Monograph

01 August 2001

Pyraclostrobin

Volume 1

Report and
Proposed Decision

Rapporteur Member State: Germany

Contents

1 Statement of subject matter and purpose for which the monograph was prepared 3

1.1 Purpose for which the monograph was prepared (Dossier Document A) 3
1.2 Summary and assessment of information relating to collective provision of dossiers (Dossier Document B) 3
1.3 Identity of the active substance (Annex IIA 1) (Dossier Documents J, K-II and L-II) 3
1.3.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1) 3
1.3.2 Common name and synonyms (Annex IIA 1.3) 3
1.3.3 Chemical name (Annex IIA 1.4) 3
1.3.4 Manufacturer's development code number (Annex IIA 1.5) 4
1.3.5 CAS, EEC and CIPAC numbers (Annex IIA 1.6) 4
1.3.6 Molecular and structural formulae, molecular mass (Annex IIA 1.7) 4
1.3.7 Manufacturer or manufacturers of the active substance (Annex IIA 1.2) 4
1.3.8 Method or methods of manufacture (Annex IIA 1.8) 4
1.3.9 Specification of purity of the active substance (Annex IIA 1. 9) 4
1.3.10 Identity of isomers, impurities and additives (Annex IIA 1.10) 5
1.3.11 Analytical profile of batches (Annex IIA 1.11) 5
1.4 Identity of the plant protection product (Annex IIA 3.1; Annex IIIA 1) (Dossier Documents J, K-II, L-II, K-III, and L- III) (to be included for each preparation for which an Annex III dossier was submitted) 5
1.4.1 Current, former and proposed trade names and development code numbers (Annex IIIA 1.3) 5
1.4.2 Manufacturer or manufacturers of the plant protection product (Annex IIIA 1.2) 5
1.4.3 Type of the preparation and code (Annex IIIA 1.5) 5
1.4.4 Function (Annex IIA 3.1; Annex IIIA 1.6) 6
1.4.5 Composition of the preparation (Annex IIIA 1.4) 6
1.5 Use of the plant protection product (Annex IIA 3.2 to 3.4; Annex IIIA 3.1 to 3.7, 3.9, 12.1) (Dossier Documents C, D, and E) (to be included for each preparation for which an Annex III dossier was submitted) 6
1.5.1 Field of use (Annex IIA 3.3; Annex IIIA 3.1) 6
1.5.2 Effects on harmful organisms (Annex IIA 3.2; Annex IIIA 3.2) 6
1.5.3 Summary of intended uses (Annex IIA 3.4; Annex IIIA 3.3 to 3. 7, 3.9) 6
1.5.4 Information on authorisations in EU Member States (Annex IIIA 12.1) 6

2 Reasoned statement of the overall conclusions 9

2.1 Identity 9
2.1.1 Identity 9
2.1.2 Physical and chemical properties 9
2.1.3 Details of uses and further information 9
2.1.3.1 Details of uses 9
2.1.3.2 Further information 10
2.1.4 Classification and labelling 10
2.2 Methods of analysis 11
2.2.1 Analytical methods for analysis of the active substance as manufactured 11
2.2.2 Analytical methods for formulation analysis 11

2.2.3 Analytical methods for residue analysis 11
2.3 Impact on human and animal health 13
2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products 13
2.3.1.1 Metabolism / Toxicokinetics 13
2.3.1.2 Acute toxicity studies, local irritation and skin sensitising properties 14
2.3.1.3 Short-term toxicity 14
2.3.1.4 Genotoxicity studies 15
2.3.1.5 Long-term toxicity / carcinogenicity studies 15
2.3.1.6 Reproductive toxicity / developmental (teratogenicity) studies 16
2.3.1.7 Neurotoxicity / Delayed neurotoxicity studies 17
2.3.1.8 Further toxicological studies 17
2.3.1.9 Human Data 17
2.3.2 ADI 18
2.3.3 AOEL 18
2.3.4 ARfD (acute reference dose) 18
2.3.5 Drinking water limit 18
2.3.6 Impact on human or animal health arising from exposure to the active substance or to impurities contained in it 19
2.4 Residues 19
2.4.1 Definition of the residues relevant to MRLs 19
2.4.2 Residues relevant to consumer safety 20
2.4.3 Residues relevant to worker safety 20
2.4.4 Proposed EU MRLs and compliance with existing MRLs 21
2.4.5 Proposed EU import tolerances and compliance with existing import tolerances 21
2.4.6 Basis for differences, if any, in conclusion reached having regard to established or proposed CAC MRLs 21
2.5 Fate and behaviour in the environment 21
2.5.1 Definition of the residues relevant to the environment 21
2.5.2 Fate and behaviour in soil 21
2.5.3 Fate and behaviour in water 22
2.5.4 Fate and behaviour in air 23
2.6 Effects on non-target species 23
2.6.1 Effects on terrestrial vertebrates 23
2.6.2 Effects on aquatic species 23
2.6.3 Effects on bees and other arthropod species 24
2.6.3.1 Effects on bees 24
2.6.3.2 Effects on other arthropod species 24
2.6.4 Effects on earthworms and other soil macro-organisms 24
2.6.5 Effects on soil micro-organisms 25
2.6.6 Effects on other non-target organisms (flora and fauna) 25
2.6.7 Effects on biological methods of sewage treatment 25
2.7 Overall conclusion (metabolism schemes) 25
2.7.1 Toxicology (laboratory animals) 25
2.7.2 Residues (plant, plant products, livestock animals) 27
2.7.3 Fate and behaviour in the environment (soil, water, air) 32
2.8 Appendices 39
2.8.1 Appendix I: Standard terms and abbreviations 39
2.8.2 Appendix II: Specific terms and abbreviations 51

2.8.3 Appendix III: Listing of end points 55
2.8.3.1 Appendix III.1: Chapter 1 (identity, physical and chemical properties, details of uses, further information, classification and labelling) 55
2.8.3.2 Appendix III.2: Chapter 2 (methods of analysis) 59
2.8.3.3 Appendix III.3: Chapter 3 (impact on human and animal health) 60
2.8.3.4 Appendix III.4: Chapter 4 (residues) 62
2.8.3.5 Appendix III.5: Chapter 5 (fate and behaviour in the environment) 66
2.8.3.6 Appendix III.6: Chapter 6 (effects on non-target species) 73

3 Proposed decision with respect to the application for inclusion of the active substance in Annex I 81

3.1 Background to the proposed decision 81
3.2 Proposed decision concerning inclusion in Annex I 82
3.3 Rational for the postponement of the decision to include the active substance in Annex I, or for the conditions and restrictions to be associated with a proposed inclusion in Annex I, as appropriate 82

4 Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex I 85

4.1 Data which are necessary for an unrestricted inclusion in Annex I of Council Directive 91/414/EEC 85
4.2 Data which should be submitted for an assessment on Member State level 86

Level 1

Pyraclostrobin

Statement of Subject Matter and
Purpose of Monograph

1 Statement of subject matter and purpose for which the monograph was prepared

1.1 Purpose for which the monograph was prepared (Dossier Document A)

This monograph is submitted to support first inclusion of the new active substance pyraclostrobin in Annex I of the Council Directive 91/414/EEC.

1.2 Summary and assessment of information relating to collective provision of dossiers (Dossier Document B)

As BASF is the only notifier of the active ingredient Pyraclostrobin, there is no relevance.

1.3 Identity of the active substance (Annex IIA 1) (Dossier Documents J, K-II and L-II)

1.3.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1)

BASF Aktiengesellschaft
Agricultural Center
Product Registration Management
P.O. Box 120
D-67141 Limburgerhof

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Fax: +49 621 60 276 04

1.3.2 Common name and synonyms (Annex IIA 1.3)

Pyraclostrobin (ISO, proposed).

