Faeces, 0–120 h	82.08	13.5
Total excreted in milk, urine and faeces	87.78	–
Total Recovery	88.78	

[a] % of total dose assuming 30% of the body weight for total body muscle and 12% for total body fat

Table 5 Time course of total radioactive residues in milk and plasma after administration of pyrazole-^{14}C-bixafen at 2.0 mg/kg bw and day (34.7 ppm in the diet)

Time after first administration [h]	administration no.	TRR [mg eq/kg] plasma	TRR [mg eq/kg] milk
0.5	1	0.003	–
2		0.039	–
4		0.066	–
6		0.081	–
8		0.081	0.109
24	2	0.065	0.054
32		0.144	0.219
48	3	0.044	0.041
56		0.153	0.205
72	4	0.066	0.055
80		0.144	0.197
96	5	0.042	0.039
104		0.108	0.143
120		0.041	0.040

Samples of tissues and milk were further analysed for the composition of the radioactivity. Extraction rates using acetonitrile/water were generally > 90%, except for liver, which required additional exhaustive extraction including hydrolysis. Parent bixafen was the major residue in milk (73.8% TRR, 0.127 mg eq/kg), muscle (55.8% TRR, 0.032 mg eq/kg), fat (88.5% TRR, 0.413 mg eq/kg) and kidney (44.1% TRR, 0.089 mg eq/kg). In liver bixafen was more strongly degraded being present at levels of 17.6% of the TRR (0.207 mg eq/kg).

Besides the parent compound M21 (bixafen-desmethyl) was a major metabolite in all matrices investigated: 17.6% TRR in milk (0.03 mg eq/kg), 43.2% TRR in muscle (0.025 mg eq/kg), 10.6% TRR in fat (0.05 mg eq/kg) and 37.9% TRR in kidney (0.077 mg eq/kg). In liver, the conventional and exhaustive extraction released a total of 33.6% of the TRR (0.397 mg eq/kg) as bixafen-desmethyl.

The only other major metabolite found was M23 in milk, liver and kidney. The sum of both isomers reached levels of 13.8% of the TRR in liver (isomer 1: 8.6% TRR, 0.102 mg eq/kg; isomer 2: 5.2% TRR, 0.062 mg eq/kg) and of 14.5% of the TRR in kidney (isomer 1: 4.3% TRR, 0.009 mg eq/kg; isomer 2: 10.2% TRR, 0.021 mg eq/kg). In milk M23 was present in lower amounts (isomer 2 only: 1.4% TRR, 0.002 mg/kg)

In the following table the extraction rates and the metabolites identified are summarised.

Table 6 Characterisation and identification of compounds in milk and tissues of the lactating goats after administration of pyrazole-^{14}C-bixafen at 2.0 mg/kg bw and day (34.7 ppm in the diet)

Compound / Fraction	Milk		Muscle		Fat		Liver		Kidney	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR [mg/kg]		0.172		0.057		0.466		1.178		0.203
Conventional extraction										
Bixafen	73.8	0.127	55.8	0.032	88.5	0.413	17.6	0.207	44.1	0.089
M14	–	–	–	–	–	–	2.2	0.026	–	–
M23	–	–	–	–	–	–	8.6	0.102	4.3	0.009
M23	1.4	0.002	–	–	–	–	5.2	0.062	10.2	0.021
M21 (bixafen-desmethyl)	17.6	0.030	43.2	0.025	10.6	0.050	21.0	0.248	37.9	0.077
Subtotal identified	92.9	0.159	99.0	0.057	99.1	0.462	54.7	0.644	96.5	0.196
Exhaustive extraction										
M42	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	7.8	0.091	n.a.	n.a.
M21 (BYF 00587-desmethyl)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	12.6	0.149	n.a.	n.a.

Compound / Fraction	Milk		Muscle		Fat		Liver		Kidney	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Subtotal identified	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	20.4	0.240	n.a.	n.a.
Total identified	92.9	0.159	99.0	0.057	99.1	0.462	75.0	0.884	96.5	0.196
Total characterised by HPLC, but unidentified	–	–	–	–	–	–	23.5	0.277	–	–
Fractions not analysed	6.8	0.012	–	–	–	–	1.1	0.013	–	–
Total extracted	99.7	0.064	99.0	0.057	99.1	0.462	99.6	1.174	96.5	0.196
Not extracted	0.3	0.001	1.0	0.001	0.9	0.004	0.4	0.005	3.5	0.007
Accountability	100.0	0.172	100.0	0.057	100.0	0.466	100.0	1.178	100.0	0.203

n.a. = Not analysed

In a second study by Spiegel, K and Koester, J (2007, BIXAFEN_015) the metabolism of [dichlorophenyl-UL-^{14}C]-bixafen was investigated in one lactating goat. The goat (39 kg bw) was dosed orally five times in 24 h intervals with 2.0 mg radiolabelled BYF 00587 per kg body weight per day. This amount corresponded to 46.08 ppm in the diet. The goat was sacrificed about 24 hours after the last dose.

Analysis of the total radioactive residues and the extraction of samples collected was conducted as described in the metabolism in goats study using [pyrazole-5-^{14}C]-bixafen by Spiegel, K and Koester, J (2007, BIXAFEN_014).

From the total dose administered approximated 1.75% of the AR was excreted via the urine and 71.88% via the faeces. The total radioactive residues (TRR) found were highest in liver with 0.737 mg eq/kg, followed by fat (0.611 mg eq/kg), kidney (0.143 mg eq/kg) and muscle (0.047 mg eq/kg). In milk the TRR increased from 0.03 mg eq/kg after the first administration up to a plateau of 0.126 mg eq/kg after four days. The TRR levels found are summarized in Table 7. The radioactivity in milk and plasma are presented in Tables 8 and 9.

Table 7 TRR in goats milk and tissues after administration of dichlorophenyl-^{14}C-bixafen at 2.0 mg/kg bw and day (46.08 ppm in the diet)

Matrix	% of total dose administered	TRR [mg eq/kg]
Liver	0.166	0.737
Kidney	0.004	0.143
Round muscle	0.13	0.047
Fat (composite sample perirenal and omental)	0.70	0.611
Total in organs and tissues	1.0	–
Milk, 0–120 h	0.09	0.039
Urine, 0–120 h	1.75	0.717
Faeces, 0–120 h	71.88	17.631
Total excreted in milk, urine and faeces	73.72	–
Total Recovery	74.73	–

Table 8 Time course of total radioactive residues in milk and plasma after administration of dichlorophenyl-^{14}C-bixafen at 2.0 mg/kg bw and day (46.08 ppm in the diet)

Time after first administration [h]	administration no.	TRR [mg eq/kg] plasma	TRR [mg eq/kg] milk
0.25	1	0.001	–
0.5		0.004	–
1		0.015	–
2		0.045	–
3		0.052	–
4		0.042	–
6		0.028	–
8		0.022	0.030

Time after first administration [h]	administration no.	TRR [mg eq/kg] plasma	TRR [mg eq/kg] milk
24	2	0.008	0.009
32		0.030	0.043
48	3	0.008	0.009
56		0.032	0.056
72	4	0.023	0.029
80		0.065	0.126
96	5	0.037	0.050
104		0.048	0.071
120		0.029	0.037

Samples of tissues and milk were further analysed for the composition of the radioactivity. Extraction rates using acetonitrile/water were generally > 90%, except for liver, which required additional exhaustive extraction including hydrolysis. Parent bixafen was the major residue in milk (77.2% TRR, 0.05 mg eq/kg), muscle (65.6% TRR, 0.031 mg eq/kg), fat (89.4% TRR, 0.547 mg eq/kg) and kidney (46.3% TRR, 0.066 mg eq/kg). In liver bixafen was more strongly degraded being present at levels of 22.8% of the TRR (0.168 mg eq/kg) in the conventional extract plus 3.7% of the TRR (0.027 mg eq/kg) in the exhaustive extract for a total of 26.5% of the TRR (0.195 mg eq/kg).

Besides the parent compound M21 (bixafen-desmethyl) was a major metabolite in all matrices investigated: 15.6% TRR in milk (0.001 mg eq/kg), 34.4% TRR in muscle (0.016 mg eq/kg), 10.4% TRR in fat (0.064 mg eq/kg) and 36.5% TRR in kidney (0.052 mg eq/kg). In liver, the conventional and exhaustive extraction released a total of 38.7% of the TRR (0.286 mg eq/kg) as bixafen-desmethyl.

The only other metabolite found was M23 in milk, liver and kidney. The sum of both isomers reached levels of 2.3% of the TRR in milk (isomer 1: 0.8% TRR, < 0.001 mg eq/kg; isomer 2: 1.5% TRR, 0.001 mg eq/kg), 18.9% of the TRR in liver (isomer 1: 13.1% TRR, 0.097 mg eq/kg; isomer 2: 5.8% TRR, 0.043 mg eq/kg) and of 9.3% of the TRR in kidney (isomer 1: 2.8% TRR, 0.004 mg eq/kg; isomer 2: 6.5% TRR, 0.0.009 mg eq/kg).

In the following table the extraction rates and the metabolites identified are summarised:

Table 9 Characterisation and identification of compounds in milk and tissues of the lactating goats after administration of dichlorophenyl-^{14}C-bixafen at 2.0 mg/kg bw and day (46.08 ppm in the diet)

	Milk		Muscle		Fat		Liver		Kidney	
Compound / Fraction	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR [mg/kg]	100	0.064	100	0.047	100	0.611	100	0.737	100	0.143
Conventional extraction										
Bixafen	77.2	0.050	65.6	0.031	89.4	0.547	22.8	0.168	46.3	0.066
M23	0.8	< 0.001	–	–	–	–	13.1	0.097	2.8	0.004
M23	1.5	0.001	–	–	–	–	5.8	0.043	6.5	0.009
M21 (bixafen-desmethyl)	15.6	0.010	34.4	0.016	10.4	0.064	18.7	0.138	36.5	0.052
Subtotal identified	95.1	0.061	100.0	0.047	99.8	0.610	60.4	0.445	92.0	0.132
Exhaustive extraction										
Bixafen	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.7	0.027	n.a.	n.a.
M21 (bixafen-desmethyl)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	20.0	0.148	n.a.	n.a.
Subtotal identified	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23.8	0.175	n.a.	n.a.
Total identified	95.1	0.061	100.0	0.047	99.8	0.610	84.2	0.620	92.0	0.132
Total characterised by HPLC without identification	2.4	0.002	–	–	–	–	5.4	0.040	5.4	0.008
Fractions not analysed	2.2	0.001	–	–	–	–	1.6	0.012	–	–

	Milk		Muscle		Fat		Liver		Kidney	
Compound / Fraction	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Total extracted	99.7	0.064	100.0	0.047	99.8	0.610	91.2	0.672	97.4	0.139
Not extracted	0.3	< 0.001	–	–	0.2	0.001	8.8	0.065	2.6	0.004
Accountability	100.0	0.064	100.0	0.047	100.0	0.611	100.0	0.737	100.0	0.143

n.a. = Not analysed

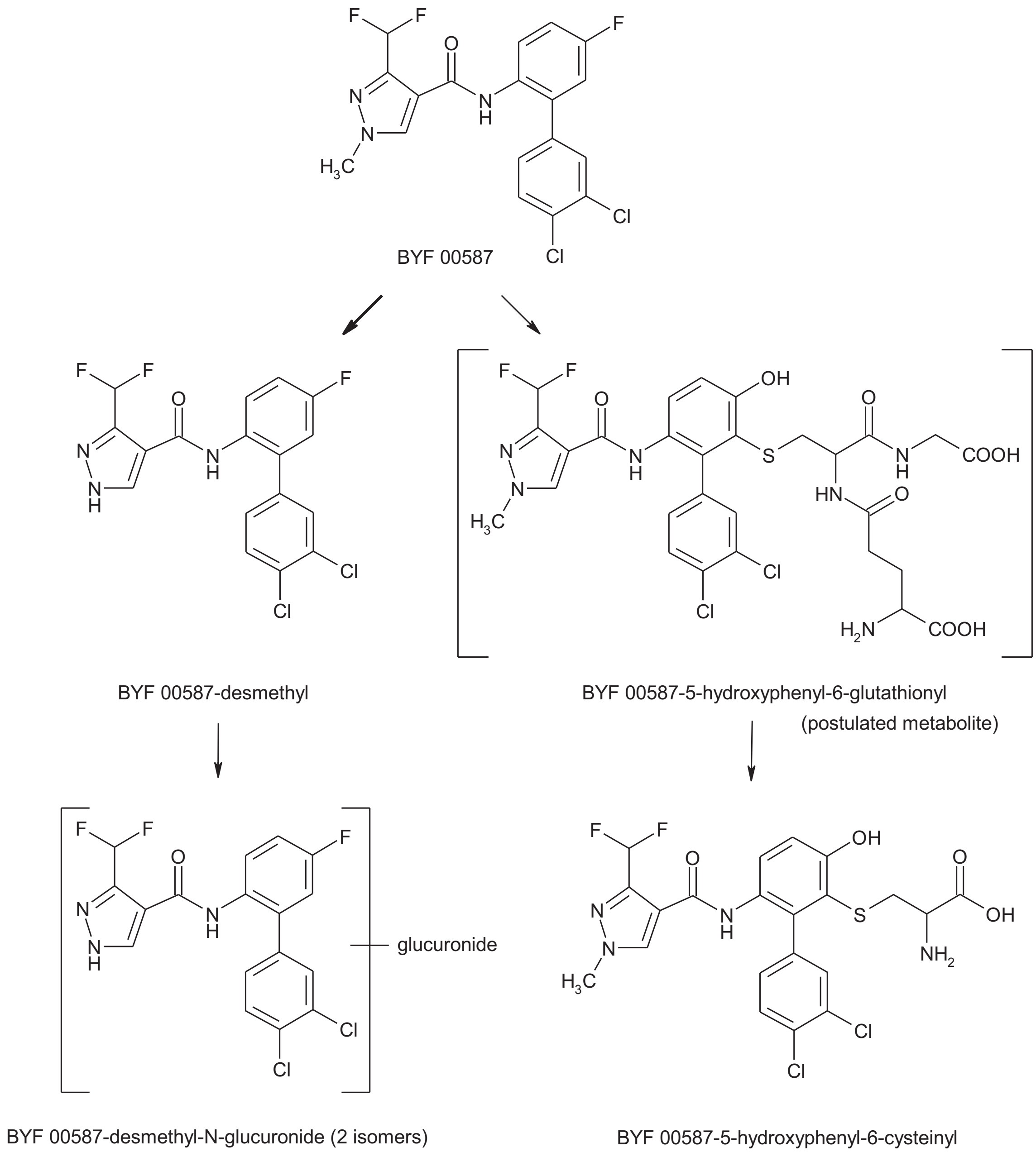


Figure 3 Proposed metabolic pathway of bixafen in lactating goats

Laying hens

The metabolism of [pyrazole-5-^{14}C]-bixafen was investigated in laying hens by Koester, J (2007, BIXAFEN_016). Six hens were orally dosed 14 times in intervals of 24 h with 2.04 mg radiolabelled bixafen per kg body weight per day (corresponding to 25.75 ppm in the diet). The animals were sacrificed about 24 hours after the last dose. The total radioactivity was measured daily in the excreta and in eggs, and at sacrifice in the dissected tissues muscle, liver, fat and skin.

Analysis of the total radioactive residues (TRR) was carried out using combustion and liquid scintillation counting (LSC).

For metabolism investigations, composite samples of muscle (leg and breast), liver, subcutaneous fat and eggs (day 1 to 6 and day 7 to 14) of all animals were used. Aliquots of egg pools, muscle, liver and excreta were extracted with acetonitrile/water mixtures. The solids obtained by conventional extraction of liver were further extracted at increased temperature (approx. +120 °C) under microwave conditions. The fat sample was extracted with acetonitrile/n-hexane mixtures and partitioned into an acetonitrile and n-hexane phase. The acetonitrile extract was concentrated and analysed.

All samples were analysed by radio-HPLC using a reversed phase column and an acidic acetonitrile/water gradient. The identity of the major components was confirmed by LC-MS/MS in the egg. The identification of parent compound and metabolites in other matrices of the laying hen was achieved by co-chromatography of original extracts with isolated metabolites or radiolabelled reference items or by comparison of known metabolic profiles.

After the dosing period a total of 88.33% of the applied radioactivity was excreted. The total radioactive residues (TRR) found were highest in eggs with up to 1.019 mg eq/kg after 13 days of dosing. A plateau of the total radioactivity in eggs was observed after approximately one week. In tissues liver contained the highest TRR (0.641 mg eq/kg), followed by fat (0.227 mg eq/kg), kidney (0.193 mg eq/kg) and muscle (0.032 mg eq/kg). The TRR levels found are summarized in Table . The time course of radioactivity in eggs is presented in Table and Table .

Table 10 TRR in laying hen eggs and tissues after administration of pyrazole-^{14}C-bixafen at 2.04 mg/kg bw and day (25.75 ppm in the diet)

Matrix	% of total dose administered (cumulative value)	TRR [mg eq/kg]
Liver	0.05	0.641
Kidney	< 0.01	0.193
Eggs from ovary/oviduct	0.16	1.464
Total body muscle	0.05	0.032
Total body skin	0.01	0.072
Total body fat (subcutaneous)	0.10	0.227
Total of organs/tissues	0.37	–
Eggs, day 1–14	1.15	0.776
Excreta, day 1–14	88.33	12.989
Total Recovery	89.95	–

Table 11 Time course of total radioactive residues in excreta after administration of pyrazole-^{14}C-bixafen at 2.04 mg/kg bw and day (25.75 ppm in the diet) to laying hens (mean of six animals)

Time after first administration [h]	administration no.	Excretion per day [% of total dose]	Cumulative excretion [% of total dose]
0	1	no excreta collected	–
1	2	5.65	12.391
2	3	12.04	14.030
3	4	18.54	13.397
4	5	24.92	13.637
5	6	31.41	17.885
6	7	38.34	17.040
7	8	45.40	16.462
8	9	50.96	10.314
9	10	57.09	11.834
10	11	63.46	12.627
11	12	70.03	12.382
12	13	76.30	12.268
13	14	82.53	11.617
14	-	88.33	10.202
Mean			12.989

Table 12 Time course of total radioactive residues in eggs after administration of pyrazole-^{14}C-bixafen at 2.04 mg/kg bw and day (25.75 ppm in the diet, mean of six animals)

Time after first administration [h]	administration no.	Cumulative secretion [% of the total dose]	TRR in eggs [mg eq/kg]
0	1	no egg collected	–
1	2	0.02	0.205
2	3	0.05	0.359
3	4	0.12	0.546
4	5	0.19	0.701
5	6	0.27	0.842
6	7	0.36	0.906
7	8	0.44	0.875
8	9	0.54	0.790
9	10	0.62	0.872
10	11	0.74	0.997
11	12	0.84	0.908
12	13	0.96	0.955
13	14	1.05	1.019
14	–	1.15	0.857
Mean			0.776

Samples of tissues and eggs were further analysed for the composition of the radioactivity. Extraction rates using acetonitrile/water were generally > 90%, except for liver, which required additional exhaustive extraction including hydrolysis.

Parent bixafen was the major residue in eggs found at levels of 68.6% of the TRR after 1–6 days (0.416 mg eq/kg) and 55.4% of the TRR after 7–14 days (0.498 mg eq/kg). Besides the parent compound M21 (bixafen-desmethyl) was a major metabolite being present at levels of 26% TRR after 1–6 days (0.157 mg eq/kg) and 35.4% TRR (0.318 mg eq/kg) after 7–14 days. M24 and M26 were also detected in eggs (7–14 days only) with 0.6% TRR (0.006 mg eq/kg) and 1.0% TRR (0.009 mg eq/kg), respectively.

Fat mainly contained unchanged parent as major residue (79.6% TRR, 0.181 mg eq/kg). M21 (bixafen-desmethyl) was the only other metabolite with 19.9% TRR (0.045 mg eq/kg).

In muscle M21 (bixafen-desmethyl) gave the highest residue (35.4% of the TRR, 0.011 mg eq/kg). The unchanged parent substance was the only other substance identified at levels of 23.4% of the TRR (0.008 mg eq/kg).

In liver a total of 42.3% of the TRR were identified as bixafen-desmethyl (conventional extract: 24.1% TRR, 0.155 mg eq/kg; exhaustive extract: 18.2% TRR, 0.116 mg eq/kg). The unchanged parent was present mainly in conjugated form cleaved by hydrolysis, resulting in a total of 0.14 mg eq/kg or 21.9% of the TRR (conventional extraction: 4.5% TRR, 0.029 mg eq/kg; exhaustive extraction: 17.4% TRR as conjugate, 0.111 mg eq/kg). Besides parent bixafen and M21 liver contained several minor metabolites all being present at levels below 10% of the TRR. M14 and M18 represented 2.3% TRR (0.015 mg eq/kg) and 1.5% TRR (0.01 mg eq/kg), respectively. In addition hydroxylated and conjugated metabolites following bixafen-desmethyl were identified: M24 with 1.8% TRR (0.012 mg eq/kg), M25 with 1.0% TRR (0.007 mg eq/kg), M26 with 8.8% TRR (0.057 mg eq/kg), M27 with 4.6% TRR (0.029 mg eq/kg) and M37 with 2.7% TRR (0.017 mg eq/kg).

In the following table the extraction rates and the metabolites identified are summarised:

Table 13 Characterisation and identification of compounds in eggs and tissues of the laying hens after administration of pyrazole-^{14}C-bixafen at 2.04 mg/kg bw and day (25.75 ppm in the diet)

Compound / Fraction	Eggs day 1–6		Eggs day 7–14		Muscle		Fat		Liver	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR [mg/kg]	100	0.606	100	0.900	100	0.032	100	0.227	100	0.641
Conventional extraction										
Bixafen	68.6	0.416	55.4	0.498	23.4	0.008	79.6	0.181	4.5	0.029
M14	–	–	–	–	–	–	–	–	2.3	0.015
M18	–		–	–	–	–	–	–	1.5	0.010
M21 (bixafen-desmethyl)	26.0	0.157	35.4	0.318	35.4	0.011	19.9	0.045	24.1	0.155
M24	–	–	0.6	0.006	–	–	–	–	1.8	0.012
M25	–	–	–	–	–	–	–	–	1.0	0.007
M26	–	–	1.0	0.009	–	–	–	–	8.8	0.057
M27	–	–	0.5	0.005	–	–	–	–	4.6	0.029
M37	–	–	–	–	–	–	–	–	2.7	0.017
Exhaustive extraction of solids of liver										
M21 (bixafen-desmethyl)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	18.2	0.116
Bixafen-conjugate [a]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	17.4	0.111
Total identified	94.6	0.573	92.8	0.835	58.7	0.019	99.5	0.226	87.0	0.557
Total characterised by HPLC without identification	3.4	0.020	3.2	0.029	34.9	0.011	–	–	4.2	0.027
Total extracted	98.0	0.593	96.0	0.864	93.6	0.030	99.5	0.226	91.2	0.584
Unextracted	2.0	0.012	4.0	0.036	6.4	0.002	0.5	0.001	8.8	0.056
Accountability	100	0.606	100	0.900	100	0.032	100	0.227	100	0.641

[a] Cleaved conjugate during exhaustive extraction. Type of conjugate not identified.

In a second study on laying hens conducted by Koester, J (2007, BIXAFEN_017) [dichlorophenyl-UL-^{14}C]-bixafen was administered to five hens orally for 14 days with 2.03 mg radiolabelled bixafen per kg body weight per day (corresponding to 32.42 ppm in the diet). The animals were sacrificed about 24 hours after the last dose. The total radioactivity was measured daily in the excreta and in eggs, and at sacrifice in the dissected tissues muscle, liver, fat and skin.

Analysis of the total radioactive residues and the extraction of samples collected was conducted as described in the metabolism in laying hens study using [pyrazole-5-^{14}C]-bixafen by Koester, J (2007, BIXAFEN_016).

At the end of the dosing period approximately 92.54% of the administered doses were excreted. The total radioactive residues (TRR) found were highest in eggs with up to 0.826 mg eq/kg after 7 days of dosing. A plateau of the total radioactivity in eggs was observed after approximately one week. In tissues liver contained the highest TRR (0.807 mg eq/kg), followed by fat (0.365 mg eq/kg), kidney (0.331 mg eq/kg) and muscle (0.037 mg eq/kg). The TRR levels found are summarized in Table . The time course of radioactivity in eggs and excreta is presented in Table and

Table .

Table 14 TRR in laying hen eggs and tissues after administration of dichlorophenyl-^{14}C-bixafen at 2.03 mg/kg bw and day (32.42 ppm in the diet)

Matrix	% of total dose administered (cumulative value)	TRR [mg eq/kg]
Liver	0.06	0.807
Kidney	0.01	0.331
Eggs from ovary/oviduct	0.10	1.449
Total body muscle	0.05	0.037
Total body skin	0.01	0.070
Total body fat (subcutaneous)	0.03	0.365
Total of organs/tissues	0.25	–
Eggs, day 1–14	0.98	0.640
Excreta, day 1–14	92.54	18.605
Total Recovery	93.77	–

Table 15 Time course of total radioactive residues in excreta after administration of dichlorophenyl-^{14}C-bixafen at 2.03 mg/kg bw and day (32.42 ppm in the diet) to laying hens (mean of five animals)

Time after first administration [h]	administration no.	Excretion per day [% of total dose]	Cumulative excretion [% of total dose]
0	1	no excreta collected	–
1	2	5.50	17.272
2	3	12.43	21.039
3	4	19.02	16.638
4	5	25.59	19.990
5	6	32.75	21.211
6	7	38.72	18.312
7	8	46.79	20.532
8	9	52.57	17.556
9	10	59.74	19.665
10	11	66.43	18.724
11	12	73.15	20.322
12	13	79.37	18.059
13	14	85.57	15.919
14	–	92.54	17.338
Mean			18.605

Table 16 Time course of total radioactive residues in eggs after administration of dichlorophenyl-^{14}C-bixafen at 2.03 mg/kg bw and day (32.42 ppm in the diet, mean of five animals)

Time after first administration [h]	administration no.	Cumulative secretion [% of the total dose]	TRR in eggs [mg eq/kg]
0	1	no egg collected	–
1	2	0.039	0.301
2	3	0.053	0.347
3	4	0.100	0.442
4	5	0.158	0.545
5	6	0.222	0.607
6	7	0.289	0.650
7	8	0.375	0.826
8	9	0.454	0.752
9	10	0.530	0.807
10	11	0.615	0.816

Time after first administration [h]	administration no.	Cumulative secretion [% of the total dose]	TRR in eggs [mg eq/kg]
11	12	0.683	0.804
12	13	0.763	0.759
13	14	0.843	0.767
14	–	0.976	0.788
Mean			0.640

Samples of tissues and eggs were further analysed for the composition of the radioactivity. Extraction rates using acetonitrile/water were generally > 90%, except for liver, which required additional exhaustive extraction including hydrolysis.

Parent bixafen was the major residue in eggs found at levels of 62.3% of the TRR after 1–7 days (0.327 mg eq/kg) and 51.1% of the TRR after 8–14 days (0.405 mg eq/kg). Besides the parent compound M21 (bixafen-desmethyl) was a major metabolite being present at levels of 33.2% TRR after 1–6 days (0.174 mg eq/kg) and 39.1% TRR (0.31 mg eq/kg) after 8–14 days. M24 and M26 were also detected in eggs with 0.9% TRR (0.007 mg eq/kg, 8–14 days only) and 0.9 to 1.2% TRR (0.005 to 0.009 mg eq/kg), respectively.

Fat mainly contained unchanged parent as major residue (80.3% TRR, 0.305 mg eq/kg). M21 (bixafen-desmethyl) was the only other metabolite with 18.6% TRR (0.071 mg eq/kg).

In muscle M21 (bixafen-desmethyl) gave the highest residue (50.8% of the TRR, 0.019 mg eq/kg). The unchanged parent substance was the only other substance identified at levels of 40.8% of the TRR (0.015 mg eq/kg).

In liver a total of 45.7% of the TRR were identified as bixafen-desmethyl (conventional extract: 25.7% TRR, 0.207 mg eq/kg; exhaustive extract: 20% TRR, 0.161 mg eq/kg). The unchanged parent was present mainly in conjugated form cleaved by hydrolysis, resulting in a total of 0.27 mg eq/kg or 31.5% of the TRR (conventional extraction: 6.7% TRR, 0.054 mg eq/kg; exhaustive extraction: 26.8% TRR as conjugate, 0.216 mg eq/kg). Besides parent bixafen and M21 liver contained several minor metabolites all being present at levels below 10% of the TRR. M14 representedM14 (bixafen-5-hydroxyphenyl-6-cysteinyl) was found with 3.5% TRR (0.029 mg eq/kg). In addition hydroxylated and conjugated metabolites following bixafen-desmethyl were identified: M24 with 3.1% TRR (0.025 mg eq/kg), M26 with 8.4% TRR (0.067 mg eq/kg) and M27 with 5.1% TRR (0.041 mg eq/kg).

In the following table the extraction rates and the metabolites identified are summarised.

Table 17 Characterisation and identification of compounds in eggs and tissues of the laying hens after administration of dichlorophenyl-^{14}C-bixafen at 2.03 mg/kg bw and day (32.42 ppm in the diet)

Compound / Fraction	Eggs day 1–7		Eggs day 8–14		Muscle		Fat		Liver	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR [mg/kg]	100	0.525	100	0.791	100	0.037	100	0.38	100	0.806
Conventional extraction										
Bixafen	62.3	0.327	51.1	0.405	40.8	0.015	80.3	0.305	6.7	0.054
M14	–	–	–	–	–	–	–	–	3.5	0.029
M21 (bixafen-desmethyl)	33.2	0.174	39.1	0.310	50.8	0.019	18.6	0.071	25.7	0.207
M24	–	–	0.9	0.007	–	–	–	–	3.1	0.025
M26	0.9	0.005	1.2	0.009	–	–	–	–	8.4	0.067
M27	–	–	0.6	0.005	–	–	–	–	5.1	0.041
Exhaustive extraction of solids of liver										
M21 (bixafen-desmethyl)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	20.0	0.161
Bixafen-conjugate [a]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	26.8	0.216

Compound / Fraction	Eggs day 1–7		Eggs day 8–14		Muscle		Fat		Liver	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Total identified	96.4	0.506	92.9	0.736	91.6	0.034	98.9	0.376	99.4	0.801
Total characterised by HPLC without identification	–	–	1.9	0.015	–	–	–	–	–	–
Total extracted	96.4	0.506	94.9	0.751	91.6	0.034	98.9	0.376	99.4	0.801
Unextracted	3.6	0.019	5.1	0.041	8.4	0.003	1.1	0.004	0.6	0.005
Accountability	100	0.525	100	0.791	100	0.037	100	0.380	100	0.806

[a] Cleaved conjugate during exhaustive extraction. Type of conjugate not identified.

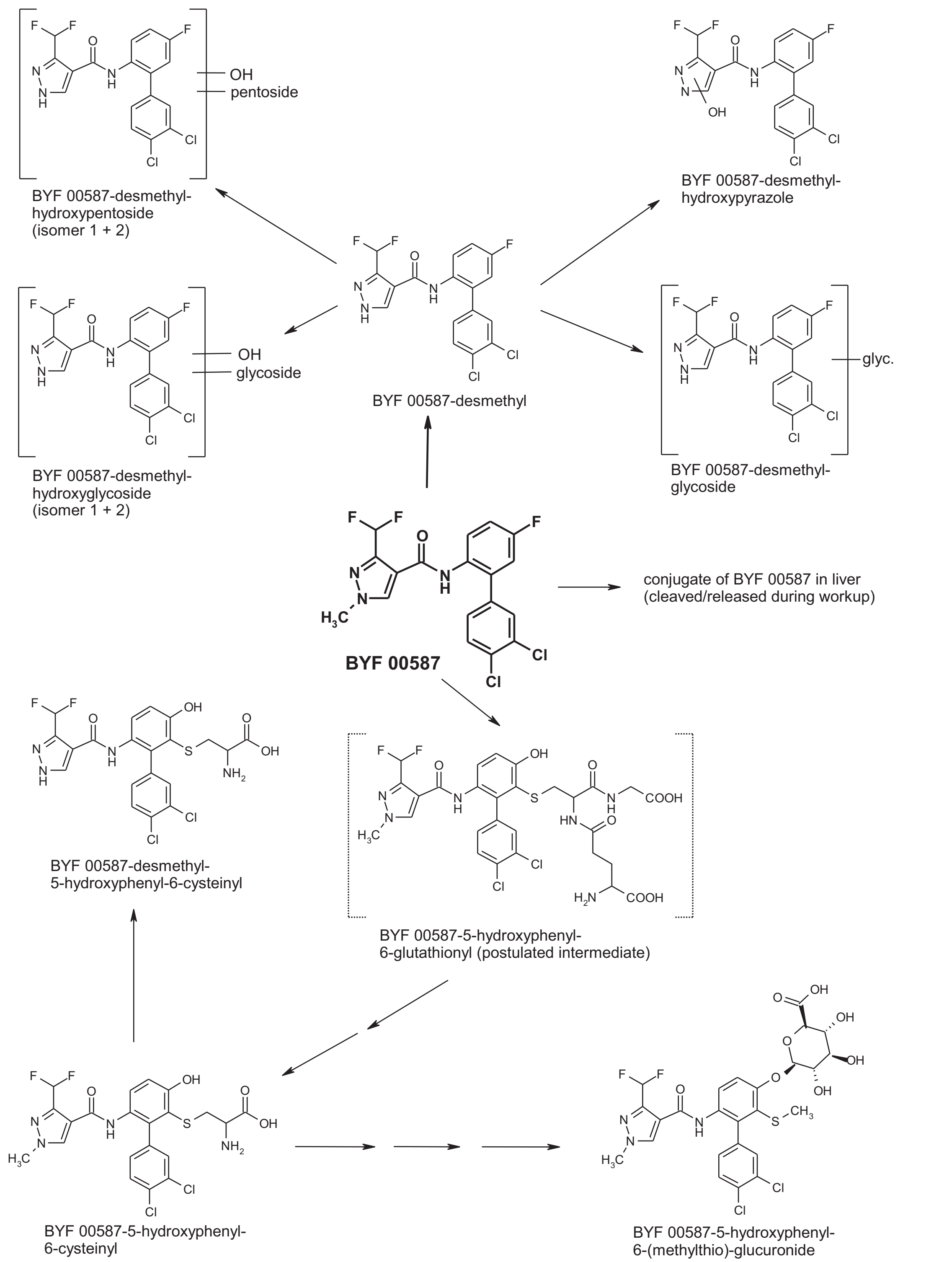


Figure 4 Proposed metabolic pathway of bixafen in laying hens

Plant metabolism

The fate of bixafen in plants was investigated following foliar application of ^{14}C-radiolabelled active substance to soya beans and wheat. In all samples unchanged bixafen was the major residue, normally amounting nearly 90% of the TRR or more. Except for soya bean seeds, the only other metabolite found was M21 (bixafen-desmethyl), however its levels were below 3% of the TRR in all samples.

In soya bean seeds, which were not directly affected by the spray solution, parent bixafen was still the main residue but represented only 29.5% of the TRR. In addition the tautomers M44 & M45 (BYF 00587-desmethyl-pyrazole-4-carboxylic acid, 18.8% TRR, 0.004 mg eq/kg) and M47 (pyrazolone-4-carboxylic acid, 12.1% TRR, 0.003 mg eq/kg) were identified. No M21 (bixafen-desmethyl) was found in soya bean seeds.

Soya beans

The metabolism of bixafen was investigated (Spiegel, K 2007, BIXAFEN_018) in soya beans (var. Merlin) after three spray applications with [pyrazole-5-^{14}C]-bixafen formulated as an EC 125. The first application was performed when the first flowers opened (BBCH 60), the second at the end of flowering (BBCH 69) and the third when approximately 80% of the pods were ripe (BBCH 88). Each application was conducted with a nominal amount of 0.06 kg ai/ha, however a total amount of 0.188 kg ai/ha was actually applied. The plants were grown in a greenhouse.

Forage was harvested at growth stage BBCH 70–71 (5 days after the 2nd application), hay at growth stage BBCH 75 (29 days after the 2nd application), and straw and seeds at maturity (BBCH 96, 26 days after the 3rd application).

The samples were homogenised with liquid nitrogen and extracted three times with a mixture of acetonitrile/water (4/1, v/v) using an Ultra-Turrax homogeniser. After each extraction step, the extracts were separated from the solids by centrifugation. The radioactivity of each extract was determined by LS measurement. The extracts were combined, diluted or, if necessary concentrated, for HPLC analysis. The remaining solids were lyophilised, and the radioactivity was determined by combustion followed by LSC. The TRR was calculated as the sum of the radioactivity determined in the extracts and in the solids.

For seeds, a subsequent exhaustive extraction was conducted with acetonitrile/water 4/1 (v/v) and with acetonitrile/acetic acid 4/1 (v/v) with microwave assistance, both at 80 °C. The microwave extracts were concentrated to the aqueous remainder and partitioned three times with heptane. The heptane phase contained only negligible amounts of radioactivity and was discarded. The aqueous phase was concentrated and analysed by HPLC.

Parent compound and metabolites were identified by HPLC and TLC co-chromatography (by spiking) or HPLC and TLC comparison with reference compounds (peak retention times, comparison of chromatographed zones).

The TRRs found in the samples collected amounted 5.32 mg eq/kg in forage, 4.0 mg eq/kg in hay, 12.9 mg eq/kg in straw, and 0.024 mg eq/kg in the seed. In forage, hay and straw unchanged bixafen was the major residue being present at levels > 89.9% of the TRR. The only other metabolite present was M21 (bixafen-desmethyl) found at 0.5% to 2.6% of the TRR.

In the seeds parent bixafen was also identified as major residue; however, the levels of 29.7% of the TRR (0.007 mg eq/kg) were lower compared to other plant parts. Besides the parent substance the tautomers M44 & M45 (BYF 00587-desmethyl-pyrazole-4-carboxylic acid, 18.8% TRR, 0.004 mg eq/kg) and M47 (pyrazolone-4-carboxylic acid, 12.1% TRR, 0.003 mg eq/kg) were found as major metabolites. M21 (bixafen-desmethyl) was not present in soya bean seeds.

An overview of the TRR levels found in the collected samples and the composition of the residue is presented in the following table.

Table 18 Total radioactive residues and their composition after application of [pyrazole-5-^{14}C]-bixafen to soya beans

Metabolite Fraction	Forage (5 d after 2nd application)		Hay (29 d after 2nd application)		Straw (26 d after 3rd application)		Seeds (26 d after 3rd application)	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR	100	5.32	100	4.00	100	12.9	100	0.024
Bixafen	95.8	5.10	91.8	3.67	89.9	11.59	29.7	0.007
M44 & M45	–	–	–	–	–	–	18.8	0.004
M47	–	–	–	–	–	–	12.1	0.003
M21 (bixafen-desmethyl)	1.5	0.08	2.6	0.10	0.5	0.06	–	–
Total identified	97.4	5.18	94.4	3.78	90.4	11.66	60.6	0.014
Total characterised	0.6	0.03	0.7	0.03	0.6	0.08	16.2	0.004
Fractions not analysed	–	–	–	–	–	–	0.7	< 0.001
Total extracted	98.0	5.21	95.1	3.80	91.0	11.74	77.5	0.018
Unextracted	2.0	0.11	4.9	0.20	9.0	1.16	22.5	0.005
Accountability	100.0	5.32	100.0	4.00	100.0	12.90	100.0	0.024

A second study investigating the metabolism of bixafen in soya beans was conducted by Spiegel, K (2007, BIXAFEN_019). [Dichlorophenyl-UL-^{14}C]-bixafen was formulated as an EC 125. The first application was performed when the first flowers opened (BBCH 60), the second at the end of flowering (BBCH 69) and the third when approximately 80% of the pods were ripe (BBCH 88). Each application was conducted with a nominal amount of 0.06 kg ai/ha, however a total amount of 0.187 kg ai/ha was actually applied. The plants were grown in a greenhouse.

Forage was harvested at growth stage BBCH 70–71 (5 days after the 2nd application), hay at growth stage BBCH 75 (29 days after the 2nd application), and straw and seeds at maturity (BBCH 96, 26 days after the 3rd application).

The samples were homogenised with liquid nitrogen and extracted three times with a mixture of acetonitrile/water (4/1, v/v) using an Ultra-Turrax homogeniser. After each extraction step, the extracts were separated from the solids by centrifugation. The radioactivity of each extract was determined by LS measurement. The extracts were combined, diluted or, if necessary concentrated, for HPLC analysis. The remaining solids were lyophilised, and the radioactivity was determined by combustion followed by LSC. The TRR was calculated as the sum of the radioactivity determined in the extracts and in the solids.

Parent compound and metabolites were identified by HPLC and TLC co-chromatography (by spiking) or HPLC and TLC comparison with reference compounds (peak retention times, comparison of chromatographed zones).

The TRRs found in the samples collected amounted 3.98 mg eq/kg in forage, 2.81 mg eq/kg in hay, 9.52 mg eq/kg in straw, and 0.005 mg eq/kg in the seed. In forage, hay and straw unchanged bixafen was the major residue being present at levels > 91.8% of the TRR. The only other metabolite present was M21 (bixafen-desmethyl) found at 0.6% to 1.9% of the TRR.

In the seeds total radioactive residues were too low for identification. The described extraction methods released 53% of the TRR.

An overview of the TRR levels found in the collected samples and the composition of the residue is presented in the following table.

Table 19 Total radioactive residues and their composition after application of [dichlorophenyl-UL-^{14}C]-bixafen to soya beans

Metabolite Fraction	Forage (5 d after 2nd application)		Hay (29 d after 2nd application)		Straw (26 d after 3rd application)		Seeds (26 d after 3rd application)	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR	100	3.98	100	2.81	100	9.52	100	0.005
Bixafen	97.7	3.89	91.8	2.58	92.3	8.79	n.a.	n.a.
M21 (bixafen-desmethyl)	1.1	0.04	1.9	0.05	0.6	0.06	n.a.	n.a.

Metabolite Fraction	Forage (5 d after 2nd application)		Hay (29 d after 2nd application)		Straw (26 d after 3rd application)		Seeds (26 d after 3rd application)	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Total identified	98.8	3.93	93.7	2.63	92.9	8.84	n.a.	n.a.
Total extracted	98.8	3.93	93.7	2.63	92.9	8.84	53.0	0.002
Unextracted	1.2	0.05	6.3	0.18	7.1	0.67	47.0	0.002
Accountability	100.0	3.98	100.0	2.81	100.0	9.52	100.0	0.005

n.a. = Not analysed

Wheat

The metabolism of bixafen in wheat (var. Thassos) was investigated by Miebach, D and Bongartz, R (2007, BIXAFEN_020) after two spray applications with [pyrazole-5-^{14}C]-bixafen formulated as an EC 200. The first application with 0.125 kg ai/ha (actual 132 g ai/ha) was performed at the end of tillering / beginning of stem elongation (BBCH 29–31), and the second with 0.15 kg ai/ha (actual 154 g ai/ha) at the end of flowering (BBCH 69). Plants were grown in plastic containers under natural temperature and light conditions in Monheim, Germany. The glass roof of the vegetation area was open during the sunshine periods and was automatically closed during rainfall. Plants were irrigated as needed.

Forage was harvested at growth stage BBCH 38 (9 days after 1st application), hay at growth stage BBCH 77 (9 days after 2nd application), and straw and grain at maturity (BBCH 89, 50 days after 2nd application).

The homogenised samples were extracted three times with a mixture of acetonitrile/water (4/1, v/v) and once with acetonitrile using an Ultra-Turrax homogeniser. The extracts were separated from the solids by suction through a filter. The ^{14}C-radioactivity of liquid samples was determined by liquid scintillation counting (LSC). The remaining solids were air dried. Solid samples were combusted and the released $^{14}CO_2$ was absorbed in an alkaline scintillation cocktail and radio-assayed by LSC. The TRR of each RAC was determined by summation of the radioactivity in the combined extracts and in the solids.

The corresponding extracts of wheat RAC were combined and concentrated to a volume suitable for HPLC chromatography. Parent compound and metabolites were quantified by HPLC. The TRRs and the quantified amounts of compounds were expressed as mg bixafen-equivalents per kg sample material.

Parent compound in the extracts of straw and grain was identified by LC-MS-spectroscopy. Metabolites in straw and grain were identified by HPLC co-chromatography with radiolabelled reference compounds. Parent compound and metabolites in forage and hay were assigned by comparison of the metabolite patterns obtained for straw and grain.

The TRRs found in the samples collected amounted 1.67 mg eq/kg in forage, 6.57 mg eq/kg in hay, 24.27 mg eq/kg in straw, and 0.162 mg eq/kg in grain. In all samples unchanged bixafen was the major residue being present at levels > 89.5% of the TRR. The only other metabolite present was M21 (bixafen-desmethyl) found at 0.8% to 2.4% of the TRR.

In the samples one further analyte (BYF 00587-4'-deschloro) was identified, which was also found at levels of 1% to 1.3% of the active ingredient in the spray solution, suggesting impurities of the tank-mix used.

An overview of the TRR levels found in the collected samples and the composition of the residue is presented in the following table.

Table 20 Total radioactive residues and their composition after application of [pyrazole-5-^{14}C]-bixafen to wheat

Compound	Forage (9 d after 1st application)		Hay (9 d after 2nd application)		Straw (50 d after 2nd application)		Grain (50 d after 2nd application)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	1.67	100	6.57	100	24.27	100	0.162
Bixafen	92.9	1.55	91.9	6.04	92.6	22.47	89.5	0.145
M21 (bixafen-desmethyl)	0.8	0.01	2.3	0.15	1.8	0.43	2.4	0.004
Total identified	93.7	1.57	94.2	6.19	94.4	22.90	91.8	0.148
Total characterised [a]	1.6	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BYF 00587-4'-deschloro [b]	3.5	0.06	2.2	0.14	1.4	0.35	2.0	0.003
Total extractable (combined extracts)	98.7	1.65	96.4	6.33	95.8	23.25	93.8	0.152
Unextracted	1.3	0.02	3.6	0.24	4.2	1.02	6.2	0.010

[a] Extracted and characterised by chromatographic behaviour

[b] Impurity in spraying solution

An additional plant metabolism study on wheat (var. Thassos) using [dichlorophenyl-UL-^{14}C]-bixafen was conducted by Miebach, D and Bongartz, R (2007, BIXAFEN_021). The active ingredient was formulated as an EC 200. The first application with 0.125 kg ai/ha (actual 128 g ai/ha) was performed at the end of tillering / beginning of stem elongation (BBCH 29–31), and the second with 0.15 kg ai/ha (actual 158 g ai/ha) at the end of flowering (BBCH 69). Plants were grown in plastic containers under natural temperature and light conditions in Monheim, Germany. The glass roof of the vegetation area was open during the sunshine periods and was automatically closed during rainfall. Plants were irrigated as needed.

Forage was harvested at growth stage BBCH 38 (9 days after 1st application), hay at growth stage BBCH 77 (9 days after 2nd application), and straw and grain at maturity (BBCH 89, 50 days after 2nd application).

All samples were analysed according to the procedures described for the wheat metabolism study conducted with [pyrazole-5-^{14}C]-bixafen by Miebach, D and Bongartz, R (2007, BIXAFEN_020).

The TRRs found in the samples collected amounted 1.57 mg eq/kg in forage, 7.64 mg eq/kg in hay, 22.85 mg eq/kg in straw, and 0.229 mg eq/kg in grain. In all samples unchanged bixafen was the major residue being present at levels > 91.7% of the TRR. The only other metabolite present was M21 (bixafen-desmethyl) found at 0.8% to 2.1% of the TRR.

In the samples one further analyte (BYF 00587-4'-deschloro) was identified, which was also found at levels of 1% to 1.3% of the active ingredient in the spray solution, suggesting impurities of the tank-mix used.

An overview of the TRR levels found in the collected samples and the composition of the residue is presented in the following table.

Table 21 Total radioactive residues and their composition after application of [dichlorophenyl-UL-^{14}C]-bixafen to wheat

Compound	Forage (9 d after 1st application)		Hay (9 d after 2nd application)		Straw (50 d after 2nd application)		Grain (50 d after 2nd application)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	1.57	100	7.64	100	22.85	100	0.229
Bixafen	97.1	1.53	91.7	7.01	93.2	21.29	92.9	0.213
M21 (bixafen-desmethyl)	0.8	0.01	2.1	0.16	1.7	0.39	2.1	0.005
Total identified	97.9	1.54	93.8	7.17	94.9	21.68	95.0	0.218

Compound	Forage (9 d after 1st application)		Hay (9 d after 2nd application)		Straw (50 d after 2nd application)		Grain (50 d after 2nd application)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
BYF 00587-4'-deschloro [a]	1.1	0.02	1.7	0.13	1.2	0.27	2.0	0.005
Total extractable (combined extracts)	99.0	1.56	95.5	7.30	96.1	21.95	97.0	0.222
Unextracted	1.0	0.02	4.5	0.34	3.9	0.90	3.0	0.007

[a] Impurity in spraying solution

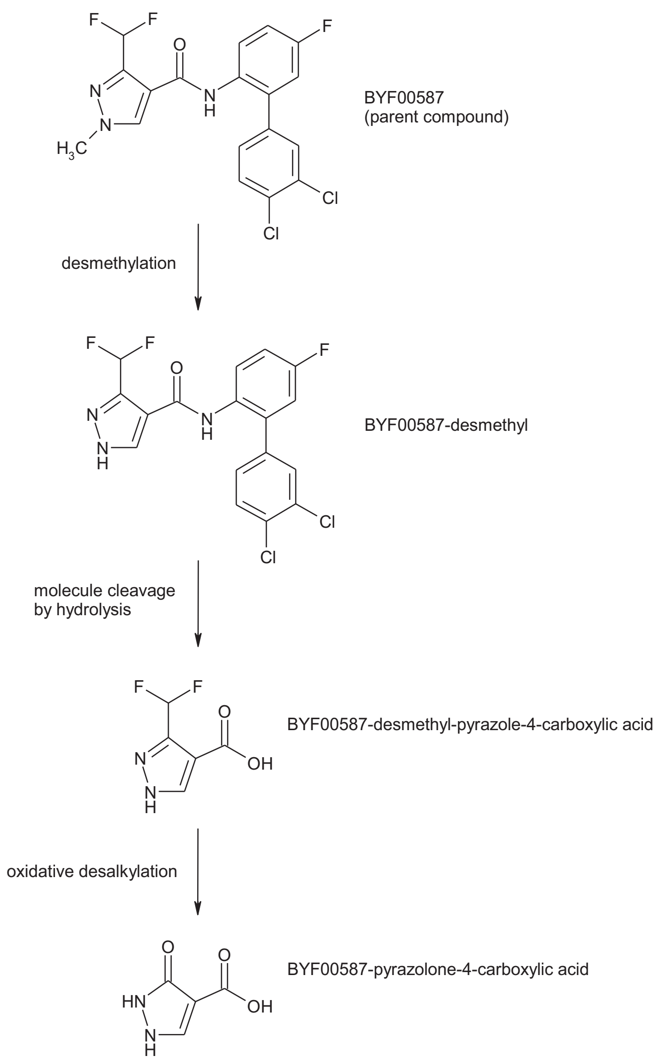


Figure 5 Proposed metabolic pathway of bixafen in plants

Environmental fate in soil

For the investigation of the environmental fate of bixafen the Meeting received studies on photolysis on soil, the aerobic soil metabolism, the behaviour in confined and field rotational crops and short- and long-term field dissipation studies.

Soil photolysis of bixafen was not observed. In the soil bixafen is highly persistent, showing about 80–90% of the applied dose remaining after 120 days observed in aerobic soil metabolism studies. The only metabolites found in minor proportions were M44 (BYF 00587-desmethyl-pyrazole-4-carboxylic acid) and M21 (Bixafen-desmethyl), the tautomers M44 & M45 later in soil of below mentioned confined rotational crop studies.

In confined rotational crop studies conducted at rates equivalent to 0.785 and 0.847 kg ai/ha bixafen and M21 (bixafen-desmethyl) were the major residue. After application of the [pyrazole-5-^{14}C]-labelled active substance, the labelled cleavage products M43 (BYF 00587-pyrazole-4-carboxamide), M44 (BYF 00587-desmethyl-pyrazole-4-carboxylic acid (tautomer 1)) and M45 (BYF 00587-desmethyl-pyrazole-4-carboxylic acid (tautomer 2)) were also identified as major metabolites.

In field rotational crop studies conducted on four locations in Europe, residues of bixafen and M21 were mostly below the LOQs of 0.01 mg/kg, except for two single detects in wheat straw (0.03 mg/kg total residue) and lettuce of pre-mature growth stage (0.06 mg/kg total residue).

Field dissipation studies confirmed the slow degradation of bixafen in the soil observed in aerobic metabolism studies. Over 5 years of annual treatment the bixafen peak concentrations in soil under field conditions still represented approximately 32–47% of the total dose applied within these years. Most of the residue was present as unchanged parent substance.

Photolysis on soil

The photodegradation of [^{14}C] bixafen (pyrazole and dichlorophenyl labels) on the soil surface was investigated under artificial sunlight (Muehmel, T and Fliege, R 2007, BIXAFEN_022). The two labels of the test item were individually applied to a 3 mm thick layer of soils (see Table) on glass dishes at a rate equivalent to 0.25 kg ai/ha. The 8 days of irradiation corresponded to about 30 days of midsummer sunlight at Phoenix, Arizona (USA).

The soil moisture was maintained at 75% field capacity at a temperature of 20 °C. Irradiated and dark control samples were taken at a range of intervals during the 8 days irradiation/incubation periods.

Table 22 Soil characteristics used in soil photolysis experiments

	Dichlorophenyl label test	Pyrazole label test
Soil ID	‘Laacher Hof AXXa’	‘Laacher Hof Wurmwiese’
Texture Class (USDA)	Sandy Loam	Sandy Loam
Sand [50 µm–2 mm] Silt [2 µm–50 µm] Clay [< 2 µm]	73% 20% 7%	53% 28% 19%
pH in 0.01 M $CaCl_2$ (1:1) pH in 1 N KCl (1:1) pH in Water (1:1) pH in Water (Saturated Paste)	6.2 6.2 6.6 6.7	5.6 5.2 5.9 6.0
Organic Matter [%OM]	4.0%	2.4%
Organic Carbon [%OC]	2.3%	1.4%
Soil Microbial Biomass	day 0, untreated: 785 mg C/kg soil	day 0, untreated: 550 mg C/kg soil
Cation Exchange Capacity [CEC]	11.4 meq/100 g	10.3 meq/100 g
Water Holding Capacity at 0.33 bar [1/3 bar WHC]	20.6 g/100 g	20.1 g/100 g
Maximum Water Holding Capacity [MWHC]	55.7 g/100 g	59.1 g/100 g

In irradiated samples no degradation of bixafen was observed. A summary of the metabolic spectrum of irradiated and dark samples is presented in the following tables.

Table 23 Photodegradation of [dichlorophenyl-^{14}C]-labelled bixafen on soil

	Amount (% of applied radioactivity) at days after treatment								
	irradiated samples						dark samples		
	0	1	2	4	6	8	1	4	8
Bixafen	98.9	95.9	96.8	94.6	94.4	91.8	97.5	97.8	98.6
Unidentified radioactivity (sum)	1.9	2.7	2.8	3.0	4.4	4.3	1.8	1.5	1.6
Greatest single peak	0.6	0.9	0.9	1.1	1.4	1.4	0.6	0.7	0.6
Total extracted residue	> 99	99	99	98	98	96	> 99	99	99
$^{14}CO_2$	n.m.	n.m.	n.m.	n.m.	n.m.	0.4	n.m.	n.m.	0.1
Volatile organic radioactivity	n.m.	n.m.	n.m.	n.m.	n.m.	< 0.1	n.m.	n.m.	0.1
Unextracted residue	0.1	1.2	1.3	1.7	2.0	3.3	0.6	1.0	1.5
Total recovery	100.9	99.8	100.9	99.3	100.9	99.9	99.6	100.3	101.5

n.m. = Not measured

Table 24 Photodegradation of [pyrazole-^{14}C]-labelled bixafen on soil

	Amount (% of applied radioactivity) at days after treatment							
	AXXa (air dried)				Wurmwiese (moist)			
	irradiated			dark	irradiated			dark
	0	4	8	8	0	4	8	8
Bixafen	99.2	94.6	89.9	98.5	98.7	94.5	92.2	96.7
Unidentified radioact. (sum)	1.7	3.5	6.7	1.7	1.8	2.7	4.4	1.5
Greatest single peak	0.7	1.1	1.6	0.7	0.8	0.9	1.2	0.6
Total extracted residue	101	99.6	99.5	100.7	100.6	99.3	99.8	100.3
Unextracted residue	0.1	1.5	2.9	0.5	0.1	2.1	3.2	2.1
Total recovery	100.0	99.6	99.5	100.7	100.6	99.3	99.8	100.3

$^{14}CO_2$ and volatile organic radioactivity not measured

Soil metabolism

Aerobic soil metabolism

The aerobic soil metabolism of bixafen was investigated in several soil types using [pyrazole]- and [dichlorophenyl]-^{14}C-bixafen.

In the study conducted by Sneikus, J and Koehn, D (2005, BIXAFEN_023) soil samples were extracted several times by shaking at ambient temperature and microwave extraction with acetonitrile/water (80/20, v/v), and the bixafen residues were analysed by TLC. HPLC was used as confirmation method. The identity of the test item was confirmed by co-chromatography using non-labelled reference item. Identification of transformation products was performed by LC/MS/MS.

Table 25 Distribution of radioactive residues after incubation of 0.7 mg/kg [pyrazole]-^{14}C-bixafen in soil "Laacherhof AXXa" (sandy loam, 55% MWHC, 1.3% OC, pH 6.6)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	94.8	96.2	93.6	93.4	95.2	92.4	93.0	91.2	90.8	89.8
M44 & M45	n.d.	n.d.	n.d.	n.d.	0.2	0.3	0.5	0.3	0.4	0.4
Unidentified radioactivity	< 0.1	0.5	0.6	0.5	0.6	0.3	0.3	0.2	0.3	0.2
Total extracted	94.8	96.7	94.2	93.9	96.0	93.0	93.9	91.7	91.5	90.3
$^{14}CO_2$	n.a.	0.1	0.1	0.1	0.1	0.2	0.3	0.4	0.5	0.7
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	5.2	5.7	6.9	6.7	6.9	6.5	8.3	7.7	8.7	8.9
Total recovery	100.0	102.5	101.2	100.7	103.0	99.6	102.4	99.8	100.8	99.9

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 26 Distribution of radioactive residues after incubation of 0.7 mg/kg [dichlorophenyl]-^{14}C-bixafen in soil "Laacherhof AXXa" (sandy loam, 55% MWHC, 1.3% OC, pH 6.6)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	95.7	95.2	94.2	94.4	92.7	92.3	91.9	93.7	90.8	91.6
Unidentified radioactivity	< 0.1	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.4	0.2
Total extracted	95.7	95.6	94.6	94.8	93.0	92.5	92.2	93.9	91.3	91.9
$^{14}CO_2$	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1	0.1	0.1	0.2
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	4.3	5.2	6.0	6.3	6.2	5.3	7.6	7.1	8.2	9.0
Total recovery	100.0	100.8	100.6	101.1	99.2	97.9	99.9	101.1	99.6	101.1

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 27 Distribution of radioactive residues after incubation of 0.7 mg/kg [pyrazole]-^{14}C-bixafen in soil "Laacherhof AIIIa" (silt loam, 55% MWHC, 1.1% OC, pH 6.9)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	94.3	95.1	94.2	92.9	88.5	90.0	89.3	88.9	87.2	86.6
M44 & M45	n.d.	n.d.	n.d.	n.d.	0.6	0.9	0.8	1.5	2.1	2.9
Unidentified radioactivity	< 0.1	0.7	0.5	0.8	0.5	0.3	0.3	0.2	0.4	0.2
Total extracted	94.3	95.8	94.7	93.7	89.6	91.2	90.5	90.7	89.7	89.7
$^{14}CO_2$	n.a.	0.1	0.1	0.1	0.2	0.2	0.1	0.4	0.6	0.8
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	5.7	4.7	5.5	6.2	5.6	7.1	7.5	7.5	8.9	10.4
Total recovery	100.0	100.5	100.3	100.0	95.4	98.5	98.0	98.6	99.3	101.0

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 28 Distribution of radioactive residues after incubation of 0.7 mg/kg [dichlorophenyl]-^{14}C-bixafen in soil "Laacherhof AIIIa" (silt loam, 55% MWHC, 1.1% OC, pH 6.9)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	94.2	95.8	94.4	93.9	92.4	92.6	89.8	90.8	88.7	86.4
Unidentified radioactivity	< 0.1	0.2	0.3	0.9	0.5	0.2	0.6	0.3	0.7	0.6
Total extracted	94.2	96.0	94.7	94.7	92.9	92.9	90.4	91.1	89.4	87.0
$^{14}CO_2$	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.2	0.2	0.3	0.4
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	5.8	4.4	5.9	6.0	6.2	7.5	9.0	8.2	9.9	12.0
Total recovery	100.0	100.5	100.6	100.7	99.2	100.4	99.6	99.4	99.6	99.5

MWHC = Maximum water holding capacity

OC = organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 29 Distribution of radioactive residues after incubation of 0.7 mg/kg [pyrazole]-^{14}C-bixafen in soil "Laacherhof Wurmwiese" (loam, 55% MWHC, 2.07% OC, pH 6.0)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	89.2	92.9	91.1	92.1	89.8	92.6	90.3	90.3	88.0	86.8
M44 & M45	n.d.	n.d.	n.d.	< 0.1	0.2	0.1	0.3	0.3	0.4	0.4
Unidentified radioactivity	4.6	0.5	0.4	0.4	0.3	0.3	0.6	0.3	1.4	0.6
Total extracted	93.8	93.4	91.5	92.5	90.3	93.0	91.3	90.9	89.8	87.8
$^{14}CO_2$	n.a.	< 0.1	0.1	0.1	0.3	0.5	0.7	1.1	1.3	1.5
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	6.2	5.3	6.5	6.2	5.3	6.6	7.9	7.0	7.6	7.7
Total recovery	100.0	98.7	98.1	98.8	95.9	100.1	99.9	99.1	98.7	97.0

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 30 Distribution of radioactive residues after incubation of 0.7 mg/kg [dichlorophenyl]-^{14}C-bixafen in soil "Laacherhof Wurmwiese" (loam, 55% MWHC, 2.07% OC, pH 6.0)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	94.4	93.0	90.7	92.9	90.5	90.3	89.7	87.2	88.1	87.3
Unidentified radioactivity	0.4	0.2	0.2	0.3	0.2	0.3	0.5	0.3	0.9	0.6
Total extracted	94.8	93.2	90.9	93.3	90.8	90.6	90.2	87.5	89.0	88.0
$^{14}CO_2$	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.1	0.1	0.2	< 0.1
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	5.2	5.0	5.2	6.2	6.1	6.8	7.2	7.0	8.9	9.0
Total recovery	100.0	98.1	96.1	99.5	96.9	97.5	97.5	94.6	98.0	96.9

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 31 Distribution of radioactive residues after incubation of 0.7 mg/kg [pyrazole]-^{14}C-bixafen in soil "Hoefchen am Hohenseh" (silt loam, 55% MWHC, 2.62% OC, pH 6.7)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	91.2	96.4	93.3	94.2	92.2	91.1	92.3	89.8	89.3	89.1
M44 & M45	n.d.	n.d.	n.d.	< 0.1	0.2	0.2	0.2	0.5	0.6	0.6
Unidentified radioactivity	2.1	0.4	0.7	0.5	0.3	0.2	0.3	0.3	0.9	0.5
Total extracted	93.3	96.8	94.0	94.7	92.7	91.5	92.9	90.6	90.8	90.2
$^{14}CO_2$	n.a.	< 0.1	0.1	0.1	0.2	0.3	0.5	0.8	1.1	1.6
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	6.7	5.6	7.1	6.9	6.7	7.0	7.6	7.7	7.9	7.5
Total recovery	100.0	102.4	101.1	101.7	99.6	98.8	101.0	99.1	99.8	99.3

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 32 Distribution of radioactive residues after incubation of 0.7 mg/kg [dichlorophenyl]-^{14}C-bixafen in soil "Hoefchen am Hohenseh" (silt loam, 55% MWHC, 2.62% OC, pH 6.7)

	Residues (% of applied radioactivity) at days after treatment									
	0	1	3	7	14	21	38	62	90	120
Bixafen	94.5	93.6	91.0	92.1	91.1	89.9	90.7	88.5	90.8	88.7
Unidentified radioactivity	0.3	0.2	0.1	0.4	0.2	0.1	0.2	0.2	0.6	0.2
Total extracted	94.7	93.8	91.1	92.5	91.3	90.0	90.9	88.7	91.3	89.0
$^{14}CO_2$	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.1	0.1	0.2	0.3
Volatile organic radioactivity	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Unextracted residue	5.3	5.2	6.1	6.2	6.9	6.9	7.2	7.1	8.5	9.0
Total recovery	100.0	99.1	97.2	98.7	98.3	97.0	98.2	96.0	100.1	98.3

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

In addition to the study presented, De Souza, TJT (2011, BIXAFEN_024) investigated the aerobic degradation of [pyrazole]-^{14}C-bixafen in four Brazilian soils. Microbial viable soil samples were collected freshly from the field and treated with a rate of 0.4909 mg ai/kg soil.

The soils were extracted with methanol/water. The quantification was performed by high pressure liquid chromatography (HPLC) with radioactivity detector. Unextracted residues were determined by combustion. The liberated $^{14}CO_2$ was absorbed in an appropriate scintillation cocktail and measured by LS counting.

Table 33 Distribution of radioactive residues after incubation of 0.49 mg/kg [pyrazole]-^{14}C-bixafen in soil "Argissolo" (clay, 50% MWHC, 3.0% OC, pH 5.9)

	Residues (% of applied radioactivity) at days after treatment						
	0	7	14	28	64	92	120
Bixafen	99.1	94.0	100.2	93.1	90.3	83.2	81.1
M44 & M45	n.d.	n.d.	n.d.	2.8	3.1	0.53	0.86
Total extracted	99.1	98.3	100.2	95.9	93.4	84.2	83.5
$^{14}CO_2$	n.a.	0.2	0.6	1.3	4.4	6.3	8.2
Volatile organic radioactivity	n.a.	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13
Unextracted residue	1.6	3.4	4.4	6.0	8.1	8.0	9.2
Total recovery	100.7	101.9	105.2	103.2	105.9	98.5	100.9

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 34 Distribution of radioactive residues after incubation of 0.49 mg/kg [pyrazole]-^{14}C-bixafen in soil "Latossolo" (clay, 50% MWHC, 1.4% OC, pH 4.9)

	Residues (% of applied radioactivity) at days after treatment						
	0	7	14	28	64	92	120
Bixafen	101.7	95.1	97.9	91.4	92.0	92.7	84.3
M44 & M45	n.d.	n.d.	0.76	3.06	1.07	0.61	2.39
Total extracted	102.2	97.2	98.3	93.0	92.6	96.4	90.0
$^{14}CO_2$	n.a.	< 0.13	0.4	1.0	3.4	3.9	4.7
Volatile organic radioactivity	n.a.	< 0.13	< 0.13	< 0.13	0.1	0.2	0.2

	Residues (% of applied radioactivity) at days after treatment						
	0	7	14	28	64	92	120
Unextracted residue	0.9	2.0	3.6	6.0	6.6	3.3	5.4
Total recovery	103.0	99.2	102.3	100.0	102.7	103.8	100.2

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 35 Distribution of radioactive residues after incubation of 0.49 mg/kg [pyrazole]-^{14}C-bixafen in soil "Neossolo" (clay, 50% MWHC, 0.5% OC, pH 5.6)

	Residues (% of applied radioactivity) at days after treatment						
	0	7	14	28	64	92	120
Bixafen	107.7	108.4	103.5	101.9	96.1	92.9	92.0
M44 (BYF 00587-desmethyl-pyrazole-4-carboxylic acid)	n.d.	n.d.	n.d.	n.d.	1.16	2.84	1.25
Total extracted	107.7	109.3	103.4	101.9	97.2	95.7	95.1
$^{14}CO_2$	n.a.	< 0.13	< 0.13	0.3	1.6	1.9	2.5
Volatile organic radioactivity	n.a.	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13
Unextracted residue	< 0.13	0.4	0.7	1.0	2.1	2.0	3.0
Total recovery	107.7	109.8	104.2	103.2	100.9	99.6	100.6

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

Table 36 Distribution of radioactive residues after incubation of 0.49 mg/kg [pyrazole]-^{14}C-bixafen in soil "Gleissolo" (clay, 50% MWHC, 5.5% OC, pH 4.4)

	Residues (% of applied radioactivity) at days after treatment						
	0	7	14	28	64	92	120
Bixafen	97.9	86.6	89.2	88.9	82.8	84.0	84.0
M44 & M45	n.d.	n.d.	n.d.	n.d.	n.d.	0.43	0.42
Total extracted	97.9	92.0	89.3	88.9	82.8	84.7	85.1
$^{14}CO_2$	n.a.	< 0.13	< 0.13	0.2	1.0	1.2	1.3
Volatile organic radioactivity	n.a.	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13
Unextracted residue	3.1	5.4	5.6	8.6	8.6	10.8	10.5
Total recovery	101.1	97.4	95.0	97.6	92.4	96.7	96.9

MWHC = Maximum water holding capacity

OC = Organic carbon

n.a. = Not analysed

n.d. = Not detected

A third study investigating the aerobic soil metabolism of bixafen was presented by Menke, U (2008, BIXAFEN_025). In this study soils (0–15 cm depth) obtained from a confined rotational crop metabolism study involving application of [pyrazole]- and [dichlorophenyl]-^{14}C-bixafen at target rates of 0.75 kg ai/ha.

Aliquots of acetonitrile/water extracts were analysed and radioactivity quantified by HPLC ($_{14}$C-radio- and UV-detector). The limit of quantification (LOQ) was 0.4% TRR. TLC was used as a method to confirm formation of metabolites. Unextracted residues were determined by combustion followed by LSC and were not characterised further.

Table 37 Distribution of radioactive residues in soil from rotational crop metabolism studies after application of 0.75 kg ai/ha [pyrazole-^{14}C]-bixafen (sandy loam, 38.4% MWHC, 1.36% OC, pH 7.3)

	Residues (% of applied radioactivity) at days after treatment			
	30	138	285	418
Bixafen	85.3	83.5	77.1	73.6
M21 (bixafen-desmethyl)	0.5	1.2	2.2	1.9
Unidentified	2.3	1.5	1.0	0.6
Total extracted	88.1	86.2	80.3	76.1
Unextracted residue	4.9	7.4	9.8	15.6
Total recovery	93.0	93.7	90.0	91.7

MWHC = Maximum water holding capacity

OC = Organic carbon

Table 38 Distribution of radioactive residues in soil from rotational crop metabolism studies after application of 0.75 kg ai/ha [dichlorophenyl-^{14}C]-bixafen (sandy loam, 38.4% MWHC, 1.36% OC, pH 7.3)

	Residues (% of applied radioactivity) at days after treatment			
	30	138	285	418
Bixafen	104.9	90.7	80.5	84.4
M21 (bixafen-desmethyl)	0.6	1.1	1.6	2.7
Unidentified	2.3	1.0	0.2	0.4
Total extracted	107.7	92.8	83.0	81.7
Unextracted residue	5.3	8.3	10.4	16.7
Total recovery	113.0	101.1	93.4	104.1

MWHC = Maximum water holding capacity

OC = Organic carbon

Confined rotational crop studies

The metabolism of [pyrazole-5-^{14}C]-bixafen was investigated by Weber, E, Spiegel, K and Koehn, D (2007, BIXAFEN_029) in the rotational crops wheat, Swiss chard and turnips from three consecutive rotations. The active ingredient was applied uniformly to the soil of a planting container by spray application (day 0) at a rate of 0.785 kg ai/ha. Crops of the first, second and third rotation were sown at day 30, day 138 and day 285. Immature samples investigated were wheat forage and hay. Wheat straw and grain, Swiss chard, turnip leaves and roots were harvested at maturity. Residues in soil were reported in the aerobic metabolism section by Menke, U (2008, BIXAFEN_025).

The samples were extracted with acetonitrile/water (4/1, v/v) subjected to a clean-up step using a C_{18}-SPE cartridge (conventional extraction). For the exhaustive extraction (only conducted for hay and straw), the solids were extracted in a first step with acetonitrile/water (4/1, v/v) and in a second step with acetonitrile/acetic acid (4/1, v/v), both with microwave assistance at increased temperature (80 °C). Each microwave extract was purified using a C_{18}-SPE cartridge. The purified fractions were combined, evaporated and the aqueous remainder was partitioned with dichloromethane.

The total radioactivity was determined by LSC (LOQ: 0.01 mg eq/kg, LOD: 0.001–0.009 mg eq/kg based on background noise level). The extracts were analysed by HPLC. Major metabolites were identified by HPLC and TLC co-chromatography using authentic reference compounds. The conventional extract of Swiss chard of the first rotation showed nearly all metabolites of the other RACs and was used to isolate the polar metabolites which were identified by HPLC-MS/MS. The metabolites in all other samples were assigned by comparison of the HPLC profiles.

In the following table the TRR found in plant samples are summarized.

Table 39 Total radioactive residues in confined rotational crops (three rotations) after application of [pyrazole-5-^{14}C]-bixafen onto soil at a rate of 0.785 kg ai/ha

	TRR (mg eq/kg)						
	wheat				Swiss	turnip	
	forage	Hay	straw	grain	chard	leaves	roots
first rotation (PBI 30 days)	0.045	0.288	0.434	< 0.01	0.064	0.077	0.047
second rotation (PBI 138 days)	0.058	0.176	0.337	< 0.01	0.059	0.027	0.012
third rotation (PBI 285 days	0.025	0.153	0.217	n.e.	0.040	0.021	0.015

n.e. = Not extracted

The identification of the radioactive residues revealed the parent substance and seven other metabolites, most of them cleavage products characteristic for the radiolabelled side of the parent molecule. In Table –42 the extracted and identified radioactivity is presented. In wheat grain the TRR was too low for an identification of the residue.

Table 40 Identification and characterisation of radioactive residues in rotational crops (1st rotation, 30 d) following one soil application of [pyrazole-5-^{14}C]-bixafen at a total field rate of 0.785 kg ai/ha

Metabolite Fraction	Wheat forage (DAT 71)		Wheat hay (DAT 99)		Wheat grain [a] (DAT 138)		Wheat straw (DAT 138)		Swiss chard (DAT 84)		Turnip leaves (DAT 104)		Turnip roots (DAT 104)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	0.045	100	0.288	100	0.008	100	0.434	100	0.064	100	0.077	100	0.047
Bixafen	18.5	0.008	32.3	0.093			22.9	0.100	25.5	0.016	36.7	0.028	59.2	0.028
M20	–	–	–	–			–	–	14.6	0.009	–	–	–	–
M21	31.2	0.014	32.0	0.092			43.6	0.189	–	–	6.2	0.005	13.9	0.007
M42	19.8	0.009	–	–			2.4	0.011	1.9	0.001	8.5	0.007	4.3	0.002
M43	11.0	0.005	7.7	0.022			4.7	0.020	13.8	0.009	11.5	0.009	3.1	0.001
M44	–	–	–	–			0.3	0.001	15.2	0.010	5.3	0.004	–	–
M45	6.8	0.003	–	–			3.6	0.016	22.9	0.015	3.4	0.003	3.1	0.001
M47	5.9	0.003	–	–			–	–	4.0	0.003	5.5	0.004	4.3	0.002
Total identified	93.2	0.042	72.0	0.207			77.6	0.337	97.8	0.062	77.1	0.059	87.8	0.041
Total characterised without identification	–	–	12.5	0.036			7.2	0.031	–	–	20.4	0.016	10.9	0.005
Fractions not analysed	–	–	5.5	0.016	45.6	0.004	10.3	0.045	–	–	–	–	–	–
Total extracted	93.2	0.042	90.1	0.259	45.6	0.004	95.1	0.413	97.8	0.062	97.5	0.075	98.7	0.047
Total unextracted	6.8	0.003	10.0	0.029	54.4	0.004	4.9	0.022	2.2	0.001	2.5	0.002	1.3	0.001

DAT = Days after treatment

[a] The TRR found in wheat grain was too low for further analysis

Table 41 Identification and characterisation of radioactive residues in rotational crops (2nd rotation, 138 d) following one soil application of [pyrazole-5-^{14}C]-bixafen at a total field rate of 0.785 kg ai/ha

Metabolite Fraction	Wheat forage (DAT 187)		Wheat hay (DAT 236)		Wheat grain [a] (DAT 285)		Wheat straw (DAT 285)		Swiss chard (DAT 198)		Turnip leaves (DAT 212)		Turnip roots (DAT 212)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	0.058	100	0.176	100	0.007	100	0.337	100	0.059	100	0.027	100	0.012
Bixafen	18.1	0.011	11.7	0.021			13.9	0.047	36.7	0.022	21.8	0.006	68.8	0.008
M20	–	–	–	–			–	–	19.8	0.012	–	–	–	–
M21	51.0	0.030	48.7	0.086			62.3	0.210	3.4	0.002	6.1	0.002	19.3	0.002
M42	2.9	0.002	7.2	0.013			–	–	–	–	3.5	0.001	4.3	0.001
M43	3.2	0.002	14.0	0.025			3.5	0.012	7.1	0.004	5.6	0.002	–	–
M44	–	–	–	–			2.0	0.007	19.2	0.011	36.9	0.010	–	–
M45	–	–	–	–			1.8	0.006	7.6	0.005	12.3	0.003	–	–
M47	3.1	0.002	–	–			–	–	2.6	0.002	4.8	0.001	3.0	< 0.001
Total identified	78.4	0.046	81.7	0.144			83.5	0.281	96.3	0.057	91.1	0.025	95.5	0.011
Total characterised without identification	10.3	0.006	5.0	0.009			1.2	0.004	–	–	4.6	0.001	–	–
Fractions not analysed	–	–	8.4	0.015	22.2	0.001	7.9	0.026	–	–	–	–	–	–
Total extracted	88.7	0.052	95.1	0.167	22.2	0.001	92.5	0.311	96.3	0.057	95.7	0.026	95.5	0.011
Total unextracted	11.3	0.007	5.0	0.009	77.8	0.005	7.5	0.025	3.7	0.002	4.3	0.001	4.5	0.001

DAT = days after treatment

[a] The TRR found in wheat grain was too low for further analysis

Table 42 Identification and characterisation of radioactive residues in rotational crops (3rd rotation, 285 d) following one soil application of [pyrazole-5-^{14}C]-bixafen at a total field rate of 0.785 kg ai/ha

Metabolite Fraction	Wheat forage (DAT 330)		Wheat hay (DAT 380)		Wheat straw (DAT 418)		Swiss chard (DAT 348)		Turnip leaves (DAT 357)		Turnip roots (DAT 357)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	0.025	100	0.153	100	0.217	100	0.040	100	0.021	100	0.015
Bixafen	11.3	0.003	18.8	0.029	16.6	0.036	34.9	0.014	28.0	0.006	62.9	0.009
M20	–	–	–	–	–	–	18.4	0.007	–	–	–	–
M21	45.8	0.011	50.4	0.077	53.5	0.116	2.5	0.001	7.6	0.002	26.8	0.004
M42	–	–	4.1	0.006	–	–	–	–	6.7	0.001	4.0	0.001
M43	15.0	0.004	14.0	0.021	4.9	0.011	9.9	0.004	14.1	0.003	–	–
M44	–	–	–	–	–	–	14.8	0.006	–	–	–	–
M45	–	–	–	–	–	–	4.9	0.002	16.1	0.003	–	–
M47	–	–	–	–	–	–	–	–	4.9	0.001	3.9	0.001
Total identified	72.0	0.018	87.3	0.133	74.9	0.162	85.4	0.034	77.3	0.016	97.6	0.014
Total characterised without identification	16.9	0.004	–	–	13.9	0.030	9.0	0.004	19.0	0.004	–	–
Fractions not analysed	–	–	7.0	0.011	7.0	0.015	–	–	–	–	–	–

Metabolite Fraction	Wheat forage (DAT 330)		Wheat hay (DAT 380)		Wheat straw (DAT 418)		Swiss chard (DAT 348)		Turnip leaves (DAT 357)		Turnip roots (DAT 357)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Total extracted	88.9	0.022	94.3	0.144	95.8	0.208	94.4	0.038	96.3	0.020	97.6	0.014
Total unextracted	11.1	0.003	5.7	0.009	4.2	0.009	5.6	0.002	3.7	0.001	2.4	< 0.001

DAT = Days after treatment

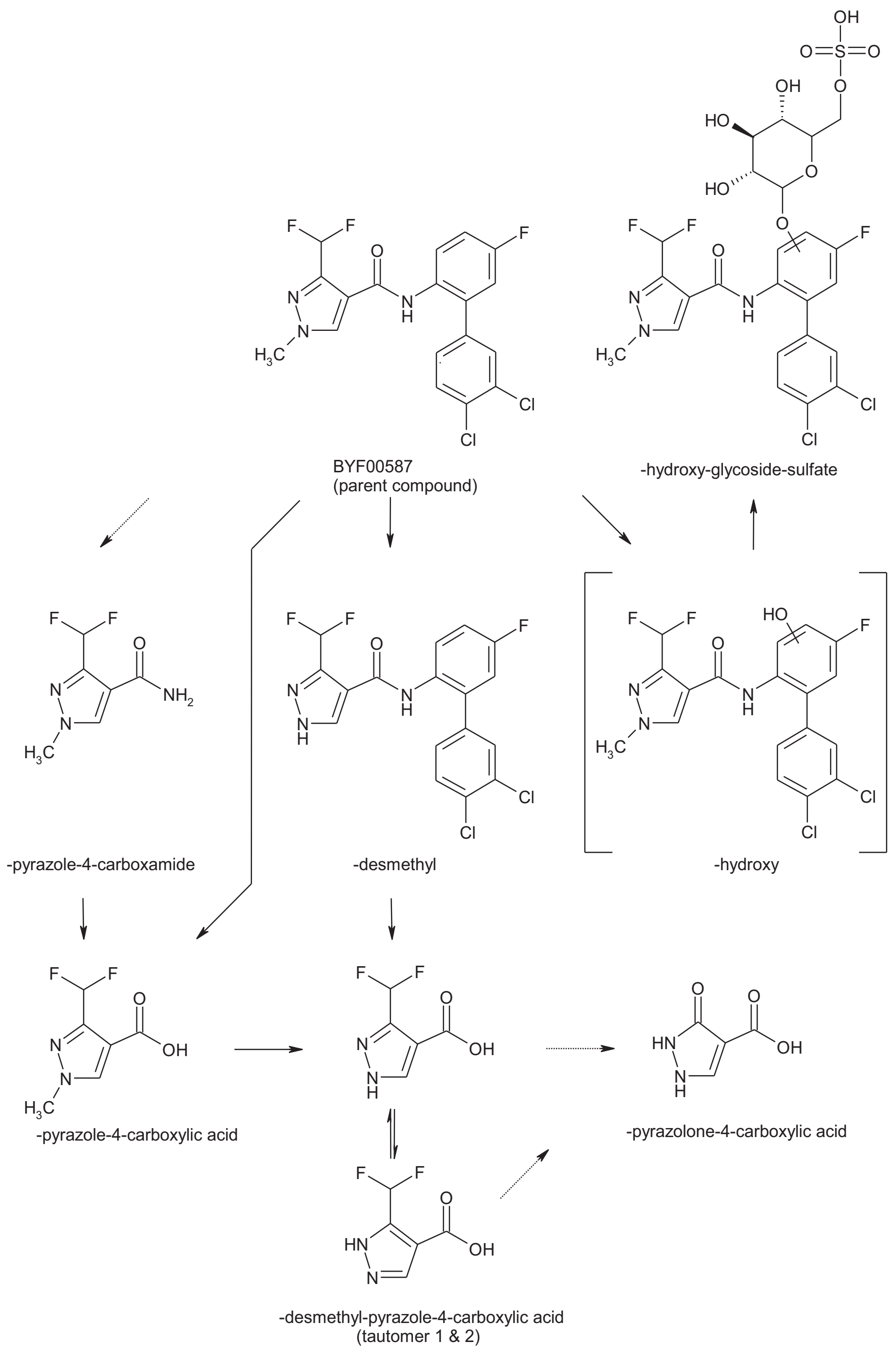


Figure 6 Proposed metabolic pathway of bixafen in rotational crops following application of [pyrazole-5-^{14}C]-bixafen

For [dichlorophenyl-UL-^{14}C]-bixafen the metabolism was investigated in the rotational crops wheat, Swiss chard and turnips from three consecutive rotations by Kuhnke, G, Weber, E., and Koehn, D (2007, BIXAFEN_028). The active ingredient was applied uniformly to the soil of a planting container by spray application (day 0) at a rate of 847 g ai/ha. Crops of the first, second and third rotation were sown at day 30, day 138 and day 285. Immature samples investigated were wheat forage and hay. Wheat straw and grain, Swiss chard, turnip leaves and roots were harvested at maturity. No residues in soil were reported.