1.3.3 Chemical name (Annex IIA 1.4)

IUPAC: methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate

CAS: Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester

1.3.4 Manufacturer's development code number (Annex IIA 1.5)

BAS 500 F, LAB 304428, Reg. No. 304428, PS 304428.

1.3.5 CAS, EEC and CIPAC numbers (Annex IIA 1.6)

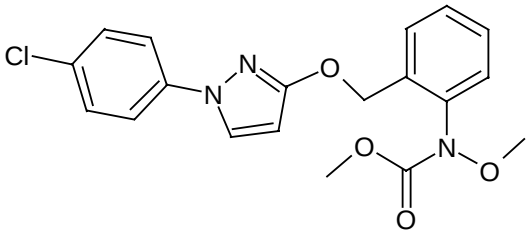
CAS: 175013-18-0
CIPAC: 657
EEC: not assigned
EINECS: not assigned

1.3.6 Molecular and structural formulae, molecular mass (Annex IIA 1.7)

Molecular formular: $C_{19}\ H_{18}\ Cl\ N_3\ O_4$

Molecular mass: 387.82 g/mol

Structural formula:



1.3.7 Manufacturer or manufacturers of the active substance (Annex IIA 1.2)

BASF Aktiengesellschaft
Agricultural Center
Product Registration Management
P.O. Box 120
D-67141 Limburgerhof

Contact person: Dr. Karl Zoller
Production Crop protection
Tel.: +49 621 60 791 46
Fax: +49 621 60 795 19

1.3.8 Method or methods of manufacture (Annex IIA 1.8)

Confidential information, see Annex C.

1.3.9 Specification of purity of the active substance (Annex IIA 1. 9)

≥ 950 g/kg (based on the analysis of material produced in a pilot plant).

1.3.10 Identity of isomers, impurities and additives (Annex IIA 1.10)

Confidential information, see Annex C.

1.3.11 Analytical profile of batches (Annex IIA 1.11)

Confidential information, see Annex C.

1.4 Identity of the plant protection product (Annex IIA 3.1; Annex IIIA 1) (Dossier Documents J, K-II, L-II, K-III, and L- III) (to be included for each preparation for which an Annex III dossier was submitted)

1.4.1 Current, former and proposed trade names and development code numbers (Annex IIIA 1.3)

Trade name: "BAS 500 00 F", preliminary designator

(country specific alternatives are under consideration)

Code number:	Plant protection product:	BAS 500 00 F
	Preliminary descriptor:	BAS 500 AE F
	Active Substance:	BAS 500 F
	BASF internal No.:	Reg. No. 304428

1.4.2 Manufacturer or manufacturers of the plant protection product (Annex IIIA 1.2)

BASF Aktiengesellschaft
Crop Protection Division
P.O. Box 1 20
67114 Limburgerhof
Germany

Contact person: Dr. Karl Zoller
Production Crop Protection
Tel. No.: (0)6 21/60-7 91 46
Fax No.: (0)6 21/60-7 95 19

1.4.3 Type of the preparation and code (Annex IIIA 1.5)

Emulsifiable concentrate (EC)

1.4.4 Function (Annex IIA 3.1; Annex IIIA 1.6)

Fungicide.

1.4.5 Composition of the preparation (Annex IIIA 1.4)

Confidential information, see Annex C.

1.5 Use of the plant protection product (Annex IIA 3.2 to 3.4; Annex IIIA 3.1 to 3.7, 3.9, 12.1) (Dossier Documents C, D, and E) (to be included for each preparation for which an Annex III dossier was submitted)

1.5.1 Field of use (Annex IIA 3.3; Annex IIIA 3.1)

Pyraclostrobin will be used as a fungicide in viticulture and on turf.

1.5.2 Effects on harmful organisms (Annex IIA 3.2; Annex IIIA 3.2)

Pyraclostrobin is a fungicide belonging to the group of strobilurins. The biochemical mode of action of the strobilurins is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) to the cytochrome bc_1 complex (Complex III). This leads to a reduction of energy-rich ATP that is available to support a range of essential processes in the fungal cell.

Pyraclostrobin is active against fungal development stages both on the plant surface and within the tissues. Pyraclostrobin has a protective as well as an eradicative/curative action. Pyraclostrobin is selective on a wide range of dicotyledonous and monocotyledonous crop species.

1.5.3 Summary of intended uses (Annex IIA 3.4; Annex IIIA 3.3 to 3. 7, 3.9)

List of uses supported by data (see Vol. 1, Chapter 2.8.3.1)

1.5.4 Information on authorisations in EU Member States (Annex IIIA 12.1)

Pyraclostrobin is a new active substance in the EU Member States.

Level 2

Pyraclostrobin

Overall Conclusions

2 Reasoned statement of the overall conclusions

2.1 Identity

2.1.1 Identity

All points (Annex II and III) have been addressed and the information supplied is acceptable.

2.1.2 Physical and chemical properties

Pyraclostrobin, chemical name (IUPAC) methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate is a new fungicidal active ingredient. It represents a modification of the structure pattern of natural fungicides called strobilurins.
The TAS is a dark brown solid (solidified melt) at room temperature with a moderate aromatic odour, the PAS is a white to light beige odourless crystalline solid with a melting point of 63.7-65.2 °C. The active substance is soluble in water (1.9 mg/L) without dissociation. The solubility in organic solvents is: slightly in n-heptane, moderately in 2-propanol, 1-octanol and olive oil, readily soluble in methanol acetone, ethyl acetate, acetonitrile, dichloromethane and toluene.
There are no high flammability, explosivity, or oxidizing properties of any concern.

BAS 500 00 F is a dark yellow emulsifiable concentrate with a moderate naphthalene like odour. It has neither explosive nor oxidising properties. The flash point is 98 °C. Its pH-value of 6.35 ± 0.5 lies within the naturally occurring range. The results of the accelerated storage test and the shelf life test confirm its stability at least two years under practical and commercial conditions. Its technical properties indicate no particular problems when used as recommended.

2.1.3 Details of uses and further information

2.1.3.1 Details of uses

Pyraclostrobin is a fungicide belonging to the group of strobilurins. The biochemical mode of action of the strobilurins is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) to the cytochrome bc_1 complex (Complex III). This leads to a reduction of energy-rich ATP that is available to support a range of essential processes in the fungal cell.

Pyraclostrobin is active against fungal development stages both on the plant surface and within the tissues. Pyraclostrobin has a protective as well as an eradicative/curative action. Pyraclostrobin is selective on a wide range of dicotyledonous and monocotyledonous crop species.

2.1.3.2 Further information

Information on handling, storage, transport or fire, destruction or decontamination, and emergency measures for the active substance as manufactured and information on packaging, cleaning procedures, handling, storage, transport or fire, emergency measures, and procedures for destruction or decontamination for the emulsion concentrate have been supplied and are acceptable.

2.1.4 Classification and labelling

The following is proposed in accordance with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

Pyraclostrobin (= BAS 500 F): active substance

Hazard symbol:	T; N	
Indication of danger:	Toxic; dangerous to the (aquatic) environment	
Risk phrases:	R 23	Toxic by inhalation
	R 38	Irritating to skin
	R 50/53	Very toxic to aquatic organisms
	May cause long-term adverse effects in the aquatic environment	

Reasons for classification
For justification of R 23 see B 6.2.3 INHALATION
For justification of R 38 see B.6.2.4 SKIN IRRITATION

The following is proposed in accordance with Directive 78/631/EEC in combination with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

BAS 500 00 F: preparation

Hazard symbol:	Xn, N	
Indication of danger:	Harmful	
Risk phrases:	R 20	Harmful by inhalation
	R 22	Harmful if swallowed
	R 36	Irritating to eyes
	R 38	Irritating to skin
	R 50/53	Very toxic to aquatic organisms
	May cause long-term adverse effects in the aquatic environment	
	(R 65	Harmful: may cause lung damage if swallowed)

Reasons for classification

For justification of R 20 see B 6.11.3	INHALATION
For justification of R 22 see B 6.11.1	ORAL
For justification of R 36 see B 6.11.5	EYE IRRITATION
For justification of R 38 see B.6.11.6	SKIN IRRITATION
For justification of R 65 see B.6.13	TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES (The notifier stated that, based on physical properties of the product, the risk phrase R65 from Solvesso should not be transferred to the classification of the product.)