The samples were extracted with acetonitrile/water (4/1, v/v) subjected to a clean-up step using a C_{18}-SPE cartridge (conventional extraction). For the exhaustive extraction (only conducted for hay and straw), the solids were extracted in a first step with acetonitrile/water (4/1, v/v) and in a second step with acetonitrile/acetic acid (4/1, v/v), both with microwave assistance at increased temperature (80 °C). Each microwave extract was purified using a C_{18}-SPE cartridge. The purified fractions were combined, evaporated and the aqueous remainder was partitioned with dichloromethane.

The total radioactivity was determined by LSC (LOQ: 0.01 mg eq/kg, LOD: 0.001–0.009 mg eq/kg based on background noise level). The extracts were analysed by HPLC. Major metabolites were identified by HPLC and TLC co-chromatography using authentic reference compounds.

In the following table the TRR found in plant samples are summarized:

Table 43 Total radioactive residues in confined rotational crops (three rotations) after application of [dichlorophenyl-UL-^{14}C]-bixafen at a total field rate of 0.847 kg ai/ha

	TRR (mg eq/kg)						
	wheat				Swiss	turnip	
	forage	Hay	straw	grain	chard	leaves	roots
first rotation (PBI 30 days)	0.020	0.195	0.492	0.001	0.033	0.025	0.033
second rotation (PBI 138 days)	0.035	0.193	0.269	0.002	0.041	0.013	0.012
third rotation (PBI 285 days)	0.013	0.129	0.241	n.a.	0.027	0.007	0.011

n.a. = Not analysed

The identification of the radioactive residues revealed the parent substance, M20 (BYF 00587-hydroxy-glycoside-sulfate) and M21 (bixafen-desmethyl) as major metabolites. Further degradation or breakdown products were not identified. In Table –46 the extracted and identified radioactivity is presented. In wheat grain the TRR was too low for an identification of the residue.

Table 44 Identification and characterisation of radioactive residues in rotational crops (1st rotation, 30 d) following one soil application of [dichlorophenyl-UL-14C]-bixafen at a total field rate of 0.847 kg ai/ha

Metabolite Fraction	Wheat forage (DAT 70)		Wheat hay (DAT 98)		Wheat grain [a] (DAT 137)		Wheat straw (DAT 137)		Swiss chard (DAT 83)		Turnip leaves (DAT 103)		Turnip roots (DAT 103)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	0.020	100	0.195	100	0.001	100	0.492	100	0.033	100	0.025	100	0.033
Bixafen	27.0	0.006	43.0	0.084			36.9	0.181	70.5	0.023	62.7	0.016	77.8	0.026
M20	–	–	–	–			–	–	27.6	0.009	–	–	–	–
M21	61.7	0.013	45.4	0.089			57.2	0.281	–	–	20.4	0.005	20.3	0.007
Total identified	88.7	0.018	88.4	0.172			94.1	0.462	70.5	0.032	83.1	0.021	98.2	0.033
Total characterised without identification	–	–	–	–			–	–	–	–	12.6	0.003	–	–
Fractions not	–	–	4.4	0.009	39.5	< 0.001	1.3	0.006	–	–	–	–	–	–

Metabolite Fraction	Wheat forage (DAT 70)		Wheat hay (DAT 98)		Wheat grain [a] (DAT 137)		Wheat straw (DAT 137)		Swiss chard (DAT 83)		Turnip leaves (DAT 103)		Turnip roots (DAT 103)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
analysed														
Total extracted	88.7	0.018	92.8	0.181	39.5	< 0.001	95.3	0.469	98.1	0.032	95.7	0.024	98.2	0.033
Total unextracted	11.3	0.002	7.2	0.014	60.5	0.001	4.7	0.023	1.9	0.001	4.3	0.001	1.8	0.001

DAT = Days after treatment

[a] The TRR found in wheat grain was too low for further analysis

Table 45 Identification and characterisation of radioactive residues in rotational crops (2nd rotation, 138 d) following one soil application of [dichlorophenyl-UL-^{14}C]-bixafen at a total field rate of 0.847 kg ai/ha

Metabolite Fraction	Wheat forage (DAT 186)		Wheat hay (DAT 235)		Wheat grain [a] (DAT 284)		Wheat straw (DAT 284)		Swiss chard (DAT 197)		Turnip leaves (DAT 211)		Turnip roots (DAT 211)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	0.035	100	0.193	100	0.002	100	0.269	100	0.041	100	0.013	100	0.012
Bixafen	20.7	0.007	37.8	0.073			13.8	0.037	51.7	0.021	41.9	0.005	74.9	0.009
M20	–	–	–	–			–	–	38.3	0.016	–	–	–	–
M21	61.9	0.022	55.3	0.107			72.1	0.194	5.0	0.002	14.6	0.002	21.2	0.003
Total identified	82.7	0.029	93.1	0.180			85.9	0.231	95.0	0.039	56.5	0.007	96.1	0.012
Total characterised without identification	–	–	1.9	0.004			5.8	0.016	–	–	33.6	0.004	–	–
Fractions not analysed	–	–	1.9	0.004	10.7	< 0.001	5.8	0.016	–	–	–	–	–	–
Total extracted	82.7	0.029	95.1	0.184	10.7	< 0.001	91.7	0.247	95.0	0.039	90.0	0.012	96.1	0.012
Total unextracted	17.3	0.006	4.9	0.010	89.3	0.001	8.4	0.023	5.0	0.002	10.0	0.001	3.9	< 0.001

DAT = Days after treatment

[a] The TRR found in wheat grain was too low for further analysis

Table 46 Identification and characterisation of radioactive residues in rotational crops (3rd rotation, 285 d) following one soil application of [dichlorophenyl-UL-^{14}C]-bixafen at a total field rate of 0.847 kg ai/ha

Metabolite Fraction	Wheat forage (DAT 329)		Wheat hay (DAT 379)		Wheat straw (DAT 417)		Swiss chard (DAT 347)		Turnip leaves (DAT 356)		Turnip roots (DAT 356)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100	0.013	100	0.129	100	0.241	100	0.027	100	0.007	100	0.011
Bixafen	17.2	0.002	32.8	0.042	22.0	0.053	56.4	0.015	59.3	0.004	72.7	0.008
M20	–	–	–	–	–	–	24.7	0.007	–	–	–	–
M21	65.9	0.009	58.2	0.075	72.8	0.175	3.1	0.001	18.1	0.001	24.2	0.003
Total identified	83.1	0.011	90.9	0.117	94.8	0.228	84.3	0.022	77.3	0.006	96.9	0.011
Total characterised without identification	–	–	2.5	0.003	1.5	0.004	10.7	0.003	17.1	0.001	–	–
Fractions not analysed	–	–	2.5	0.003	1.5	0.004	–	–	–	–	–	–
Total extracted	83.1	0.011	93.5	0.121	96.3	0.232	95.0	0.025	94.4	0.007	96.9	0.011
Total	16.9	0.002	6.5	0.009	3.7	0.009	5.0	0.001	5.6	< 0.001	3.1	< 0.001

Metabolite Fraction	Wheat forage (DAT 329)		Wheat hay (DAT 379)		Wheat straw (DAT 417)		Swiss chard (DAT 347)		Turnip leaves (DAT 356)		Turnip roots (DAT 356)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
unextracted												

DAT = Days after treatment

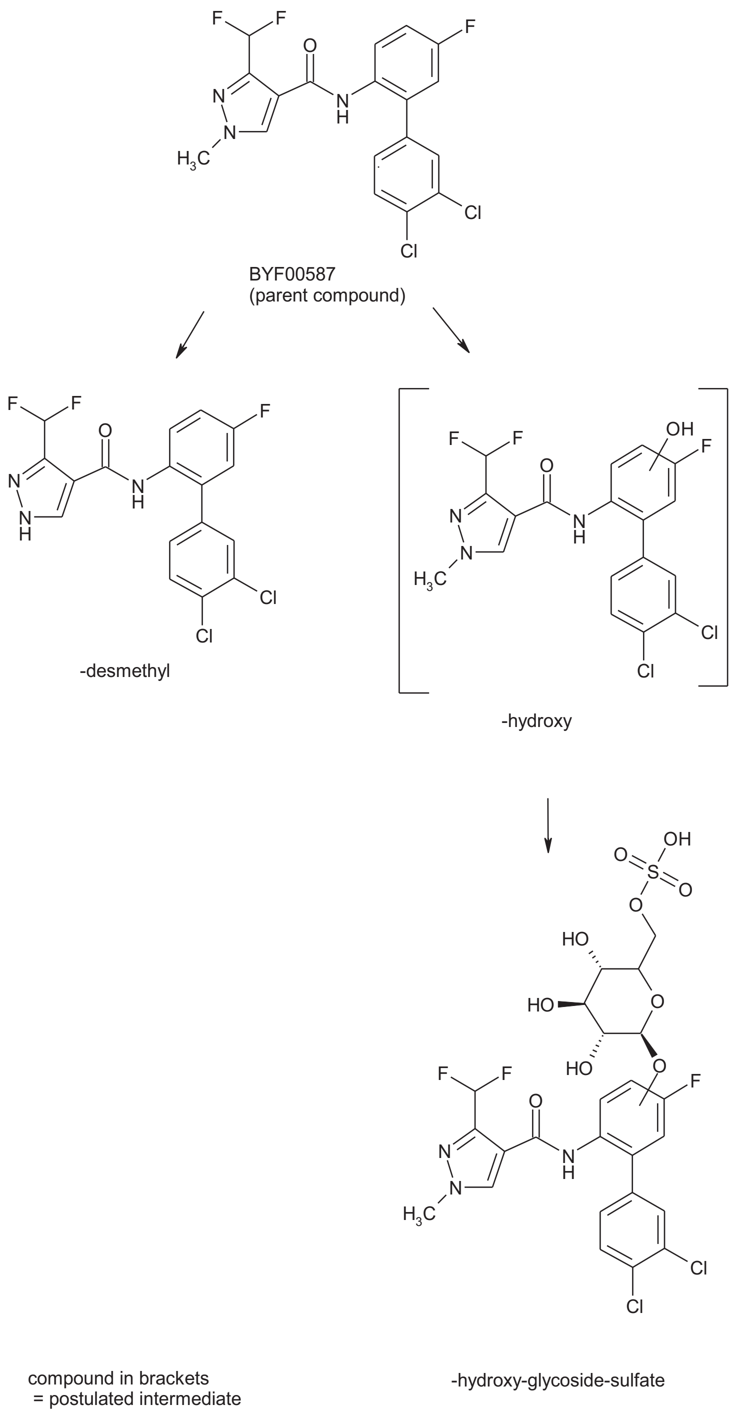


Figure 7 Proposed metabolic pathway of bixafen in rotational crops after application of [dichlorophenyl-UL-^{14}C]-bixafen

Field crop rotation studies

Four rotational crop studies (limited field studies) were carried out on bixafen during the 2006 and 2007 season. Two studies were performed in Germany and one study each in northern France and Spain.

For investigation of the residue levels that may arise in succeeding crops after a short plant back interval simulating crop failure (30 days), the bixafen was applied once as a spray application at a rate of 0.281 kg ai/ha to bare soil. For longer rotations split spray applications to barley as primary crops were carried out. At all locations, the first spray application on barley was performed at a rate of 0.156 kg ai/ha at growth stage of BBCH 47 ("flag leaf sheath opening"). The second application was carried out at a rate of 0.125 kg ai/ha at growth stage BBCH 69 ("end of flowering"). The grain was harvested normally and the straw was left on the field and ploughed. Until plating/sowing of succeeding crops, no other crop was cultivated.

In all plots turnips/carrots, lettuce and wheat were planted as succeeding crops with plant back intervals of 30, 60–180 and 270–365 days.

In the following table an overview of the studies, involved plots and cultivated crops is presented.

Table 47 Overview of field crop rotation studies for bixafen

Study Report, Location, Reference	Succeeding crop, plant back interval		
Rotation 1: 0.281 kg ai/ha to bare soil			
RA-2143/06, Northern France, Fresnoy les Roye, Schoening, R and Erler, S (2008, BIXAFEN_031)	Turnip, 30 days	Lettuce, 30 days	Wheat, 30 days
RA-2145/06, Spain, Llorona, Schoening, R and Erler, S (2008, BIXAFEN_033)	Carrot, 32 days	Lettuce, 32 days	Wheat, 32 days
RA-2144/06, Germany, Monheim, Schoening, R and Erler, S (2008, BIXAFEN_032)	Turnip, 30 days	Lettuce, 27 days	Wheat, 28 days
RA-2139/06, Germany, Burscheid, Schoening, R and Erler, S (2008, BIXAFEN_030)	Turnip, 30 days	Lettuce, 27 days	Wheat, 30 days
Rotation 2: primary crop: barley; 0.156 kg ai/ha at BBCH 47 plus 0.125 kg ai/ha at BBCH 69 (RA-2145/06 at BBCH 71)			
RA-2143/06, Northern France, Fresnoy les Roye, Schoening, R and Erler, S (2008, BIXAFEN_031)	Turnip/carrot, 60 days	Lettuce, 60 days	Wheat, 120 days
RA-2145/06, Spain, Llorona, Schoening, R and Erler, S (2008, BIXAFEN_033)	Turnip/carrot, 70 days	Lettuce, 70 days	Wheat, 184 days
RA-2144/06, Germany, Monheim, Schoening, R and Erler, S (2008, BIXAFEN_032)	Turnip/carrot, 60 days	Lettuce, 60 days	Wheat, 136 days
RA-2139/06, Germany, Burscheid, Schoening, R and Erler, S (2008, BIXAFEN_030)	Turnip/carrot, 61 days	Lettuce, 61 days	Wheat, 140 days
Rotation 3: primary crop: barley; 0.156 kg ai/ha at BBCH 47 plus 0.125 kg ai/ha at BBCH 69 (RA-2145/06 at BBCH 71)			
RA-2143/06, Northern France, Fresnoy les Roye, Schoening, R and Erler, S (2008, BIXAFEN_031)	Turnip/carrot, 331 days	Lettuce, 298 days	Wheat, 298 days
RA-2145/06, Spain, Llorona, Schoening, R and Erler, S (2008, BIXAFEN_033)	Turnip/carrot, 302 days	Lettuce, 302 days	Wheat, 278 days
RA-2144/06, Germany, Monheim, Schoening, R and Erler, S (2008, BIXAFEN_032)	not sampled—damaged by animals	Lettuce, 331 days	Wheat, 304 days
RA-2139/06, Germany, Burscheid, Schoening, R and Erler, S (2008, BIXAFEN_030)	Turnip/carrot, 314 days	Lettuce, 328 days	Wheat, 300 days

Sampling was done as follows: Samples of lettuce, turnip body and leaf (northern Europe) or carrot root and leaf (southern Europe) were taken 14 days prior to harvest date and at commercial harvest following all plant back-intervals. Wheat green material was sampled at growth stage BBCH 29/30, while samples of grain and straw were collected at full maturity of the crop (BBCH 89) from all rotations.

Samples of plant material were analysed for bixafen and M21 (bixafen-desmethyl) using method no. 01012 (see analytical methods).

In nearly all samples collected not residues of bixafen or its metabolite M21 (bixafen-desmethyl) above the LOQ of 0.01 mg/kg for each analyte were found. The only exceptions were residues 0.05 mg/kg for bixafen in lettuce (early growth stage, BBCH 46) grown in Burscheid, Germany (1st rotation, sampled 60 days after treatment) and of 0.02 mg/kg for M21 in wheat straw grown Llorona, Spain (1st rotation, sampled 262 days after treatment). In the following table a summary of all residue data is presented:

Rotation	Crop, variety	Treatment	Portion analysed	DALT (d)	Bixafen (mg/kg)	M21 (mg/kg)	Total (mg/kg)
RA-2143/06, Northern France, Fresnoy les Roye, Schoening, R and Erler, S (2008, BIXAFEN_031)							
1st	Turnip, BI.6I Collet Violet	1 × 0.281 kg ai/ha	leaf body	78 93 78 93	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce, Beurre-Hardy		head	61 75	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat, Perfector		forage straw grain	204 336 336	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
2nd	Turnip, Collet Violett	1 × 0.156 kg ai/ha + 1 × 0.125 kg ai/ha	leaf body	111 125 111 125	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce, Beurre Hardy, Butterhead variety		head	90 104	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat, Isengrain		forage straw grain	293 419 419	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
3rd	Turnip, Collet Violett	1 × 0.156 kg ai/ha + 1 × 0.125 kg ai/ha	leaf body	406 421 406 421	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce, Madras, Loose leaf		head	344 358	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Spring wheat, Lona		grain straw	453 453	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
RA-2145/06, Spain, Llorona, Schoening, R and Erler, S (2008, BIXAFEN_033)							
1st	Carrot, Touchon	1 × 0.281 kg ai/ha	leaf body	179 193 179 193	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce, Batavia, Butterhead variety		head	70 83	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat		forage straw grain	155 262 262	< 0.01 < 0.01 < 0.01	< 0.01 0.02 < 0.01	< 0.02 0.03 < 0.02
2nd	Carrot	1 × 0.156 kg ai/ha + 1 × 0.125 kg ai/ha	leaf body	173 187 173 187	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02

Rotation	Crop, variety	Treatment	Portion analysed	DALT (d)	Bixafen (mg/kg)	M21 (mg/kg)	Total (mg/kg)
	Lettuce		head	97 110	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat		forage straw grain	307 394 394	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
3rd	Carrot	1 × 0.156 kg ai/ha + 1 × 0.125 kg ai/ha	leaf body	421 435 421 435	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce		head	358 371	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat		forage straw grain	371 442 442	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
RA-2144/06, Germany, Monheim, Schoening, R and Erler, S (2008, BIXAFEN_032)							
1st	Turnip, Rondo	1 × 0.281 kg ai/ha	leaf body	86 100 86 100	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce		head	59 73	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat, Skater		forage straw grain	216 304 304	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
2nd	Turnip, Rondo	1 × 0.156 kg ai/ha + 1 × 0.125 kg ai/ha	leaf body	122 136 122 136	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce, Gisela		head	94 108	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat, Skater		forage straw grain	324 412 412	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
3rd	Lettuce, Gisela	1 × 0.156 kg ai/ha + 1 × 0.125 kg ai/ha	head	354 368	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Spring wheat, Thasos		forage straw grain	368 448 448	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
RA-2139/06, Germany, Burscheid, Schoening, R and Erler, S (2008, BIXAFEN_030)							
1st	Turnip, Rondo	1 × 0.281 kg ai/ha	leaf body	90 104 90 104	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce		head	60 74	0.05 < 0.01	< 0.01 < 0.01	0.06 < 0.02
	Winter wheat, Tommy		forage straw grain	190 314 314	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
2nd	Turnip, Rondo	1 × 0.156 kg ai/ha + 1 × 0.125 kg ai/ha	leaf body	151 135 121 135	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02
	Lettuce, Gisela		head	97 111	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat, Tommy		forage straw grain	302 426 426	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02
3rd	Turnip, Mairuebe	1 × 0.156 kg ai/ha + 1 ×	leaf body	421 435 421	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02

Rotation	Crop, variety	Treatment	Portion analysed	DALT (d)	Bixafen (mg/kg)	M21 (mg/kg)	Total (mg/kg)
		0.125 kg ai/ha		435	< 0.01	< 0.01	< 0.02
	Lettuce, Gisela		head	359 373	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02
	Winter wheat, Tommy		forage straw grain	350 446 446	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02

Field dissipation studies

The dissipation of bixafen in soil was investigated at six sites under European field conditions (Heinemann, O, 2007, BIXAFEN_026). The active ingredient was applied at nominal rates of 0.125 kg ai/ha as a 450 SC formulation onto the ground in Burscheid (Germany), Little Shelford (Great Britain), Esloev (Sweden), Vatteville (Northern France), Vilobi d'Onyar (Spain) and Albaro (Italy).

Soil samples were taken at day 0 to approximately 730 days post application at a maximum depth of 50 cm. The samples were extracted in a microwave extractor using acetonitrile/water (4/1, v/v). Identification and quantitation of the analytes was achieved by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode. The limit of quantitation (LOQ) of the method was 5.0 µg/kg for bixafen and bixafen-desmethyl. The limit of detection (LOD) of the method was 1.5 µg/kg for both analytes.

In the soil only the first 0–10 cm contained significant residues above the LOQ. Other layers are not summarized. The soil characteristics investigated are summarized in Table .

Table 48 Soil characteristics (0–30 cm depth) used for bixafen soil dissipation studies

Soil		Burscheid, Germany	Little Shelford, Great Britain	Esloev, Sweden	Vatteville, Northern France	Vilobi d'Onyar, Spain	Albaro, Italy
Soil type (USDA)	Dim	silt loam	sandy loam	silt loam	silt loam	loam	silt loam
Clay (< 0.002 mm)	[%]	22.9	15.6	27.4	16.5	14.8	20.7
Silt (0.002-0.050 mm)	[%]	70.5	20.8	50.2	66.0	37.8	53.5
Sand (0.050-2.00 mm)	[%]	6.6	63.6	22.4	17.5	47.4	25.8
CEC	[meq/100 g]	12.9	16.1	29.9	12.5	7.4	20.0
Chalk	[% $CaCO_3$]	< 0.1	10.8	3.0	< 0.1	< 0.1	12.9
Organic carbon	[% Carbon]	1.01	1.21	3.48	0.80	0.68	1.14
pH ($CaCl_2$)		6.3	7.4	7.4	6.7	6.1	7.5
pH (H_2O)		6.9	8.0	8.1	7.3	6.7	8.3
Wk max	[g/100 g]	45.6	43.2	52.5	43.3	36.2	48.8
Wk pF 2	[Vol%]	45.5	30.5	43.5	42.1	26.3	41.3

For M21 (bixafen-desmethyl) no residues above the LOQ were found in the samples investigated. In the following tables the bixafen residue found in soil and the percent remaining are summarized.

Table 49 Residues of bixafen found in different soils treated with nominal rates of 0.125 kg ai/ha after 0–740 days

Burscheid, Germany		Little Shelford, Great Britain		Esloev, Sweden		Vatteville, North. France		Vilobi d'Onyar, Spain		Albaro, Italy	
DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg
0	97.4	0	107.8	0	132	0	89.9	0	85.1	0	92.3

Burscheid, Germany		Little Shelford, Great Britain		Esloev, Sweden		Vatteville, North. France		Vilobi d'Onyar, Spain		Albaro, Italy	
DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg
12	83.1	13	71.3	14	79.6	14	60.2	13	56.7	14	53.8
25	85.1	27	72.6	28	94.0	28	58.7	27	64.0	27	44.4
56	52.4	55	59.2	56	67.5	56	45.6	55	65.8	57	23.4
104	45.8	102	65.8	100	63.0	107	56.7	100	36.0	100	48.6
151	47.6	146	53.5	150	66.3	153	46.7	154	24.8	150	22.3
201	54.9	211	49.5	209	60.7	210	43.6	223	21.1	209	32.6
266	40.4	270	44.7	276	66.5	267	50.4	273	34.7	272	42.5
363	51.6	365	52.2	365	65.6	364	36.8	377	26.5	366	19.9
481	47.2	481	40.7	480	60.3	484	35.2	504	24.8	479	12.4
592	49.5	613	34.8	578	57.1	607	36.7	603	26.6	601	18.3
726	46.1	740	31.0	724	52.1	734	37.2	729	18.3	730	15.9

Table 50 Percent bixafen remaining in different soils treated with nominal rates of 0.125 kg ai/ha after 0–740 days

Burscheid, Germany		Little Shelford, Great Britain		Esloev, Sweden		Vatteville, North. France		Vilobi d'Onyar, Spain		Albaro, Italy	
DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg	DAT [days]	µg/kg
0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0
12	85.3	13	66.1	14	60.3	14	67.0	13	66.6	14	58.3
25	87.4	27	67.3	28	71.2	28	65.3	27	75.2	27	48.1
56	53.8	55	54.9	56	51.1	56	50.7	55	77.3	57	25.4
104	47.0	102	61.0	100	47.7	107	63.1	100	42.3	100	52.7
151	48.9	146	49.6	150	50.2	153	51.9	154	29.1	150	24.2
201	56.4	211	45.9	209	46.0	210	48.5	223	24.8	209	35.3
266	41.5	270	41.5	276	50.4	267	56.1	273	40.8	272	46.0
363	53.0	365	48.4	365	49.7	364	40.9	377	31.1	366	21.6
481	48.5	481	37.8	480	45.7	484	39.2	504	29.1	479	13.4
592	50.8	613	32.3	578	43.3	607	40.8	603	31.3	601	19.8
726	47.3	740	28.8	724	39.5	734	41.4	729	21.5	730	17.2

Long-term soil accumulation

The possibility of the accumulation of bixafen and M21 (bixafen-desmethyl) in soil under European field conditions was investigated after annual applications of bixafen on bare soil plots at two sites in Monheim am Rhein (Germany) and Tarascon (France) by Heinemann, O and Weuthen, M (2013, BIXAFEN_027). The sites were located in the Monheim, Germany and Les Cayades, Southern France. Each plot received annual treatments to bare soil equivalent to 0.138 kg ai/ha. During the year grass was sown on these plots and incorporated before the next application.

Table 51 Soil properties for the long-term soil dissipation study with bixafen

		Depth (cm)			
		Monheim		Les Cayades	
Soil property	Units	0–30	30–50	0–30	30–50
Soil type (USDA)		sandy loam	sandy loam	silt loam	silt loam
lay (< 0.002 mm)	[%]	10.5	8.5	19.5	27.1
Silt (0.002–0.050 mm)	[%]	18.2	21.3	62.8	53.9
Sand (0.050–2.00 mm)	[%]	71.3	70.2	17.7	19.0
CEC	[meq/100 g]	7.1	3.8	12.1	12.8
Chalk	[% $CaCO_3$]	< 0.1	< 0.1	40.6	38.6
Organic carbon	[% Carbon]	1.28	0.43	0.74	0.89
pH ($CaCl_2$)		6.3	6.6	7.5	7.7

		Depth (cm)			
		Monheim		Les Cayades	
Soil property	Units	0–30	30–50	0–30	30–50
pH (H_2O)		6.9	7.3	8.4	8.7
Max water capacity	[g/100 g]	39.7	33.8	40.3	42.7
pF 2	[Vol%]	26.9	23.5	36.4	35.9

Since 2005 soil samples were collected and analysed for residues of bixafen and M21. Soil samples were extracted with acetonitrile/water (4/1, v/v). Identification and quantitation of the analytes was done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode. The limit of quantitation (LOQ) of the method was 5.0 µg/kg, the limit of detection (LOD) was 1.5 µg/kg for both analytes.

Following the 1st application in spring 2005, the experiments were either terminated when a plateau concentration was reached (France in autumn 2010), or due to technical reasons (Germany in autumn 2012).

Following eight consecutive annual treatments in Germany, the total soil residue of Bixafen was degraded to 47% (based on a nominal concentration of 734 µg/kg resulting from eight applications of 137.7 g/ha each and the highest peak concentration of 347.5 µg/kg soil). However, a plateau in the soil concentration of bixafen was not observed.

Following six consecutive annual treatments in France, the total soil residue of Bixafen in autumn, i.e. prior to winter dormancy, was degraded to 32.5% (based on a nominal concentration of 551 µg/kg resulting from six applications of 137.7 g/ha each and the highest peak concentration of 179.2 µg/kg soil). A plateau was reached at the end of the study.

At both sites, the residues of M21 were low and represented less than 5% of the nominal Bixafen concentration applied until the respective sampling point in time.

In the following tables the residues of bixafen and M21 (bixafen-desmethyl) in soil over 2720 days are summarized. Although soil samples up to a depth of 100 cm were collected, residues are only presented for soil layers containing detectable residues.

Table 52 Residues of bixafen and bixafen-desmethyl after annual application in Monheim, Germany at rates of 0.138 kg ai/ha

Days & appl.	Bixafen in µg/kg soil																							
	0 [a]	0 /1st	194	353 /2nd	354	565	710	711 /3rd	914	1082	1082 /4th	1284	1460	1460 /6th	1665	1797	1804 /7th	2021	2161	2176 /8th	2392	2524	2526 /9th	2720
Layer in cm	Bixafen																							
0–10	n.d.	81.5	51.8	59.9	115.6	101	90.3	144	146.1	137.6	212.3	161.2	159.3	239.8	177.2	240.6	280.6	226.9	223.5	319.5	273	261.5	347.5	292
10–20	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	7.09	18.27	12.97	8.68	16.81	12.27	9.7	18.35	15.11	21.5	24.7	19.7	34.9	26.9	32.6	28.1
20–30	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	5.5	5.1	5.3	10.3	7.4	8.4	7.4
30–40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
40–50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
50–60	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	< 5	n.a.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
60–70	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	< 5	< 5	n.d.	n.d.	< 5	< 5	< 5
70–80	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	< 5	n.d.	n.d.	< 5	< 5	< 5
80–90	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5
90–100	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.d.	< 5	< 5	< 5	8	< 5
	M21 (bixafen-desmethyl)																							
0–10	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	5.2	5.3	5.2	6.2	7.5	7.6	9.0	10.2	9.5	13.1	14.0	14.6	17.4	16.2	16.1	24.7
10–20	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
20–30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	n.d.	n.d.	< 5

n.a. = Not analysed

n.d. = Not detected (LOD: 1.5 µg/kg soil)

Table 53 Residues of bixafen and bixafen-desmethyl after annual application in Les Cayades, Southern France at rates of 0.138 kg ai/ha

Days & appl.	Bixafen in µg/kg soil																
	0 [a]	0/1st	215	334	335	570	697	698	913	1062	1063	1293	1426	1427	1655	1795	1797	2015
Layer in cm	Bixafen																	
0–10	n.d.	79.5	27.1	34.5	120.8	52.3	54.5	132.3	93.3	91.2	140.4	73.6	106.4	154.6	91.5	106.7	179.2	101.4
10–20	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	6.3	< 5	< 5	6.2	< 5	< 5	5.5	7.4
20–30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
30–40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	n.d.	< 5
40–50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	n.d.	n.d.	n.d.
50–60	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	< 5	n.a.	n.a.	n.a.	n.a.
	M21 (bixafen-desmethyl)																	
0–10	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	5.6	< 5	< 5	5.2	< 5	< 5
10–20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

n.a. = Not analysed

n.d. = Not detected (LOD: 1.5 µg/kg soil)

RESIDUE ANALYSIS

Analytical methods

For bixafen and M21 bixafen-desmethyl analytical method were provided following a comparable pattern. The samples (plant, animal and soil) are extracted using acetonitrile/water (4/1, v/v) or in case of fatty matrices n-hexane (saturated with acetonitrile). For some matrices the extraction is supported by using microwaves. After filtration and a clean-up with C18 SPE the residue is measured by HPLC-MS/MS with two specific mass transitions for each analyte. All methods were validated with an LOQ of 0.01 mg/kg.

The applicability of multi residue methods was investigated for the DFG S19; however, the results did not confirm sufficient applicability.

Table 54 Overview of analytical methods for bixafen

Method	Matrix	Extraction	Clean-Up	Detection, LOQ
Method 00983	Plants (high acid, high, starch, high oil, high water)	acetonitrile/water (4/1, v/v), using microwaves	filtration	HPLC-MS/MS Bixafen: m/z 414 → 394 and m/z 414→ 266 LOQ: 0.01 mg/kg
Method 01063	Animals (eggs, milk, muscle, kidney, fat, liver)	eggs, muscle, kidney and milk: acetonitrile/water (4/1, v/v) liver: acetonitrile/water (4/1, v/v), using microwaves fat: n-hexane (saturated with acetonitrile), partitioning with acetonile (saturated with n-hexane)	filtration & C18 SPE	HPLC-MS/MS Bixafen: m/z 414 → 394 and m/z 414→ 266 LOQ: 0.01 mg/kg M21: m/z 398 → 378 and m/z 398→ 358 LOQ: 0.01 mg/kg
Method 00959 & Method 00959/M001	Soil	acetonitrile/water (4/1, v/v), using microwaves	centrifugation	HPLC-MS/MS Bixafen: m/z 414 → 394 and m/z 414→ 266 LOQ: 5 µg/kg M21: m/z 398 → 378 and m/z 398→ 358 LOQ: 5 µg/kg
Method 01012	Plants (high, starch, high water)	acetonitrile/water (4/1, v/v), using microwaves	filtration	HPLC-MS/MS with isotopically-labelled ISTD Bixafen: m/z 414 → 394 and m/z 414→ 266 LOQ: 0.01 mg/kg M21: m/z 398 → 378 and m/z 398→ 358 LOQ: 0.01 mg/kg
Method 01013	Plants (high acid, high, starch, high oil, high water)	acetonitrile/water (4/1, v/v) containing cysteine hydrochloride	filtration	HPLC-MS/MS with isotopically-labelled ISTD Bixafen: m/z 414 → 394 and m/z 414→ 266 LOQ: 0.01 mg/kg M21: m/z 398 → 378 and

Method	Matrix	Extraction	Clean-Up	Detection, LOQ
				m/z 398→ 358 LOQ: 0.01 mg/kg
Method 01036	Animals (eggs, milk, muscle, kidney, fat, liver)	eggs, muscle, kidney and milk: acetonitrile/water (4/1, v/v) liver: acetonitrile/water (4/1, v/v), using microwaves fat and milk cream: n-hexane (saturated with acetonitrile), partitioning with acetonile (saturated with n-hexane)	filtration & C18 SPE	HPLC-MS/MS with isotopically-labelled ISTD Bixafen: m/z 414 → 394 and m/z 414→ 266 LOQ: 0.01 mg/kg M21: m/z 398 → 378 and m/z 398→ 358 LOQ: 0.01 mg/kg

ISDT = Internal standard

Plant materials

For plant materials a suitable enforcement method (method 00983) was developed by Bardel, P and Schoening, R (2006, BIXAFEN_034). The residues are extracted from 5 g of plant material with acetonitrile/water (4/1, v/v), using microwaves. After filtration and dilution, the solution was analysed by HPLC-MS/MS (m/z 414 → 394 and m/z 414→ 266). Residues were quantified against external bracketing matrix-matched standards.

The applicability of the method was confirmed in an independent laboratory by Ballesteros C and Portet, M (2007, BIXAFEN_035). In both laboratories parent bixafen was analysed with a validated LOQ of 0.01 mg/kg (see Table).

The applicability of the DFG method S 19 multi-residue method was investigated by Class, T (2006, BIXAFEN_036). However, even though GC gave acceptable peak shapes for bixafen, it was assessed that the DFG method S 19 used in combination with GC/MS is not applicable as a multi-residue enforcement method due to a lack of sensitivity, even when the most prominent fragment ion observed was monitored, and a lack of specifity, as no other ions were observed with sufficient sensitivity to serve as confirmatory ions. Thus, it was shown that DFG method S 19 is not suitable for the determination of bixafen residues.

Table 55 Recovery data for the determination of bixafen in plant commodities

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Wheat grain	0.01	5	98–102	101	1.7	Bixafen,
	0.1	5	87–100	95	5.2	Bardel, P and Schoening, R
Wheat, green material	0.01	5	80–100	90	9.6	(2006, BIXAFEN_034)
	0.1	5	90–103	96	4.8	m/z 414 → 394
Orange, fruit	0.01	5	88–98	93	4.9	
	0.1	5	78–95	86	8.0	
Rape, seed	0.01	5	86–978	92	5.0	
	0.1	5	92–95	93	1.2	
Wheat grain	0.01	5	94–107	102	4.9	Bixafen,
	0.1	5	81–101	94	8.3	Bardel, P and Schoening, R
Wheat, green material	0.01	5	81–105	94	12.1	(2006, BIXAFEN_034)
	0.1	5	88–99	96	4.8	m/z 414 → 266 (confirmation)
Orange, fruit	0.01	5	85–998	92	7.0	
	0.1	5	77–90	84	6.9	
Rape, seed	0.01	5	91–101	95	4.9	

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
	0.1	5	93–97	95	1.9	
Wheat grain	0.01	5	89–98	93	3.6	Bixafen,
	0.1	5	98–101	99	1.1	Ballesteros, C and Portet, M
Orange, fruit	0.01	5	93–97	94	2.3	(2007, BIXAFEN_035)
	0.1	5	99–106	101	2.8	m/z 414 → 394
Rape, seed	0.01	5	86–93	88	3.1	Independent laboratory validation
	0.1	5	89	89	0	

Animal materials

For the enforcement of bixafen and M21 (bixafen-desmethyl) in animal commodities method 01063 was developed by Billian, P and Druskus, M (2007, BIXAFEN_037). The residues were extracted from 5 g of milk, muscle, liver, kidney and egg with acetonitrile/water (4/1, v/v) using an ultra-turax (for liver with microwave assistance).

The extraction of fat samples was performed with n-hexane (saturated with acetonitrile) in a blender. After filtration, addition of acetonitrile (saturated with n-hexane) and subsequent shaking, the acetonitrile phase was separated.

The solutions were cleaned up on a C18 cartridge, made up to volume, diluted, and subjected to reversed phase HPLC-MS/MS in positive (parent compound, M21 bixafen-desmethyl) or negative (bixafen-desmethyl) ion modes. Two MRM transitions were monitored for bixafen (m/z 414 → 394 and m/z 414→ 266) and M21 (bixafen-desmethyl, m/z 398 → 378 and m/z 398→ 358). Residues were quantified against matrix-matched standards (without using the ISTD signal for calculation of the results).

The applicability of the method was confirmed in an independent laboratory by Ballesteros, C (2007, BIXAFEN_038). In both laboratories parent bixafen and M21 (bixafen-desmethyl) were validated with a LOQ of 0.01 mg/kg (see Table & Table).

Table 56 Recovery data for the determination of bixafen in animal commodities

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Egg (without shell)	0.01	5	80–103	92	9.4	Bixafen,
	0.1	5	91–101	96	4.1	Billian, P and Druskus, M
Milk	0.01	5	85–98	91	5.9	(2007, BIXAFEN_037)
	0.1	5	86–98	91	6.4	m/z 414 → 394
Muscle	0.01	5	90–105	97	6.1	
	0.1	5	92–100	95	3.6	
Kidney	0.01	5	83–98	93	6.4	
	0.1	5	95–113	103	7.4	
Fat	0.01	5	90–99	95	3.5	
	0.1	5	93–104	98	4.6	
Liver	0.01	5	88–100	95	4.5	
	0.1	5	98–104	101	2.8	
Egg (without shell)	0.01	5	84–104	93	8.5	Bixafen,
	0.1	5	91–100	96	3.6	Billian, P and Druskus, M
Milk	0.01	5	86–99	92	5.8	(2007, BIXAFEN_037)
	0.1	5	85–99	91	7.0	m/z 414 → 266 (confirmation)
Muscle	0.01	5	90–105	96	5.8	
	0.1	5	92–100	95	3.8	
Kidney	0.01	5	84–96	93	5.4	
	0.1	5	94–113	102	7.6	
Fat	0.01	5	90–96	93	2.6	
	0.1	5	91–102	96	4.8	
Liver	0.01	5	89–100	96	4.5	
	0.1	5	98–103	100	2.3	

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Egg (without shell)	0.01	5	89–97	94	3.9	Bixafen,
	0.1	5	88–95	92	3.2	Ballesteros C
Milk	0.01	5	111–123	115	4.2	(2007, BIXAFEN_038)
	0.1	5	106–114	110	3.3	m/z 414 → 394
Fat	0.01	5	101–106	103	1.8	Independent laboratory validation
	0.1	5	93–100	95	3.2	
Liver	0.01	5	80–100	92	8.1	
	0.1	5	84–116	97	13.6	
Egg (without shell)	0.01	5	92–97	94	2.4	Bixafen,
	0.1	5	87–94	91	3.2	Ballesteros C
Milk	0.01	5	106–121	113	5.1	(2007, BIXAFEN_038)
	0.1	5	104–113	108	3.6	m/z 414 → 266 (confirmation)
Fat	0.01	5	100–104	102	2.0	Independent laboratory validation
	0.1	5	91–97	93	2.8	
Liver	0.01	5	81–98	93	7.6	
	0.1	5	82–115	95	13.7	

Table 57 Recovery data for the determination of M21 (bixafen-desmethyl) in animal commodities

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Egg (without shell)	0.01	5	78–104	90	11.1	M21 (bixafen-desmethyl),
	0.1	5	90–97	94	3.2	Billian, P and Druskus, M
Milk	0.01	5	90–101	94	4.9	(2007, BIXAFEN_037)
	0.1	5	88–108	97	9.0	m/z 398 → 378
Muscle	0.01	5	88–95	92	3.0	
	0.1	5	90–102	95	4.8	
Kidney	0.01	5	76–101	91	10.8	
	0.1	5	74–87	80	7.3	
Fat	0.01	5	84–92	89	3.5	
	0.1	5	96–111	103	5.8	
Liver	0.01	5	93–99	96	2.7	
	0.1	5	95–101	98	2.4	
Egg (without shell)	0.01	5	77–102	89	10.5	M21 (bixafen-desmethyl),
	0.1	5	89–97	94	3.6	Billian, P and Druskus, M
Milk	0.01	5	88–100	92	5.3	(2007, BIXAFEN_037)
	0.1	5	89–108	98	8.9	m/z 398 → 358 (confirmation)
Muscle	0.01	5	88–93	90	2.4	
	0.1	5	90–102	95	4.7	
Kidney	0.01	5	76–98	91	9.4	
	0.1	5	73–87	80	7.2	
Fat	0.01	5	83–93	89	4.4	
	0.1	5	97–111	103	5.5	
Liver	0.01	5	91–99	95	3.2	
	0.1	5	96–102	99	2.4	
Egg (without shell)	0.01	5	89–99	94	3.9	M21 (bixafen-desmethyl),
	0.1	5	91–98	94	3.3	Ballesteros C
Milk	0.01	5	95–107	99	4.8	(2007, BIXAFEN_038)
	0.1	5	105–116	112	3.9	m/z 398 → 378
Fat	0.01	5	92–101	97	3.4	Independent laboratory validation
	0.1	5	95–103	99	3.4	
Liver	0.01	4	102–108	106	2.7	
	0.1	5	106–114	112	2.9	
Egg (without shell)	0.01	5	90–97	94	2.7	M21 (bixafen-desmethyl),
	0.1	5	91–99	94	3.3	Ballesteros C
Milk	0.01	5	94–111	101	6.5	(2007, BIXAFEN_038)
	0.1	5	104–119	113	5.0	m/z 398 → 358 (confirmation)
Fat	0.01	5	94–102	98	3.8	Independent laboratory validation
	0.1	5	96–104	100	3.3	

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Liver	0.01	4	104–112	109	3.1	
	0.1	5	109–116	113	2.4	

Soil

For the analysis of bixafen in soil method 00959 was developed by Brumhard, B and Freitag, T (2006, BIXAFEN_039). Residues of bixafen are extracted from approx. 20 g of soil in a microwave extractor with 40 mL of a mixture of acetonitrile/water (4+1, v+v). Then a subsample is centrifuged to remove fine particles of the soil.

Identification and quantitation of the test items is done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode (MRM). The first MRM transition of bixafen is the quantification ion with the mass 394 [m/z 414 → 394] and the second MRM transition is the confirmatory ion with the mass 266 [m/z 414 → 266]. The analytical method was successfully validated with a LOQ of 5 µg/kg soil.

An extension of this method for the determination of the metabolite M21 (bixafen-desmethyl) was reported by Brumhard, B and Koch, V (2008, BIXAFEN_048). The extraction of the residue is identical to the methodology described for parent bixafen. For the HPLC-MS/MS measurement the following mass transitions are used for bixafen-desmethyl: m/z 398 → 378 and m/z 398→ 358.

Table 58 Recovery data for analytical method 00959 measuring bixafen in soil

Matrix	Fortification level (µg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Soil "Höfchen"	5	15	79–108	97	7.2	Bixafen,
	50	15	69–101	82	11.5	Brumhard, B and Freitag, T
Soil "Laacher Hof"	5	10	79–103	87	8.1	(2006, BIXAFEN_039)
	50	10	68–94	79	9.8	m/z 414 → 394
Soil "Höfchen"	5	15	84–109	98	7.6	Bixafen,
	50	15	69–100	82	11.5	Brumhard, B and Freitag, T
Soil "Laacher Hof"	5	10	82–113	89	10.3	(2006, BIXAFEN_039)
	50	10	68–94	79	10.2	m/z 414 → 266 (confirmation)

Table 59 Recovery data for analytical method 00959/M001 measuring M21 (bixafen-desmethyl) in soil

Matrix	Fortification level (µg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Soil "Höfchen"	5	5	89–100	95	4.3	Bixafen,
	50	5	90–95	94	2.3	Brumhard, B and Koch, V
Soil "Laacher Hof"	5	5	86–97	91	4.9	(2008, BIXAFEN_48)
	50	5	91–96	93	1.9	m/z 398 → 378
Soil "Höfchen"	5	5	93–98	95	2.6	Bixafen,
	50	5	92–96	93	1.6	Brumhard, B and Koch, V
Soil "Laacher Hof"	5	5	94–99	97	2.4	(2008, BIXAFEN_48)
	50	5	89–94	92	2.2	m/z 398 → 358 (confirmation)

Specialised methods

For the data generation in supervised field trials and processing studies method 01012 was developed by Schoening, R (2006, BIXAFEN_040). Residues were extracted from 5 g of plant material with acetonitrile/water (4/1, v/v), using microwaves. After filtration of the extract, the stable isotopically-labelled analytes were added as internal standards (ISTD). The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS in positive (bixafen, bixafen-desmethyl) or negative (bixafen-desmethyl) ion modes without further clean-up. Two MRM transitions for quantitation and confirmation were monitored for each analyte and in each matrix tested (bixafen: m/z 414→ 394 and 414→ 266; bixafen-desmethyl: m/z 398→ 378 and 398→ 358).

The extraction efficiency of method 01012 was investigated on wheat samples from the [dichlorophenyl-UL-^{14}C]-wheat metabolism study (Sur, R and Kuhnke, G, 2007, BIXAFEN_041) and on samples from the confined rotational crop study (Justus, K and Kuhnke, G, 2007, BIXAFEN_042). In summary method 01012 was validated with an LOQ of 0.01 mg/kg for each analyte. Validation data and the extraction efficiencies are summarised in Table

Table 60 Recovery data for analytical method 01012 measuring bixafen in plant matrices

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Wheat, grain	0.01	5	93–97	95	1.4	Bixafen,
	0.1	5	94–95	95	0.3	Schoening, R
Wheat, straw	0.01	5	87–92	89	1.9	(2006, BIXAFEN_040)
	0.1	5	83–90	87	3.3	m/z 414 → 394
Wheat, green material	0.01	5	88–99	92	4.9	
	0.1	5	88–93	90	2.2	
Lettuce, head	0.01	5	96–99	98	1.5	
	0.1	5	95–98	96	1.5	
Turnip, body	0.01	5	94–96	95	1.3	
	0.1	5	94–96	95	0.6	
Wheat, grain	0.01	5	96–99	98	1.1	Bixafen,
	0.1	5	95–96	96	0.6	Schoening, R
Wheat, straw	0.01	5	85–90	88	2.5	(2006, BIXAFEN_040)
	0.1	5	84–90	87	2.8	m/z 414 → 266 (confirmation)
Wheat, green material	0.01	5	87–95	90	4.1	
	0.1	5	86–94	90	3.5	
Lettuce, head	0.01	5	91–95	93	1.9	
	0.1	5	88–100	95	5.7	
Turnip, body	0.01	5	92–100	95	3.4	
	0.1	5	94–99	96	1.8	

Table 61 Recovery data for analytical method 01012 measuring M21 (bixafen-desmethyl) in plant matrices

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Wheat, grain	0.01	5	93–100	96	2.9	M21 (bixafen-desmethyl),
	0.1	5	94–99	97	1.8	Schoening, R
Wheat, straw	0.01	5	84–90	86	2.8	(2006, BIXAFEN_040)
	0.1	5	81–88	86	3.0	m/z 398 → 378
Wheat, green material	0.01	5	86–93	90	2.9	
	0.1	5	88–92	90	1.8	
Lettuce, head	0.01	5	90–95	92	2.2	
	0.1	5	94–98	96	1.3	
Turnip, body	0.01	5	92–98	95	2.5	
	0.1	5	94–97	96	1.5	
Wheat, grain	0.01	5	92–97	94	1.9	M21 (bixafen-desmethyl),
	0.1	5	89–95	92	2.3	Schoening, R
Wheat, straw	0.01	5	86–92	90	2.6	(2006, BIXAFEN_040)
	0.1	5	82–87	85	2.5	m/z 398 → 358 (confirmation)
Wheat, green material	0.01	5	87–93	89	3.2	
	0.1	5	88–93	90	1.9	
Lettuce, head	0.01	5	93–97	95	1.6	
	0.1	5	92–95	94	1.2	
Turnip, body	0.01	5	95–103	98	3.1	

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
	0.1	5	96–100	97	1.6	

Table 62 Extraction efficiency of method 01012

Matrix	Reference	Method	% of TRR (mg/kg)					
			TRR	combined extracts	unextracted	bixafen	M21 (bixafen-desmethyl)	Total [a]
Wheat, forage (from wheat metabolism)	Sur, R and Kuhnke, G, 2007, BIXAFEN_041	metabolism study	100 (1.57)	99.0 (1.56)	1.0 (0.02)	97.1 (1.53)	0.8 (0.01)	97.9 (1.54)
		method 01012	100 (1.53)	97.7 (1.50)	2.3 (0.04)	96.1 (1.47)	0.9 (0.01)	96.9 (1.49)
Wheat, grain (from wheat metabolism)	Sur, R and Kuhnke, G, 2007, BIXAFEN_041	metabolism study	100 (0.229)	97.0 (0.222)	3.0 (0.007)	92.9 (0.213)	2.1 (0.005)	95.0 (0.218)
		method 01012	100 (0.174)	95.1 (0.166)	4.9 (0.009)	91.7 (0.160)	1.6 (0.003)	93.3 (0.163)
Wheat, straw (from wheat metabolism)	Sur, R and Kuhnke, G, 2007, BIXAFEN_041	metabolism study	100 (22.85)	96.1 (21.95)	3.9 (0.90)	93.2 (21.29)	1.7 (0.39)	94.9 (21.68)
		method 01012	100 (32.23)	95.3 (30.73)	4.7 (1.50)	92.2 (29.71)	1.9 (0.62)	94.1 (30.34)
Wheat, straw (from confined rotation crops study)	Justus, K and Kuhnke, G, 2007, BIXAFEN_042	metabolism study	100 (0.492)	95.3 (0.469)	4.7 (0.023)	36.9 (0.181)	57.2 (0.281)	94.1 (0.462)
		method 01012	100 (0.536)	68.0 (0.365)	32.0 (0.171)	28.2 (0.151)	39.8 (0.213)	68.0 (0.365)
Wheat, forage (from confined rotation crops study)	Justus, K and Kuhnke, G, 2007, BIXAFEN_042	metabolism study	100 (0.035)	82.7 (0.029)	17.3 (0.006)	20.7 (0.007)	61.9 (0.022)	82.7 (0.029)
		method 01012	100 (0.037)	72.6 (0.027)	27.4 (0.010)	19.6 (0.007)	53.0 (0.020)	72.6 (0.027)

[a] Sum of bixafen and M21 (bixafen-desmethyl), expressed as bixafen equivalents

For plant commodities a second study used in supervised field trials was conducted by Brumhard, B and Stuke, S (2008, BIXAFEN_043). Multi method 01013 was developed for the determination of residues of bixafen, prothioconazole, tebuconazole, trifloxystrobin and their metabolites bixafen-desmethyl, prothioconazole-desthio and CGA321113 in/on plant material (citrus fruit, pea green seed, wheat grain, rape seed and corn green material). All analytes are extracted from plant materials using a mixture of acetonitrile/water (4/1; v/v) containing cysteine hydrochloride by using a blender. After filtration of the extract, the stable isotopically labelled analytes were added. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS without a further clean-up step.

Bixafen was detected using electrospray ionization in the positive ion mode (ESI+), while M21 (bixafen-desmethyl) was detected using electrospray ionization in the negative ion mode (ESI-). Two MRM transitions for quantitation and confirmation were monitored for each analyte and in each matrix tested (bixafen: m/z 414→ 394 and 414→ 266; bixafen-desmethyl: m/z 398→ 378 and 398→ 358). Residues were quantified using internal stable-labelled standards.

In the following tables the validation data for bixafen and M21 (bixafen-desmethyl) are summarised. The analytical method was successfully validated with LOQs of 0.01 mg/kg for bixafen and M21 (bixafen-desmethyl).

Table 63 Recovery data for analytical method 01013 measuring bixafen in plant matrices

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Citrus fruit	0.01	5	95–114	105	6.5	Bixafen,
	0.1	5	90–100	97	4.4	Brumhard, B and Stuke, S
Pea, green seed	0.01	5	97–106	101	3.5	(2008, BIXAFEN_043)
	0.1	5	98–101	99	1.2	m/z 414 → 394
Rape, seed	0.01	5	101–117	106	6.2	
	0.1	5	96–103	100	3.1	
Wheat, grain	0.01	5	100–104	102	1.6	
	0.1	5	96–102	99	2.3	
Maize, green material	0.01	5	104–114	109	3.5	
	0.1	5	100–110	105	4.0	
Citrus fruit	0.01	5	96–113	106	6.0	Bixafen,
	0.1	5	96–108	104	4.6	Brumhard, B and Stuke, S
Pea, green seed	0.01	5	80–95	85	7.1	(2008, BIXAFEN_043)
	0.1	5	90–101	96	4.5	m/z 414 → 266 (confirmation)
Rape, seed	0.01	5	95–104	101	4.1	
	0.1	5	95–100	97	2.2	
Wheat, grain	0.01	5	97–108	103	4.8	
	0.1	5	91–100	94	4.5	
Maize, green material	0.01	5	101–118	110	6.4	
	0.1	5	102–114	107	4.4	

Table 64 Recovery data for analytical method 01013 measuring M21 (bixafen-desmethyl) in plant matrices

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Citrus fruit	0.01	5	98–107	103	3.2	M21 (bixafen-desmethyl),
	0.1	5	91–102	98	4.8	Brumhard, B and Stuke, S
Pea, green seed	0.01	5	92–106	99	5.3	(2008, BIXAFEN_043)
	0.1	5	94–103	98	3.4	m/z 398 → 378
Rape, seed	0.01	5	100–110	104	4.1	
	0.1	5	98–107	101	3.9	
Wheat, grain	0.01	5	96–103	100	2.7	
	0.1	5	97–101	99	1.8	
Maize, green material	0.01	5	103–115	111	4.3	
	0.1	5	100–109	104	3.5	
Citrus fruit	0.01	5	98–108	103	4.1	M21 (bixafen-desmethyl),
	0.1	5	90–102	97	4.6	Brumhard, B and Stuke, S

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Pea, green seed	0.01	5	91–102	97	5.2	(2008, BIXAFEN_043)
	0.1	5	92–102	99	4.2	m/z 398 → 358 (confirmation)
Rape, seed	0.01	5	98–112	103	5.6	
	0.1	5	93–102	97	3.5	
Wheat, grain	0.01	5	97–103	99	2.6	
	0.1	5	96–102	99	2.4	
Maize, green material	0.01	5	102–114	110	4.6	
	0.1	5	101–111	106	4.4	

In the livestock animal feeding study the analytical method 01036 was used, which was described by Schoening, R and Willmes, J (2008, BIXAFEN_044).

Skim milk and cream first were separated by using a centrifuge. The residues were extracted from 5 g of whole milk, skim milk, muscle, kidney, and egg with acetonitrile/water (4/1, v/v) using an ultra-turax. After filtration and clean-up on a C18 Cartridge the stable labelled internal standards were added to the extracts.

Residues in fat and milk cream were extracted from 5 g sample material with n-hexane (saturated with acetonitrile) first in an ultra-turax. After filtration and clean-up through a syringe tube, acetonitrile (saturated with n-hexane) was added, and, after shaking, the acetonitrile phase was separated. Subsequently the stable isotopically labelled standards were added.

Residues in liver were extracted from 5 g sample material with acetonitrile/water (4/1, v/v), either directly by using a microwave (cattle liver) or for chicken liver by using a blender first and a subsequent microwave extraction of the solids with acetonitrile/water (7/3, v/v). After filtration and clean-up on a C18 cartridge, the stable isotopically labelled standards were added to the extracts.

The solutions were made up to volume, diluted, filtered through a membrane filter and subjected to reversed phase HPLC-MS/MS in positive (parent compound, BYF00587-desmethyl) or negative (BYF00587-desmethyl) ion modes. Two MRM transitions for quantitation and confirmation were monitored for each analyte and in each matrix tested (bixafen: m/z 414→ 394 and 414→ 266; bixafen-desmethyl: m/z 398→ 378 and 398→ 358). Residues were quantified using internal stable-labelled standards.

In the following tables the validation data for bixafen and M21 (bixafen-desmethyl) are summarised. The analytical method was successfully validated with LOQs of 0.01 mg/kg for bixafen and M21 (bixafen-desmethyl).

Table 65 Recovery data for analytical method 01036 measuring bixafen in animal matrices

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Egg (without shell)	0.01	5	97–107	102	3.6	Bixafen,
	0.1	5	98–104	101	2.3	Schoening, R and Willmes, J
Egg yolk	0.01	3	91–98	95	3.8	(2008, BIXAFEN_044)
	0.1	3	101–102	101	0.6	m/z 414 → 394
Egg white	0.01	3	91–105	99	7.3	
	0.1	3	100–105	102	2.5	
Milk, whole	0.01	5	87–105	98	6.8	

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
	0.1	5	99–104	101	2.1	
Skim milk	0.01	3	91–104	97	6.8	
	0.1	3	97–102	100	2.9	
Milk cream	0.01	3	93–95	94	1.1	
	0.1	3	94–95	94	0.6	
Muscle	0.01	5	92–105	98	5.4	
	0.1	5	89–100	96	4.4	
Kidney	0.01	5	91–99	94	3.3	
	0.1	5	97–99	98	0.7	
Poultry liver	0.01	5	95–100	91	4.8	
	0.1	5	96–100	97	2.1	
Cattle liver	0.01	5	96–102	99	2.9	
	0.1	5	96–100	97	1.7	
Fat	0.01	5	83–101	92	7.8	
	0.1	5	93–99	96	2.6	
Egg (without shell)	0.01	5	92–99	95	3.2	Bixafen,
	0.1	5	99–104	101	1.9	Schoening, R and Willmes, J
Egg yolk	0.01	3	80–99	91	11.0	(2008, BIXAFEN_044)
	0.1	3	95–98	96	1.6	m/z 414 → 266 (confirmation)
Egg white	0.01	3	96–115	104	9.7	
	0.1	3	100–104	102	2.0	
Milk, whole	0.01	5	97–113	103	6.3	
	0.1	5	105–111	107	2.1	
Skim milk	0.01	3	103–119	110	7.4	
	0.1	3	96–102	99	3.0	
Milk cream	0.01	3	69–103	99	3.6	
	0.1	3	94–96	95	1.1	
Muscle	0.01	5	93–104	100	5.0	
	0.1	5	95–108	103	5.1	
Kidney	0.01	5	86–103	97	6.8	
	0.1	5	95–98	97	1.3	
Poultry liver	0.01	5	85–96	90	4.9	
	0.1	5	98–103	100	2.1	
Cattle liver	0.01	5	73–104	90	12.7	
	0.1	5	95–97	96	0.9	
Fat	0.01	5	84–108	99	9.3	
	0.1	5	96–104	101	3.2	

Table 66 Recovery data for analytical method 01036 measuring M21 (bixafen-desmethyl) in animal matrices

Matrix	Fortification level (mg/kg)	n	Recovery range (%)	Recovery, mean (%)	RSD (%)	Analyte, reference, MRM transition
Egg (without shell)	0.01	5	91–102	97	4.1	M21 (bixafen-desmethyl),
	0.1	5	94–101	97	2.7	Schoening, R and Willmes, J
Egg yolk	0.01	3	85–103	95	9.6	(2008, BIXAFEN_044)
	0.1	3	94–103	99	4.6	m/z 398 → 378
Egg white	0.01	3	102–105	103	1.7	
	0.1	3	103–107	105	2.0	
Milk, whole	0.01	5	94–104	99	4.0	
	0.1	5	96–102	99	2.4	
Skim milk	0.01	3	91–104	98	6.7	
	0.1	3	97–101	99	2.0	
Milk cream	0.01	3	91–99	94	4.6	
	0.1	3	98–102	100	2.0	
Muscle	0.01	5	95–109	101	5.1	
	0.1	5	89–98	96	4.0	
Kidney	0.01	5	89–100	95	5.2	
	0.1	5	94–97	95	1.4	
Poultry liver	0.01	5	83–100	93	7.5	
	0.1	5	93–96	94	1.5	
Cattle liver	0.01	5	92–99	94	3.1	
	0.1	5	94–96	95	1.4	
Fat	0.01	5	84–100	94	5.6	
	0.1	5	88–98	93	4.0	
Egg (without shell)	0.01	5	89–102	96	6.1	M21 (bixafen–desmethyl),
	0.1	5	91–96	94	2.8	Schoening, R and Willmes, J
Egg yolk	0.01	3	90–102	98	6.8	(2008, BIXAFEN_044)
	0.1	3	97–103	99	3.2	m/z 398 → 358 (confirmation)
Egg white	0.01	3	99–109	103	5.0	
	0.1	3	100–104	102	2.0	
Milk, whole	0.01	5	90–104	99	5.8	
	0.1	5	93–99	97	2.6	
Skim milk	0.01	3	91–110	99	9.9	
	0.1	3	92–97	94	2.7	
Milk cream	0.01	3	93–95	94	1.2	
	0.1	3	86–92	89	3.4	
Muscle	0.01	5	93–112	100	7.3	
	0.1	5	88–99	95	4.8	
Kidney	0.01	5	90–102	98	5.0	
	0.1	5	97–105	102	3.1	
Poultry liver	0.01	5	87–96	91	3.9	
	0.1	5	93–96	95	1.2	
Cattle liver	0.01	5	88–100	94	5.3	
	0.1	5	93–97	95	1.6	
Fat	0.01	5	81–101	90	8.1	
	0.1	5	90–99	94	4.1	

Stability of pesticides in stored analytical samples

The storage stability of bixafen and M21 (bixafen-desmethyl) has been investigated in wheat grain, wheat straw, wheat green material, lettuce head, potato tuber, rape seed and soil for a storage period of up to 24 months. No significant degradation of the residues was observed.

For animal matrices no storage stability data were provided. Samples in livestock metabolism or feeding studies were analysed within one month.

The storage stability of bixafen and its primary metabolite M21 (bixafen-desmethyl) was investigated in plant matrices (wheat grain, wheat straw, wheat green material, lettuce head, potato tuber and rape seed) for up to 24 months by Schoening, R and Billian, P (2009, BIXAFEN_045).

Homogenised samples were weighed into glass bottles and fortified individually at levels of 0.1 mg/kg for each analyte. After fortification, the solvent was allowed to evaporate. In addition, untreated samples of each sample material were prepared for control and recovery experiments. Subsequently the bottles were closed and stored deep frozen until analysis (except for the day 0 samples). At each sampling interval, three fortified and three control samples were removed from the deep-freezer. Subsequently, two of the control samples of each sample material were freshly fortified with a mixture of the test items to determine the concurrent recoveries. Fortification levels were at the same magnitude as the spiked storage samples. Bixafen and desmethyl-bixafen were spiked separately to separate control material.

The analytical method 01012 was used for the determination of bixafen and its metabolite desmethyl-bixafen in/on plant material. The analytes were extracted with acetonitrile/water (4/1, v/v) using a microwave. After filtration of the extract, the stable isotopically-labelled analytes were added as internal standards. The solution was made up to volume, diluted and subjected to reversed phase HPLC-MS/MS in positive and negative ion modes without further clean-up. Residues were quantified using internal stable-labelled standards. The LOQ was 0.01 mg/kg for all matrices and for both analytes investigated.

In the following table the recovered residues in stored samples are summarised:

Table 67 Storage stability of bixafen and M21 (bixafen-desmethyl) in plant commodities fortified at levels of 0.1 mg/kg

Matrix	Analyte	Storage period (months)	Residue level in stored samples (% nominal level)			Procedural recovery (% nominal level)	
			Individual values	Mean	RSD (%)	Individual values	Mean
Wheat, grain	bixafen	0	97, 94, 93, 96, 92	94	2.2	–	–
		1	87, 100, 92	93	7.1	99, 99	99
		2	101, 91, 101	98	5.9	94, 95	95
		3	74, 88, 90	84	10.4	97, 104	101
		6	100, 101, 103	101	1.5	98, 107	103
		12	84, 84, 86	85	1.4	95, 98	97
		18	92, 100, 101	98	5.1	97, 105	101
		24	100, 98, 99	99	1.0	95, 96	96
	M21 (bixafen-desmethyl)	0	88, 86, 90, 89, 86	88	2.0	–	–
		1	120, 123, 129	124	3.7	98, 98	98
		2	115, 116, 117	116	0.9	98, 96	97
		3	118, 118, 127	121	4.3	100, 98	99
		6	115, 119, 124	119	3.8	113, 97	105
		12	111, 112, 111	111	0.5	98, 97	98
		18	118, 118, 114	117	2.0	104, 109	107
		24	99, 104, 102	102	2.5	89, 86	88
Wheat, green material	bixafen	0	93, 86, 94, 96, 95	93	4.3	–	–
		1	101, 103, 100	101	1.5	102, 103	103
		2	101, 102, 99	101	1.5	98, 100	99
		3	105, 108, 111	108	2.8	101, 103	102
		6	95, 100, 102	99	3.6	102, 98	100
		12	91, 94, 94	93	1.9	93, 96	95
		18	99, 99, 101	100	1.2	96, 98	97
		24	96, 96, 102	98	3.5	97, 102	100
	M21 (bixafen-desmethyl)	0	87, 87, 89, 87, 83	87	2.5	–	–
		1	112, 114, 116	114	1.8	98, 98	98

Matrix	Analyte	Storage period (months)	Residue level in stored samples (% nominal level)			Procedural recovery (% nominal level)	
			Individual values	Mean	RSD (%)	Individual values	Mean
		2	113, 120, 121	118	3.7	95, 97	96
		3	115, 120, 121	119	2.7	103, 101	102
		6	110, 110, 111	110	0.5	96, 100	98
		12	106, 113, 117	112	5.0	101, 99	100
		18	108, 105, 108	107	1.6	98, 93	96
		24	96, 93, 97	95	2.2	81, 84	83
Wheat, straw	bixafen	0	93, 95, 98, 95, 96	95	1.9	–	–
		1	89, 92, 92	91	1.9	87, 88	88
		2	86, 90, 93	90	3.9	84, 85	85
		3	90, 91, 93	91	1.7	108, 104	106
		6	104, 104, 105	104	0.6	95, 97	96
		12	84, 87, 88	86	2.4	87, 85	86
		18	96, 96, 96	96	0.0	93, 93	93
		24	98, 93, 93	95	3.1	90, 88	89
	M21 (bixafen-desmethyl)	0	85, 90, 86, 84, 88	87	2.8	–	–
		1	104, 106, 107	106	1.4	85, 88	87
		2	102, 103, 105	103	1.5	86, 85	86
		3	115, 116, 118	116	1.3	103, 109	106
		6	98, 101, 103	101	2.5	87, 87	87
		12	104, 104, 104	104	0.0	90, 91	91
		18	107, 117, 112	112	4.5	93, 95	94
		24	100, 98, 100	99	1.2	78, 80	79
Potato, tuber	bixafen	0	90, 92, 91, 91, 91	91	0.8	–	–
		1	104, 105, 104	104	0.6	102, 107	105
		2	74, 76, 76	75	1.5	75, 73	74
		3	129, 129, 129	129	0.0	100, 100	100
		6	102, 104, 104	103	1.1	102, 103	103
		12	95, 94, 96	95	1.1	97, 93	95
		18	102, 103, 100	102	1.5	113, 96	105
		24	93, 94, 95	94	0.6	96, 94	95
	M21 (bixafen-desmethyl)	0	87, 89, 93, 90, 92	90	2.6	–	–
		1	124, 125, 126	125	0.8	98, 106	102
		2	94, 97, 95	95	1.6	80, 76	78
		3	125, 130, 135	130	3.8	99, 97	98
		6	118, 123, 121	121	2.1	96, 100	98
		12	113, 113, 113	113	0.0	96, 95	96
		18	114, 114, 114	114	0.0	111, 93	102
		24	106, 107, 106	106	0.5	84, 83	84
Lettuce, head	bixafen	0	95, 96, 97, 103, 97	98	3.2	–	–
		1	103, 104, 102	103	1.0	99, 100	100
		2	101, 105, 111	106	4.8	99, 104	102
		3	112, 114, 115	114	1.3	97, 103	100
		6	104, 104, 117	108	6.9	114, 100	107
		12	100, 98, 100	99	1.2	94, 100	97
		18	99, 100, 101	100	1.0	97, 99	98
		24	101, 100, 101	101	0.6	93, 94	94
	M21 (bixafen-desmethyl)	0	91, 93, 96, 93, 89	92	2.8	–	–
		1	62, 75, 76	71	11.0	101, 103	102
		2	100, 101, 104	102	2.0	95, 100	98
		3	84, 81, 85	83	2.5	103, 106	105
		6	79, 84, 90	84	6.5	99, 113	106
		12	92, 93, 95	93	1.6	96, 93	95
		18	92, 92, 93	92	0.6	95, 92	94
		24	97, 99, 93	96	3.2	91, 87	89
Rape, seed	bixafen	0	87, 87, 91, 89, 89	89	1.9	–	–
		1	91, 90, 93	91	1.7	100, 99	100
		2	63, 66, 67	65	3.2	69, 69	69

Matrix	Analyte	Storage period (months)	Residue level in stored samples (% nominal level)			Procedural recovery (% nominal level)	
			Individual values	Mean	RSD (%)	Individual values	Mean
		3	109, 113, 114	112	2.4	100, 101	101
		6	94, 89,88	90	3.6	98, 99	99
		12	77, 89, 92	86	9.2	92, 92	92
		18	103, 96, 95	98	4.5	93, 93	93
		24	93, 91, 91	92	1.3	89, 90	90
	M21 (bixafen-desmethyl)	0	90, 89, 86, 91, 91	89	2.3	–	–
		1	123, 123, 122	123	0.5	103, 100	102
		2	86, 86, 86	86	0.0	71, 74	73
		3	114, 120, 121	118	3.2	90, 98	94
		6	111, 112, 113	112	0.9	96, 95	96
		12	117, 117, 117	117	0.0	96, 96	96
		18	113, 113, 121	116	4.0	89, 89	89
		24	105, 104, 104	104	0.6	84, 89	87

In addition to plant matrices the storage stability of bixafen (Brumhard, B and Freitag, T, 2008, BIXAFEN_046) and bixafen-desmethyl (Freitag, T and Hoffmann, M, 2009, BIXAFEN_047) in stored soil samples was investigated.

Untreated soil from Laacher Hof, Germany (sandy loam) and Höfchen, Germany (silt loam) was used for the storage stability study and individually fortified at levels .of 50 µg/kg with both analytes. After 273/279, 456 and 587 days for bixafen and 134, 183, and 721 days for M21 (bixafen-desmethyl) samples were analysed for the remaining residue.

Soil samples were analysed for bixafen according to method 00952. The residue of M21 (bixafen-desmethyl) in soil was measured with the modified version 00950/M001. The LOQ for both methods was 5 µg/kg soil (see analytical methods).

Table 68 Storage stability of bixafen and M21 (bixafen-desmethyl) in soil fortified at levels of 50 µg/kg

Soil	Analyte, Reference	Storage period (days)	Residue level in stored samples (% nominal level)			Procedural recovery (% nominal level)	
			Individual values	Mean	RSD (%)	Individual values	Mean
Laacher Hof	bixafen, Brumhard, B and Freitag, T 2008, BIXAFEN_046	0	95, 98, 97, 94	96	1.7	–	–
		273	92, 93, 95, 94	93	1.3	91.9	–
		456	94, 94, 93, 94	94	0.6	97.4	–
		587	104, 95, 97, 96	98	3.8	92.2	–
	M21 (bixafen-desmethyl) Freitag, T and Hoffmann, M, 2009, BIXAFEN_047	0	95, 96, 95, 96	96	0.8	–	–
		134	92, 98, 107, 96	98	6.2	102	–
		183	112, 111, 107, 102	108	4.4	107	–
		721	97, 95, 99, 91	96	3.6	101	–
Höfchen	bixafen, Brumhard, B and Freitag, T 2008, BIXAFEN_046	0	96, 99, 100, 97	98	1.9	–	–
		279	97, 98, 97, 98	97	0.6	100	–
		456	93, 95, 97, 94	95	1.7	97.8	–
		587	100, 96, 97, 97	98	2.0	100	–
	M21 (bixafen-desmethyl) Freitag, T and Hoffmann, M, 2009, BIXAFEN_047	0	94, 92, 94, 95	94	1.5	–	–
		134	100, 99, 107, 103	102	3.7	98	–
		183	99, 107, 113, 103	105	5.7	105	–
		721	95, 98, 102, 98	98	3.0	105	–

USE PATTERN

Bixafen is a protectant fungicide. The Meeting received uses involving foliar spray applications in the field to rape plants and cereal grains.

Table 69 List of uses of bixafen

Crop	Country	Application detail					
		Form	Type	kg ai/ha	Growth stage at last treatment	No.	Pre harvest interval (PHI) in days
Oilseeds							
Oilseed rape	United Kingdom	EC	spraying	0.075	–	2	56
Cereals							
Barley	Austria	EC	spraying	0.075	BBCH 69	2	35
Barley	Belgium	EC	spraying	0.075	BBCH 49	2	not established—covered by growth stage
Barley	Estonia	EC	spraying	0.075	BBCH 61	2	35
Barley	France	EC	spraying	0.075	BBCH 61	1	35
Barley	Germany	EC	spraying	0.075	BBCH 61	2	not established—covered by growth stage
Barley	Hungary	EC	spraying	0.075	flowering	2	35
Barley	Ireland	EC	spraying	0.06	BBCH 61	2	not established—covered by growth stage
Barley	Latvia	EC	spraying	0.06	BBCH 61	2	35
Barley	Lithuania	EC	spraying	0.06	BBCH 61	2	35
Barley	Netherlands	EC	spraying	0.075	BBCH 61	2	not established—covered by growth stage
Barley	Romania	EC	spraying	0.05	BBCH 59	2	35
Barley	Switzerland	EC	spraying	0.075	BBCH 51	1	not established—covered by growth stage
Barley	United Kingdom	EC	spraying	0.125	BBCH 61	2	not established—covered by growth stage
Oats	Belgium	EC	spraying	0.075	BBCH 59	2	not established—covered by growth stage
Oats	Estonia	EC	spraying	0.075	BBCH 61	2	35
Oats	France	EC	spraying	0.094	BBCH 61	1	35
Oats	Hungary	EC	spraying	0.075	flowering	2	35
Oats	Ireland	EC	spraying	0.06	BBCH 61	2	not established—covered by growth stage
Oats	Netherlands	EC	spraying	0.075	BBCH 61	2	not established—covered by growth stage
Oats	United Kingdom	EC	spraying	0.125	BBCH 61	2	not established—covered by growth stage
Rye	Austria	EC	spraying	0.094	BBCH 69	2	35
Rye	Belgium	EC	spraying	0.094	BBCH 59	2	covered by growth stage
Rye	Estonia	EC	spraying	0.075	BBCH 69	2	35
Rye	France	EC	spraying	0.094	BBCH 69	1	35
Rye	Germany	EC	spraying	0.094	BBCH 69	2	covered by growth stage
Rye	Hungary	EC	spraying	0.075	flowering	2	35
Rye	Ireland	EC	spraying	0.094	BBCH 73	2	not established—covered by growth stage
Rye	Latvia	EC	spraying	0.06	BBCH 69	2	35
Rye	Lithuania	EC	spraying	0.06	BBCH 69	2	35
Rye	Netherlands	EC	spraying	0.094	BBCH 69	2	not established—covered by growth stage
Rye	Switzerland	EC	spraying	0.094	BBCH 61	1	not established—covered by growth stage
Rye	United Kingdom	EC	spraying	0.125	BBCH 73	2	not established—covered by growth stage
Spelt	Belgium	EC	spraying	0.094	BBCH 65	2	not established—covered by growth stage
Spelt	Netherlands	EC	spraying	0.094	BBCH 69	2	not established—covered by growth

Crop	Country	Application detail					
		Form	Type	kg ai/ha	Growth stage at last treatment	No.	Pre harvest interval (PHI) in days
							stage
Spelt	Switzerland	EC	spraying	0.075	BBCH 61	1	not established—covered by growth stage
Triticale	Austria	EC	spraying	0.094	BBCH 69	2	35
Triticale	Belgium	EC	spraying	0.094	BBCH 65	2	covered by growth stage
Triticale	Estonia	EC	spraying	0.075	BBCH 69	2	35
Triticale	France	EC	spraying	0.094	BBCH 69	1	35
Triticale	Germany	EC	spraying	0.094	BBCH 69	2	covered by growth stage
Triticale	Hungary	EC	spraying	0.075	flowering	2	35
Triticale	Ireland	EC	spraying	0.094	BBCH 73	2	not established—covered by growth stage
Triticale	Latvia	EC	spraying	0.06	BBCH 69	2	35
Triticale	Lithuania	EC	spraying	0.06	BBCH 69	2	35
Triticale	Netherlands	EC	spraying	0.094	BBCH 69	2	not established—covered by growth stage
Triticale	Switzerland	EC	spraying	0.094	BBCH 61	1	not established—covered by growth stage
Triticale	United Kingdom	EC	spraying	0.125	BBCH 73	2	not established—covered by growth stage
Wheat	Austria	EC	spraying	0.094	BBCH 69	2	35
Wheat	Belgium	EC	spraying	0.094	BBCH 65	2	covered by growth stage
Wheat	Estonia	EC	spraying	0.075	BBCH 69	2	35
Wheat	France	EC	spraying	0.094	BBCH 69	1	35
Wheat	Germany	EC	spraying	0.094	BBCH 69	2	covered by growth stage
Wheat	Hungary	EC	spraying	0.075	flowering	2	35
Wheat	Ireland	EC	spraying	0.094	BBCH 73	2	not established—covered by growth stage
Wheat	Latvia	EC	spraying	0.06	BBCH 69	2	35
Wheat	Lithuania	EC	spraying	0.06	BBCH 69	2	35
Wheat	Netherlands	EC	spraying	0.094	BBCH 69	2	not established—covered by growth stage
Wheat	Romania	EC	spraying	0.06	BBCH 69	2	35
Wheat	Switzerland	EC	spraying	0.094	BBCH 61	1	not established—covered by growth stage
Wheat	United Kingdom	EC	spraying	0.125	BBCH 73	2	not established—covered by growth stage

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

Residue levels were reported as measured. Application rates were always reported as bixafen equivalents. When residues were not detected they are shown as below the LOQ, e.g., < 0.01 mg/kg. Application rates and spray concentrations have generally been rounded to two significant figures. HR and STMR values from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels. These results are underlined.

Laboratory reports included method validation including batch recoveries with spiking at residue levels similar to those occurring in samples from the supervised trials. Dates of analyses or duration of residue sample storage were also provided. Field reports provided data on the sprayers used and their calibration, plot size, residue sample size and sampling date. Although trials included control plots, no control data are recorded in the tables except where residues in control samples exceeded the LOQ. Residue data are recorded unadjusted for % recovery.