2.2 Methods of analysis

2.2.1 Analytical methods for analysis of the active substance as manufactured

Analytical methodology is available for the determination of the active substance and the impurities in the technical material as manufactured.

Pyraclostrobin in the technical active substance is determined by a HPLC external standard method on a reversed phase column with UV detection.

10 impurities in the technical active substance are determined by a HPLC method on a reversed phase column with UV detection. The residual solvent is quantified by head space gas chromatography with flame ionisation detection. Another impurity is quantified by solid-phase microextraction coupled to GC/MS.

All methods are fully validated.

2.2.2 Analytical methods for formulation analysis

Analytical methodology is available for the determination of the active substance in the EC formulation.

Pyraclostrobin in the EC formulation is determined by a HPLC external standard method on a normal phase column with UV detection.
The method is fully validated.

2.2.3 Analytical methods for residue analysis

For the assessment of the analytical methods for the determination of pyraclostrobin residues the following criteria were used:

- The submitted methods enable the enforcement of the following relevant residue limits (at the time of evaluation):

plants and plant products	0.05	mg/kg	proposed MRL for other products of plant origin
milk	0.01	mg/kg	proposed MRL
meat, fat, eggs	0.05	mg/kg	proposed MRL
soil	0.05	mg/kg	general limit
drinking water	0.1	µg/L	EU drinking water limit
surface water	3	µg/L	NOEC of Daphnia magna as most sensitive species
air	6	µg/m³	based on a proposed systemic AOEL of 0.02 mg/kg bw

- Mean recovery rates at each fortification level in the range of 70 to 110% with a relative standard deviation of ≤ 20%
- No interfering blanks (< 30% of the LOQ)
- Methods must employ the simplest approach, involve the minimum cost, and require commonly available equipment.
- The enforcement method for food must be suitable for the determination of all compounds included in the residue definition (see 2.4.1), using an additional confirmatory method if appropriate.
- The enforcement methods for environmental matrices must be able to analyse for all compounds of toxicological and/or ecotoxicological significance in soil, water and air (see 2.5.1), using an additional confirmatory method if appropriate.

Methods for the determination of metabolites are not needed, because pyraclostrobin is considered as the only relevant analyte for monitoring purposes.

According to these criteria adequate analytical methods are available for the determination of pyraclostrobin in plant material, food of animal origin, soil, drinking water, surface water and air.
Analytical methods for body fluids are not submitted. Because of the classification of the active substance as T the lack of an appropriate method is considered as an essential data gap.

Table B.2.2-1: Analytical methods for the determination of residues

Matrix		Method	Limit of quantification		Reference
crops	wheat (grain, straw, forage), grapes, peanut, orange	LC-MS-MS	0.02	mg/kg	Reinhard and Mackenroth, 1999
	wheat (grain, straw, forage), grapes, peanut, orange	HPLC-UV	0.02	mg/kg	Abdel-Baky and Riley, 2000
products	yeast, malt, pot barley	LC-MS-MS	0.02	mg/kg	Reinhard and Mackenroth, 1999
	milk	HPLC-UV	0.01	mg/kg	Kampke-Thiel, 1999
	muscle, liver, kidney, fat, eggs	HPLC-UV	0.05	mg/kg	Kampke-Thiel, 1999

Matrix		Method	Limit of quantification		Reference
soil		HPLC-UV	0.01	mg/kg	Ziegler, 1998
		LC-MS	0.01	mg/kg	Zangmeister,1999
water	drinking, surface	HPLC-UV	0.05	µg/l	Zangmeister, 2000
	drinking, surface	LC-MS-MS	0.05	µg/l	Zangmeister, 1999
air		HPLC-UV	0.3	µg/m^3	Zangmeister, 1999

2.3 Impact on human and animal health

2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products

2.3.1.1 Metabolism / Toxicokinetics

Following oral administration of either a single low (5 mg/kg bw) or a high dose (50 mg/kg bw) to rats, pyraclostrobin [Methyl-N-(2-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxymethyl-phenyl)-N-methoxy carbamate; BAS 500 F] was rapidly absorbed from the gastrointestinal tract. However, oral absorption is incomplete and accounts for approximately 50% or even less of the dose. This percentage was estimated by summing up the amount of urinary and biliary excretion.
The elimination process was nearly completed after 120 hours with the major part of radioactivity being excreted within the first 48 hours irrespective of dose, dosing regimen (single versus repeated administration) or sex. About 11-15% of the applied radioactivity was eliminated via the urine while excretion via the faeces accounted for 80 – 90% of the dose. However, 35% of the radioactivity was actually eliminated from the body via the bile. Initial half-lives were approximately 10 h, terminal half-lives ranged between 20 and 37 h. A comparison of AUC values for both dose levels suggests nearly linear kinetics.
Tissue distribution determination revealed highest amounts of radioactivity in the GI tract, followed by liver. All other tissues had residues similar to or less than the concentrations in plasma. Although this compound is lipophilic, there is no evidence of accumulation. Most likely, this is due to extensive metabolism and rapid and effective excretion.
The systemically available portion was rapidly and intensively metabolised with N-demethoxylation being the quantitatively most important pathway. Phase I biotransformation was further characterised by various hydroxylations, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to a large number of observed metabolites. No major differences were observed with regard to sex and dose level.
Dermal absorption of pyraclostrobin is poor. A skin penetration rate of 1% in humans should be used for calculations but is still considered an overestimation.

2.3.1.2 Acute toxicity studies, local irritation and skin sensitising properties

In rats, pyraclostrobin was of low acute oral and dermal toxicity. No mortality occurred up to the highest oral dose of 5,000 mg/kg bw although some signs of intoxication were observed. The dermal LD_{50} was greater than 2,000 mg/kg bw with no signs of systemic toxicity occurring. In contrast, clear toxic properties of the active ingredient were noted in the inhalation study in rats including severe symptoms of systemic poisoning and deaths. Classification and labelling (T, R23) is considered necessary although, due to the physico-chemical properties of pyraclostrobin, an acute inhalative risk for humans is not expected.
The compound produced mild skin irritation and should be labelled accordingly (Xi, R38). Pyraclostrobin was not irritant to the eyes and was not a skin sensitiser in the Maximisation test.

2.3.1.3 Short-term toxicity

The oral short-term toxicity of pyraclostrobin was investigated in rats, mice, and dogs. Additionally, subacute dermal toxicity was examined in rats. The results of these studies are summarised in Table 2.3-1.

Table 2.3-1: Summary of short-term toxicity studies

Study type / species / dose levels	NOAEL (mg/kg bw/d)	LOAEL / Critical effects
4-week feeding Wistar rat 0, 20, 100, 500, 1500 ppm	9/9.6 m/f [100 ppm]	500 ppm: Effects on body weight, red blood cells, duodenum and liver.
4-week dermal Wistar rat 0, 40, 100, 250 mg/kg bw/d	>250 (systemic)	250 mg/kg bw/d: No systemic toxicity. 40 mg/kg bw/d: Signs of local irritation.
3-month feeding Wistar rat 0, 50, 150, 500, 1000, 1500 ppm	10.7/12.6 m/f [150 ppm]	500 ppm: Reduced body weight and food consumption, effects on clinical-chemical parameters, liver hypertrophy, and mucosal hypertrophy of the duodenum.
3-month feeding B6C3F1 mouse 0, 50, 150, 500, 1000, 1500 ppm	<9.2/12.9 m/f [<50/50 ppm m/f] (*ca. 4; m [30 ppm], carcinogenicity study)*	50 ppm: Reduced body weight (gain) and increased urea values in males. At higher dose levels, adverse effects in the gastrointestinal tract, on red blood cells, on white blood cells and lymphatic organs, as well as on adrenals, liver and kidney.
3-month feeding Beagle dog 0, 100, 200, 450 ppm	5.8/6.2 m/f [200 ppm]	450 ppm: Body weight loss in females, vomitus, diarrhoea, clinical-chemical and hematological changes in females, hypertrophy of the duodenal mucosa.
12- month feeding Beagle dog 0, 100, 200, 400 ppm	5.4/5.4 m/f [200 ppm]	400 ppm: Reduced body weight and food consumption (females); vomitus, diarrhoea; hemoglobin and hematocrit decreased (females); increase of white blood cells and platelets (males).

m: males; f: females

The signs of toxicity, as observed in the three species tested, were comparable. The critical clinical effects were reduction of body weight and body weight gain in all three species. In dogs, vomitus and diarrhoea occurred additionally. The target organ in all three species was the duodenum, showing mucosal hypertrophy, which was characterised by an increased ratio of cytoplasm to the nuclei in the villi, and by hyperplastic changes in the epithelial cells. Furthermore, some clinical chemical and haematological parameters were affected suggesting, together with pathological findings, minor adverse effects on the blood and the liver.
For rats, a short-term NOAEL of 150 ppm (10.7 mg/kg bw/d) has been established. For dogs, the short-term NOAEL is 200 ppm, equivalent to about 6 mg/kg bw/d, based on the 3-month and 1-year feeding study in this species.
For female mice, the no observed adverse effect level was 50 ppm (12.9 mg/kg bw/d).
For males, the NOAEL in the 3-month mouse study was lower than 50 ppm (9.2 mg/kg bw/d). Taking into consideration all available data for this species, a NOAEL of 30 ppm (ca 4 mg/kg bw/d) can be established for male mice based on the body weight data after 91 days from the carcinogenicity study. This dose is also considered the lowest relevant NOAEL for overall short-term oral toxicity.
In a 4-week dermal toxicity study in rats, no substance related systemic toxicity was detected up to the highest dose tested (250 mg/kg bw/d) whereas local effects were seen at all dose levels.

2.3.1.4 Genotoxicity studies

Pyraclostrobin was evaluated for its potential genotoxicity *in vitro* using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis (UDS) test. *In vivo*, the test substance was assessed for the induction of micronuclei in mice. The results of these studies unequivocally demonstrated the absence of a genotoxic effect.

2.3.1.5 Long-term toxicity / carcinogenicity studies

A chronic toxicity study in rats and two carcinogenicity studies in rats and mice were performed with pyraclostrobin and failed to provide evidence of a carcinogenic effect.
Restricted to the high doses, body weight and body weight gain were decreased in all studies, mostly accompanied by a diminished food consumption.
In the carcinogenicity study in rats, liver cell necrosis occurred in males. Additionally, in the chronic toxicity study in rats, alanine aminotransferase and alkaline phosphatase values were decreased at the high dose. A toxicological significance of these reduced enzyme activities is equivocal.
The overall NOAEL as obtained from the chronic/carcinogenicity studies in rats (males and females combined) and the carcinogenicity study in mice is about 4 mg/kg bw/d (see Table 2.3-2).

Table 2.3-2: Summary of long-term toxicity studies

Study type / species / dose levels	NOAEL (mg/kg bw/d)	LOAEL / Critical effects
24-month chronic toxicity Wistar rats 0, 25, 75, 200 ppm	3.4/4.6 m/f [75 ppm]	200 ppm: Reduced body weight. No increase in tumour incidences.
24-month carcinogenicity Wistar rats 0, 25, 75, 200 ppm	3.4/4.7 m/f [75 ppm]	200 ppm: Reduced body weight, reduced food consumption (m), liver cell necrosis (m). Not carcinogenic.
18-month carcinogenicity B6C3F1 mice 0, 10, 30, 120 ppm (m, f), 180 ppm (f)	4.1/4.8 m/f [30 ppm]	120 ppm (m, f) & 180 ppm (f): Reduced body weight. Not carcinogenic.

2.3.1.6 Reproductive toxicity / developmental (teratogenicity) studies

The reproduction toxicity of pyraclostrobin was investigated in a two-generation reproduction study in rats as well as in teratogenicity studies in rats and rabbits.

The results of the reproduction toxicity studies are summarised in Table 2.3-3.

Table 2.3-3: Summary of reproduction toxicity studies

Study type / species / dose levels	NOAEL	LOAEL / Critical effects
2-generation study Wistar rats 0, 25, 75, 300 ppm	Parental toxicity: ca 8.2 mg/kg bw/d [75 ppm] Reproductive toxicity: ca 8.2 mg/kg bw/d [75 ppm]	300 ppm: Parental toxicity: reduced food consumption and body weight gain; Reproductive toxicity: reduced pup body weight gain, organ weight changes and a delay in female pup development (F1 litters only). No adverse effects on fertility.
Developmental toxicity Wistar rats 0, 10, 25 and 50 mg/kg bw/d; days 6-19	Maternal toxicity: 10 mg/kg bw/d Developmental toxicity: 25 mg/kg bw/d	25 mg/kg bw/d: Maternal toxicity: reduced food consumption and body weight (gain). 50 mg/kg bw/d: Developmental toxicity: increased variations.
Developmental toxicity Himalayan rabbits (1st study) 0, 5, 10 and 20 mg/kg bw/d; days 7-28	Maternal toxicity: <5 mg/kg bw/d Developmental toxicity: 5 mg/kg bw/d	5 mg/kg bw/d: Maternal toxicity: reduced food consumption, reduced body weight gain. 10 mg/kg bw/d: Developmental toxicity: skeletal malformations increased; at 20 mg/kg bw/d increase in resorptions and postimplantation losses; reduced number of live fetuses.
Developmental toxicity Himalayan rabbits (2nd study with special regard to maternal effects) 0, 1, 3, 5 mg/kg bw/d; days 7-28	Maternal toxicity: 3 mg/kg bw/d Developmental toxicity: 5 mg/kg bw/d	5 mg/kg bw/d: Maternal toxicity: reduced food consumption, reduced body weight gain. No evidence of developmental toxicity (limited range of parameters investigated).

Fertility was not affected up to the highest dose level of 300 ppm (ca 32.6 mg/kg bw/d) in the two-generation study. The NOAEL concerning parental toxicity in this study was 75 ppm (approximately 8.2 mg/kg bw/d) for F0 and F1 animals, and this was also the NOAEL for reproductive toxicity in the F1 and F2 litters. Offspring effects were confined to reduced body weight gain and associated changes of organ weights. A single developmental landmark (vaginal opening) was delayed in F1 pups only at 300 ppm suggesting a possible retardation in female pup development.

In the prenatal toxicity study in rats, developmental toxicity was observed at the highest dose tested (50 mg/kg bw/d), based on increased incidences of several soft tissue and skeletal variations inside the range of the historical control values. The high and intermediate dosages were clearly toxic to the dams as demonstrated by a marked reduction in body weight gain. Thus, the NOAEL for maternal toxicity was established at 10 mg/kg bw/d, and the developmental NOAEL was 25 mg/kg bw/d.
In the first rabbit prenatal toxicity study, developmental toxicity was observed in the presence of severe maternal toxicity suggesting a higher vulnerability of this species. Maternal toxicity was proven by clear reduction of body weight gain and a lower food consumption at 5 mg/kg bw/d and above. Prenatal toxicity was substantiated by embryolethality resulting in elevated postimplantation losses and a reduction in the mean number of live fetuses/doe at 20 mg/kg bw/d. At 10 and 20 mg/kg bw/d, increased incidences of skeletal malformations outside the historical control range of this laboratory were observed although this finding did not reach statistical significance. The NOAEL for developmental toxicity was 5 mg/kg bw/d. A clear NOAEL for maternal toxicity could not be established and, therefore, a further rabbit study was performed by the notifier on request of the Rapporteur. In this second study, a lower food consumption and an initial impact on body weight gain was confirmed in the does receiving the highest dose of 5 mg/kg bw/d. The medium dose of 3 mg/kg bw/d was considered the maternal NOAEL. There were no indications of fetal effects in this study, however, the range of parameters investigated was rather limited since the focus of this additional study was on maternal toxicity.