Bixafen—supervised residue trials

Commodity	Indoor/Outdoor	Treatment	Countries	Table
Rape seed	outdoor	foliar	Belgium, France, Germany, Italy, Spain,	Table 70

Commodity	Indoor/Outdoor	Treatment	Countries	Table
			The Netherlands, United Kingdom	
Barley	outdoor	foliar	Belgium, France, Germany, Italy, Portugal, Spain, Sweden, United Kingdom	Table 71
Wheat	outdoor	foliar	France, Germany, Greece, Italy, Portugal, Spain, Sweden, United Kingdom	Table 72Table
Rape forage	outdoor	foliar	Belgium, France, Germany, Italy, Spain, The Netherlands, United Kingdom	Table 73
Barley, forage	outdoor	foliar	Belgium, France, Germany, Italy, Portugal, Spain, Sweden, United Kingdom	Table 74
Wheat, forage	outdoor	foliar	France, Germany, Greece, Italy, Portugal, Spain, Sweden, United Kingdom	Table 75
Barley, straw	outdoor	foliar	Belgium, France, Germany, Italy, Portugal, Spain, Sweden, United Kingdom	Table 76
Wheat, straw	outdoor	foliar	France, Germany, Greece, Italy, Portugal, Spain, Sweden, United Kingdom	Table 77

Table 70 Residues of bixafen and M21 (bixafen-desmethyl) in rape seeds following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
cGAP UK	2	0.075	0.025–0.075	100–300	n.s.						
France (North), Braslou 2008 (Grizzly)	2	0.075 0.075	0.025 0.025	300 300	63 67	pod seed	42 55	0.02 0.01	0.01 < 0.01	0.03 0.02	08-2116-01 BIXAFEN_057
The Netherlands, Hoofddorp 2008 (Maximus)	2	0.075 0.075	0.038 0.038	200 200	64 69	seed	64	< 0.01	< 0.01	< 0.02	08-2116-02 BIXAFEN_057 Processing trial
Belgium, Cortil-Noirmont 2008 (Exocet)	2	0.075 0.075	0.038 0.038	200 200	64 73	pod seed	42 56	0.09 < 0.01	0.09 0.01	0.18 0.02	08-2116-03 BIXAFEN_057
Germany, Werl 2008 (Astrid)	2	0.075 0.075	0.038 0.038	200 200	65 69	seed	56	0.01	0.01	0.02	08-2116-04 BIXAFEN_057
The Netherlands, Nieuw Beerta 2009 (not specified)	2	0.075 0.075	0.025 0.025	300 300	69 77	seed	49	< 0.01	< 0.01	< 0.02	09-2053-02 BIXAFEN_058
Belgium, Cortil-Noirmont	2	0.075 0.075	0.038 0.038	200 200	65 73	pod seed	30 42 55	0.20 0.14 < 0.01	0.03 0.03 < 0.01	0.23 0.17 < 0.02	09-2053-03 BIXAFEN_058

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
2009 (Monalisa)											
France (North), Lignieres les Roye 2009 (Kador)	2	0.075 0.075	0.025 0.025	300 300	67 79	seed	55	< 0.01	< 0.01	< 0.02	09-2053-04 BIXAFEN_058
United Kingdom, Bishop Burton 2009 (Castille)	2	0.075 0.075	0.039 0.038	192 200	65 69	pod seed	30 42 56	0.19 0.05 < 0.01	0.05 0.02 < 0.01	0.24 0.07 < 0.02	09-2244-01 Bixafen_059
Germany, Burscheid 2011 (Elektra)	2	0.06 0.06	0.02 0.02	300 300	71 78	pod seed	31 50	0.18 0.017	0.11 0.011	0.29 0.028	11-2013-03 BIXAFEN_060
France (North), Cahmbourg sur Indre 2008 (Flash)	2	0.06 0.06	0.02 0.02	300 300	76 80	pod seed	21 33	0.24 < 0.01	0.04 < 0.01	0.28 < 0.02	08-2112-01 BIXAFEN_066
Germany, Burscheid 2008 (Titan)	2	0.06 0.06	0.02 0.02	300 300	69 73	seed	35	0.02	< 0.01	0.03	08-2112-02 BIXAFEN_066 Processing trial
Belgium, Cortil-Noirmont 2008 (Exocet)	2	0.06 0.06	0.02 0.02	300 300	77 80	pod seed	21 28	0.32 < 0.01	0.09 < 0.01	0.41 < 0.02	08-2112-03 BIXAFEN_066
Germany, Werl 2008 (Astrid)	2	0.06 0.06	0.02 0.02	300 300	75 78	seed	30	0.02	< 0.01	0.03	08-2112-04 BIXAFEN_066
United Kingdom, Bishop Burton 2009 (Castille)	2	0.06 0.06	0.03 0.03	200 200	67 75	pod seed	29 44	< 0.01 0.01	< 0.01 < 0.01	< 0.02 0.02	09-2245-01 BIXAFEN_067 Processing trial
United Kingdom, Banbury 2011 (D.K. Cabernet)	2	0.06 0.06	0.03 0.03	200 200	80 83	Seed	28	0.052	< 0.01	0.062	11-2137-01 BIXAFEN_073
Spain, Les Franqueses del Valles	2	0.063 0.06	0.02 0.02	315 300	74 78	pod seed	(14) 21 30	– < 0.01 0.017	– < 0.01 < 0.01	– < 0.02 0.027	11-2013-01 BIXAFEN_060

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
2011 (Pacific)											
France (South), Bouloc 2011 (NK Alamir)	2	0.06 0.06	0.02 0.02	300 300	72 79	seed	30	< 0.01	< 0.01	< 0.02	11-2013-02 BIXAFEN_060
Italy, Tarquinia 2011 (Hybristar)	2	0.06 0.06	0.02 0.02	300 300	73 75	pod seed	14 21 30	0.48 0.51 < 0.01	0.063 0.087 < 0.01	0.543 0.597 < 0.02	11-2013-04 BIXAFEN_060
Spain, La Luisiana 2011 (Eswilliams)	2	0.06 0.069	0.02 0.02	300 344	77 82	seed	31	< 0.01	< 0.01	< 0.02	11-2013-05 BIXAFEN_060
France (South), Velleron 2011 (Hybrilux)	2	0.06 0.06	0.02 0.02	300 300	79 80	seed	30	< 0.01	< 0.01	< 0.02	11-2013-06 BIXAFEN_060

[a] Expressed as bixafen

DALT = Days after last treatment

BBCH 63 = 30% of flowers on main raceme open

BBCH 64 = 40% of flowers on main raceme open

BBHC 65 = Full flowering: 50% of flowers on main raceme open

BBCH 67 = Flowering declining: majority of petals fallen

BBCH 69 = End of flowering

BBCH 71–78 = 10% to 80% of pods have reached final size

BBCH 79 = Nearly all pods have reached final size

BBCH 82 = 20% of pods ripe, seeds dark and hard

Table 71 Residues of bixafen and M21 (bixafen-desmethyl) in barley grain following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), St. Cyr en Arthies 2006 (Carafe)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	34	<u>0.04</u>	< 0.01	<u>0.05</u>	R 2006 0432/7 BIXAFEN_049
France (North), Chambourg sur Indre 2006 (Vanessa)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	49	<u>0.08</u>	0.02	<u>0.10</u>	R 2006 0433/5 BIXAFEN_049
Sweden, Staffanstorp	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	36 45	<u>0.09</u> 0.06	0.02 0.01	<u>0.11</u> 0.07	R 2006 0434/3 BIXAFEN_049

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
2006 (Pasadena)											
United Kingdom, Hoxne 2006 (Sequel)	2	0.125 0.125	0.042 0.042	300 300	39 61	grain	62	0.04	< 0.01	0.05	R 2006 0435/1 BIXAFEN_049
Germany, Swisttal-Heimerzheim 2006 (Class)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35	0.07	0.01	0.08	R 2006 0437/8 BIXAFEN_049
France (North), St. Cyr en Arthies 2007 (Heinley)	2	0.125 0.125	0.042 0.042	300 300	37 61	ear grain	34 58	0.16 0.04	0.04 0.01	0.20 0.05	R 2007 0081/4 BIXAFEN_050
France (North), Carrépuis 2007 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	41 61	ear grain	35 60	0.09 0.02	0.02 < 0.01	0.11 0.03	R 2007 0082/0 BIXAFEN_050
Germany, Vechta-Lanförden 2007 (Tocada)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35	0.10	0.01	0.11	R 2007 0160/8 BIXAFEN_050
United Kingdom, Sandringham 2007 (Tippel)	2	0.125 0.125	0.042 0.042	300 300	39 69	grain	35 66	0.04 0.05	0.01 0.01	0.05 0.06	R 2007 0161/6 BIXAFEN_050
Belgium, Villers-Perwin 2007 (Beatrix)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	34 51	0.07 0.09	0.02 0.01	0.09 0.10	R 2007 0162/4 BIXAFEN_050
France (South), Villeneuve lés Bouloc 2006 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35	0.10	0.01	0.11	R 2006 0438/6 BIXAFEN_051
Italy, Bologna 2006 (Federal)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35 46	0.04 0.04	< 0.01 < 0.01	0.05 0.05	R 2006 0439/4 BIXAFEN_051
France (South), Quincieux 2006 (Scarlett)	2	0.125 0.125	0.042 0.042	300 300	37 71	grain	35	0.14	0.02	0.16	R 2006 0440/8 BIXAFEN_051

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
Spain, Llerona 2006 (Graphic)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35 48	0.08 0.06	0.02 0.02	0.10 0.08	R 2006 0441/6 BIXAFEN_051
Portugal, Azambuja 2006 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35 57	0.02 0.03	< 0.01 < 0.01	0.03 0.04	R 2006 0442/4 BIXAFEN_051
France (South), Villeneuve Lés Bouloc 2007 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	ear grain	34 60	0.17 0.06	0.07 0.02	0.24 0.08	R 2007 0083/0 BIXAFEN_052
Italy, Bologna 2007 (Tunica)	2	0.125 0.125	0.042 0.042	300 300	39 61	grain	39 56	0.06 0.06	0.02 0.02	0.08 0.08	R 2007 0084/9 BIXAFEN_052
Spain, Caldes de Montbui 2007 (Grafit)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35 40	0.25 0.25	0.04 0.04	0.29 0.29	R 2007 0085/7 BIXAFEN_052
France (South), Cherves 2007 (Scarlett)	2	0.125 0.125	0.042 0.042	300 300	37 61	ear grain	35 50	0.08 0.04	0.04 < 0.01	0.12 0.05	R 2007 0158/6 BIXAFEN_052
Italy, Senetica di Bondeno 2007 (Tunica)	2	0.125 0.125	0.042 0.042	300 300	37 61	grain	35	0.34	0.04	0.38	R 2007 0159/4 BIXAFEN_052
Sweden, Staffanstorp 2006 (Prestige)	2	0.25 0.25	0.084 0.084	300 300	37 61	grain	40	0.23	0.03	0.26	R 2006 0444/0 BIXAFEN_062 processing trial
Germany, Swisttal- Heimerzheim 2006 (Class)	2	0.25 0.25	0.084 0.084	300 300	37 61	grain	35	0.13	0.02	0.15	R 2006 0445/9 BIXAFEN_062 processing trial
France (North), Fresnoy les Roye 2006 (Scarlet)	2	0.25 0.25	0.084 0.084	300 300	37 61	grain	46	0.20	0.02	0.22	R 2006 0446/7 BIXAFEN_062 processing trial
Germany, Burscheid 2006 (Barke)	2	0.25 0.25	0.084 0.084	300 300	37 61	grain	43	0.03	< 0.01	0.04	R 2006 0447/5 BIXAFEN_062 processing trial

[a] Expressed as bixafen

DALT = Days after last treatment

BBCH 37 = Flag leaf just visible, still rolled

BBCH 39 = Flag leaf stage: flag leaf fully unrolled, ligule just visible

BBCH 41 = Early boot stage: flag leaf sheath extending

BBCH 61 = Beginning of flowering: first anthers visible

BBCH 69 = End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain

BBCH 71 = Watery ripe: first grains have reached half their final size

Table 72 Residues of bixafen and M21 (bixafen-desmethyl) in wheat grain following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), Chambourg sur Indre 2006 (Tecnico)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	34	0.01	< 0.01	0.02	R 2006 0421/1 BIXAFEN_053
France (North), Chaussy 2006 (Isengrain)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	37	< 0.01	< 0.01	< 0.02	R 2006 0423/8 BIXAFEN_053
Sweden, Staffanstorp 2006 (Vinjett)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	35 47	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02	R 2006 0424/6 BIXAFEN_053
United Kingdom, Thetford 2006 (Paragon)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	34 38	0.03 0.01	< 0.01 < 0.01	0.04 0.02	R 2006 0425/4 BIXAFEN_053
Germany, Leverkusen 2006 (Batis)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	35	0.01	< 0.01	0.02	R 2006 0426/2 BIXAFEN_053
France (North), Braslou 2007 (Mendel)	2	0.125 0.125	0.042 0.042	300 300	47 69	ear grain	35 44	0.17 < 0.01	0.12 < 0.01	0.29 < 0.02	R 2007 0091/1 BIXAFEN_054
United Kingdom, Diss 2007 (Belvoir)	2	0.125 0.125	0.039 0.042	318 300	47 69	ear grain	35 73	0.06 < 0.01	0.05 < 0.01	0.11 < 0.02	R 2007 0093/8 BIXAFEN_054
France (North), Chambourg sur Indre 2007	2	0.125 0.125	0.042 0.042	300 300	47 69	ear grain	35 56	0.14 < 0.01	0.10 < 0.01	0.24 < 0.02	R 2007 0094/6 BIXAFEN_054

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
(Apache)											
Sweden, Staffanstorp 2007 (Vinjett)	2	0.125 0.125	0.042 0.042	300 300	47 69	ear grain	35 69	0.18 < 0.01	0.17 < 0.01	0.35 < 0.02	R 2007 0095/4 BIXAFEN_054
Germany, Burscheid 2007 (Thasos)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	35 56	0.03 < 0.01	0.01 < 0.01	0.04 < 0.02	R 2007 0155/1 BIXAFEN_054
Greece, Thiva 2006 (Claudio)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	35 43	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02	R 2006 0427/0 BIXAFEN_055
Italy, Palidoro Fiumicino 2006 (Claudio)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	35 52	0.01 < 0.01	< 0.01 < 0.01	0.02 < 0.02	R 2006 0428/9 BIXAFEN_055
France (South), Lagardelle/ Lèze 2006 (not specified)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	35	< 0.01	< 0.01	< 0.02	R 2006 0429/7 BIXAFEN_055
Spain, Paradas Sevilla 2006 (Italo)	2	0.125 0.125	0.042 0.042	300 300	53 69	grain	35 47	0.03 0.02	< 0.01 < 0.01	0.04 0.03	R 2006 0430/0 BIXAFEN_055
France (South), Vouille 2006 (Technico)	2	0.125 0.125	0.042 0.042	300 300	47 69	grain	35 35	< 0.01 < 0.01	< 0.01 < 0.01	< 0.02 < 0.02	R 2006 0431/9 BIXAFEN_055
France (South), Villeneuve lès Bouloc 2007 (Panifor)	2	0.125 0.125	0.042 0.042	300 300	47 69	ear grain	35 35	0.37 < 0.01	0.24 < 0.01	0.61 < 0.02	R 2007 0086/5 BIXAFEN_056
Italy, Spinazzola 2007 (Simeto)	2	0.125 0.125	0.042 0.042	300 300	47 69	ear grain	35 44	0.61 0.02	0.15 < 0.01	0.76 0.03	R 2007 0087/3 BIXAFEN_056
France (South), Les Chères 2007 (Autan)	2	0.125 0.125	0.042 0.042	300 300	52 69	ear grain	35 44	0.14 < 0.01	0.17 < 0.01	0.31 < 0.02	R 2007 0088/1 BIXAFEN_056
Spain, Alcala de Guadaira	2	0.125 0.125	0.045 0.042	279 300	47 69	ear grain	36 54	0.38 0.02	0.13 < 0.01	0.51 0.03	R 2007 0090/3 BIXAFEN_056

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
Sevilla 2007 (Bolido R1)											
Portugal, Ereira 2007 (Galeira)	2	0.125 0.125	0.042 0.042	300 300	47 69	ear grain	35 53	0.47 < 0.01	0.25 < 0.01	0.72 < 0.02	R 2007 0157/8 BIXAFEN_056
United Kingdom, Bury St. Edmunds 2006 (Cordiale)	2	0.23 0.25	0.082 0.083	279 300	47 69	grain	40	0.05	< 0.01	0.06	R 2006 0527/7 BIXAFEN_064 processing trial
Sweden, Staffanstorp 2006 (Tommi)	2	0.25 0.25	0.083 0.083	300 300	47 61	grain	75	0.03	0.01	0.04	R 2006 0528/5 BIXAFEN_064 processing trial
France (North), Fresnoy les Roye 2006 (Chango)	2	0.25 0.25	0.083 0.083	300 300	47 69	grain	53	0.02	0.01	0.03	R 2006 0529/3 BIXAFEN_064 processing trial
France (North), Chambourg sur Indre 2006 (Apache)	2	0.25 0.25	0.083 0.083	300 300	47 69	grain	39	0.04	< 0.01	0.05	R 2006 0530/7 BIXAFEN_064 processing trial

[a] Expressed as bixafen

DALT = Days after last treatment

BBCH 47 = Flag leaf sheath opening

BBCH 52 = 20% of inflorescence emerged

BBCH 53 = 30% of inflorescence emerged

BBCH 69 = End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain

Table 73 Residues of bixafen and M21 (bixafen-desmethyl) in rape forage following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), Braslou 2008 (Grizzly)	2	0.075 0.075	0.025 0.025	300 300	63 67	forage rest of plant	–0 0 42	0.44 1.5 0.08	0.04 0.04 0.02	0.48 1.54 0.10	08-2116-01 BIXAFEN_057
The Netherlands,	2	0.075 0.075	0.038 0.038	200 200	64 69	forage	–0 0	0.19 2.5	0.03 0.03	0.22 2.53	08-2116-02 BIXAFEN_057

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
Hoofddorp 2008 (Maximus)											Processing trial
Belgium, Cortil-Noirmont 2008 (Exocet)	2	0.075 0.075	0.038 0.038	200 200	64 73	forage rest of plant	–0 0 42	0.17 1.3 0.07	0.05 0.05 0.02	0.22 1.35 0.09	08-2116-03 BIXAFEN_057
Germany, Werl 2008 (Astrid)	2	0.075 0.075	0.038 0.038	200 200	65 69	forage	–0 0	0.27 1.5	0.04 0.04	0.31 1.54	08-2116-04 BIXAFEN_057
The Netherlands, Nieuw Beerta 2009 (not specified)	2	0.075 0.075	0.025 0.025	300 300	69 77	forage	–0 0	0.17 0.99	0.03 0.02	0.20 1.01	09-2053-02 BIXAFEN_058
Belgium, Cortil-Noirmont 2009 (Monalisa)	2	0.075 0.075	0.038 0.038	200 200	65 73	forage rest of plant	–0 0 30 42	0.17 1.0 0.31 0.19	0.01 0.01 0.02 0.02	0.18 1.01 0.33 0.21	09-2053-03 BIXAFEN_058
France (North), Lignieres les Roye 2009 (Kador)	2	0.075 0.075	0.025 0.025	300 300	67 79	forage	–0 0	0.11 0.74	0.01 0.02	0.12 0.76	09-2053-04 BIXAFEN_058
United Kingdom, Bishop Burton 2009 (Castille)	2	0.075 0.075	0.039 0.038	192 200	65 69	forage rest of plant	–0 0 30 42	0.28 1.7 0.09 0.18	0.03 0.03 0.02 0.02	0.31 1.73 0.11 0.20	09-2244-01 Bixafen_059
Germany, Burscheid 2011 (Elektra)	2	0.06 0.06	0.02 0.02	300 300	71 78	forage	–0 0	0.23 1.1	0.028 0.028	0.258 1.128	11-2013-03 BIXAFEN_060
France (North), Cahmbourg sur Indre 2008 (Flash)	2	0.06 0.06	0.02 0.02	300 300	76 80	forage rest of plant	–0 0 21	0.23 1.3 0.29	0.02 0.02 0.02	0.25 1.35 0.31	08-2112-01 BIXAFEN_066
Germany,	2	0.06	0.02	300	69	forage	–0	0.14	0.01	0.15	08-2112-02

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
Burscheid 2008 (Titan)		0.06	0.02	300	73		0	1.3	0.02	1.32	BIXAFEN_066 Processing trial
Belgium, Cortil-Noirmont 2008 (Exocet)	2	0.06 0.06	0.02 0.02	300 300	77 80	forage rest of plant	–0 0 21	0.23 0.88 0.25	0.02 0.02 0.04	0.25 0.90 0.29	08-2112-03 BIXAFEN_066
Germany, Werl 2008 (Astrid)	2	0.06 0.06	0.02 0.02	300 300	75 78	forage	–0 0	0.27 1.5	0.03 0.03	0.30 1.53	08-2112-04 BIXAFEN_066
United Kingdom, Bishop Burton 2009 (Castille)	2	0.06 0.06	0.03 0.03	200 200	67 75	forage rest of plant	–0 0 29	0.17 0.69 0.06	0.01 0.01 0.01	0.18 0.70 0.07	09-2245-01 BIXAFEN_067 Processing trial
United Kingdom, Banbury 2011 (D.K. Cabernet)	2	0.06 0.06	0.03 0.03	200 200	80 83	Forage	–0 0	0.54 1.7	0.03 0.029	0.57 1.7	11-2137-01 BIXAFEN_073
Spain, Les Franqueses del Valles 2011 (Pacific)	2	0.063 0.06	0.02 0.02	315 300	74 78	forage rest of plant	–0 0 14 21	0.12 0.26 0.087 < 0.01	0.012 0.017 < 0.01 < 0.01	0.132 0.277 0.097 < 0.02	11-2013-01 BIXAFEN_060
France (South), Bouloc 2011 (NK Alamir)	2	0.06 0.06	0.02 0.02	300 300	72 79	forage	–0 0	0.39 1.8	0.065 0.074	0.455 1.874	11-2013-02 BIXAFEN_060
Italy, Tarquinia 2011 (Hybristar)	2	0.06 0.06	0.02 0.02	300 300	73 75	forage rest of plant	–0 0 14 21	0.22 0.87 0.20 0.18	0.013 0.01 < 0.01 0.01	0.233 0.88 0.21 0.19	11-2013-04 BIXAFEN_060
Spain, La Luisiana 2011 (Eswilliams)	2	0.06 0.069	0.02 0.02	300 344	77 82	forage	–0 0	0.47 2.1	0.093 0.077	0.563 2.177	11-2013-05 BIXAFEN_060
France (South), Velleron	2	0.06 0.06	0.02 0.02	300 300	79 80	forage	–0 0	0.31 1.1	0.035 0.041	0.345 1.141	11-2013-06 BIXAFEN_060

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
2011 (Hybrilux)											

–0 = Sampling directly before last treatment

[a] Expressed as bixafen

DALT = Days after last treatment

BBCH 63 = 30% of flowers on main raceme open

BBCH 64 = 40% of flowers on main raceme open

BBHC 65 = Full flowering: 50% of flowers on main raceme open

BBCH 67 = Flowering declining: majority of petals fallen

BBCH 69 = End of flowering

BBCH 71–78 = 10% to 80% of pods have reached final size

BBCH 79 = Nearly all pods have reached final size

BBCH 82 = 20% of pods ripe, seeds dark and hard

Table 74 Residues of bixafen and M21 (bixafen-desmethyl) in barley forage following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), St. Cyr en Arthies 2006 (Carafe)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0	1.4 4.4	0.08 0.08	1.48 4.48	R 2006 0432/7 BIXAFEN_049
France (North), Chambourg sur Indre 2006 (Vanessa)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0	1.0 3.9	0.03 0.04	1.03 3.94	R 2006 0433/5 BIXAFEN_049
Sweden, Staffanstorp 2006 (Pasadena)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0 7 14 28	1.7 7.0 4.1 3.7 4.6	0.23 0.25 0.51 0.60 0.49	1.93 7.25 4.61 4.3 5.09	R 2006 0434/3 BIXAFEN_049
United Kingdom, Hoxne 2006 (Sequel)	2	0.125 0.125	0.042 0.042	300 300	39 61	forage	–0 0 8 14 28	0.42 2.1 0.80 0.59 0.38	0.03 0.04 0.05 0.06 0.04	0.45 2.14 0.85 0.65 0.42	R 2006 0435/1 BIXAFEN_049
Germany, Swisttal-Heimerzheim 2006 (Class)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0 7 13 28	0.88 3.9 2.0 2.7 2.8	0.11 0.10 0.12 0.21 0.20	0.99 4.0 2.12 2.91 3.0	R 2006 0437/8 BIXAFEN_049

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), St. Cyr en Arthies 2007 (Heinley)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage rest of plant	–0 0 7 14 28 34	0.47 3.4 2.1 1.5 0.41 0.54	0.07 0.07 0.13 0.14 0.08 0.11	0.54 3.47 2.23 1.64 0.49 0.64	R 2007 0081/4 BIXAFEN_050
France (North), Carrépuis 2007 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	41 61	forage rest of plant	–0 0 35	1.5 4.3 0.66	0.09 0.10 0.09	1.59 4.4 0.75	R 2007 0082/0 BIXAFEN_050
Germany, Vechta-Lanförden 2007 (Tocada)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0 7 14 28	0.37 2.5 1.5 0.90 1.1	0.08 0.07 0.11 0.09 0.07	0.45 2.57 1.61 0.99 1.17	R 2007 0160/8 BIXAFEN_050
United Kingdom, Sandringham 2007 (Tippel)	2	0.125 0.125	0.042 0.042	300 300	39 69	forage	–0 0 8 14 28	0.36 2.4 1.3 0.84 0.39	0.07 0.07 0.08 0.11 0.09	0.43 2.47 1.38 0.95 0.48	R 2007 0161/6 BIXAFEN_050
Belgium, Villers-Perwin 2007 (Beatrix)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0	0.18 2.8	0.07 0.09	0.25 2.89	R 2007 0162/4 BIXAFEN_050
France (South), Villeneuve lés Bouloc 2006 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0	0.84 3.6	0.12 0.10	0.96 3.7	R 2006 0438/6 BIXAFEN_051
Italy, Bologna 2006 (Federal)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0	0.58 3.4	0.01 0.01	0.59 3.41	R 2006 0439/4 BIXAFEN_051
France (South), Quincieux 2006 (Scarlett)	2	0.125 0.125	0.042 0.042	300 300	37 71	forage	–0 0 7 14 27	0.89 3.8 2.5 1.6 2.2	0.21 0.20 0.34 0.28 0.26	1.1 4.0 2.84 1.88 2.46	R 2006 0440/8 BIXAFEN_051
Spain, Llerona 2006 (Graphic)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0 7 14 28	1.2 3.3 2.8 2.1 2.0	0.10 0.11 0.16 0.18 0.27	1.3 3.41 2.96 2.28 2.27	R 2006 0441/6 BIXAFEN_051
Portugal,	2	0.125	0.042	300	37	forage	–0	1.1	0.08	1.18	R 2006 0442/4

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
Azambuja 2006 (Prestige)		0.125	0.042	300	61		0 7 14 28	2.6 1.5 1.4 1.1	0.09 0.11 0.15 0.11	2.69 1.61 1.55 1.21	BIXAFEN_051
France (South), Villeneuve Lés Bouloc 2007 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage rest of plant	–0 0 7 13 27 34	0.85 3.1 1.4 0.96 0.51 0.17	0.12 0.14 0.16 0.18 0.16 0.07	0.96 3.24 1.56 1.14 0.67 0.24	R 2007 0083/0 BIXAFEN_052
Italy, Bologna 2007 (Tunica)	2	0.125 0.125	0.042 0.042	300 300	39 61	forage	–0 0	0.46 3.6	0.22 0.20	0.68 3.8	R 2007 0084/9 BIXAFEN_052
Spain, Caldes de Montbui 2007 (Grafit)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0 7 13 28	0.13 3.0 1.8 1.5 2.1	0.03 0.04 0.11 0.17 0.20	0.16 3.04 1.91 1.67 2.3	R 2007 0085/7 BIXAFEN_052
France (South), Cherves 2007 (Scarlett)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage rest of plant	–0 0 7 14 28 35	0.91 4.2 2.8 1.4 0.50	0.06 0.07 0.14 0.15 0.08	0.97 4.27 2.94 1.55 0.58	R 2007 0158/6 BIXAFEN_052
Italy, Senetica di Bondeno 2007 (Tunica)	2	0.125 0.125	0.042 0.042	300 300	37 61	forage	–0 0	0.69 5.8	0.18 0.17	0.87 5.97	R 2007 0159/4 BIXAFEN_052
Sweden, Staffanstorp 2006 (Prestige)	2	0.25 0.25	0.084 0.084	300 300	37 61	forage	0	9.1	0.18	9.28	R 2006 0444/0 BIXAFEN_062 processing trial
Germany, Swisttal-Heimerzheim 2006 (Class)	2	0.25 0.25	0.084 0.084	300 300	37 61	forage	0	6.1	0.18	6.28	R 2006 0445/9 BIXAFEN_062 processing trial
France (North), Fresnoy les Roye 2006 (Scarlet)	2	0.25 0.25	0.084 0.084	300 300	37 61	forage	0	7.8	0.17	7.97	R 2006 0446/7 BIXAFEN_062 processing trial
Germany,	2	0.25	0.084	300	37	forage	0	6.3	0.07	6.37	R 2006 0447/5

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
Burscheid 2006 (Barke)		0.25	0.084	300	61						BIXAFEN_062 processing trial

–0 = Sampling directly before last treatment

[a] Expressed as bixafen

DALT = Days after last treatment

BBCH 37 = Flag leaf just visible, still rolled

BBCH 39 = Flag leaf stage: flag leaf fully unrolled, ligule just visible

BBCH 41 = Early boot stage: flag leaf sheath extending

BBCH 61 = Beginning of flowering: first anthers visible

BBCH 69 = End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain

BBCH 71 = Watery ripe: first grains have reached half their final size

Table 75 Residues of bixafen and M21 (bixafen-desmethyl) in wheat forage following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), Chambourg sur Indre 2006 (Tecnico)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0	1.9 7.1	0.19 0.21	2.09 7.31	R 2006 0421/1 BIXAFEN_053
France (North), Chaussy 2006 (Isengrain)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0	1.1 3.0	0.09 0.09	1.19 3.09	R 2006 0423/8 BIXAFEN_053
Sweden, Staffanstorp 2006 (Vinjett)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0 7 14 28	1.8 4.6 4.1 3.2 3.2	0.12 0.13 0.36 0.39 0.50	1.92 4.73 4.46 3.59 3.7	R 2006 0424/6 BIXAFEN_053
United Kingdom, Thetford 2006 (Paragon)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0 7 14 28	0.83 2.7 2.1 1.8 1.8	0.07 0.08 0.13 0.13 0.13	0.90 2.78 2.23 1.93 1.93	R 2006 0425/4 BIXAFEN_053
Germany, Leverkusen 2006 (Batis)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0 7 14 28	0.31 2.2 1.3 0.92 0.59	0.05 0.05 0.06 0.10 0.11	0.36 2.25 1.36 1.02 0.70	R 2006 0426/2 BIXAFEN_053
France (North), Braslou	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0 7 14	2.6 1.4 0.88 0.65	0.09 0.14 0.18 0.26	2.69 1.54 1.06 0.91	R 2007 0091/1 BIXAFEN_054

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
2007 (Mendel)						 rest of plant	28 35	0.45 0.97	0.09 0.33	0.54 1.3	
United Kingdom, Diss 2007 (Belvoir)	2	0.125 0.125	0.039 0.042	318 300	47 69	forage rest of plant	–0 0 35	0.57 2.7 0.36	0.16 0.17 0.25	0.73 2.87 0.61	R 2007 0093/8 BIXAFEN_054
France (North), Chambourg sur Indre 2007 (Apache)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage rest of plant	–0 0 7 14 28 35	0.70 3.6 1.8 1.2 1.1 1.5	0.16 0.19 0.25 0.28 0.45 0.63	0.86 3.79 2.05 1.48 1.55 2.13	R 2007 0094/6 BIXAFEN_054
Sweden, Staffanstorp 2007 (Vinjett)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage rest of plant	–0 0 35	2.8 3.2 1.4	0.14 0.15 0.70	2.94 3.35 2.1	R 2007 0095/4 BIXAFEN_054
Germany, Burscheid 2007 (Thasos)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0 7 14 28	1.5 4.5 2.0 1.4 1.0	0.24 0.26 0.29 0.30 0.31	1.74 4.76 2.29 1.7 1.31	R 2007 0155/1 BIXAFEN_054
Greece, Thiva 2006 (Claudio)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0	1.0 3.8	0.07 0.07	1.07 3.87	R 2006 0427/0 BIXAFEN_055
Italy, Palidoro Fiumicino 2006 (Claudio)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0	0.48 2.5	0.09 0.08	0.57 2.58	R 2006 0428/9 BIXAFEN_055
France (South), Lagardelle/ Lèze 2006 (not specified)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0 7 14 28	1.2 5.3 2.7 2.9 3.4	0.13 0.15 0.18 0.24 0.34	1.33 5.45 2.88 3.14 3.74	R 2006 0429/7 BIXAFEN_055
Spain, Paradas Sevilla 2006 (Italo)	2	0.125 0.125	0.042 0.042	300 300	53 69	forage	–0 0 7 14 28	1.0 2.8 1.6 1.1 1.7	0.08 0.07 0.09 0.09 0.17	1.08 2.87 1.69 1.19 1.87	R 2006 0430/0 BIXAFEN_055
France (South), Vouille	2	0.125 0.125	0.042 0.042	300 300	47 69	forage	–0 0 7 14	1.2 5.1 3.6 3.0	0.07 0.09 0.22 0.31	1.27 5.19 3.82 3.31	R 2006 0431/9 BIXAFEN_055

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
2006 (Technico)							28	2.9	0.30	3.2	
France (South), Villeneuve lès Bouloc 2007 (Panifor)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage rest of plant	–0 0 7 14 28 35	0.53 4.3 1.5 0.86 0.77 1.1	0.22 0.24 0.31 0.31 0.37 0.42	0.75 4.54 1.81 1.17 1.14 1.52	R 2007 0086/5 BIXAFEN_056
Italy, Spinazzola 2007 (Simeto)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage rest of plant	–0 0 7 14 28 35	0.58 0.76 2.8 2.0 1.7 3.1	0.09 0.16 0.17 0.20 0.19 0.33	0.67 0.92 2.97 2.20 1.89 3.43	R 2007 0087/3 BIXAFEN_056
France (South), Les Chères 2007 (Autan)	2	0.125 0.125	0.042 0.042	300 300	52 69	forage rest of plant	–0 0 7 14 28 35	0.56 2.5 1.3 0.96 0.46 0.79	0.17 0.16 0.26 0.31 0.24 0.33	0.73 2.66 1.56 1.27 0.70 1.12	R 2007 0088/1 BIXAFEN_056
Spain, Alcala de Guadaira Sevilla 2007 (Bolido R1)	2	0.125 0.125	0.045 0.042	279 300	47 69	forage rest of plant	–0 0 36	0.48 3.5 1.8	0.05 0.05 0.26	0.53 3.55 2.06	R 2007 0090/3 BIXAFEN_056
Portugal, Ereira 2007 (Galeira)	2	0.125 0.125	0.042 0.042	300 300	47 69	forage rest of plant	–0 0 35	0.60 4.0 2.7	0.14 0.18 0.55	0.74 4.18 3.25	R 2007 0157/8 BIXAFEN_056
United Kingdom, Bury St. Edmunds 2006 (Cordiale)	2	0.23 0.25	0.082 0.083	279 300	47 69	forage	0	4.3	0.11	4.41	R 2006 0527/7 BIXAFEN_064 processing trial
Sweden, Staffanstorp 2006 (Tommi)	2	0.25 0.25	0.083 0.083	300 300	47 61	forage	0	10	0.17	10.17	R 2006 0528/5 BIXAFEN_064 processing trial
France (North), Fresnoy les Roye 2006 (Chango)	2	0.25 0.25	0.083 0.083	300 300	47 69	forage	0	4.2	0.13	4.33	R 2006 0529/3 BIXAFEN_064 processing trial

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), Chambourg sur Indre 2006 (Apache)	2	0.25 0.25	0.083 0.083	300 300	47 66	forage	0	5.8	0.13	5.93	R 2006 0530/7 BIXAFEN_064 processing trial

–0 = Sampling directly before last treatment

[a] expressed as bixafen

DALT = Days after last treatment

BBCH 47 = Flag leaf sheath opening

BBCH 52 = 20% of inflorescence emerged

BBCH 53 = 30% of inflorescence emerged

BBCH 69 = End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain

Table 76 Residues of bixafen and M21 (bixafen-desmethyl) in barley straw following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), St. Cyr en Arthies 2006 (Carafe)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	34	5.4	0.18	5.58	R 2006 0432/7 BIXAFEN_049
France (North), Chambourg sur Indre 2006 (Vanessa)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	49	3.7	0.20	3.9	R 2006 0433/5 BIXAFEN_049
Sweden, Staffanstorp 2006 (Pasadena)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	36 45	10 4.3	1.4 0.66	11.4 4.96	R 2006 0434/3 BIXAFEN_049
United Kingdom, Hoxne 2006 (Sequel)	2	0.125 0.125	0.042 0.042	300 300	39 61	straw	62	1.1	0.08	1.18	R 2006 0435/1 BIXAFEN_049
Germany, Swisttal-Heimerzheim 2006 (Class)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35	4.8	0.37	5.17	R 2006 0437/8 BIXAFEN_049
France (North),	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	58	0.64	0.08	0.72	R 2007 0081/4 BIXAFEN_050

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
St. Cyr en Arthies 2007 (Heinley)											
France (North), Carrépuis 2007 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	41 61	straw	60	0.77	0.08	0.85	R 2007 0082/0 BIXAFEN_050
Germany, Vechta-Lanförden 2007 (Tocada)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35	0.70	0.04	0.74	R 2007 0160/8 BIXAFEN_050
United Kingdom, Sandringham 2007 (Tippel)	2	0.125 0.125	0.042 0.042	300 300	39 69	straw	35 66	0.45 1.1	0.13 0.14	0.58 1.24	R 2007 0161/6 BIXAFEN_050
Belgium, Villers-Perwin 2007 (Beatrix)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	34 51	0.68 0.86	0.17 0.14	0.85 1.0	R 2007 0162/4 BIXAFEN_050
France (South), Villeneuve lés Bouloc 2006 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35	5.2	0.40	5.6	R 2006 0438/6 BIXAFEN_051
Italy, Bologna 2006 (Federal)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35 46	0.46 0.32	0.04 0.03	0.50 0.35	R 2006 0439/4 BIXAFEN_051
France (South), Quincieux 2006 (Scarlett)	2	0.125 0.125	0.042 0.042	300 300	37 71	straw	35	3.7	0.37	4.07	R 2006 0440/8 BIXAFEN_051
Spain, Llerona 2006 (Graphic)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35 48	4.2 5.7	0.41 0.42	4.61 6.12	R 2006 0441/6 BIXAFEN_051
Portugal, Azambuja 2006 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35 57	1.4 1.5	0.11 0.14	1.51 1.64	R 2006 0442/4 BIXAFEN_051

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (South), Villeneuve Lés Bouloc 2007 (Prestige)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	60	<u>1.2</u>	0.10	<u>1.3</u>	R 2007 0083/0 BIXAFEN_052
Italy, Bologna 2007 (Tunica)	2	0.125 0.125	0.042 0.042	300 300	39 61	straw	39 56	0.75 <u>0.76</u>	0.25 0.25	1.0 <u>1.01</u>	R 2007 0084/9 BIXAFEN_052
Spain, Caldes de Montbui 2007 (Grafit)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35 40	<u>3.1</u> 2.5	0.22 0.18	<u>3.32</u> 2.68	R 2007 0085/7 BIXAFEN_052
France (South), Cherves 2007 (Scarlett)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	50	<u>1.9</u>	0.23	<u>2.13</u>	R 2007 0158/6 BIXAFEN_052
Italy, Senetica di Bondeno 2007 (Tunica)	2	0.125 0.125	0.042 0.042	300 300	37 61	straw	35	<u>6.2</u>	0.48	<u>6.68</u>	R 2007 0159/4 BIXAFEN_052

a Expressed as bixafen

DALT = Days after last treatment

BBCH 37 = Flag leaf just visible, still rolled

BBCH 39 = Flag leaf stage: flag leaf fully unrolled, ligule just visible

BBCH 41 = Early boot stage: flag leaf sheath extending

BBCH 61 = Beginning of flowering: first anthers visible

BBCH 69 = End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain

BBCH 71 = Watery ripe: first grains have reached half their final size

Table 77 Residues of bixafen and M21 (bixafen-desmethyl) in wheat straw following foliar spray with an EC formulation

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), Chambourg sur Indre 2006 (Tecnico)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	34	<u>10</u>	0.78	<u>10.78</u>	R 2006 0421/1 BIXAFEN_053

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
France (North), Chaussy 2006 (Isengrain)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	37	1.8	0.27	2.07	R 2006 0423/8 BIXAFEN_053
Sweden, Staffanstorp 2006 (Vinjett)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	35 47	8.4 7.8	1.2 1.3	9.6 9.1	R 2006 0424/6 BIXAFEN_053
United Kingdom, Thetford 2006 (Paragon)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	34 38	3.6 3.1	0.26 0.18	3.86 3.28	R 2006 0425/4 BIXAFEN_053
Germany, Leverkusen 2006 (Batis)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	35	1.3	0.20	1.5	R 2006 0426/2 BIXAFEN_053
France (North), Braslou 2007 (Mendel)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	44	0.95	0.35	1.3	R 2007 0091/1 BIXAFEN_054
United Kingdom, Diss 2007 (Belvoir)	2	0.125 0.125	0.039 0.042	318 300	47 69	straw	73	0.52	0.26	0.78	R 2007 0093/8 BIXAFEN_054
France (North), Chambourg sur Indre 2007 (Apache)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	56	1.9	0.6	2.5	R 2007 0094/6 BIXAFEN_054
Sweden, Staffanstorp 2007 (Vinjett)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	69	0.93	0.31	1.24	R 2007 0095/4 BIXAFEN_054
Germany, Burscheid 2007 (Thasos)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	35 56	4.1 0.55	0.30 0.21	4.4 0.76	R 2007 0155/1 BIXAFEN_054
Greece, Thiva 2006	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	35 43	2.6 3.2	0.47 0.50	3.07 3.7	R 2006 0427/0 BIXAFEN_055

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 [a]	Total [a]	Reference
(Claudio)											
Italy, Palidoro Fiumicino 2006 (Claudio)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	35 52	0.90 1.8	0.27 0.37	1.17 2.17	R 2006 0428/9 BIXAFEN_055
France (South), Lagardelle/ Lèze 2006 (not specified)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	35	5.7	0.52	6.22	R 2006 0429/7 BIXAFEN_055
Spain, Paradas Sevilla 2006 (Italo)	2	0.125 0.125	0.042 0.042	300 300	53 69	straw	35 47	1.7 1.5	0.18 0.21	1.88 1.71	R 2006 0430/0 BIXAFEN_055
France (South), Vouille 2006 (Technico)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	35 35	5.4 4.9	0.59 0.60	5.99 5.5	R 2006 0431/9 BIXAFEN_055
France (South), Villeneuve lès Bouloc 2007 (Panifor)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	45	1.4	0.51	1.91	R 2007 0086/5 BIXAFEN_056
Italy, Spinazzola 2007 (Simeto)	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	44	3.6	0.45	4.05	R 2007 0087/3 BIXAFEN_056
France (South), Les Chères 2007 (Autan)	2	0.125 0.125	0.042 0.042	300 300	52 69	straw	44	0.79	0.36	1.15	R 2007 0088/1 BIXAFEN_056
Spain, Alcala de Guadaira Sevilla 2007 (Bolido R1)	2	0.125 0.125	0.045 0.042	279 300	47 69	straw	54	2.6	0.64	3.24	R 2007 0090/3 BIXAFEN_056
Portugal, Ereira 2007	2	0.125 0.125	0.042 0.042	300 300	47 69	straw	53	3.3	0.61	3.91	R 2007 0157/8 BIXAFEN_056

Location,	Application					Residues, mg/kg					Trial No.,
Year (variety)	no	kg ai/ha	kg ai/hL	water L/ha	BBCH	Sample	DALT	Bixafen	M21 a	Total a	Reference
(Galeira)											

a Expressed as bixafen

DALT = Days after last treatment

BBCH 47 = Flag leaf sheath opening

BBCH 52 = 20% of inflorescence emerged

BBCH 53 = 30% of inflorescence emerged

BBCH 69 = End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain

FATE OF RESIDUES IN STORAGE AND PROCESSING

Nature of residue during processing

The hydrolysis of bixafen under processing conditions was investigated by Justus, K and Kuhnke, G (2008, BIXAFEN_061). [Pyrazole-5-^{14}C]-bixafen was diluted in buffered drinking water at 0.25 mg ai/L, which corresponds to approximately 50% of the water solubility. The test solutions contained 0.7% acetonitrile. Incubation was done at three representative sets of hydrolysis conditions: 90 °C, pH 4 for 20 minutes (pasteurisation); 100 °C, pH 5 for 60 minutes (baking, brewing and boiling) and 120 °C, pH 6 for 20 minutes (sterilisation).

Parent compound and potential hydrolysis products were identified and quantified by HPLC. Thin layer chromatography was used for confirmation of the identity of the test item by co-chromatography with the non-labelled reference item. Material balances were established for each set of hydrolysis conditions.