2.3.1.7 Neurotoxicity / Delayed neurotoxicity studies

No indications of a specific neurotoxic potential of pyraclostrobin were observed in rats neither in an acute nor in a subchronic neurotoxicity study. There was also no evidence of neurotoxicity coming from any other of the toxicological studies.

2.3.1.8 Further toxicological studies

Three groundwater metabolites of pyraclostrobin were tested for their ability to cause gene mutations and proved all negative in the Ames test.

2.3.1.9 Human Data

Since pyraclostrobin is a new compound, human data and experience are very limited.
No poisoning incidents have been reported so far. With regard to the toxicological profile of pyraclostrobin, serious health problems are not anticipated.

2.3.2 ADI

The ADI should be based on the overall chronic NOAEL of 4 mg/kg bw/day as established in the long-term studies in rats and mice. According to the comprehensive toxicological database, the compound has no genotoxic or carcinogenic potential, is not teratogenic and does not affect fertility. Therefore, the standard assessment factor of 100 is considered appropriate.

The calculation results in a proposed ADI of:

0.04 mg/kg bw.

This ADI value is in agreement with the proposal of the notifier.

2.3.3 AOEL

The AOEL is usually derived on the basis of so-called mid-term toxicity studies, i.e. the subacute/subchronic or teratogenicity studies. For pyraclostrobin, the lowest relevant oral NOAEL established in studies of these types was 4 mg/kg bw/day which is mainly based on body weight data in male mice but also supported by results obtained in the other subchronic studies as well as by the maternal findings in the developmental toxicity studies in rabbits.
For establishing the systemic AOEL, the oral absorption rate of approximately 50% must be taken into account. Because of the toxicological profile of pyraclostrobin and in accordance with current EU assessment practice, the standard assessment factor of 100 should be applied resulting in a **systemic AOEL of 0.02 mg/kg bw/day.**
The notifier had proposed a systemic AOEL of 0.08 mg/kg bw/day using a lower assessment factor of 25.

2.3.4 ARfD (acute reference dose)

The long-term as well as oral short-term toxicity studies with pyraclostrobin suggest an overall NOAEL of 4 mg/kg bw/day. This is also supported by the outcome of the teratogenicity studies in rabbits since the LOEL for maternal toxicity was 5 mg/kg bw/day. Thus, it is considered most appropriate to derive also the ARfD on this basis resulting in a proposed value of

0.04 mg/kg bw.

In contrast, the notifier had proposed to derive the ARfD from the 4-week study in rats resulting in a numerical value of of 0.09 mg/kg bw. However, the range of parameters investigated in this study was rather limited. Moreover, the evidence of maternal effects in both rabbit developmental toxicity studies at a dose level of 5 mg/kg bw/day does not allow to accept this proposal.

2.3.5 Drinking water limit

The determination of a MAC value is not necessary, because according to Directive 91/414/EC only the ADI and AOEL values have to be determined. Therefore, the

establishment of a maximum admissible concentration for drinking water from an ADI value is not yet confirmed by a harmonised EU proposal. In addition to that, the maximum admissible concentration of an active substance is 0.1 µg/l, as established by the Directive 80/778/EEC.

2.3.6 Impact on human or animal health arising from exposure to the active substance or to impurities contained in it

A comprehensive toxicological database was developed for pyraclostrobin. The acute oral and dermal toxicity was low. In contrast, inhalative toxicity was high but is not considered a significant hazard. There was no evidence of a mutagenic, carcinogenic, neurotoxic or teratogenic potential, and reproductive performance was not affected. The NOAEL in chronic studies and the lowest NOAEL in short-term studies were 4 mg/kg bw.
Harmful effects on the health of operators, bystanders, workers, or consumers, are not expected when the plant protection product is used in accordance with good plant protection practice.
The potential operator exposure was estimated for the intended use of BAS 500 00 F. On the basis of the German model without PPE, the estimated systemic exposure to pyraclostrobin accounts for up to 14.4% of the proposed systemic AOEL. In the calculation on the basis of the UK-POEM without PPE, the exposure was 54.3% of the AOEL. In view of the recommended application technique in combination with Good Agricultural Practice (GAP) bystanders may be exposed only incidental, i.e. briefly and to relatively low quantities of spray compared to an operator. Therefore, it is not likely that the potential exposure of bystanders will exceed the AOEL.
The active substance intake by consumers was estimated according to the BBA guideline. The theoretical maximum daily intake (TMDI) accounted for only a part of the ADI which represents a large margin of safety for consumers.
In view of the recommended uses and application techniques, harmful effects on the health of domestic or wild animals are not to be expected.

2.4 Residues

2.4.1 Definition of the residues relevant to MRLs

Plants

The metabolism in plants was investigated in grapes, wheat and potatoes. The metabolic pattern is similar in all three crop groups. Therefore the metabolism in plants is considered to be proofed.
The residue consists only of two quantitative relevant compounds: parent pyraclostrobin and the desmethoxymetabolite BF 500-3 (500 M07)
Due to the fact that the residue level is significant higher for pyraclostrobin than for the metabolite and that BF 500-3 (500 M07) is main metabolite in the animal metabolism too, the residue definition for plants is proposed as parent compound only.

Residue definition: Pyraclostrobin

Livestock

The metabolism and distribution of radioactive labelled pyraclostrobin was investigated in lactating goats and laying hens.
After administration of the test compound, most of the radioactivity was excreted. There was no indication of accumulation of ^{14}C-pyraclostrobin in goat milk, eggs and tissues. The total radioactive residues in the edible portions were low.
The parent compound pyraclostrobin was main residue in nearly all samples under investigation. First step in the metabolism is the desmethoxylation at the oxime ether bond (metabolite BF 500-3 (500 M07)). Further minor metabolites are the result of reactions on BF 500-3 (500 M07).

Residue definition: Pyraclostrobin

2.4.2 Residues relevant to consumer safety

Chronic risk assessment

Estimates of the chronic dietary intake for pyraclostrobin were carried out on basis of the WHO European regional diet and of the German diet.

The intake was compared with the proposed ADI (0.04 mg/kg bw / day)

TMDI (WHO European diet 1998): 0.0048 mg/kg bw/day – 11.9 % of the proposed ADI
TMDI (German diet, 4-6 years old girl): 0.0034 mg/kg bw/day – 8.5 % of the proposed ADI

Acute risk assessment

Estimates of the acute dietary intake for pyraclostrobin were carried out for relevant uses using consumption data from UK. For pyraclostrobin the only use relevant for acute risk assessment is the use in grapes.

The intake was compared with the proposed ARfD (0.04 mg/kg bw / day).

Grapes:
NESTI (UK, adult): 0.0097 mg/kg bw - 24.4 % of the proposed ARfD
NESTI (UK, toddler): 0.0392 mg/kg bw – 98.1 % of the proposed ARfD

2.4.3 Residues relevant to worker safety

The possible amount of residues to which a worker may be exposed was calculated (B.6.14.3). Based on the given assumption, the systemic worker exposure would be 0.0048 mg/kg bw/d for the person not wearing PPE and 0.00024 mg/kg bw/d for the person wearing PPE. This corresponds to 24% and 1.2% of the proposed AOEL, respectively.
Therefore, the estimated exposure to pyraclostrobin during re-entry operations does not present an undue risk to the worker also if no PPE is worn. But it should be noted that a re-entry of treated areas/crops should not be performed before the spray deposit is completely dry.

2.4.4 Proposed EU MRLs and compliance with existing MRLs

Proposed MRL's based on an assessment of the GAP and residue data submitted		
Commodity	**proposed MRL [mg/kg]**	**Data requirements**
grapes	2	none
wheat, rye, triticale	0.1	none
barley, oats	0.2	further trials with barley from southern Europe
other products of plant origin	0.02 *	none
milk	0.01 *	none
other products of animal origin	0.05 *	none

* indicates lower limit of quantification

2.4.5 Proposed EU import tolerances and compliance with existing import tolerances

Banana
For bananas a MRL on the LOD of 0.02 mg/kg can be proposed because no residues were detected in all residue trials submitted.

2.4.6 Basis for differences, if any, in conclusion reached having regard to established or proposed CAC MRLs

There are currently (2001) no CODEX-MRL`s fixed.

2.5 Fate and behaviour in the environment

2.5.1 Definition of the residues relevant to the environment

According to the results presented, the parent compound is the only relevant residue for quantification in soil, water, and air. pyraclostrobin is rapidly degraded in the environment forming a number of metabolites. Some metabolites reached concentrations greater than 10% TAR in some studies, however, they are transient and not biologically, ecotoxicologically or toxicologically active.

2.5.2 Fate and behaviour in soil

The degradation of pyraclostrobin in aerobic soil studies is characterised by a rather low mineralisation rate (about 5% TAR within 100 days) and a formation of high amounts of bound residues (about 55% TAR within 100 days). The same metabolites, BF 500-6 and BF 500-7, were found in all soil types. BF 500-6 generally exceeded 10% TAR (maximum 31% TAR), whereas BF 500-7 slightly exceeded 10% TAR only in one of all investigated

soils. Bound residues increased with time and the major portion of radioactivity was associated with insoluble humins and high-molecular humic acids. A release of pyraclostrobin or metabolites could not be observed, neither with harsh extraction methods (NaOH) nor with intensive activity of soil-eating animals (earthworms). Photolytical degradation leads to the same degradation products, however, all metabolites were formed in amounts less than 10% TAR. Under anaerobic conditions, a very fast de-methoxylation took place, forming the metabolite BF 500-3 in high amounts (max. 96% TAR within 7 days). This reaction is supposed to be the first step also in the aerobic soil degradation, however, in aerobic soil the further reaction to BF 500-6, BF 500-7 and bound residues is too fast to detect this short-lived intermediate. In anaerobic soil, however, the further reactions of BF 500-3 are slowed down considerably.
Pyraclostrobin is degraded in soil under laboratory conditions with DT_{50}-values ranging from 12 to 137 days for five microbially active soils. Higher soil moisture generally accelerates the degradation. Photolysis does not significantly influence the degradation rate, however, reduces the amounts of the metabolites BF 500-6 and BF 500-7. In field studies, the DT_{50}-values of pyraclostrobin are lower, in a range between 14 and 85 days and mean of 26.1 days (1^{st} order estimations). The DT_{90}-values in the field were 49 to 114 days (mean of 86.6 d).
The soil metabolites have DT_{50}-values in the laboratory studies ranging from 60 to 166 days for the BF 500-6 and 38 to 159 days for the BF 500-7. (The high values for the BF 500-7 were calculated for soils, in which the metabolite was formed in amounts <10% TAR).
Under field conditions however, the metabolites BF 500-3 and BF 500-7 could not be detected at all. Only the metabolite BF 500-6 was found sporadically in trace amounts close to the quantification limit. All the results presented indicate that pyraclostrobin and its metabolites are moderately stable in an active soil environment.
Adsorption and column leaching studies clearly show that there is no risk of displacement into deeper soil layers or leaching into the groundwater neither for pyraclostrobin nor for the metabolites BF 500-3, BF 500-6 and BF 500-7. The minimum K_{oc}-value found during all experiments for all compounds and tested soil types was 3000 ml/g. Even under worst case laboratory leaching conditions (unaged as well as aged soil column leaching), all radioactivity remained in the upper soil layer and no radioactivity could be found in the leaches. Pyraclostrobin as well as its metabolites can be classified as non-mobile in soil and therefore, no lysimeter or field leaching study was performed.

2.5.3 Fate and behaviour in water

Pyraclostrobin is hydrolytically stable at pH 5 and 7. Only with pH 9, a very slightly degradation could be observed.
Since pyraclostrobin is very UV-susceptible, the degradation pathways obtained with the water/sediment study performed in the dark and the aqueous photolysis study are very different. In the aqueous photolysis, numerous breakdown and rearrangement products are formed (about 38), whereas in the water/sediment study a very fast binding to the sediment occurred. Under outdoor conditions, both factors together (photolysis and sediment adsorption) are influencing the degradation of pyraclostrobin in a natural water body. Therefore a study was performed, where a water/sediment system was incubated under conditions simulating the light and temperature regime of the major application period for the active substance (May-July). This study showed that besides about 15 minor degradation products, 3 metabolites were formed in the water in amounts >10% TAR (BF 500-11, chlorophenyl-ring split off; BF 500-13, des-methoxy-derivative of BF 500-11; BF 500-14, rearrangement product of the active substance). In the sediment, only the metabolite BF 500-3,

known from the anaerobic soil studies, exceeded 10% TAR. All metabolites are degrading with half-life times of 20 days (BF 500-11), 31 days (BF 500-13, value taken from the aqueous photolysis study), 14 days (BF500-14) and 99 days (BF 500-3). The active substance pyraclostrobin had a DT_{50}-value of 5 days in the water and 4 days in the sediment.
For the evaluation of the active substance the water/sediment study conducted according to the current guideline (in the dark) was considered. The active substance disappears quickly from the water phase at the begin of the incubation period. This process slows down with time. The DT50 values established according to 1st order kinetics was 8.7 days.
All these results show that in case pyraclostrobin reaches the water, it disappears from the water phase either by fast photolytical reactions or by adsorption to the sediment.

2.5.4 Fate and behaviour in air

Volatilisation studies from plant and soil surfaces showed a very low potential for the active substance to be displaced into the atmosphere. Even if small amounts of pyraclostrobin reach the troposphere, the half-life would be very short. The photochemical half-life for reactions with OH-radicals was determined to be less than 2 hours.

2.6 Effects on non-target species

2.6.1 Effects on terrestrial vertebrates

The toxicity of pyraclostrobin to mammals and birds is low. Taking into account the intended use then even under worst case assumptions all toxicity-exposure-ratios are above the Annex-VI-triggers, i.e. the risk to terrestrial vertebrates is acceptable.

Acute toxicity to mammals:	LD_{50} >5000 mg/kg bw
Long-term toxicity to mammals:	NOAEL 75 ppm
	(Reproductive NOAEL from rat multi-generation study)
Acute toxicity to birds:	LD_{50} >2000 mg/kg bw
Dietary toxicity to birds:	LC_{50} >5000 ppm
Reproductive toxicity to birds:	NOEL 1000 ppm

2.6.2 Effects on aquatic species

A data package in accordance with the requirements of Annexes II and III of Directive 91/414/EEC has been submitted for the active substance pyraclostrobin (BAS 500 F), relevant metabolites and the formulated product (BAS 500 00 F). The data are considered sufficient for a final risk assessment.

The active substance pyraclostrobin is highly toxic to fish and aquatic invertebrates, whereas green algae are less sensitive. The formulated product BAS 500 00 F has a comparable acute toxicity to *Oncorhynchus mykiss*, *Daphnia magna* and *Scenedesmus subspicatus* when compared to the effects of the active substance. Fish are clearly identified as the most sensitive test organisms. The dose/response relationship observed in acute toxicity tests with seven fish species is very steep. The ratio of acute to chronic effects is almost 1. Therefore the

lowest NOEC of 2.3 µg as/L (98 d early life-stage study with *Oncorhynchus mykiss*) is considered to be relevant for the risk assessment.
The TER value calculated on the basis of a $PEC_{sw, initial}$ of 3.827 µg as/L in a distance of 3 m to a waterbody (scenario: grapevines, 3 x 0.1 kg as/ha in 12 d interval) indicates a risk to fish species, which requires adequate risk mitigation measures (i.e. buffer zones) to be set at Member State level.