In the following table the recovered radioactivity and its composition is summarised:

Table 78 Hydrolysis of bixafen under simulated processing conditions

Hydrolysis conditions	Incubation time (min)	Bixafen	
		mg/L	% applied radioactivity
pH 4, 90 °C	0	0.240	100
	20	0.236	98.2
pH 5, 100 °C	0	0.222	100
	60	0.222	100
pH 6, 120 °C	0	0.245	100
	20	0.240	980

Residues after processing

The fate of bixafen and its metabolite M21 (bixafen-desmethyl) during processing of raw agricultural commodity (RAC) was investigated in rapeseeds, barley grain and wheat grain using important processing procedures. As a measure of the transfer of residues into processed products, a processing factor (PF) was used, which is defined as:

$$PF = \frac{\text{Total residue in processed product (mg kg}^{-1}\text{)}}{\text{Total residue in raw agricultural commodity (mg kg}^{-1}\text{)}}$$

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

A summary of all processing factors for bixafen relevant for the estimation of maximum residue levels of the dietary intake is given in Table 79.

Rape seed

For the processing of oilseed, rape seed collected from three locations in Northern Europe (Freitag, T, Reineke, A and Krusell, L, 2010, BIXAFEN_057; Freitag, T, Reineke, A and Krusell, L, 2010, BIXAFEN_066 and Noss, G and Teubner, L, 2010, BIXAFEN_067) were processed into pomace, meal and different oils simulating the industrial practice at a laboratory scale (Freitag, T and Hoffmann, M, 2011, BIXAFEN_68; Freitag, T and Hoffmann, M, 2011, BIXAFEN_69 and Hoffmann, M and Teubner, L, 2012, BIXAFEN_70). The treatment program consisted of two spray applications at 0.06 or 0.075 kg ai/ha for each treatment. The first treatment was carried out at the growth stage BBCH 64 to 69 (end of flowering), and the second one at BBCH 69 to 75. The sampling dates were 35, 44 or 64 days after the final application was carried out.

The rape seeds were dried and cleaned using a sieve. The conditioned and cleaned rape seeds were pressed in a screw press yielding oil, screw-pressed and pomace.

An aliquot of the pomace was milled and then extracted with addition of n-hexane. In the rotary evaporator the n-hexane was removed, yielding oil, solvent extracted. The solvent-extracted meal was sampled after storing at room temperature or drying.

The oil fractions (screw-pressed oil and solvent extracted oil) were mixed yielding the sample of crude oil. The crude oil was filtered or hydrated and deslimed by adding water and phosphorus acid, heating and stirring, yielding the sample crude oil, preclarified.

The oily phase was heated while stirring and a sodium hydroxide solution was added. Water was added and the phases were allowed to separate. After phase separation the watery phase was removed. Crude oil, neutralised was sampled.

For bleaching, the oil was heated up to 90–100 °C while stirring. After addition of 1% pod sol the oil was bleached for 5 min without vacuum and 20 min with vacuum. Afterwards the pod sol was removed (filtration).

The oil was heated under vacuum up to160 °C while stirring. After reaching 160 °C, steam was transferred through the oil for deodorization. After cooling to 160 °C the steam supply was stopped and then the oil was dried under vacuum until a temperature of ≤ 80 °C was reached. The refined oil was sampled.

All samples were analysed for bixafen and M21 (bixafen-desmethyl) according to method 01013. The limit of quantification (LOQ) was 0.01 mg/kg for the individual analytes, and consequently 0.02 mg/kg for the sum of both in all matrices. In the following table the residues found in the processed products are summarised:

Table 79 Residues of bixafen and M21 (bixafen-desmethyl) in processed rape commodities and calculation of processing factors

Location, year, reference (variety)	No.	kg ai/ha	BBCH	Sample	DALT	Bixafen		M21 (bixafen-desmethyl)		Total	
						mg/kg	PF	mg/kg	PF	mg/kg	PF
The Netherlands, Hoofddorp 2008 (Maximus)	2	0.075 0.075	64 69	seed	64	< 0.01	–	< 0.01	–	< 0.01	–
				oil, screw-pressed	64	< 0.01	–	< 0.01	–	< 0.01	–
				pomace	64	< 0.01	–	< 0.01	–	< 0.01	–

Location, year, reference (variety)	No.	kg ai/ha	BBCH	Sample	DALT	Bixafen		M21 (bixafen-desmethyl)		Total	
						mg/kg	PF	mg/kg	PF	mg/kg	PF
08-2116-02				meal	64	< 0.01	–	< 0.01	–	< 0.01	–
Field part: BIXAFEN_057				oil, solv. extracted	64	< 0.01	–	< 0.01	–	< 0.01	–
Processing part: BIXAFEN_068				oil, crude	64	< 0.01	–	< 0.01	–	< 0.01	–
				crude oil, preclarified	64	< 0.01	–	< 0.01	–	< 0.01	–
				crude oil, neutralised	64	< 0.01	–	< 0.01	–	< 0.01	–
				oil, refined	64	< 0.01	–	< 0.01	–	< 0.01	–
Germany, Burscheid	2	0.06 0.06	69 73	seed	35	0.02	–	< 0.01	–	0.03	–
2008 (Titan)				oil, screw-pressed	35	< 0.01	< 0.5	< 0.01	–	< 0.02	< 0.66
08-2112-02				pomace	35	0.01	0.5	0.01	–	0.02	0.66
Field part: BIXAFEN_056				meal	35	< 0.01	< 0.5	0.01	–	0.02	0.66
Processing part: BIXAFEN_069				oil, solv. extracted	35	< 0.01	< 0.5	< 0.01	–	< 0.02	< 0.66
				oil, crude	35	0.01	0.5	< 0.01	–	0.02	0.66
				crude oil, preclarified	35	< 0.01	< 0.5	< 0.01	–	< 0.02	< 0.66
				crude oil, neutralised	35	< 0.01	< 0.5	< 0.01	–	< 0.02	< 0.66
				oil, refined	35	< 0.01	< 0.5	< 0.01	–	< 0.02	< 0.66
United KIngdom, Bishop Burton	2	0.06 0.06	67 75	seed	44	0.01	–	< 0.01	–	0.02	–
2008 (Castille)				oil, screw-pressed	44	< 0.01	< 1	< 0.01	–	< 0.02	< 1
09-2245-01				pomace	44	0.01	1	< 0.01	–	0.02	1
Field part: BIXAFEN_067				meal	44	0.02	2	< 0.01	–	0.03	1.5
Processing part: BIXAFEN_070				oil, solv. extracted	44	0.02	2	< 0.01	–	0.03	1.5
				oil, crude	44	0.01	1	< 0.01	–	0.02	1
				crude oil, preclarified	44	0.01	1	< 0.01	–	0.02	1
				crude oil, neutralised	44	0.02	2	< 0.01	–	0.03	1.5
				oil, refined	44	0.02	2	< 0.01	–	0.03	1.5

DALT = Days after last treatment

PF = Processing factor

BBCH 64 = 40% of flowers on main raceme open

BBCH 67 = Flowering declining: majority of petals fallen
BBCH 69 = End of flowering
BBCH 71–78 = 10% to 80% of pods have reached final size

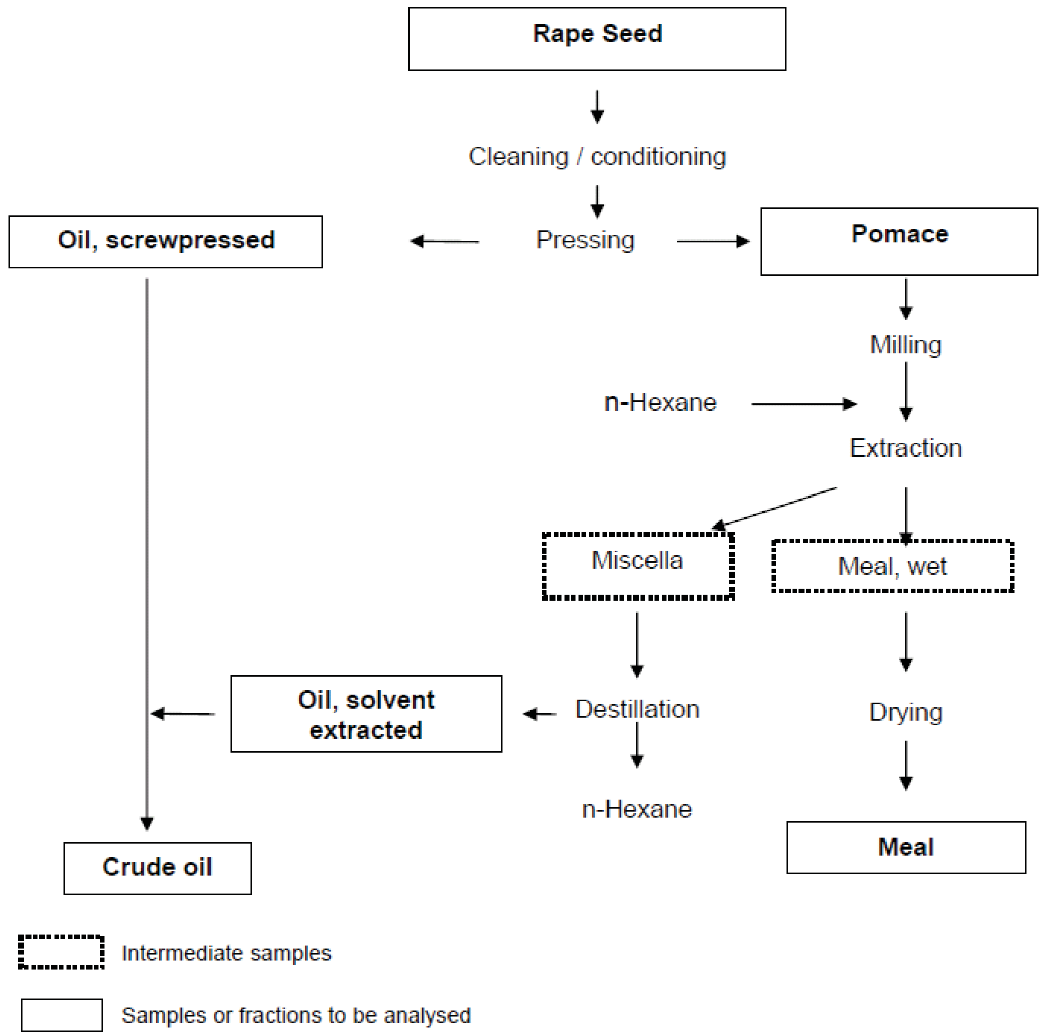


Figure 8 Flow chart of rape seeds processed into pomace, oil, screw-pressed, crude oil, oil, solvent extracted and meal

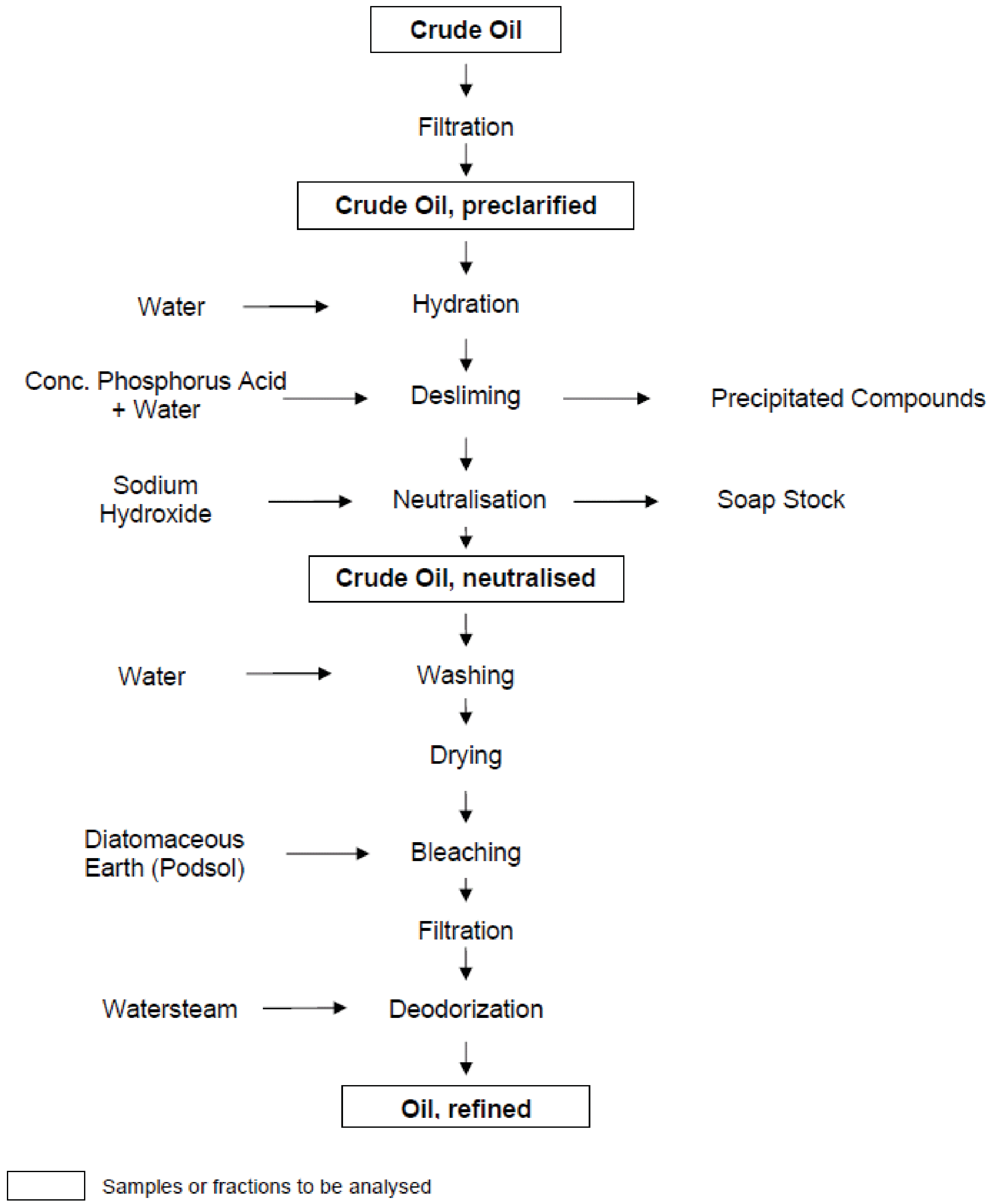


Figure 9 Flow chart for rape seeds processed into crude oil, preclarified, crude oil, neutralised and oil, refined

Barley

In Northern Europe four supervised field trials on barley were conducted involving two treatments at BBCH 37 and 61 with 0.25 kg ai/ha each (Schoening, R and Wolters, A, 2007, BIXAFEN_062). Samples were collected 35 to 46 days after the last treatment.

The production of both beer and pearl barley from grain of these trials was performed on a technical scale simulating commercial procedures (Schoening, R, Billian, P and Wolters, A, 2007, BIXAFEN_063).

In order to prepare pearl barley, the grain was first cleaned to separate husks and other impurities from the barley. Then the seeds were conditioned by adjusting the moisture content to about 14–16%. Depending on the initial moisture content this was done by damping prior to further processing for two of the four samples. Finally, the seeds were decorticated in a mill where they were passed through a vertically rotating cylinder. An abrasion of 30–35% was reached. For analysis, samples of pearl barley and pearl barley rub-off were collected (see Figure).

The beer brewing process starts with sieving and cleaning of the specimen. Before the beginning of the malting, the barley grains undergo a 3-step steeping procedure (combination of wet and dry steeping) at a temperature of 13–14 °C, over 2 days, until a final steeping degree of 44.8–44.9% was reached. Germination took place at a temperature of 14 ± 1 °C for 5 days at a relative air humidity of > 90%. After germination, the malting process is terminated by kiln-drying at maximum 82 °C. At the end of drying, samples of brewing malt and malt culms (malt sprouts) were taken.

In a next step, the malt was ground. Mash was produced by adding water, and heating the mixture at stepwise increasing temperatures with a defined temperature and time regime. The mash was then lautered in a 2-step process (filtering and washing). This process resulted in brewer's grain, of which samples were taken, and wort. The wort was cooked and commercially bought hops were added. After boiling, the flocs (hops draff) were separated in a whirlpool. The deposited hops draff was sampled. After cooling and ventilating of the wort, yeast was added. After a period of 6–-7 days, the yeast deposited on the tank bottom and was sampled as brewer's yeast. At the beginning of maturation the young beer was stored at room temperature (warm maturation) for 2 days in casks. Subsequently, the young beer was stored under pressure at 2 °C for about 4 weeks (cold maturation). During this time the remaining extract was fermented and sludge particles and yeast settled at the bottom. The rack beer was then filtered and filled into bottles. Following filtration, beer samples were taken (see Figure & 12).

All samples were analysed for bixafen and M21 (bixafen-desmethyl) according to method 01012. The limit of quantification (LOQ) was 0.01 mg/kg for the individual analytes, and consequently 0.02 mg/kg for the sum of both in all matrices. In the following table the residues found in the processed products are summarised:

Table 80 Residues of bixafen and M21 (bixafen-desmethyl) in processed barley commodities and calculation of processing factors

Location, year, reference (variety)	No.	kg ai/ha	BBCH	Sample	DALT	Bixafen		M21 (bixafen-desmethyl)		Total	
						mg/kg	PF	mg/kg	PF	mg/kg	PF
Sweden,	2	0.25	37	grain	40	0.23	–	0.03	–	0.26	–
Staffanstorp		0.25	61	brewer's malt	40	0.22	0.96	0.06	2	0.27	1
				malt culms	40	0.17	0.74	0.06	2	0.23	0.88
2006				beer	40	< 0.01	< 0.04	< 0.01	< 0.33	< 0.02	< 0.08
(Prestige)				brewer's	40	0.04	0.17	0.01	0.33	0.05	0.19
				yeast	40	0.23	1	0.04	1.3	0.27	1
R 2006 0444/0				brewer's	40	0.17	0.74	0.03	1	0.20	0.77
				grain	40	0.04	0.17	0.01	0.33	0.05	0.19
Field part:				hops draff	40	0.74	3.2	0.09	3	0.84	3.2
BIXAFEN_062				pearl barley							
				pearl barley							
Processing part:				rub-off							
BIXAFEN_063											
Germany,	2	0.25	37	grain	35	0.13	–	0.02	–	0.15	–
Swisttal-		0.25	61	brewer's malt	35	0.12	0.92	0.03	1.5	0.15	1
Heimerzheim				malt culms	35	0.14	1.1	0.04	2	0.18	1.2

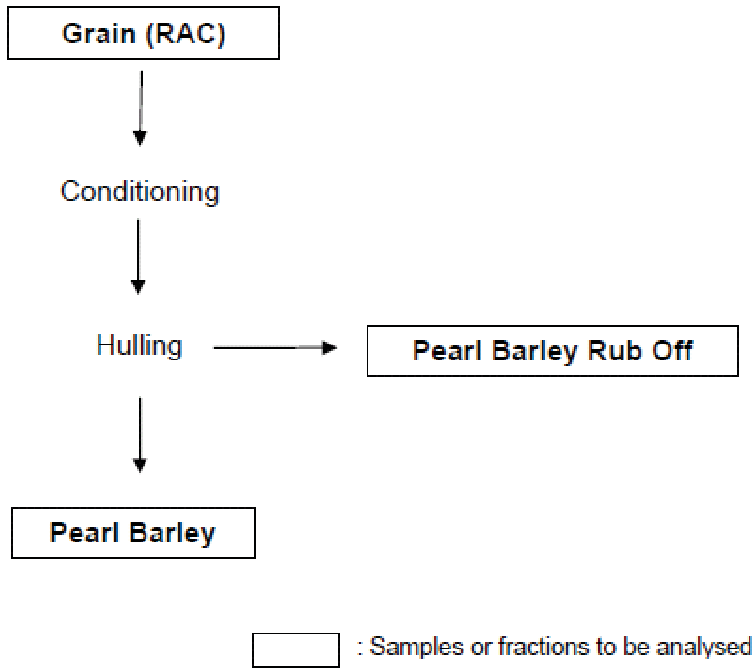
Location, year, reference (variety)	No.	kg ai/ha	BBCH	Sample	DALT	Bixafen		M21 (bixafen-desmethyl)		Total	
						mg/kg	PF	mg/kg	PF	mg/kg	PF
2006 (Class) R 2006 0445/9 Field part: BIXAFEN_062 Processing part: BIXAFEN_063				beer brewer's yeast brewer's grain hops draff pearl barley pearl barley rub-off	35 35 35 35 35 35	< 0.01 0.01 0.14 0.09 0.03 0.67	< 0.08 0.08 1.1 0.69 0.23 5.2	< 0.01 < 0.01 0.03 0.02 0.01 0.07	< 0.5 < 0.5 1.5 1 0.5 3.5	< 0.02 0.02 0.16 0.10 0.04 0.74	< 0.13 0.13 1.1 0.67 0.27 4.9
France (North), Fresnoy les Roye 2006 (Scarlet) R 2006 0446/7 Field part: BIXAFEN_062 Processing part: BIXAFEN_063	2	0.25 0.25	37 61	grain brewer's malt malt culms beer brewer's yeast brewer's grain hops draff pearl barley pearl barley rub-off	46 46 46 46 46 46 46 46 46	0.20 0.16 0.14 < 0.01 0.04 0.17 0.13 0.04 0.91	– 0.8 0.7 < 0.05 0.2 0.85 0.65 0.2 4.6	0.02 0.04 0.03 < 0.01 < 0.01 0.03 0.02 0.01 0.06	– 2 1.5 < 0.5 < 0.5 1.5 1 0.5 3	0.22 0.20 0.17 < 0.02 0.05 0.19 0.15 0.05 0.97	– 0.91 0.77 < 0.09 0.23 0.86 0.68 0.23 4.4
Germany, Burscheid 2006 (Barke) R 2006 0447/5 Field part: BIXAFEN_062 Processing part: BIXAFEN_063	2	0.25 0.25	37 61	grain brewer's malt malt culms beer brewer's yeast brewer's grain hops draff pearl barley pearl barley rub-off	43 43 43 43 43 43 43 43 43	0.03 0.01 0.01 < 0.01 < 0.01 0.01 0.01 < 0.01 0.11	– 0.33 0.33 < 0.33 < 0.33 0.33 0.33 < 0.33 3.7	< 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 0.01	– – – – – – – – –	0.04 0.02 0.02 < 0.02 < 0.02 0.02 0.02 < 0.02 0.12	– 0.5 0.5 < 0.5 < 0.5 0.5 0.5 < 0.5 3

DALT = Days after last treatment

PF = Processing factor

BBCH 37 = Flag leaf just visible, still rolled

BBCH 61 = Beginning of flowering: first anthers visible



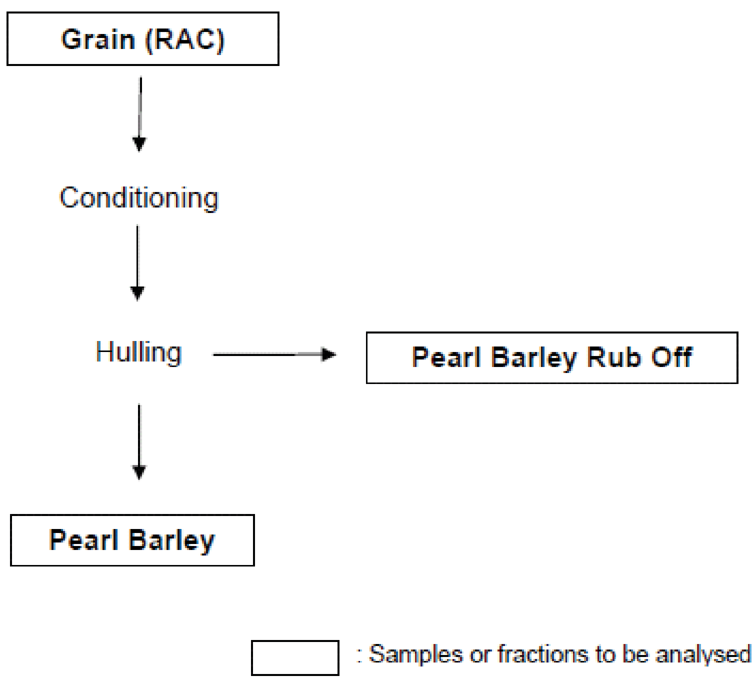


Figure 10 Flow chart of the processing of barley to pearl barley

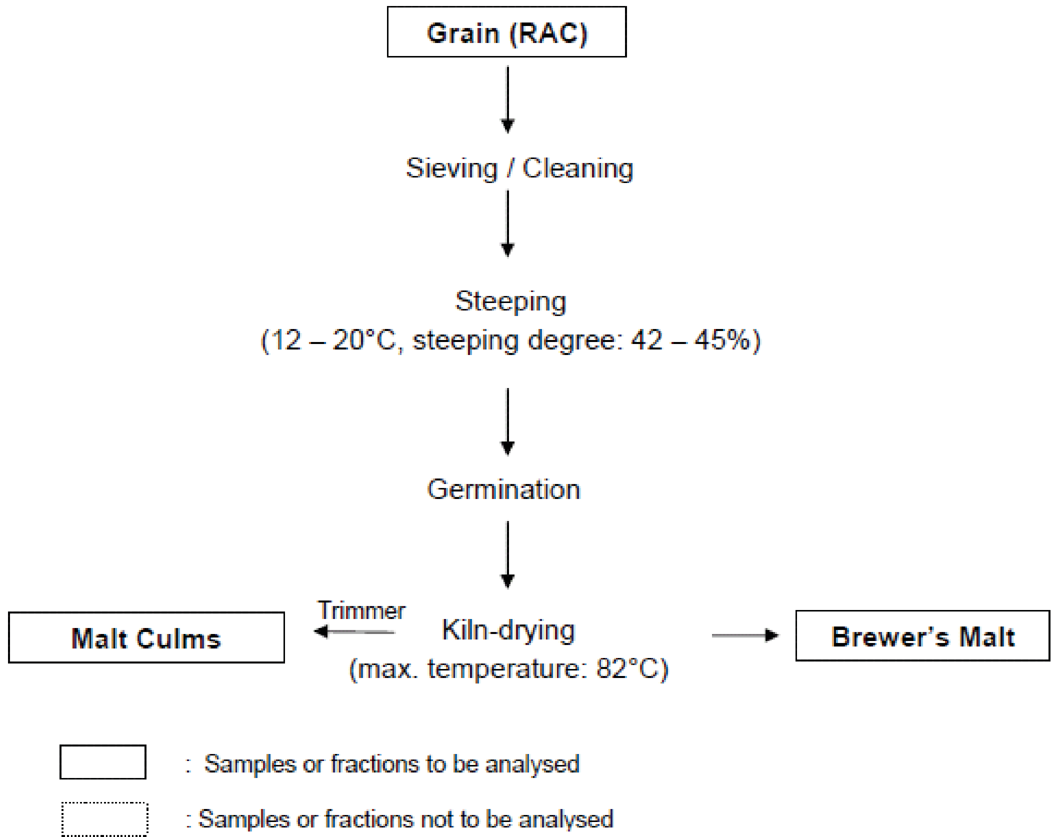


Figure 11 Flow chart of the processing of barley to malt

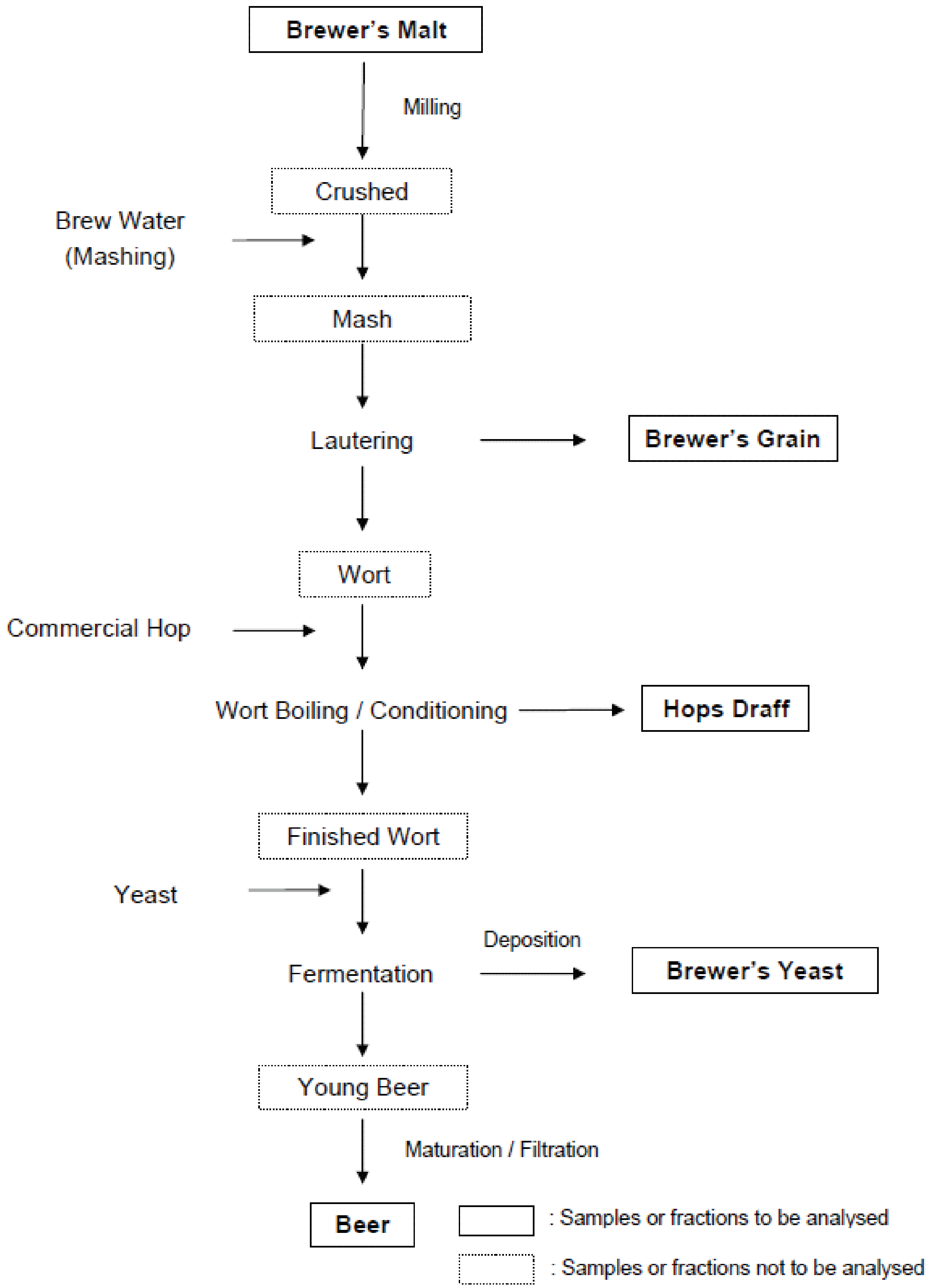


Figure 12 Flow chart of the processing of malt to beer

Wheat

In 2006, four residue studies on winter wheat were conducted in Northern Europe by Schoening, R and Erler, S (2007, BIXAFEN_064). The treatment program consisted of two spray applications at BBCH 47 and BBCH 69 with application rates of 0.232 to 0.250 kg ai/ha each. The sampling dates were 39 to 53 days after the final application.

The processing of wheat grain samples collected from three of the locations into processed fractions (white flour, white flour bran, semolina, semolina bran, white bread, whole-meal, whole-meal bread and wheat germs) was performed in specialised pilot plants to simulate industrial procedures of the milling and bakery industry (Schoening, R and Erler, S, 2007, BIXAFEN_065). The sample originating from the United Kingdom was divided in two portions (A and B). The second portion (B) was processed at a different processing site than portion A.

In order to prepare flour and semolina, the grain was first cleaned and a moisture content of about 17% was adjusted. The cleaned grain was then milled to either white flour and white flour bran, to whole meal flour, or to semolina and semolina bran.

For preparation of white bread the ingredients of the dough were weighed, combined and kneaded. After kneading the dough was placed into the fermentation chamber for fermentation. The bread was baked on one level in the baking oven at a constant heat of 230 °C (oven setting) for 30 min.

For preparation of whole-meal bread the sourdough was prepared one day before the baking process. Whole-meal and water were mixed together with the starter. The mixture was placed into the fermentation chamber. The ingredients of the dough were weighted, combined and kneaded. After kneading the dough was placed into the fermentation chamber for fermentation. The bread was baked on one level in the baking oven at a constant heat of 207 °C (oven setting) for nearly 1 hour.

All samples were analysed for bixafen and M21 (bixafen-desmethyl) according to method 01012. The limit of quantification (LOQ) was 0.01 mg/kg for the individual analytes, and consequently 0.02 mg/kg for the sum of both in all matrices. In the following table the residues found in the processed products are summarised:

Table 81 Residues of bixafen and M21 (bixafen-desmethyl) in processed wheat commodities and calculation of processing factors

Location, year, reference (variety)	No.	kg ai/ha	BBCH	Sample	DALT	Bixafen		M21 (bixafen-desmethyl)		Total	
						mg/kg	PF	mg/kg	PF	mg/kg	PF
United Kingdom,	2	0.23	47	grain	40	0.05	–	< 0.01	–	0.06	–
Bury St.		0.25	69	white flour	40	0.01	0.2	< 0.01	–	0.02	0.33
Edmunds				white flour bran	40	0.13	2.6	0.02	–	0.15	2.5
				semolina	40	< 0.01	< 0.2	< 0.01	–	< 0.02	< 0.33
2006				semolina	40	< 0.01	< 0.2	< 0.01	–	< 0.02	< 0.33
(Cordiale)				bran	40	< 0.01	< 0.2	< 0.01	–	< 0.02	< 0.33
				white bread	40	0.04	0.8	< 0.01	–	0.05	0.83
R 2006 0527/7				whole meal	40	0.02	0.4	< 0.01	–	0.03	0.5
Field part: BIXAFEN_064				wholemeal bread	40	0.06	1.2	0.02	–	0.08	1.33
				wheat germ							
Processing part: BIXAFEN_065 Portion A											
United Kingdom,	2	0.23	47	grain	40	0.05	–	< 0.01	–	0.06	–
Bury St.		0.25	69	white flour	40	0.01	0.2	< 0.01	–	0.02	0.33
Edmunds				white flour bran	40	0.14	2.4	0.02	–	0.16	2.7
				semolina	40	< 0.01	< 0.2	< 0.01	–	< 0.02	< 0.33

Location, year, reference (variety)	No.	kg ai/ha	BBCH	Sample	DALT	Bixafen		M21 (bixafen-desmethyl)		Total	
						mg/kg	PF	mg/kg	PF	mg/kg	PF
2006				semolina	40	0.07	1.4	0.01	–	0.08	1.33
(Cordiale)				bran	40	0.01	0.2	< 0.01	–	0.02	0.33
				white bread	40	0.04	0.8	< 0.01	–	0.05	0.83
R 2006 0527/7				whole meal	40	0.03	0.6	< 0.01	–	0.04	0.66
Field part:				wholemeal							
BIXAFEN_064				bread	40	0.03	0.6	0.02	–	0.05	0.83
				wheat germ							
Processing part:											
BIXAFEN_065											
Portion B											
France (North),	2	0.25	47	grain	53	0.02	–	0.01	–	0.03	–
Fresnoy les		0.25	69	white flour	53	0.01	0.5	< 0.01	< 1	0.02	0.66
Roye				white flour	53	0.07	3.5	0.05	5	0.12	4
				bran							
2006				semolina	53	< 0.01	< 0.5	< 0.01	< 1	< 0.02	< 0.66
(Chango)				semolina	53	0.01	0.5	< 0.01	< 1	0.02	0.66
				bran	53	< 0.01	< 0.5	< 0.01	< 1	< 0.02	< 0.66
R 2006 0527/7				white bread	53	0.03	1.5	0.02	2	0.05	1.7
Field part:				whole meal	53	0.01	0.5	0.01	1	0.02	0.66
BIXAFEN_064				wholemeal							
				bread	53	0.03	1.5	0.05	5	0.08	2.7
Processing part:				wheat germ							
BIXAFEN_065											
France (North),	2	0.25	47	grain	39	0.04	–	< 0.01	–	0.05	–
Chambourg sur		0.25	69	white flour	39	0.01	0.25	< 0.01	–	0.02	0.4
Indre				white flour	39	0.11	2.8	0.01	–	0.12	2.4
				bran							
2006				semolina	39	< 0.01	< 0.25	< 0.01	–	< 0.02	< 0.4
(Apache)				semolina	39	0.01	0.25	< 0.01	–	0.02	0.4
				bran	39	< 0.01	< 0.25	< 0.01	–	< 0.02	< 0.4
R 2006 0527/7				white bread	39	0.04	1	< 0.01	–	0.05	1
Field part:				whole meal	39	0.02	0.5	< 0.01	–	0.03	0.6
BIXAFEN_064				wholemeal							
				bread	39	0.03	0.75	0.01	–	0.04	0.8
Processing part:				wheat germ							
BIXAFEN_065											

Portion A/B = Field samples split into two processing samples

DALT = Days after last treatment

PF = Processing factor

BBCH 47 = Flag leaf sheath opening

BBCH 69 = End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain

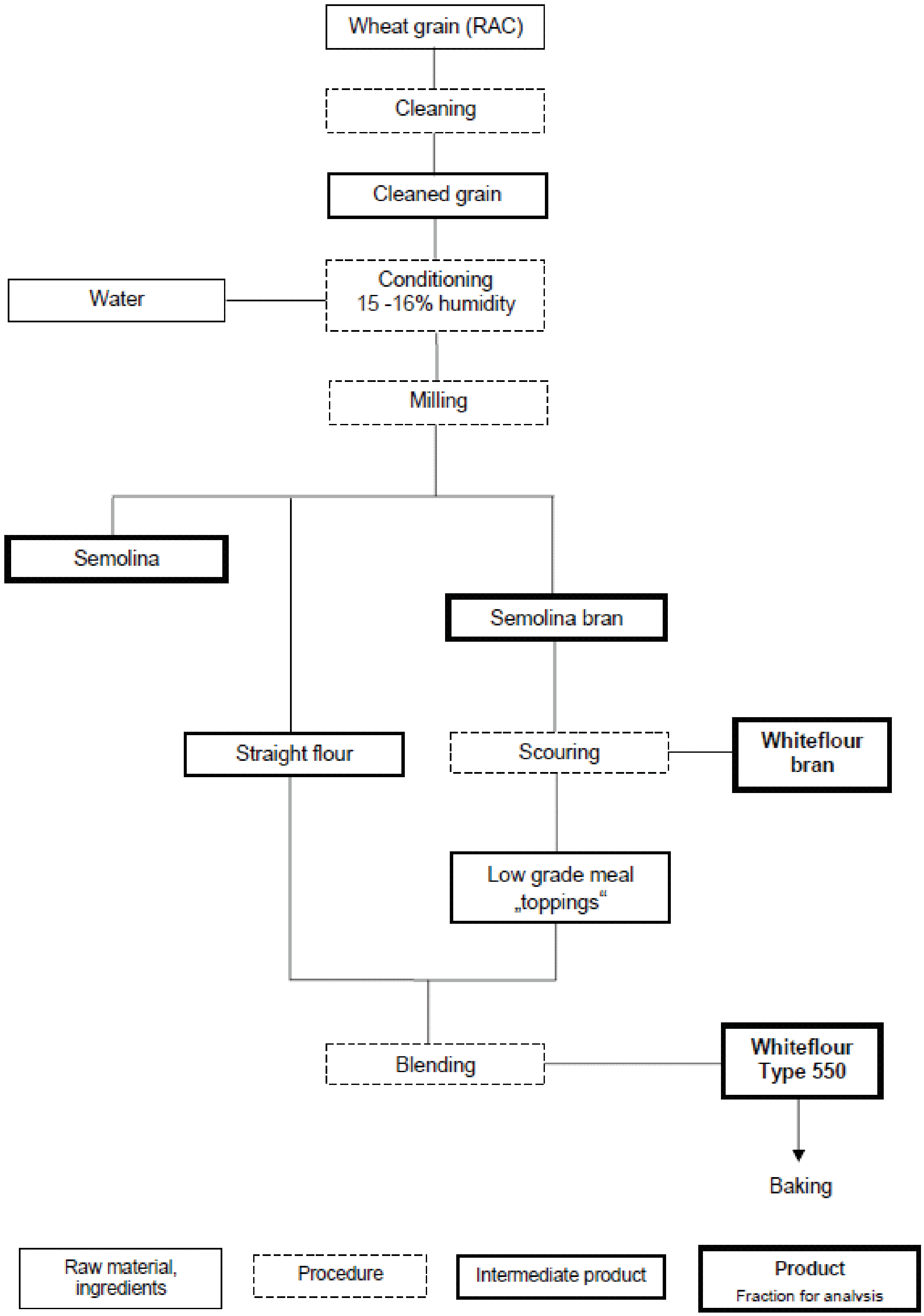


Figure 13 Flow chart for processing wheat grain into white flour, semolina and white flour bran