Compared to the active substance the three major metabolites (BF 500-11, BF 500-13 and BF 500-14) proved to be far less toxic to the standard test species. Potential effects therefore are considered to be covered by the risk mitigation measures to be set for the active substance.

2.6.3 Effects on bees and other arthropod species

2.6.3.1 Effects on bees

There has been performed a test with a formulated product (BAS 500 00F, 247.86 g as/L) and with the active substance. The obtained LD_{50}-values by oral uptake and contact are standing for a low toxicity to bees. The hazard quotients calculated on the basis of LD_{50}-values and the highest amount of as/ha indicate a low risk for bees, when the product is used as intended.

2.6.3.2 Effects on other arthropod species

According to the data submitted some sublethal effects for plant dwelling species (e.g. *Chrysopa carnea* and *Typhlodromus pyri*) cannot be ruled out for the in-field situation. However, these effects are not unacceptable as lethal effects were very low in *Chrysopa carnea* and recovery was demonstrated from field studies for *Typhlodromus pyri.*

It is therefore established in the light of current scientific and technical knowledge and as laid down in the SETAC/ESCORT "*Guidance document on regulatory testing procedures for pesticides with non-target arthropods*" (Barrett et al., 1994), that the use of pyraclostrobin as outlined in this monograph has no unacceptable influence on non-target arthropods, represented by species of four ecological groups.

2.6.4 Effects on earthworms and other soil macro-organisms

The studies on pyraclostrobin, the product BAS 500 00 F and the metabolites BAS 500-6 and BAS 500-7 indicate that the acute and the chronic toxicity to earthworms is low. The acute and long term TERs are above the relevant Annex VI triggers.
Three field studies on either the formulation BAS 500 00 F or BAS 500 01 F were conducted and resulted in the overall conclusion that the risk for earthworms was acceptable, although there were some effects on earthworms in one study. These appeared mainly with the maximum application rate, which does not take into account vegetation cover of the soil during application. Therefore effects are considered acceptable.

Acute toxicity to earthworms:	LC_{50} 565.9 mg as/kg (pyraclostrobin) LC_{50} 281.8 mg product/kg (BAS 500 00 F)
Reproductive toxicity to earthworms:	NOEC 1 L product/ha (corresponds to 0.443 mg as/kg)

A study on release of bound residues showed that no release of soil bound residues by earthworms was observed and that the concentration in earthworms was lower than the total radioactive concentration in soil.

2.6.5 Effects on soil micro-organisms

The influence of the EC formulation BAS 500 00 F on the microbial activity is in case of one- and ten-fold application rate (1 and 10 L/ha) < 25 % in comparison with untreated soil. Also the two metabolites (BF 500-6 and BF 500-7) have no lasting effects on carbon- and nitrogen conversion.
When applying BAS 500 00 F containing plant protection products according to the recommended pattern of use no lasting effects on microbial activities are to be expected.

2.6.6 Effects on other non-target organisms (flora and fauna)

Greenhouse studies on vegetative vigour were conducted with BAS 500 00 F with 3 mono- and 3 dicotyledonous species. The application rates tested corresponded to 160 g and 480 g as/l. No significant effects on weight and phytotoxicity were observed. Therefore it can be concluded that no risk for terrestrial non-target plants is likely to occur.

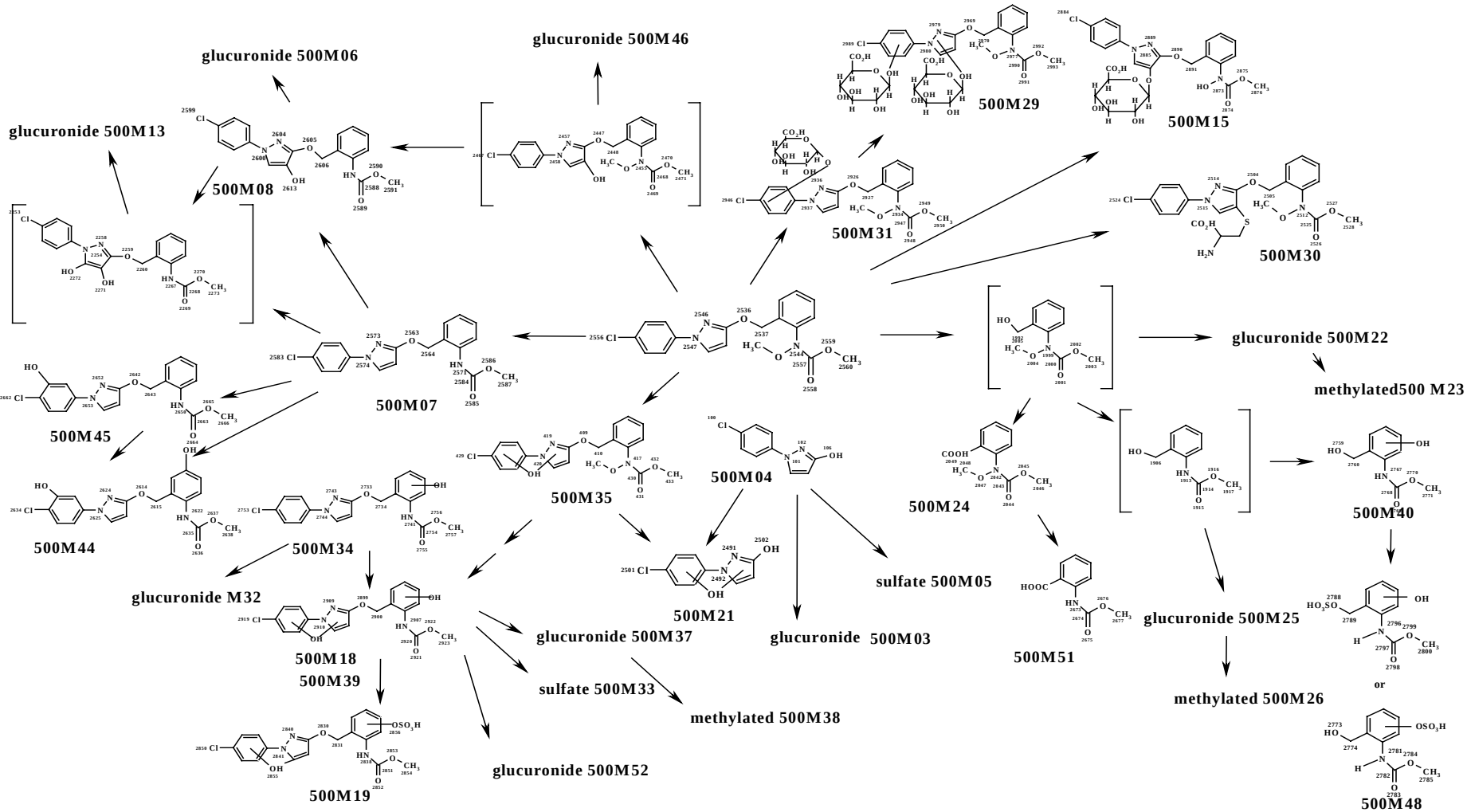
2.6.7 Effects on biological methods of sewage treatment

A significant inhibition of respiration was not observed up to the highest tested concentration of 1000 mg/L. An effect on the biodegradation process of activated sludge is not to be expected.