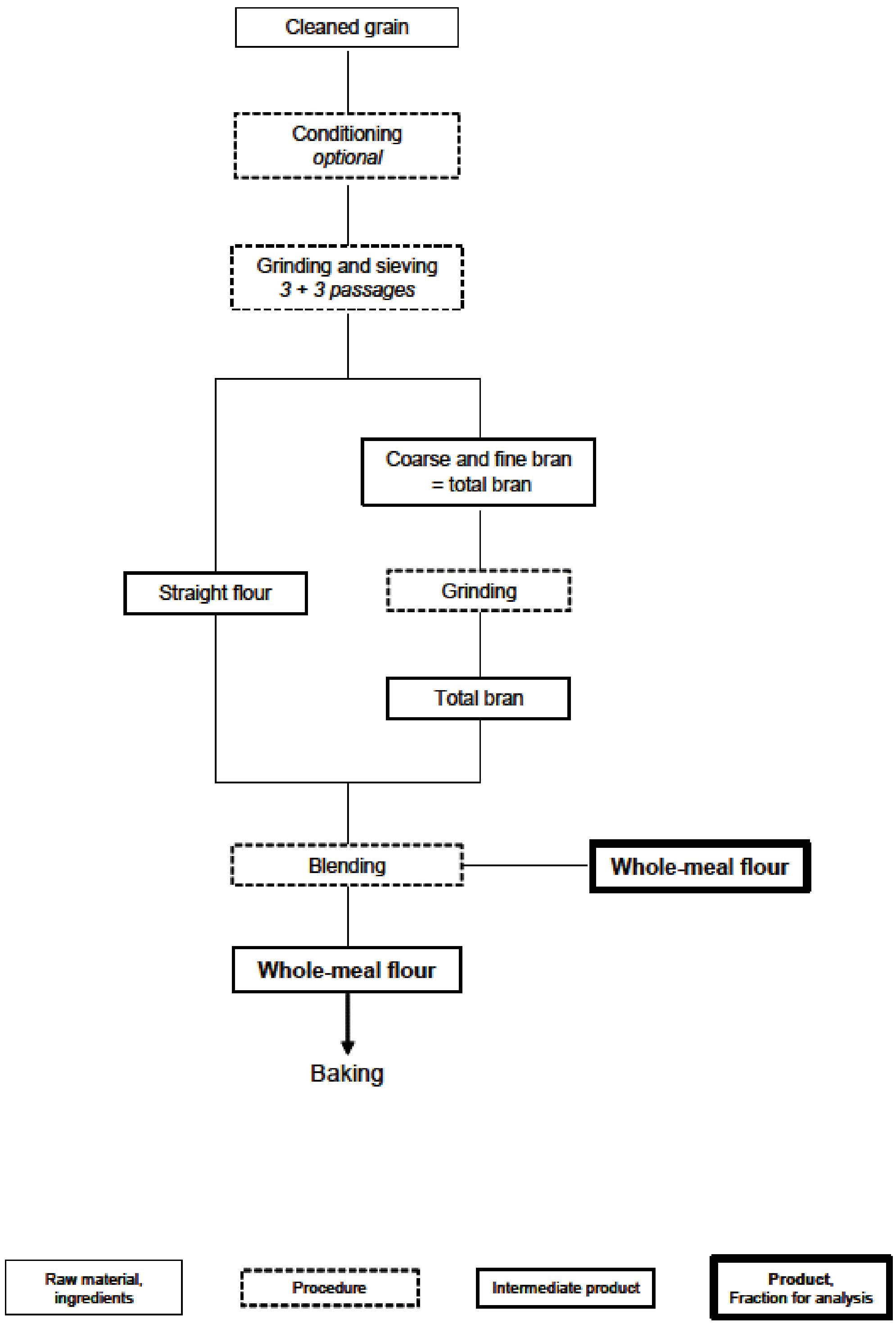


Figure 14 Flow chart for processing wheat grain into wholemeal flour

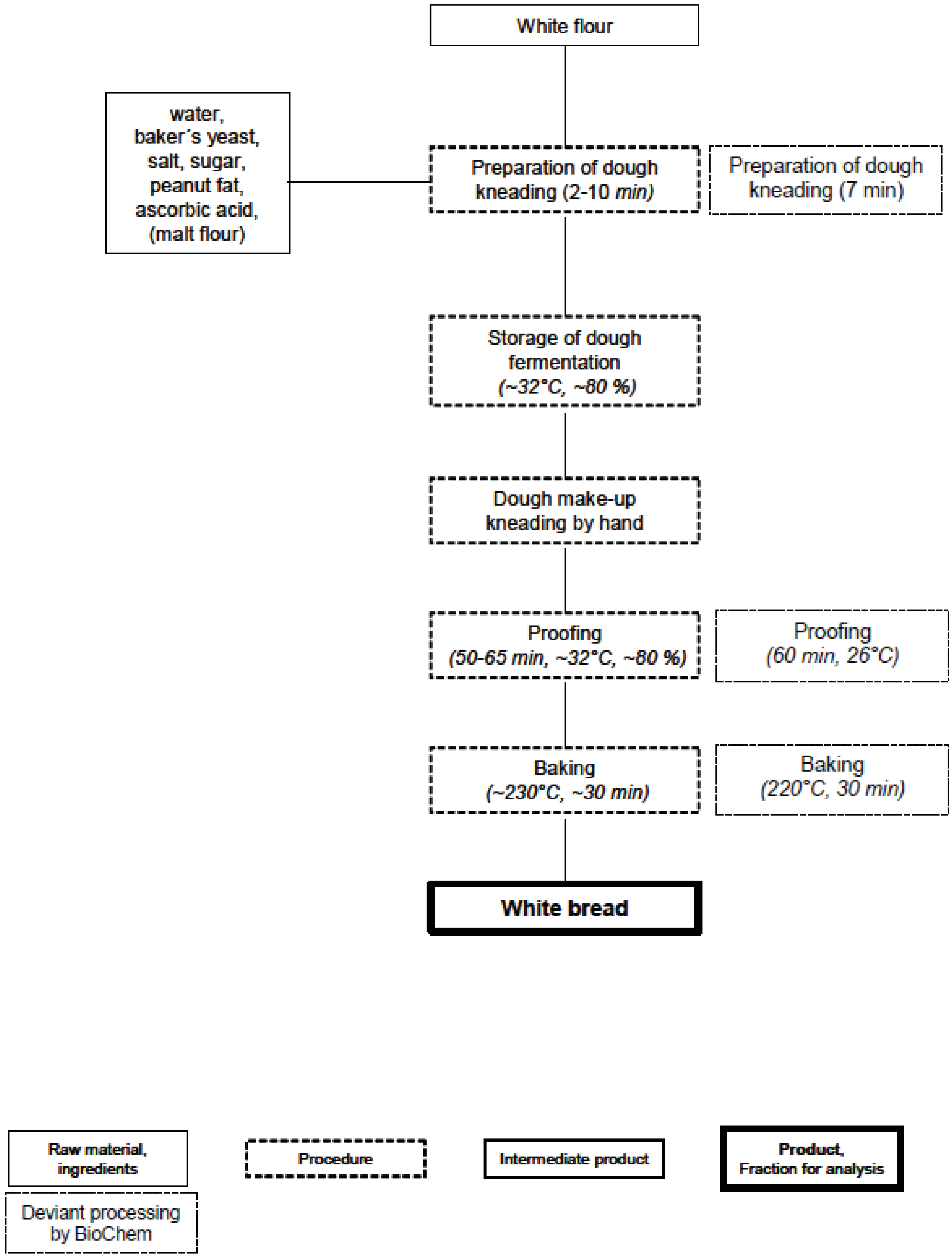


Figure 15 Flow chart for processing wheat grain into white bread

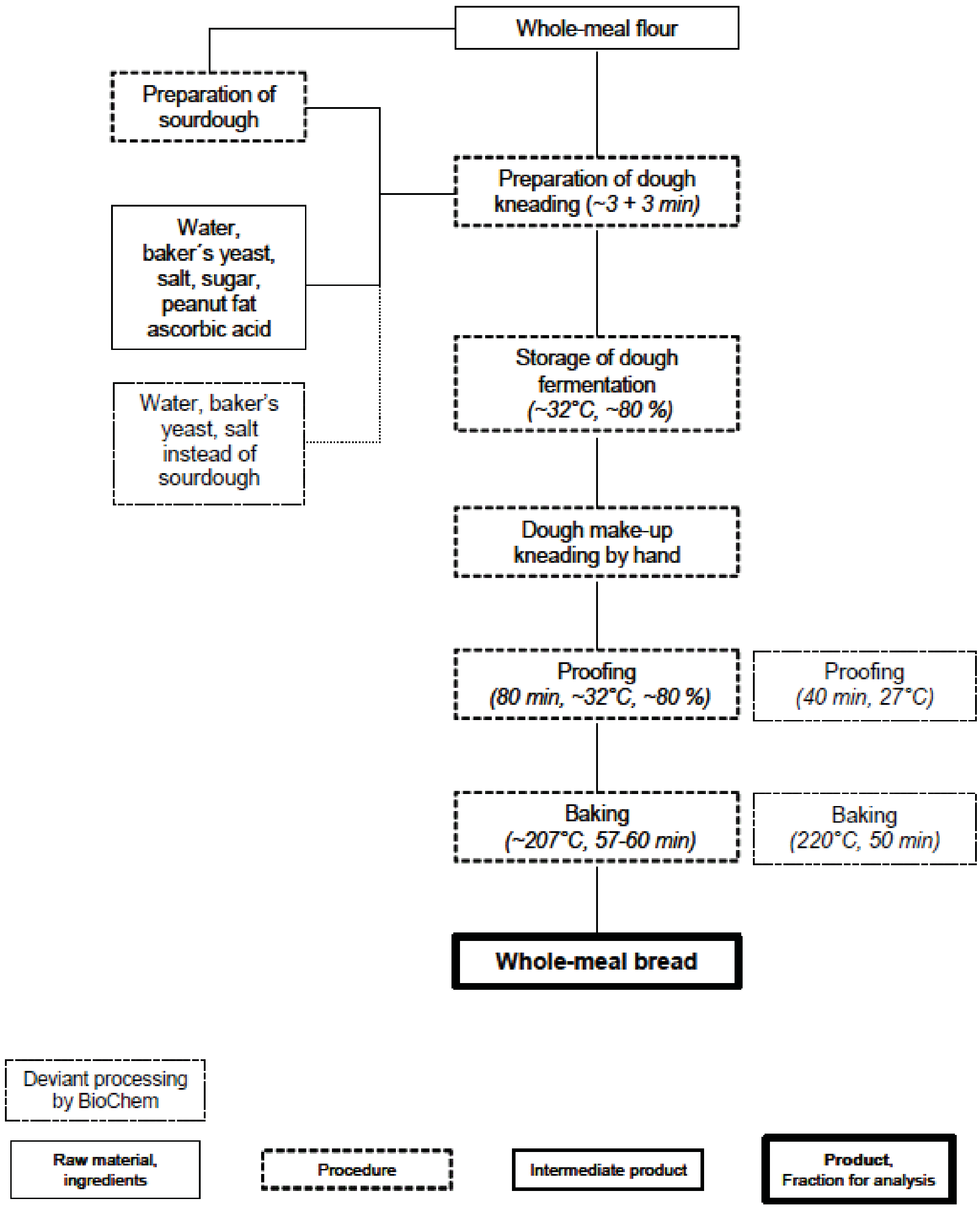


Figure 16 Flow chart for processing wheat grain into wholemeal bread

Table 82 Overview of processing factors for bixafen-derived residues

Raw commodity	Processed commodity	Bixafen		M21 (bixafen-desmethyl)		Total residue	
		Individual processing factors	Mean or best estimate processing factor	Individual processing factors	Mean or best estimate processing factor	Individual processing factors	Median or best estimate processing factor
Rape	oil, screw-pressed	< 0.5, < 1	1 (best estimate)	–	–	< 0.66, < 1	1 (best estimate)
	pomace	0.5, 1	0.75	–	–	0.66, 1	0.83
	meal	< 0.5, 2	2 (best estimate)	–	–	0.66, 1.5	1.5 (best estimate)
	oil, solv. extracted	< 0.5, 2	2 (best estimate)	–	–	< 0.66, 1.5	1.5 (best estimate)
	oil, crude	0.5, 1	0.75	–	–	0.66, 1	0.83
	crude oil, preclarified	< 0.5, 1	1 (best estimate)	–	–	< 0.66, 1	1.5 (best estimate)
	crude oil, neutralised	< 0.5, 2	2 (best estimate)	–	–	< 0.66, 1.5	1.5 (best estimate)
	oil, refined	< 0.5, 2	2 (best estimate)	–	–	< 0.66, 1.5	1.5 (best estimate)
Barley	brewer's malt	0.33, 0.8, 0.92, 0.96	0.86	1.5, 2, 2	2	0.5, 0.91, 1, 1	0.96
	malt culms	0.33, 0.7, 0.74, 1.1	0.72	1.5, 2, 2	2	0.5, 0.77, 0.88, 1.2	0.83
	beer	< 0.04, < 0.05, < 0.08, < 0.33	< 0.065	< 0.33, < 0.5, < 0.5	< 0.5	0.08, < 0.09, < 0.13, < 0.5	< 0.11
	brewer's yeast	0.08, 0.17, 0.2, < 0.33	0.19	0.33, < 0.5, < 0.5	< 0.5	0.13, 0.19, 0.23, < 0.5	0.21
	brewer's grain	0.33, 0.85, 1, 1.1	0.93	1.3, 1.5, 1.5	1.5	0.5, 0.86, 1, 1.1	0.93
	hops draff	0.33, 0.65, 0.69, 0.74	0.67	1, 1, 1	1	0.5, 0.67, 0.68, 0.77	0.68
	pearl barley	0.17, 0.2, 0.23, < 0.33	0.22	0.33, 0.5, 0.5	0.5	0.19, 0.23, 0.27, < 0.5	0.25
	pearl barley rub-off	3.2, 3.3, 4.6, 5.2	4	3, 3, 3.5	3	3, 3.2, 4.4, 4.9	3.8
Wheat	white flour	0.2, 0.2, 0.25, 0.5	0.23	< 1	< 1	0.33, 0.33, 0.4, 0.66	0.37
	White flour bran	2.4, 2.6, 2.8, 3.5	2.7	5	5	2.4, 2.5, 2.7, 4	2.6
	semolina	< 0.2, < 0.2, < 0.25, < 0.5	< 0.23	< 1	< 1	< 0.33, < 0.33, < 0.4, < 0.66	< 0.37
	semolina bran	< 0.2, 0.25, 0.5, 1.4	0.38	< 1	< 1	< 0.33, 0.4, 0.66, 1.33	0.53
	white bread	< 0.2, 0.2, < 0.25, < 0.5	0.2 (best estimate)	< 1	< 1	< 0.33, 0.33, < 0.4, < 0.66	0.33 (best estimate)
	whole meal	0.8, 0.8, 1, 1.5	0.9	2	2	0.83, 0.83, 1, 1.7	0.91
	wholemeal bread	0.4, 0.5, 0.5, 0.6	0.5	1	1	0.5, 0.6, 0.66, 0.66	0.63
	wheat germ	0.6, 0.75, 1.2, 1.5	1	5	5	0.8, 0.83, 1.3, 2.7	1.1

RESIDUES IN ANIMAL COMMODITIES

Farm animal feeding studies

For the estimation of residues of bixafen and its metabolite M21 (bixafen-desmethyl) in animal matrices laying hens and lactating cow feeding studies were submitted to the Meeting.

Poultry

The magnitude of the residue of bixafen and its metabolite M21 (bixafen-desmethyl) has been studied in laying hens by Billian, P, Barfknecht, R and Wolters, A (2008, BIXAFEN_071). Adult chickens (12 birds per diet group divided into three subgroups with four animals each, one control group with four birds) were exposed for 28 consecutive days to levels of 1.5 ppm (1× dose group), 4.5 ppm (3× dose group), or 15 ppm feed/day (10× dose group) corresponding to approximately 0.1 , 0.3 and 1.0 mg/kg bw/day. For a depuration study, three groups of hens were fed at the 10× dose rate for 28 consecutive days followed by untreated feed for another 7 days, 14 days or 21 days. Each subgroup comprised five birds.

Eggs were collected during the whole dosing period. On day 29 after the first dose, the hens were sacrificed, and liver, fat and overlaying skin, and muscle from breast and leg were collected for analysis.

Tissues and eggs were pooled and analysed using method 01036 (see analytical method section). The analytical method was validated for bixafen and M21 at a LOQ of 0.01 mg/kg for each analyte. The LOQ of the total bixafen residues (sum of bixafen and bixafen-desmethyl) was calculated to be 0.02 mg/kg. Residue data were obtained by high performance liquid chromatography-electrospray ionization/tandem mass spectrometry (HPLC-MS/MS) using isotopically labelled internal standards.

In the following table the residues found in eggs are summarised. Prior to dosing of bixafen, eggs collected contained no detectable residues of bixafen or M21 (bixafen-desmethyl). The results for these samples are not presented.

Table 83 Residues of bixafen and M21 (bixafen-desmethyl) in eggs after administration of bixafen at 0.1, 0.3 or 1.0 mg/kg bw and day

Days	Residues in mg bixafen-equivalents per kg [mean]		
	1× (1.5 ppm, 0.1 mg/kg bw)	3× (4.5 ppm, 0.3 mg/kg bw)	10× (15 ppm, 1.0 mg/kg bw)
0	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)
1	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)
2	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01, < 0.01, 0.01 [0.01] M21: < 0.01(3) Total: < 0.01, < 0.01, 0.01 [0.01]	bixafen: 0.03, 0.03, 0.04 [0.033] M21: 0.01, 0.01, 0.02 [0.013] Total: 0.04, 0.04, 0.06 [0.047]
5	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: 0.01, 0.01, 0.02 [0.013] M21: 0.01, 0.01, 0.02 [0.013] Total: 0.02, 0.02, 0.04 [0.027]	bixafen: 0.05, 0.06, 0.07 [0.06] M21: 0.05, 0.06, 0.07 [0.06] Total: 0.1, 0.12, 0.14 [0.12]
7	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: 0.02, 0.02, 0.04 [0.027] M21: 0.02, 0.03, 0.04 [0.03] Total: 0.04, 0.05, 0.07 [0.053]	bixafen: 0.05, 0.05, 0.07 [0.057] M21: 0.07, 0.07, 0.08 [0.073] Total: 0.12, 0.13, 0.14 [0.13]
9	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: 0.02, 0.02, 0.03 [0.023] M21: 0.02, 0.03, 0.03 [0.027] Total: 0.04, 0.05, 0.06 [0.05]	bixafen: 0.06, 0.07, 0.08 [0.07] M21: 0.08, 0.08, 0.09 [0.083] Total: 0.13, 0.14, 0.16 [0.143]

Days	Residues in mg bixafen-equivalents per kg [mean]		
	1× (1.5 ppm, 0.1 mg/kg bw)	3× (4.5 ppm, 0.3 mg/kg bw)	10× (15 ppm, 1.0 mg/kg bw)
12	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: 0.02, 0.02, 0.03 [0.023] M21: 0.02, 0.02, 0.03 [0.023] Total: 0.04, 0.05, 0.05 [0.047]	bixafen: 0.06, 0.07, 0.11 [0.08] M21: 0.07, 0.08, 0.12 [0.09] Total: 0.13, 0.15, 0.23 [0.17]
14	bixafen: < 0.01(3) M21: < 0.01, < 0.01, 0.01 [0.01] Total: < 0.02(2), 0.02 [0.02]	bixafen: 0.03, 0.03, 0.03 [0.03] M21: 0.03, 0.03, 0.03 [0.03] Total: 0.06, 0.06, 0.06 [0.06]	bixafen: 0.06, 0.06, 0.1 [0.073] M21: 0.07, 0.08, 0.1 [0.083] Total: 0.13, 0.14, 0.2 [0.157]
16	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: 0.03, 0.03, 0.03 [0.03] M21: 0.03, 0.03, 0.03 [0.03] Total: 0.06, 0.06, 0.06 [0.06]	bixafen: 0.05, 0.07, 0.08 [0.067] M21: 0.07, 0.09, 0.09 [0.083] Total: 0.12, 0.16, 0.17 [0.15]
21	bixafen: < 0.01(3) M21: < 0.01, < 0.01, 0.01 [0.01] Total: < 0.02(2), 0.02 [0.02]	bixafen: 0.03, 0.03, 0.03 [0.03] M21: 0.03, 0.03, 0.04 [0.033] Total: 0.06, 0.06, 0.07 [0.063]	bixafen: 0.06, 0.06, 0.08[a], 0.09[a], 0.09[a] 0.1 [0.08] M21: 0.07, 0.09, 0.09[a], 0.1[a], 0.12[a], 0.12 [0.098] Total: 0.13, 0.15, 0.18[a], 0.18[a], 0.21[a], 0.22 [0.178]
23	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: 0.02, 0.02, 0.03 [0.023] M21: 0.02, 0.03, 0.03 [0.027] Total: 0.04, 0.05, 0.06 [0.05]	bixafen: 0.06, 0.07, 0.07[a], 0.08, 0.08[a], 0.09[a] [0.075] M21: 0.09, 0.09, 0.09[a], 0.1[a], 0.1[a], 0.11 [0.097] Total: 0.15, 0.16, 0.16[a], 0.18[a], 0.19, 0.19[a] [0.172]
26	bixafen: < 0.01(3) M21: < 0.01, < 0.01, 0.01 [0.01] Total: < 0.02(2), 0.02 [0.02]	bixafen: 0.02, 0.03, 0.03 [0.027] M21: 0.02, 0.03, 0.04 [0.03] Total: 0.05, 0.06, 0.06 [0.057]	bixafen: 0.07, 0.07, 0.07[a], 0.07[a], 0.08, 0.09[a] [0.075] M21: 0.08, 0.08[a], 0.09, 0.09[a], 0.1, 0.1[a] [0.09] Total: 0.14, 0.16, 0.17[a], 0.17[a], 0.17[a], 0.18 [0.165]
28	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: 0.02, 0.03, 0.03 [0.027] M21: 0.02, 0.03, 0.03 [0.027] Total: 0.05, 0.05, 0.06 [0.057]	bixafen: 0.05, 0.07, 0.07[a], 0.08[a], 0.09, 0.09[a] [0.075] M21: 0.06, 0.08[a], 0.09, 0.09, 0.09[a], 0.1[a] [0.085] Total: 0.11, 0.16, 0.17[a], 0.17[a], 0.17[a], 0.18 [0.16]
30	not performed	not performed	bixafen: 0.04 [a], 0.05 [a], 0.05 [a] [0.047] M21: 0.06 [a], 0.08 [a], 0.08 [a] [0.073] Total: 0.11 [a], 0.12 [a], 0.13 [a] [0.12]
33	not performed	not performed	bixafen: 0.02 [a], 0.02 [a], 0.02 [a] [0.02] M21: 0.03 [a], 0.03 [a], 0.03 [a] [0.03] Total: 0.05 [a], 0.05 [a], 0.05 [a] [0.05]
35	not performed	not performed	bixafen: < 0.01 [a] (3) M21: < 0.01 [a], < 0.01 [a], 0.01 [a] [0.01] Total: < 0.02 [a], < 0.02 [a], 0.02 [a] [0.02]
37, 40, 42	not performed	not performed	bixafen: < 0.01 [a] (2) M21: < 0.01 [a] (2) Total: < 0.02 [a] (2)
44, 48, 49	not performed	not performed	bixafen: < 0.01 [a] M21: < 0.01 [a] Total: < 0.02 [a]

[a] Depuration sub-group

The distribution of the residue between egg yolk and egg white was investigated for the 10× group on eggs collected after 22, 25 and 27 days. In the following table the residues of bixafen and M21 (bixafen-desmethyl) are presented:

Table 84 Distribution of bixafen and M21 (bixafen-desmethyl) in egg yolk and egg white after administration of bixafen at 1.0 mg/kg bw and day (10×)

Day	Residues in egg yolk [mg bixafen equiv./kg]			Residues in egg white [mg bixafen equiv./kg]			Residues in whole egg [mg bixafen equiv./kg]		
	bixafen	M21	total	bixafen	M21	total	bixafen	M21	total
22	0.13	0.20	0.33	0.04	0.01	0.05	0.07	0.08	0.15
25	0.14	0.24	0.38	0.05	0.02	0.07	0.08	0.09	0.17
27	0.10	0.20	0.30	0.03	0.01	0.04	0.05	0.07	0.12

For poultry, tissues residues of bixafen and M21 (bixafen-desmethyl) found directly at the end of the dosing period are presented in the following table. In samples collected from animal after depuration, no detectable residue of bixafen or M21 (bixafen-desmethyl) were present.

Table 85 Residues of bixafen and M21 (bixafen-desmethyl) in tissues of laying hens after administration of bixafen at 0.1, 0.3 or 1.0 mg/kg bw and day (1×, 3× or 10×)

Commodity	Residues in mg bixafen-equivalents per kg [mean]		
	1× (1.5 ppm, 0.1 mg/kg bw)	3× (4.5 ppm, 0.3 mg/kg bw)	10× (15 ppm, 1.0 mg/kg bw)
Skin with fat	bixafen: < 0.01, < 0.01, 0.01 [0.01] M21: < 0.01(3) Total: < 0.02, < 0.02, 0.02 [0.02]	bixafen: 0.04, 0.04, 0.05 [0.043] M21: 0.01, 0.01, 0.02 [0.013] Total: 0.05, 0.06, 0.06 [0.057]	bixafen: 0.05, 0.05, 0.07 [0.057] M21: 0.01, 0.01, 0.02 [0.013] Total: 0.06, 0.06, 0.09 [0.07]
Liver	bixafen: < 0.01(3) M21: < 0.01, < 0.01, 0.01 [0.01]) Total: < 0.02, < 0.02, 0.02 [0.02]	bixafen: < 0.01, 0.01, 0.01 [0.01] M21: 0.01, 0.02, 0.03 [0.02] Total: 0.02, 0.03, 0.04 [0.03]	bixafen: < 0.01, < 0.01, 0.01 [0.01] M21: 0.02, 0.03, 0.04 [0.03] Total: 0.03, 0.04, 0.05 [0.04])
Muscle	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)

Lactating cows

Residues in lactating cows were investigated by Schoening, R and Wolters, A (2007, BIXAFEN_072). Thirteen lactating Holstein dairy cows (*Bos taurus*; three cows/treatment group, one control cow and a depuration group consisting of three cows) were dosed orally, via capsule, for 29 consecutive days with bixafen with either 0 ppm (control, 0×), 4 ppm (1× dose group), 12 ppm (3× dose group), or 40 ppm(10× dose group), corresponding to 0, 0.15 mg/kg bw, 0.45 mg/kg bw and 1.5 mg/kg bw, respectively.

Milk was collected twice daily. On day 29 after the administration of the first dose, the animals with the exception of the cows of the depuration group were sacrificed and liver, kidney, composite muscle, subcutaneous fat, mesenteric fat and perirenal fat were collected for analysis. For the depuration group (10×), milk for analysis was additionally sampled during the depuration phase. The animals of this group were sacrificed on day 8, 15 or 22 after the last dosing (corresponding to day 36, 43 or 50 after the first dosing), and animal tissues were collected for analysis.

Tissue and milk samples were analysed for bixafen and M21 (bixafen-desmethyl) residue by high performance liquid chromatography-electrospray ionization/tandem mass spectrometry (LC-

MS/MS) using isotopically labelled internal standards (method 01036, see section for analytical methods). The limit of quantitation (LOQ) was 0.01 mg/kg for each analyte, expressed as bixafen equivalents. The LOQ of the total residue of bixafen was calculated to be 0.02 mg/kg.

In milk residues of bixafen and M21 (bixafen-desmethyl) are presented in the following table:

Table 86 Residues of bixafen and M21 (bixafen-desmethyl) in milk after administration of bixafen at 0.15, 0.45 or 1.5 mg/kg bw and day

Days	Residues in mg bixafen-equivalents per kg [mean]		
	1× (4 ppm, 0.15 mg/kg bw)	3× (12 ppm, 0.45 mg/kg bw)	10× (40 ppm, 1.5 mg/kg bw)
0	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(6) M21: < 0.01(6) Total: < 0.02(6)
1	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)	bixafen: < 0.01(3) M21: 0.011, 0.012, 0.017 [0.013] Total: 0.021, 0.022, 0.027 [0.023]	bixafen: 0.017[a], 0.023, 0.023[a], 0.031, 0.031[a], 0.032 [0.026] M21: 0.23[a], 0.26[a], 0.03, 0.035, 0.41[a], 0.043 [0.033] Total: 0.04[a], 0.049[a], 0.058, 0.062, 0.072[a], 0.074 [0.059]
2	bixafen: < 0.01(3) M21: < 0.01, < 0.01, 0.011 [0.01] Total: < 0.02, < 0.02, 0.021 [0.02]	bixafen: < 0.01, 0.014, 0.014 [0.013] M21: 0.023, 0.027, 0.049 [0.033] Total: 0.033, 0.041, 0.063 [0.046]	bixafen: 0.033[a], 0.4[a], 0.043, 0.053[a], 0.064, 0.085 [0.053] M21: 0.072[a], 0.073[a], 0.086, 0.102[a], 0.113, 0.146 [0.099] Total: 0.105[a], 0.113[a], 0.129, 0.155[a], 0.198, 0.210 [0.152]
4	bixafen: < 0.01(3) M21: < 0.01, 0.012, 0.015 [0.012] Total: < 0.02, 0.022, 0.025 [0.022]	bixafen: 0.01, 0.017, 0.025 [0.017] M21: 0.029, 0.042, 0.076 [0.049] Total: 0.039, 0.059, 0.101 [0.066]	bixafen: 0.046[a], 0.047[a], 0.051[a], 0.061, 0.079, 0.143 [0.071] M21: 0.091[a], 0.099[a], 0.108[a], 0.131, 0.18, 0.194 [0.134] Total: 0.138[a], 0.15[a], 0.154[a], 0.192, 0.259, 0.337 [0.205]
8	bixafen: < 0.01(3) M21: < 0.01, 0.013, 0.014 [0.012] Total: < 0.02, 0.023, 0.024 [0.022]	bixafen: 0.014, 0.021, 0.023 [0.0119] M21: 0.032, 0.041, 0.067 [0.047] Total: 0.046, 0.062, 0.09[0.066]	bixafen: 0.054, 0.057[a], 0.069, 0.076[a], 0.078[a], 0.147 [0.08] M21: 0.082, 0.102[a], 0.108[a], 0.143[a], 0.176, 0.214 [0.138] Total: 0.136, 0.165[a], 0.18[a], 0.219[a], 0.245, 0.361 [0.218]
10	bixafen: < 0.01, < 0.01, 0.012 [0.011] M21: 0.013, 0.018, 0.019 [0.017] Total: 0.023, 0.028, 0.031 [0.027]	bixafen: 0.013, 0.022, 0.026 [0.020] M21: 0.028, 0.049, 0.062 [0.046] Total: 0.041, 0.071, 0.088 [0.067]	bixafen: 0.063, 0.063[a], 0.067[a], 0.072, 0.078[a], 0.138 [0.08] M21: 0.087[a], 0.107, 0.128[a], 0.136[a], 0.16, 0.19 [0.135] Total: 0.15[a], 0.17, 0.195[a], 0.214[a], 0.232, 0.328 [0.215]
13	bixafen: < 0.01(3) M21: 0.011, 0.014, 0.021 [0.015] Total: 0.021, 0.024, 0.031 [0.025]	bixafen: 0.014, 0.021, 0.023 [0.019] M21: 0.028, 0.044, 0.059 [0.044] Total: 0.042, 0.065, 0.082 [0.063]	bixafen: 0.05[a], 0.058, 0.064, 0.065[a], 0.068[a], 0.083 [0.065] M21: 0.075[a], 0.095, 0.112[a], 0.123, 0.128, 0.136[a] [0.112] Total: 0.125[a], 0.159, 0.177[a], 0.181, 0.204[a], 0.211[0.176]
17	bixafen: < 0.01, 0.011, 0.012 [0.011] M21: 0.022, 0.027, 0.034 [0.028] Total: 0.033, 0.038, 0.046 [0.039]	bixafen: 0.013, 0.018, 0.021[0.017] M21: 0.03, 0.043, 0.054 [0.042] Total: 0.043, 0.054, 0.072 [0.056]	bixafen: 0.053[a], 0.061, 0.065, 0.069[a], 0.071[a], 0.083 [0.067] M21: 0.088[a], 0.127, 0.131, 0.135[a], 0.142[a], 0.149 [0.129] Total: 0.141[a], 0.192, 0.206[a], 0.21, 0.211[a], 0.214 [0.196]

Days	Residues in mg bixafen-equivalents per kg [mean]		
	1× (4 ppm, 0.15 mg/kg bw)	3× (12 ppm, 0.45 mg/kg bw)	10× (40 ppm, 1.5 mg/kg bw)
20	bixafen: < 0.01(3) M21: 0.013, 0.02, 0.02 [0.018] Total: 0.023, 0.03, 0.03 [0.028]	bixafen: 0.012, 0.017, 0.022 [0.017] M21: 0.031, 0.042, 0.072 [0.048] Total: 0.043, 0.059, 0.094 [0.065]	bixafen: 0.029[a], 0.052, 0.056[a], 0.066[a], 0.069, 0.092 [0.061] M21: 0.047[a], 0.106, 0.11[a], 0.112, 0.123[a], 0.132 [0.105] Total: 0.076[a], 0.158, 0.166[a], 0.189[a], 0.201, 0.204 [0.166]
24	bixafen: < 0.01, < 0.01, 0.01 [0.01] M21: 0.01, 0.019, 0.024 [0.018] Total: 0.02, 0.029, 0.034 [0.028]	bixafen: 0.017, 0.021, 0.022 [0.02] M21: 0.038, 0.043, 0.058 [0.046] Total: 0.051, 0.064, 0.080 [0.065]	bixafen: 0.04, 0.051[a], 0.063[a], 0.084, 0.088[a], 0.124 [0.075] M21: 0.102[a], 0,128[a], 0.134, 0.147, 0.156, 0.172[a] [0.14] Total: 0.165[a], 0.174, 0.179[a], 0.24, 0.26[a], 0.271 [0.215]
28	bixafen: < 0.01(3) M21: 0.012, 0.015, 0.017 [0.015] Total: 0.022, 0.025, 0.027 [0.025]	bixafen: 0.01, 0.024, 0.026 [0.02] M21: 0.031, 0.052, 0.089 [0.057] Total: 0.041, 0.076, 0.115[0.077]	bixafen: 0.052[a], 0.055[a], 0.062, 0.079, 0.083, 0.084[a] [0.069] M21: 0.104[a], 0.105[a], 0.135, 0.144, 0.17[a], 0.19 [0.141] Total: 0.157[a], 0.159[a], 0.206, 0.218, 0.254[a], 0.269 [0.211]
29	bixafen: < 0.01, < 0.01, 0.012 [0.011] M21: 0.011, 0.021, 0.028 [0.02] Total: 0.021, 0.031, 0.04 [0.031]	bixafen: 0.13, 0.21, 0.22 [0.019] M21: 0.037, 0.046, 0.076 [0.053] Total: 0.05, 0.067, 0.098[0.072]	bixafen: 0.051, 0.054[a], 0.056[a], 0.059[a], 0.06, 0.091 [0.062] M21: 0.101[a], 0.103[a], 0.106, 0.118[a], 0.143, 0.147 [0.12] Total: 0.157, 0.157[a], 0.157[a], 0.177[a], 0.207, 0.234 [0.182]
31	not performed	not performed	bixafen: < 0.01 [a], < 0.01 [a], 0.01 [a] [0.01] M21: 0.035 [a], 0.039 [a], 0.049 [a] [0.041] Total: 0.045 [a], 0.049 [a], 0.059 [a] [0.051]
34, 36, 41, 43, 48, 50	not performed	not performed	bixafen: < 0.01(3) M21: < 0.01(3) Total: < 0.02(3)

The distribution of the residue between skim milk and cream was investigated for the milk from the 10× group at day 26. In the following table the residues of bixafen and M21 (bixafen-desmethyl) are presented:

Table 87 Distribution of bixafen and M21 (bixafen-desmethyl) in skim milk and cream after administration of bixafen at 1.5 mg/kg bw and day (10×, day 26)

Sample	Residue [mg bixafen equiv./kg]					
	bixafen	ratio	M21	ratio	total	ratio
Whole milk	0.072 [a]	1	0.141 [a]	1	0.213 [a]	1
Skim milk	< 0.01(3)	0.14	0.03, 0.03, 0.03 [0.03]	0.21	0.04, 0.04, 0.04 [0.04]	0.19
Cream	0.562, 0.81, 1.41 [0.927]	13	1.038, 1.163, 1.336 [1.179]	8.4	1.6, 1.973, 2.746 [2.106]	9.9
(milk fat [b]		(20)		(13)		(15)

[a] Residues for whole milk on day 26 not reported. The values represent the mean of the pooled day 24 and day 28 samples

[b] Based on average measured milk fat content in cream of 65%

For bovine tissues residues of bixafen and M21 (bixafen-desmethyl) found directly at the end of the dosing period are presented in the following table.

Table 88 Residues of bixafen and M21 (bixafen-desmethyl) in tissues of lactating cows after administration of bixafen at 0.15, 0.45 or 1.5 mg/kg bw and day (1×, 3× or 10×)

Commodity	Residues in mg bixafen-equivalents per kg [mean]		
	1× (4 ppm, 0.15 mg/kg bw)	3× (12 ppm, 0.45 mg/kg bw)	10× (40 ppm, 1.5 mg/kg bw)
Perirenal fat	bixafen: 0.059, 0.09, 0.092 [0.08] M21: 0.08, 0.114, 0.117 [0.104] Total: 0.139, 0.204, 0.209 [0.184]	bixafen: 0.135, 0.213, 0.218 [0.189] M21: 0.191, 0.263, 0.266 [0.24] Total: 0.326, 0.476, 0.484 [0.429]	bixafen: 0.326, 0.667, 1.041 [0.678] M21: 0.474, 0.767, 0.881 [0.707] Total: 0.80, 1.434, 1.922 [1.385]
Mesenteric fat	bixafen: 0.061, 0.076, 0.086 [0.074] M21: 0.075, 0.085, 0.109 [0.09] Total: 0.136, 0.161, 0.195 [0.164]	bixafen: 0.118, 0.204, 0.214 [0.179] M21: 0.178, 0.228, 0.246 [0.217] Total: 0.296, 0.432, 0.46 [0.396]	bixafen: 0.394, 0.615, 0.927 [0.645] M21: 0.503, 0.76, 0.838 [0.7] Total: 0.897, 1.375, 1.765 [1.346]
Subcutaneous fat	bixafen: 0.036, 0.06, 0.062 [0.053] M21: 0.032, 0.051, 0.059 [0.047] Total: 0.068, 0.111, 0.121 [0.1]	bixafen: 0.053, 0.08, 0.117 [0.083] M21: 0.05, 0.064, 0.095 [0.07] Total: 0.103, 0.144, 0.212 [0.153]	bixafen: 0.144, 0.3, 0.849 [0.431] M21: 0.156, 0.278, 0.662 [0.365] Total: 0.3, 0.578, 1.511 [0.796]
Liver	bixafen: 0.033, 0.05, 0.053 [0.045] M21: 0.383, 0.556, 0.632 [0.524] Total: 0.416, 0.606, 0.685 [0.569]	bixafen: 0.083, 0.169, 0.184 [0.145] M21: 1.148, 1.199, 1.523 [1.29] Total: 1.231, 1.368, 1.707 [1.435]	bixafen: 0.377, 0.443, 0.481 [0.434] M21: 4.318, 4.441, 4.889 [4.549] Total: 4.761, 4.818 5.37 [4.983]
Muscle	bixafen: < 0.01, 0.01, 0.011[0.013] M21: 0.029, 0.042, 0.054 [0.042] Total: 0.039, 0.052, 0.065 [0.052]	bixafen: 0.012, 0.029, 0.045 [0.029] M21: 0.069, 0.117, 0.215 [0.134] Total: 0.081, 0.146, 0.26 [0.162]	bixafen: 0.128, 0.135, 0.157 [0.14] M21: 0.505, 0.695, 0.84 [0.68] Total: 0.633, 0.83, 0.997 [0.82]
Kidney	bixafen: 0.012, 0.017, 0.019 [0.016] M21: 0.09, 0.133, 0.133 [0.119] Total: 0.102, 0.15, 0.152 [0.135]	bixafen: 0.03, 0.051, 0.056 [0.046] M21: 0.252, 0.314, 0.318 [0.295] Total: 0.282, 0.365, 0.374 [0.34]	bixafen: 0.134, 0.152, 0.166 [0.151] M21: 0.906, 1.07, 1.14 [1.039] Total: 1.04, 1.222, 1.306 [1.189]

For the 10× group three animals were kept for depuration for an interval of up to 21 additional days. Residues found in tissues sampled after sacrifice are presented in the following table:

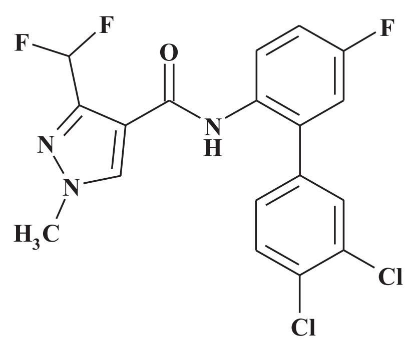
Table 89 Residues of bixafen and M21 (bixafen-desmethyl) in tissues of lactating cows after administration of bixafen at 0.15, 0.45 or 1.5 mg/kg bw and day (1×, 3× or 10×) destined for depuration of up to 21 days

Commodity	Residues in mg bixafen-equivalents per kg [mean]		
	7 day depuration (day 36)	14 day depuration (day 43)	21 day depuration (day 50
Perirenal fat	bixafen: 0.018 M21: < 0.01 Total: 0.028	bixafen: < 0.01 M21: < 0.01 Total: < 0.02	bixafen: < 0.01 M21: < 0.01 Total: < 0.02

Commodity	Residues in mg bixafen-equivalents per kg [mean]		
	7 day depuration (day 36)	14 day depuration (day 43)	21 day depuration (day 50
Mesenteric fat	bixafen: 0.02 M21: < 0.01 Total: 0.03	bixafen: < 0.01 M21: < 0.01 Total: < 0.02	bixafen: < 0.01 M21: < 0.01 Total: < 0.02
Subcutaneous fat	bixafen: 0.097 M21: < 0.01 Total: 0.107	bixafen: < 0.01 M21: < 0.01 Total: < 0.02	bixafen: < 0.01 M21: < 0.01 Total: < 0.02
Liver	bixafen: < 0.02 M21: 0.117 Total: 0.137	bixafen: < 0.01 M21: 0.089 Total: 0.099	bixafen: < 0.01 M21: 0.071 Total: 0.081
Muscle	bixafen: < 0.01 M21: < 0.01 Total: < 0.02	bixafen: < 0.01 M21: < 0.01 Total: < 0.02	bixafen: < 0.01 M21: < 0.01 Total: < 0.02
Kidney	bixafen: < 0.01 M21: < 0.01 Total: < 0.02	bixafen: < 0.01 M21: < 0.01 Total: < 0.02	bixafen: < 0.01 M21: < 0.01 Total: < 0.02

APPRAISAL

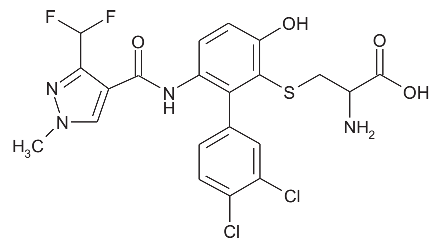
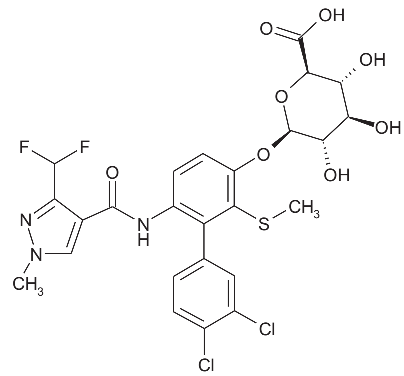
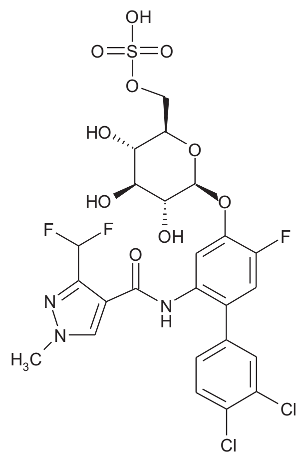
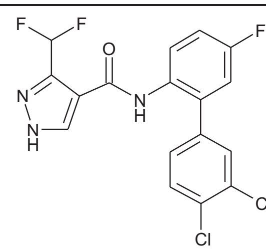
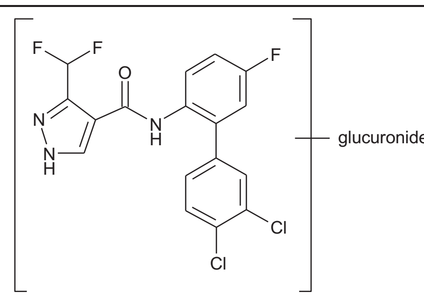
Bixafen (ISO common name) is a pyrazole-carboxamide fungicide used to control diseases on rape plants and cereals. Bixafen inhibits fungal respiration by binding to mitochondrial respiratory complex II. It was considered for the first time by the 2013 JMPR for toxicology and residues.

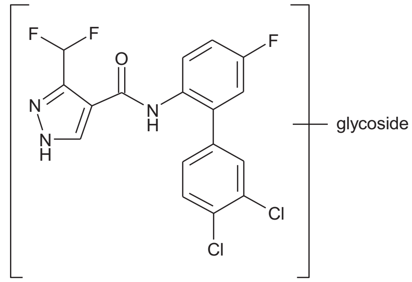
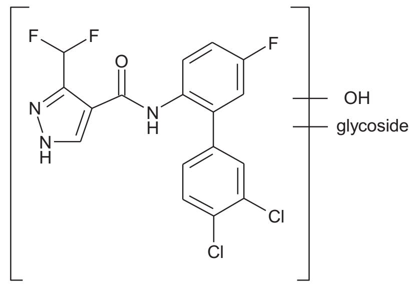
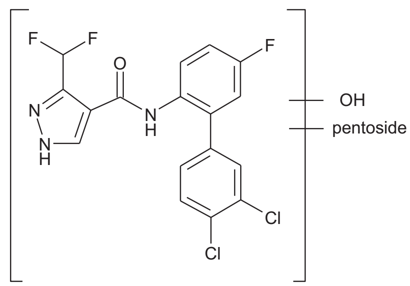
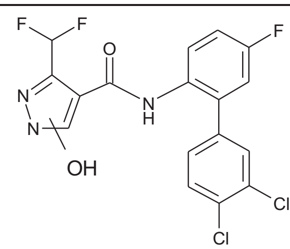
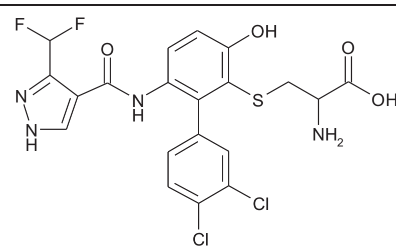
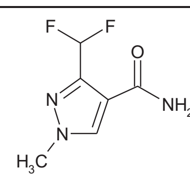


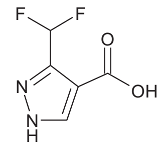
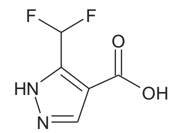
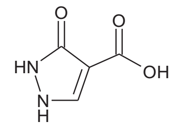
The IUPAC name of bixafen is N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide and the CA name is 1H-pyrazole-4-carboxamide, N-(3',4'-dichloro-5-fluoro[1,1'-biphenyl]-2-yl)-3-(difluoromethyl)-1-methyl-.

Bixafen labelled either in the pyrazole- or dichlorophenyl-moiety was used in the metabolism and environmental fate studies.

The following abbreviations are used for the metabolites discussed below:

M14	S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteine (IUPAC)	
M18	3',4'-dichloro-6-({[3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)-2-(methylthio)biphenyl-3-yl beta-L-glucopyranosiduronic acid (IUPAC)	
M20	not nomenclature possible - position of hydroxy group not specified	 or isomer
M21 (bixafen-desmethyl)	N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (IUPAC)	
M23	not nomenclature possible - structure not specified	

M24	not nomenclature possible - structure not specified	
M25	not nomenclature possible - structure not specified	
M26	not nomenclature possible - structure not specified	
M27	not nomenclature possible - structure not specified	
M37	S-[3',4'-dichloro-6-({[3-(difluoromethyl)-1H-pyrazol-4-yl]carbonyl}amino)-3-hydroxybiphenyl-2-yl]cysteine (IUPAC)	
M43	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (IUPAC)	

M44	3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid (IUPAC)	 proposal for tautomer 1
M45	5-(difluoromethyl)-1H-pyrazole-4-carboxylic acid (IUPAC)	 proposal for tautomer 2
M47	3-hydroxy-1H-pyrazole-4-carboxylic acid (IUPAC)	

Animal metabolism

Information was available on the metabolism of bixafen in laboratory animals, lactating goats and laying hens.

In rats given (dichlorophenyl-U-^{14}C)-labelled bixafen orally by gavage, absorption was rapid and accounted for at least 83% of the total administered radioactivity after a single low dose (2 mg/kg bw). The maximum plasma concentrations of radioactivity were reached approximately 2–4 and 8 hours after administration of the low and high doses (2 and 50 mg/kg bw), respectively. Radioactivity was widely distributed throughout the body. Elimination of the radioactivity was mainly via faeces (≥ 91%), whereas elimination via urine accounted for 1–3% of the administered dose. In bile duct–cannulated rats, extensive biliary excretion (up to 83%) was demonstrated. Elimination of the radioactivity from the body was rapid, with a half-life in plasma of 8–9 hours and a mean residence time of 13–19 hours (for the low dose). Residues in tissues at 72 hours after a single oral dose as well as after repeated oral dosing accounted for 0.1–3% of the administered radioactivity, with liver and kidneys containing the highest concentrations of residues.

Metabolism of bixafen in rats was extensive, and more than 30 metabolites were identified. The main metabolic routes included demethylation, hydroxylation of the parent and bixafen-desmethyl, and conjugation with glucuronic acid or glutathione. A minor metabolic reaction was the cleavage of the amide bridge of bixafen.

Two studies on metabolism in lactating goats were available. The goats received five daily doses of [pyrazole-^{14}C]-bixafen or [dichlorophenyl-^{14}C]-bixafen at rates equivalent to 35 ppm and 46 ppm in the diet, respectively. The animals were sacrificed approximately 24 hours after the last dose. In both studies approximately 1.3% of the total dose was recovered from milk or tissues of the animals. Most of the radioactivity was excreted via faeces (74–82% AR) and urine (1.8–5.4% AR).

The metabolic pattern in both studies was comparable. In milk (TRR: 0.064–0.17 mg eq/kg), muscle (TRR: 0.047–0.057 mg eq/kg) and fat (TRR: 0.47–0.61 mg eq/kg) unchanged bixafen was the major residue, representing 74–77%, 56–66% and 89% of the total radioactivity, respectively. M21 (bixafen-desmethyl) was the only major metabolite being present at 16–18% of the TRR in milk, 34–43% in muscle and 10–11% in fat.

For kidney (TRR: 0.14–0.2 mg eq/kg) and liver (TRR: 0.74–1.2 mg eq/kg) parent bixafen was also a major residue, representing 44–46% of the total radioactivity in kidney and 18–23% in liver. Significant metabolites identified were M21 (bixafen-desmethyl), representing 37–38% of the TRR in kidney and 19–21% TRR in liver, followed by the two M23 isomers counting for a total of 9.3–15% of the TRR in kidney and 14–19% in liver.

For laying hens groups of hens received daily doses of [pyrazole-^{14}C]-bixafen or [dichlorophenyl-^{14}C]-bixafen at rates equivalent to 26 ppm and 32 ppm in the diet for 14 consecutive days. The animals were sacrificed ca. 24 hours after the last dose. Approximately 1.5% of the total dose in both studies was recovered from eggs or tissues of the animals. Most of the radioactivity administered was found in the excreta (88–93% AR). Total radioactive residues were 0.53–0.9 mg eq/kg in eggs, 0.032–0.037 mg eq/kg in muscle, 0.23–0.38 mg eq/kg in fat and 0.64–0.81 mg eq/kg in liver.

Parent bixafen was a major residue in eggs and all tissues except liver, representing 51–69% of the TRR in eggs, 23–41% in muscle and 80% in fat. In hens liver, only minor amounts of bixafen were detected (4.5–6.7% TRR).

M21 (bixafen-desmethyl) was the only major metabolite found in poultry tissues and eggs. It was found at levels of 26–39% of the TRR in eggs, 35–51% in muscle, 19–20% in fat and 24–26% in liver. In liver, M14, M18, M24, M25, M26, M27 and M37 were identified as minor metabolites, representing 1.0–8.8% of the TRR (0.007–0.067 mg eq/kg) each.

In summary bixafen is the major residue in most tissues, milk and eggs. It is moderately metabolized in goats and hens mainly resulting in M21 (bixafen-desmethyl). All major metabolites were also identified in the rat. The metabolites M18, M25 and M26, mainly found in poultry liver, were not directly identified in the rat.

Plant metabolism

The Meeting received plant metabolism studies for bixafen following foliar application of either [pyrazole-^{14}C]-bixafen or [dichlorophenyl-^{14}C]-bixafen to soya beans or wheat.

Soya beans were independently treated with both bixafen-labels with three foliar applications of 0.06 kg ai/ha each when the first flowers opened (BBCH 60), at the end of flowering (BBCH 69) and finally when approximately 80% of the pods were ripe (BBCH 88). Samples were collected containing forage (5 days after 2nd application), hay (29 days after 2nd application), straw and seed (26 days after the 3rd application). Total radioactive residues were 4.0–5.3 mg eq/kg for forage, 2.8–4.0 mg eq/kg for hay, 9.5–13 mg eq/kg for straw and 0.005–0.024 mg eq/kg for seeds.

In all plant parts directly affected by the spray solution, unchanged bixafen was the major residue representing 96–98% of the TRR in forage, 92% in hay and 90–92% in straw. The only other metabolite identified was M21 (bixafen-desmethyl), present at 0.5–2.6% of the TRR.

For soya bean seeds, only samples following application of the pyrazole-label contained sufficient total radioactive residues for further investigation. Bixafen was the major residue with 30% of the TRR. Metabolites identified were M44 and M45 (19% TRR, 0.004 mg eq/kg) and M47 (12% TRR, 0.003 mg eq/kg). M21 (bixafen-desmethyl) was not identified in soya bean seeds.

Wheat was independently treated with both bixafen-labels using one foliar application of 0.125 kg ai/ha at the end of tillering / beginning of stem elongation (BBCH 29–31) followed by a second spraying with 0.15 kg ai/ha at the end of flowering (BBCH 69). Forage was harvested 9 days after the 1st application, hay 9 days after the 2nd application and straw and grain at maturity (50 days after the 2nd application). Total radioactive residues were 1.6–1.7 mg eq/kg for forage, 6.6–7.6 mg eq/kg for hay, 23–24 mg eq/kg for straw and 0.16–0.23 mg eq/kg for seeds.

In all samples unchanged bixafen was the major residue, representing > 90% of the TRR. The only other metabolite identified was M21 (bixafen-desmethyl) at 0.8–2.4% of the TRR.

In summary the plant metabolism of bixafen in the plants investigated is very limited. In plant parts directly affected by the spray solution, unchanged bixafen was the only residue significant. M21 (bixafen-desmethyl) was present at low levels up to 2.6% of the TRR.

In soya bean seeds, which were protected by the pod during treatment, bixafen was present at lower concentrations of 0.007 mg eq/kg (30% TRR). Major metabolites in soya bean seeds were M44, M45 and M47, probably taken up from the soil and distributed systemically, however at low levels not exceeding 0.004 mg eq/kg. All three of these metabolites were not identified in the rat.

Environmental fate in soil

The Meeting received information on the fate of bixafen after aerobic degradation in soil and after photolysis on the soil surface. In addition, the Meeting received information on the uptake and metabolism of bixafen soil residues by rotational crops, its dissipation under field conditions and long-term accumulation in soil.

In soil photolysis studies degradation of bixafen was not observed.

In aerobic soil metabolism studies under laboratory conditions bixafen was highly persistent with 80–90% remaining after 120 days. The only metabolite found was M44 (maximum 2.9% of AR), while the rest of the radioactivity remained unextracted or was recovered as $^{14}CO_2$. DT_{50} values could not be calculated due to the minimal degradation observed within 120 days.

In soil samples from the confined rotational crop metabolism studies mentioned below, bixafen was also slowly degraded. The only metabolite found was identified as M21 (bixafen-desmethyl), slowly increasing from 0.5% (day 30) to 2.3% TRR at the end of the study (day 418). M44 was not detected.

In summary it can concluded that bixafen is persistent in soil, being degraded to a very minor extent.

Confined rotational crop studies on Swiss chard, turnips and wheat were conducted at rates equivalent to 0.79 kg ai/ha (pyrazole-label) and 0.85 kg ai/ha (dichlorophenyl-label). In plant commodities bixafen (11–78% TRR) and M21 (bixafen-desmethyl, 3–73% TRR) were the major residue components found for both labels. Quantified concentrations for the sum of both analytes were 0.016–0.024 mg eq/kg for Swiss chard, 0.005–0.035 mg eq/kg in turnip roots and tops and 0.011–0.041 mg eq/kg, 0.106–0.18 mg eq/kg and 0.152–0.462 mg eq/kg for wheat forage, hay and straw, respectively. In grain TRR levels were too low for identification (0.001–< 0.01 mg eq/kg).

Following application of the pyrazole-labelled active substance, the cleavage products M43 (3–15% TRR), M44 (0.3–37% TRR) and M45 (2–23% TRR) were identified as major metabolites. Concentrations were between 0.001–0.015 mg eq/kg each.

Following treatment with the dichlorophenyl-label, M20 was found in Swiss chard only at levels of 25–38% of the TRR (0.007–0.016 mg eq/kg).

The residue concentrations of bixafen and M21 in plants declined moderately in animal feed commodities while in food commodities only a slow decline of the residue was observed over the three crop rotations investigated. In all commodities investigated, except for wheat grain, detectable residues above the LOQ of 0.01 mg/kg were found for bixafen.

Field rotational crop studies were conducted at four locations in Europe. Bixafen was either applied to bare soil to simulate crop failure (0.28 kg ai/ha) or to barley as a primary crop (0.16 kg ai/ha at BBCH 56 plus 0.125 kg ai/ha at BBCH 69). Turnip/carrots, lettuce and wheat were planted as rotational crops at three rotations. Samples analysed for residues of bixafen and M21 were below the LOQ of 0.01 mg/kg for each analyte, except for one sample of wheat straw (M21: 0.02 mg/kg) and lettuce at a pre-mature growth stage (BBCH 46; bixafen: 0.05 mg/kg).

Field dissipation studies at six locations in Europe (four in the north, two in the south) confirmed the slow degradation of bixafen in soil observed in the aerobic metabolism studies. Within the first 100 days, a significant degradation of the residue concentration in soil was observed, leaving 42–63% of the initial concentrations. However, the decline after this period up to 730 days was minimal, leaving 17–47% of the initial concentration. M21 was not found above the LOQ of the analytical method.

The Meeting observed that the degradation of bixafen in soil follows a bi-phasic kinetics, starting with a fast decline within the first 100 days. After that initial interval, bixafen is highly persistent in soil, accumulating with subsequent treatments over multiple years.

In a long-term soil accumulation study under field conditions residues of bixafen and M21 (bixafen-desmethyl) in soil were investigated involving five and seven years of annual treatment with 0.14 kg ai/ha to the ground. In the first location in France a plateau for the bixafen concentration in soil was reached after five years, resulting in concentrations of up to 0.18 mg per kg soil. In Germany, the study was terminated after 7 years, due to technical reasons, without reaching a plateau, showing a bixafen peak concentration of 0.35 mg per kg soil. Most of the residue (> 95%) was present as unchanged parent substance located in the initial 10 cm soil layer. Based on an average density of 1.5 g/cm^3 for soil these concentrations are equivalent to single application rates to the bare soil of 0.27 kg ai/ha in the French trial and 0.53 kg ai/ha in the German trial.

The Meeting concluded that bixafen residues accumulate in soil after annual treatments. Under consideration of the highest annual application rate reported in the authorised GAPs of 0.25 kg ai/ha, soil residue concentrations equivalent to single application rates to bare soil of 0.9 kg ai/ha could be reached.

In summary the Meeting concluded that bixafen is persistent in soil, accumulating after subsequent years of annual treatment. Confined rotational crop studies indicate a potential uptake of residues for bixafen and M21 (bixafen-desmethyl) into plant commodities. The Meeting also recognized that field rotational crop studies involved soil treatment rates not addressing the soil concentrations expected after subsequent annual treatment.

Methods of residue analysis

The Meeting received analytical methods for the analysis of bixafen and M21 (bixafen-desmethyl) in plant and animal matrices. The basic principle employs extraction by homogenisation with acetonitrile/water (4/1, v/v) or n-hexane with acetonitrile partitioning for fatty samples. The extracts were cleaned with filtration and C18 solid-phase extraction. Residues are determined by liquid chromatography (LC) in combination with tandem mass spectroscopy (MS/MS). Mass-transitions are m/z 414→394 (m/z 414→266 for confirmation) for bixafen and m/z 398→378 (m/z 398→358 for confirmation) for M21 (bixafen-desmethyl). The methods submitted are suitable for measuring residues of bixafen and M21 in plant and animal commodities with a LOQ of 0.01 mg/kg for each analyte.

The extraction efficiency with acetonitrile/water (4/1, v/v) was tested for wheat (forage, grain straw) obtained from plant metabolism and confined rotational crop studies. Extraction rates were of > 90% for primary treated commodities and 68–73% (corresponding to 72–99% of the TTR) for commodities from rotational crops.

For the application of multi-residue methods the DFG S-19 was tested, but found to be unsuitable for analysing bixafen or M21 in plant matrices.

Stability of residues in stored analytical samples

The Meeting received information on the storage stability of bixafen and M21 (bixafen-desmethyl) in plant matrices. In wheat grain, wheat straw, wheat green material, lettuce head, potato tuber, rape seed and in soil, no significant degradation of both analytes was observed within 24 months.

For animal matrices no storage stability data were provided. Samples in livestock metabolism or feeding studies were analysed within one month of sampling.

Definition of the residue

Livestock animal metabolism studies were conducted on laying hens (36–32 ppm) and lactating goats (35–46 ppm).

In goats parent bixafen and M21 (bixafen-desmethyl) were the major residue. Bixafen represented 74–77% TRR in milk, 56–66% TRR in muscle, 44–46% TRR in kidney, 18–23% TRR in liver and 89% TRR in fat. M21 was the major metabolite present at 16–18% TRR in milk, 34–43% TRR in muscle, 37–38% TRR in kidney, 19–21% TRR in liver and 10–11% TRR in fat. In kidney and liver the two isomers of M23 were found at 9–15% of the TRR in kidney (isomer 1: 2.8–4.3%; isomer 2: 6.5–10% TRR) and of 14–19% in liver (isomer 1: 8.6–13% TRR; isomer 2: 5.2–5.8% TRR).

In laying hens again bixafen was the major residue in eggs and all tissues except liver, representing 51–69% TRR in eggs, 23–41% in muscle and 80% in fat. In liver only minor amounts of bixafen were detected (4.5–6.7% of the TRR). M21 (bixafen-desmethyl) was the major metabolite found in poultry tissues and eggs. It accounted for 26–39% of the TRR in eggs, 35–51% in muscle, 19–20% in fat and 24–26% in liver. In liver, M14, M18, M24, M25, M26, M27 and M37 were identified as minor metabolites, representing 1.0–8.8% of the TRR (0.007–0.067 mg eq/kg) each. Of these, M18, M25 and M26 were not identified in the rat, however the exposure of M18 was below the respective acute and chronic TTCs for Cramer class III while M25 and M26 have structural similarity to bixafen-desmethyl and are covered by the ADI for bixafen. As a consequence no consideration of dietary intake is required.

M21 (bixafen-desmethyl) was identified as the major residue in rat studies, suggesting that it is covered by toxicological reference values for parent bixafen. The Meeting concluded that parent bixafen and M21 (bixafen-desmethyl) are suitable marker compounds in animal commodities and should be included into the residue definition for compliance with MRLs and for the estimation of the dietary intake. Analytical methods are capable of measuring both analytes.

In livestock feeding studies the distribution of bixafen and M21 between skim milk/cream and egg white/egg yolk was investigated. The average ratio for cream/skim milk was > 93 for bixafen and 39 for M21. Egg yolk concentrations of bixafen were three times higher than in egg white while M21 showed ratios of 12–20. For the parent substance a log P_{ow} of 3.3 was measured.

The data for milk and eggs suggests that bixafen and M21 partition in the fat portion. In addition, residues in fat tissues were about ten times higher when compared to muscle. The Meeting decided that residues of bixafen are fat-soluble.

The fate of bixafen in plants was investigated following foliar application to soya beans and wheat. In all samples unchanged bixafen was the major residue, normally representing at least 90% of the TRR. M21 was present at very low levels, not exceeding 3% of the TRR. In soya bean seeds, which were not directly exposed to the spray solution due to the pods, only 30% of the TRR (0.007 mg eq/kg) was present as bixafen. Further major metabolites in soya bean seeds were identified as the tautomers M44 and M45 (19% TRR, 0.004 mg eq/kg) and M47 (12% TRR, 0.003 mg eq/kg).

In confined rotational crop studies, plant commodities Swiss chard, wheat and turnips contained concentrations of radioactive residues as high as 0.49 mg eq/kg, in wheat straw. Bixafen (11–78% TRR) and M21 (bixafen-desmethyl, 3–73% TRR) were the major compounds identified. In addition, M43 (3–15% TRR), M44 (0.3–37% TRR) and M45 (2–23% TRR) were identified as major metabolites in all rotational crops. M20 was only found in Swiss chard (25–38% TRR, 0.007–0.016 mg eq/kg). Wheat grain did not contain TRR levels allowing further identification (0.001–< 0.01 mg eq/kg).

The Meeting concluded that parent bixafen is a suitable maker for compliance with MRLs in all plant commodities (primary treated or rotational). For the estimation of the dietary intake M21 was insignificant in wheat and soya beans directly treated, but was identified in high relative amounts in rotational crops. Therefore, the Meeting decided to include M21 (bixafen-desmethyl) into the residue definition for dietary intake with the parent substance. The metabolites M20, M44, M45 and M47, mainly found in rotational crops, were not identified in the rat. However, the estimated exposure levels based on the confined rotational crop study are below the respective acute and chronic TTCs for Cramer class III. As a result, no consideration is required for dietary intake.

Analytical methods are capable of measuring bixafen and M21 (bixafen-desmethyl) in plant matrices.

Definition of the residue for compliance with MRL for plant commodities: *bixafen*

Definition of the residue for compliance with MRL for animal commodities and (for the estimation of dietary intake) for plant and animal commodities: *sum of bixafen and N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (bixafen-desmethyl), expressed as bixafen*

The residue is fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised European trial data for applications of bixafen to rape seed, barley and wheat.

Residue values referred to as "total" describe the sum of bixafen and M21 (bixafen-desmethyl), expressed as bixafen.

The Meeting concluded that field rotational crop studies do not address residues in soil expected after subsequent annual application of bixafen. Confined rotational crop studies available are not considered representative of field conditions. In the absence of suitable data, residue concentrations in plant commodities taken up from the soil by annual crops could not be estimated. Therefore, the Meeting decided, that no recommendations on maximum residue levels and median/highest residues could be made for bixafen in non-permanent crops.

Nevertheless, for the benefit of potential future assessments of bixafen uses, the Meeting decided to evaluate GAPs and residue data following direct application.

Rape seed

Bixafen is registered in the UK for use on rape seed at rates of 2 × 0.075 kg ai/ha with a PHI of 56 days. Supervised field trials conducted in northern Europe, according to this GAP, were submitted.

For MRL compliance purposes residues of parent bixafen in rape seeds were (n=10): < 0.01(6), 0.01(3), 0.017 mg/kg.

For dietary intake purposes the total residues in rape seeds were (n=10): < 0.02(5), 0.02(4), 0.028 mg/kg.

Barley and oats

For barley and oats the maximum GAP in northern Europe was reported from the UK involving two foliar applications of up to 0.125 kg ai/ha each. The last application is conducted at BBCH 61 and the PHI is covered by the growth between treatment and harvest. Supervised field trials conducted in northern Europe according to this GAP were submitted.

For MRL compliance purposes residues of parent bixafen in barley grain in northern Europe were (n=10): 0.02, 0.04(3), 0.05, 0.07, 0.08, 0.09, 0.09, 0.1 mg/kg.

For dietary intake purposes the total residues in barley grain in northern Europe were (n=10): 0.03, 0.05(3), 0.06, 0.08, 0.1, 0.1, 0.11, 0.11 mg/kg.

In Southern Europe a GAP for barley and oats was reported from France with one application of 0.075 kg ai/ha up to BBCH 61 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for the residue data originating from southern Europe against the GAP of the UK.

For MRL compliance purposes residues of parent bixafen in barley grain in southern Europe according to the UK GAP were (n=9): 0.03, 0.04, 0.04, 0.06, 0.06, 0.08, 0.1, 0.25, 0.34 mg/kg.

For dietary intake purposes the total residues in barley grain in southern Europe according to the UK GAP were (n=9): 0.04, 0.05, 0.05, 0.08, 0.08, 0.1, 0.11, 0.29, 0.38 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for barley:

For MRL compliance purposes residues of parent bixafen in barley grain in whole Europe (n=19): 0.02, 0.03, 0.04(5), 0.05, 0.06, 0.06, 0.07, 0.08, 0.08, 0.09, 0.09, 0.1, 0.1, 0.25 and 0.34 mg/kg.

For dietary intake purposes the total residues in barley grain in whole Europe were (n=19): 0.03, 0.04, 0.05(5), 0.06, 0.08(3), 0.1(3), 0.11(3), 0.29 and 0.38 mg/kg.

Wheat, rye, triticale and spelt

For wheat, rye and triticale the maximum GAP in Northern Europe was reported from the UK and involved two foliar applications of up to 0.125 kg ai/ha each. The last application is conducted at BBCH 69 and the PHI is covered by the growth between treatment and harvest. Supervised field trials conducted in Northern Europe according to this GAP were submitted.

For MRL compliance purposes residues of parent bixafen in wheat grain in northern Europe were (n=10): < 0.01(6), 0.01, 0.01, 0.03, 0.03 mg/kg.

For dietary intake purposes the total residues in wheat in northern Europe were (n=10): < 0.02(6), 0.02, 0.02, 0.04, 0.04 mg/kg.

In southern Europe a GAP for wheat, rye and triticale was reported from France with one application of 0.094 kg ai/ha up to BBCH 69 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against UK GAP:

For MRL compliance purposes residues of parent bixafen in wheat grain in Southern Europe according to the UK GAP were (n=10): < 0.01(6), 0.01, 0.02, 0.02, 0.03 mg/kg.

For dietary intake purposes the total residues in wheat grain in southern Europe according to UK GAP were (n=10): < 0.02(6), 0.02, 0.03, 0.03, 0.04 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for wheat:

For monitoring purposes residues of parent bixafen in wheat grain in whole Europe (n=20): < 0.01(12), 0.01(3), 0.02, 0.02, 0.03, 0.03 and 0.03 mg/kg.

For dietary intake purposes the total residues in wheat grain in whole Europe were (n=20): < 0.02(12), 0.02(3), 0.03, 0.03, 0.04, 0.04 and 0.04 mg/kg.

Animal feeds

Oilseed rape, forage

The Meeting noted that the only authorisation submitted for bixafen in rape was from UK explicitly relating to oilseed rape. This GAP involves late treatment of the crop 56 days before harvest, which is normally beyond the common timeframe for utilization of oilseed rape as a forage crop, i.e., before winter and up to BBCH 39. This is supported by supervised field trials in northern Europe, where last applications were conducted at growth stages at the end of flowering or at early maturity.

The Meeting concluded that the reported GAP for bixafen is not relevant for the utilization of oilseed rape as an animal forage crop.

Barley, oats, rye, triticale and wheat – forage of cereals

GAPs for barley and oats in the UK are for a maximum of two foliar applications up to flowering (BBCH 61) with 0.125 kg ai/ha each. The PHI is covered by the interval between treatment and harvest (covered by growth stage).

For the calculation of the livestock animal dietary burden the total residues in barley forage (fresh) in northern Europe were (n=10): 2.1, 2.5, 2.6, 2.9, 3.5, 3.9, 4.0, 4.4, 4.5, 7.3 mg/kg.

In southern Europe a GAP for barley and oats was reported from France with one application of 0.075 kg ai/ha up to BBCH 61 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against the UK GAP:

For the calculation of the livestock animal dietary burden the total residues in barley forage (fresh) in southern Europe were (n=9): 2.7, 3.0, 3.2, 3.4, 3.4, 3.7, 3.8, 4.3, 6.0 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for barley and oat forage:

For the calculation of the livestock animal dietary burden the total residues in barley forage (fresh) in whole Europe were (n=19): 2.1, 2.5, 2.6, 2.7, 2.9, 3.0, 3.2, 3.4, 3.4, 3.5, 3.7, 3.8, 3.9, 4.0, 4.3, 4.4, 4.5, 6.0, 7.3 mg/kg.

For wheat, rye and triticale the maximum GAP in northern Europe was reported for the UK involving two foliar applications of up to 0.125 kg ai/ha. The last application is at BBCH 69 with the PHI covered by growth between treatment and harvest. Supervised field trials conducted in northern Europe according to this GAP were submitted.

For the calculation of the livestock animal dietary burden the total residues in wheat forage (fresh) in northern Europe were (n=10): 1.5, 2.4, 2.8, 2.9, 3.1, 3.4, 3.8, 4.7, 4.8, 7.3 mg/kg.

In southern Europe a GAP for wheat, rye and triticale was reported from France with one application of 0.094 kg ai/ha up to BBCH 69 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against the UK GAP.

For the calculation of the livestock animal dietary burden the total residues in wheat forage (fresh) in southern Europe were (n=10): 2.6, 2.7, 2.9, 3.0, 3.6, 3.9, 4.2, 4.5, 5.2, 5.5 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for wheat forage.

For the calculation of the livestock animal dietary burden the total residues in barley and wheat forage (fresh) in Europe were (n=20): 1.5, 2.4, 2.6, 2.7, 2.8, 2.9, 2.9, 3.0, 3.1, 3.4, 3.6, 3.8, 3.9, 4.2, 4.5, 4.7, 4.8, 5.2, 5.5, 7.3 mg/kg.

Barley, oats, rye, triticale and wheat – straw and fodder

GAPs for barley and oats in the UK are for a maximum of two foliar applications up to flowering (BBCH 61) with 0.125 kg ai/ha each. The PHI is covered by the interval between treatment and harvest (covered by growth stage).

For MRL compliance purposes residues of parent bixafen in barley straw (fresh) in northern Europe (n=10): 0.64, 0.7, 0.77, 0.86, 1.1, 1.1, 3.7, 4.8, 5.4, 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in barley straw (fresh) in northern Europe were (n=10): 0.72, 0.74, 0.85, 1.0, 1.2, 1.2, 3.9, 5.2, 5.6, 11 mg/kg.

In southern Europe a GAP for barley and oats was reported from France with one application of 0.075 kg ai/ha up to BBCH 61 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from Southern Europe against the UK GAP.

For MRL compliance purposes residues of parent bixafen in barley straw (fresh) in southern Europe (n=9): 0.46, 0.76, 1.2, 1.5, 1.9, 3.1, 5.2, 5.7, 6.2 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in barley straw (fresh) in southern Europe were (n=9): 0.5, 1.0, 1.3, 1.6, 2.1, 3.3, 5.6, 6.1, 6.7 mg/kg.

Since both datasets are not significantly different (Mann-Whitney-U-testing), the Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for barley and oat straw.

For MRL compliance purposes residues of parent bixafen in barley straw (fresh) in whole Europe (n=19): 0.46, 0.64, 0.7, 0.76, 0.77, 0.86, 1.1, 1.1, 1.2, 1.5, 1.9, 3.1, 3.7, 4.8, 5.2, 5.4, 5.7, 6.2 and 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in barley straw (fresh) in whole Europe were (n=19): 0.5, 0.72, 0.74, 0.85, 1.0, 1.0, 1.2, 1.2, 1.3, 1.6, 2.1, 3.3, 3.9, 5.2, 5.6, 5.6, 6.1, 6.7, 11 mg/kg.

For wheat, rye and triticale the maximum GAP in Northern Europe was reported from the UK and involved two foliar applications of up to 0.125 kg ai/ha. The last application is at BBCH 69 and the PHI is covered by growth between treatment and harvest. Supervised field trials conducted in northern Europe according to this GAP were submitted.

For MRL compliance purposes residues of parent bixafen in wheat straw (fresh) in northern Europe (n=10): 0.52, 0.93, 0.95, 1.3, 1.8, 1.9, 3.6, 4.1, 8.4 and 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in wheat straw (fresh) in northern Europe were (n=10): 0.78, 1.2, 1.3, 1.5, 2.1, 2.5, 3.9, 4.4, 9.6 and 11 mg/kg.

In southern Europe a GAP for wheat, rye and triticale was reported from France with one application of 0.094 kg ai/ha up to BBCH 69 with a 35 day PHI. However, no corresponding residue data were submitted.

The Meeting decided to explore the use of global residue data as outlined in the 2011 JMPR Report (2.4) for residue data from southern Europe against the UK GAP:

For MRL compliance purposes residues of parent bixafen in wheat straw (fresh) in southern Europe (n=10): 0.79, 1.4, 1.7, 1.8, 2.6, 3.2, 3.3, 3.6, 5.4 and 5.7 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in wheat straw (fresh) in southern Europe were (n=10): 1.2, 1.9, 1.9, 2.2, 3.2, 3.7, 3.9, 4.1, 6.0 and 6.2 mg/kg.

The Meeting decided to combine that data and evaluate all European supervised field trials against the UK GAP for wheat, rye and triticale straw:

For MRL compliance purposes residues of parent bixafen in wheat straw (fresh) in whole Europe (n=20): 0.52, 0.79, 0.93, 0.95, 1.3, 1.4, 1.7, 1.8, 1.8, 1.9, 2.6, 3.2, 3.3, 3.6, 3.6, 4.1, 5.4, 5.7, 8.4 and 10 mg/kg.

For the calculation of the livestock animal dietary burden the total residues in wheat straw (fresh) in whole Europe were (n=20): 0.78, 1.2, 1.2, 1.3, 1.5, 1.9, 1.9, 2.1, 2.2, 2.5, 3.2, 3.7, 3.9, 3.9, 41, 4.4, 6.0, 6.2, 9.6 and 11 mg/kg.

Residues in rotational crops

Bixafen is highly persistent in soil, showing accumulation over subsequent years of treatment. In field rotational crop studies conducted at rates corresponding to the highest annual application rates registered, no significant residues were found in plant commodities. However, the long-term field accumulation study submitted suggests plateau residues in soil after up to seven years of annual treatment are equivalent to 2–3 times the soil residues expected after a single treatment at the registered maximum annual application rate. In confined rotational crop studies approximating this plateau level observed in soil, residues above the LOQ of 0.01 mg/kg were found for bixafen and M21 (bixafen-desmethyl) in all plant commodities investigated except for wheat grain.

The Meeting concluded that the accumulation of bixafen in soil results in residue concentrations in follow crops which are relevant for MRL compliance, dietary intake assessment and the estimation of livestock dietary burden. However, the Meeting recognized that the available field rotational crop studies were underdosed compared to the soil concentrations following long-term use of bixafen, while confined rotational crop studies are not considered representative for field conditions.

The Meeting decided that further information on bixafen in rotational crops under field conditions are required involving application rates approximating the plateau levels in soil after subsequent years of treatment. The estimation of maximum residue levels and median or highest residues for annual crops is not possible without considering the contribution of residues taken up from soil and will be postponed to a future meeting when new data becomes available to assess the rotational crop situation.

Fate of residues during processing

The Meeting received information on the hydrolysis of radio-labelled bixafen as well as processing studies using unlabelled material on grown residues in oilseed rape, barley and wheat.

In a hydrolysis study using radio-labelled bixafen typical processing conditions were simulated (pH 4,5 and 6 with 90 °C, 100 °C and 120 °C for 20, 60 and 20 minutes). In duplicate samples of sterile buffer solution no degradation was observed.

The Meeting concluded that no recommendations on bixafen residues in plant commodities can be made (see residues in rotational crops section) and therefore no processing factors are required. For an overview of the available information on the fate of bixafen during processing please refer to the 2013 Evaluation.

Residues in animal commodities

Farm animal feeding studies

The Meeting received feeding studies involving bixafen on lactating cows and laying hens.

Three groups of lactating cows were dosed daily at levels of 4, 12 and 40 ppm in the diet (0.15, 0.45 and 1.5 mg/kg bw) for 28 consecutive days. Milk was collected throughout the whole study and tissues were collected on day 29 within 24 hours of the last dose.

In milk highest mean total residues were 0.039 mg/kg for the 4 ppm group, 0.077 mg/kg for the 12 ppm group and 0.218 mg/kg for the 40 ppm group. Investigation of the distribution of the residue in cream gave a 9.9 fold higher concentration than in whole milk (15 between whole milk and milk fat).

Total residues in muscle for the 4, 12 and 40 ppm groups were 0.039–0.065 mg/kg (mean: 0.052 mg/kg), 0.081–0.26 mg/kg (mean: 0.162 mg/kg) and 0.63–1.0 mg/kg (mean: 0.82 mg/kg), respectively. In liver residues were 0.42–0.69 mg/kg (mean: 0.57 mg/kg) for the 4 ppm group, 1.2–1.7 mg/kg (mean: 1.4 mg/kg) for the 12 ppm group and 4.8–5.4 mg/kg (mean: 5.0 mg/kg) for the 40 ppm group. Kidney contained total residues of 0.1–0.15 mg/kg (mean: 0.14 mg/kg), 0.28–0.37 mg/kg (mean: 0.34 mg/kg) and 1.0–1.3 mg/kg (mean: 1.2 mg/kg) for the for the 4, 12 and 40 ppm group.

For fat three different tissues were analysed (perirenal, mesenteric and subcutaneous fat). Highest residues were found in perirenal fat with 0.14–0.21 mg/kg (mean: 0.18 mg/kg) for the 4 ppm group, 0.33–0.48 mg/kg (mean: 0.43 mg/kg) for the 12 ppm group and 0.8–1.9 mg/kg (mean: 1.4 mg/kg) for the 40 ppm group.

For laying hens three groups of animals were dosed with rates of 1.5, 4.5 and 15 ppm in the dry weight feed (0.1, 0.3 and 1.0 mg/kg bw) for 28 consecutive days. Eggs were collected throughout the whole study and tissues were collected on day 29 after the last dose.

In eggs total residues at the plateau phase were < 0.02-0.02 mg/kg (highest daily mean: 0.02 mg/kg) for the 1.5 ppm group and ranged between 0.05 to 0.07 mg/kg (highest daily mean: 0.063 mg/kg) for the 4.5 ppm and between 0.13 to 0.22 mg/kg (highest daily mean: 0.178 mg/kg) for the 15 ppm group.

In tissues no residues above the LOQ were found in muscle. Total residues in fat for the 1.5, 4.5 and 15 ppm groups were < 0.02–0.02 mg/kg (mean: 0.02 mg/kg), 0.05–0.06 mg/kg (mean: 0.057 mg/kg) and 0.06–0.09 mg/kg (mean: 0.07 mg/kg), respectively. In liver residues were < 0.02–0.02 mg/kg (mean: 0.02 mg/kg) for the 1.5 ppm group, 0.02–0.04 mg/kg (mean: 0.03 mg/kg) for the 4.5 ppm group and 0.03–0.05 mg/kg (mean: 0.04 mg/kg) for the 15 ppm group.

Estimated maximum and mean dietary burdens of livestock and animal commodities maximum residue levels

The Meeting noted that the uptake of bixafen and M21 (bixafen-desmethyl) from soil contributes significantly to the overall residues in annual crops. Based on the information available (see residues in rotational crops), no estimation on livestock animal dietary burdens and the corresponding residue levels in animal commodities can be made.

RECOMMENDATIONS

Definition of the residue for compliance with MRL for plant commodities: *bixafen*

Definition of the residue for compliance with MRL for animal commodities and (for the estimation of dietary intake) for plant and animal commodities: *sum of bixafen and N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1H-pyrazole-4-carboxamide (bixafen-desmethyl), expressed as bixafen*

The residue is fat-soluble.

FURTHER WORK OR INFORMATION

The Meeting considered that the currently available information on residues in rotational crops was not sufficient to make recommendations on maximum residue levels in plant and animal commodities. For future recommendations field rotational crop studies approximating plateau concentrations of bixafen in soil are required.

DIETARY RISK ASSESSMENT

The Meeting concluded that the contribution of residues in plant commodities from soil uptake cannot be estimated based on the available data. Thus no estimations on median or highest residues in food commodities of plant and animal origin could be made, precluding both long and short-term dietary risk assessments for bixafen.

Consequently, the dietary risk assessment will be undertaken at a future meeting when the residues derived from both direct application and those taken up from the soil in a rotational crop situation can be evaluated together.

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