2.7 Overall conclusion (metabolism schemes)

2.7.1 Toxicology (laboratory animals)

Figure 2.7-1: Metabolic pathway of pyraclostrobin in rats



2.7.2 Residues (plant, plant products, livestock animals)

Figure 2.7-2: Metabolic pathway of pyraclostrobin in grapes

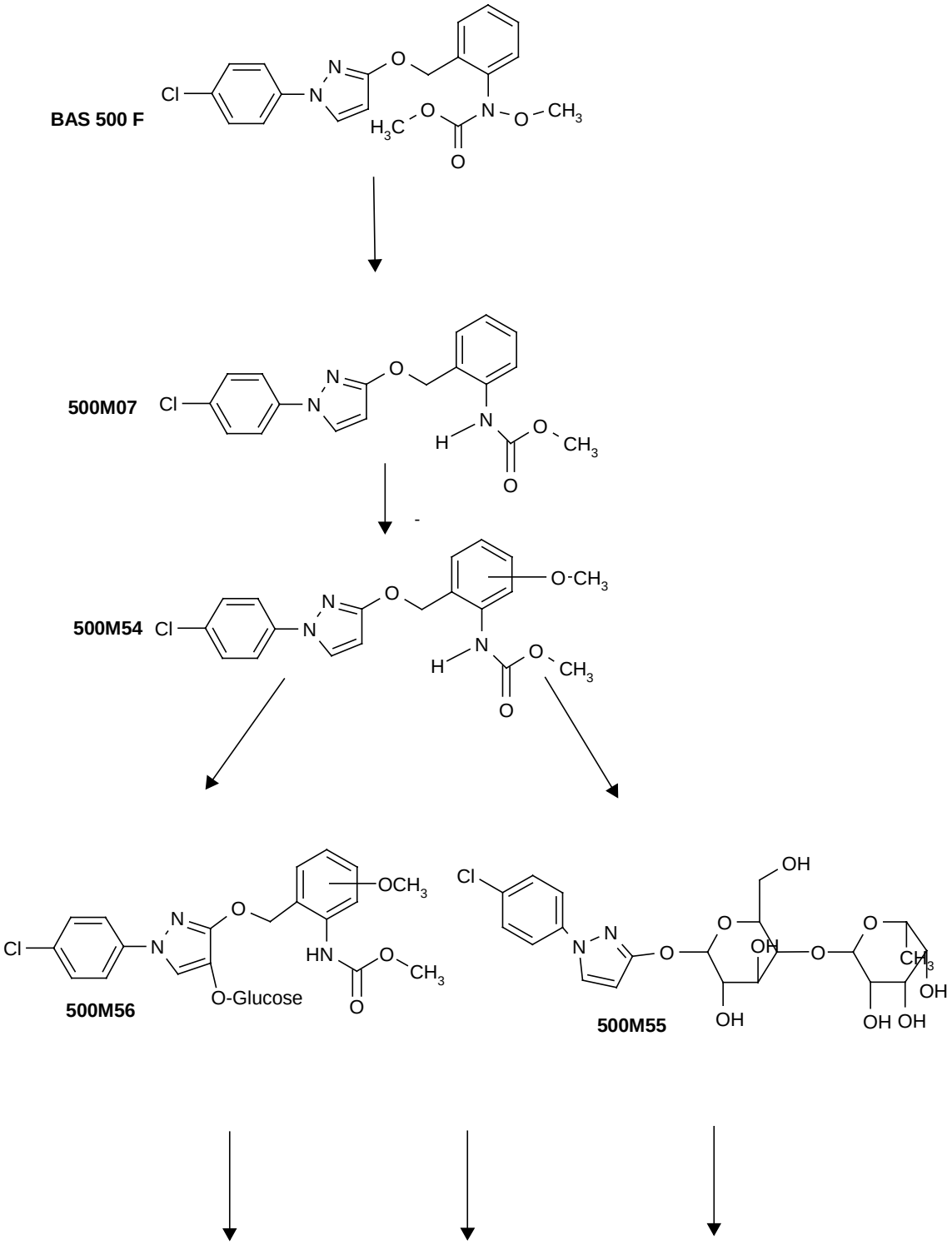


Figure 2.7-3: Metabolic pathway of pyraclostrobin in potatoes

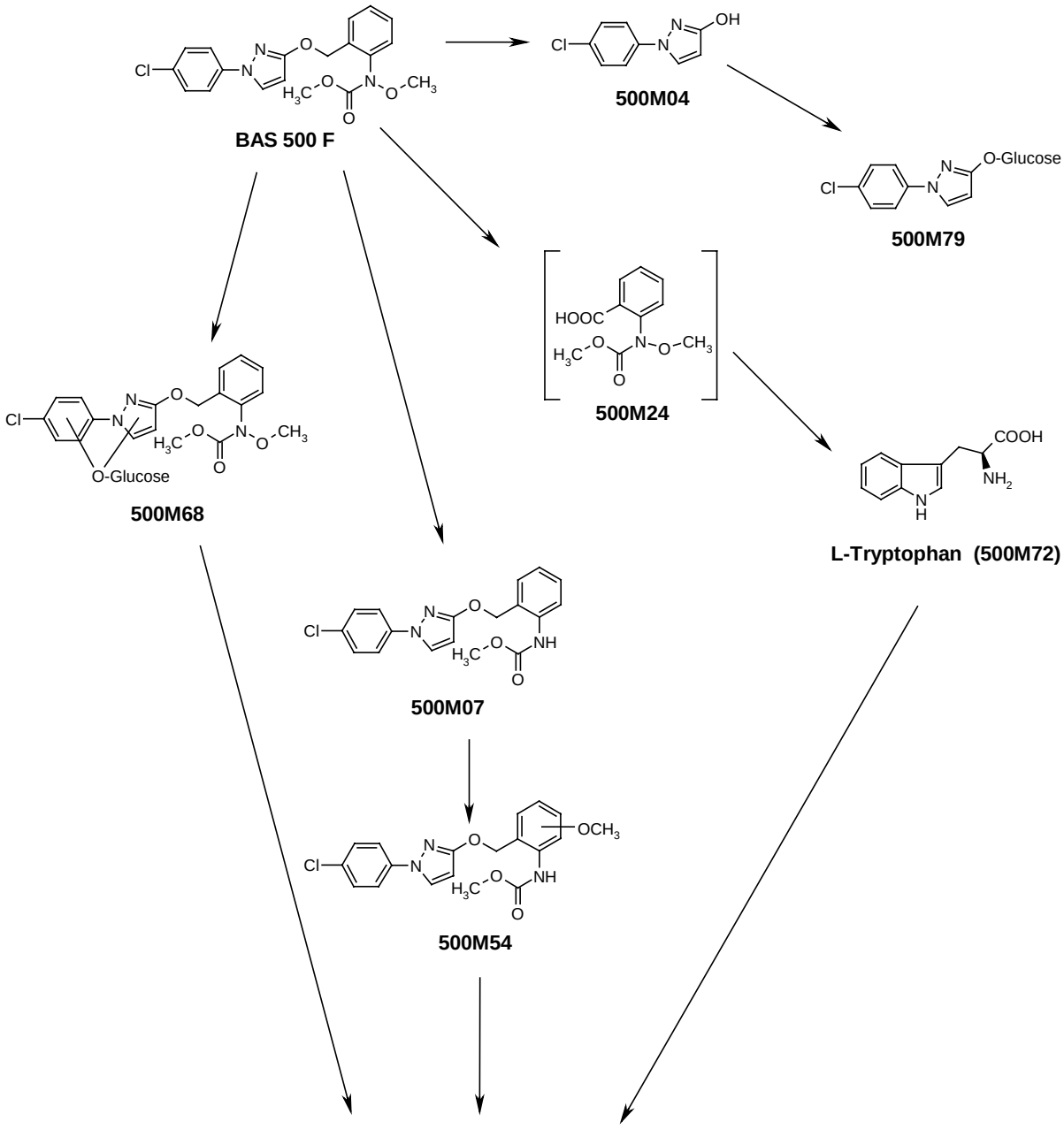


Figure 2.7-4: Metabolic pathway of pyraclostrobin in wheat

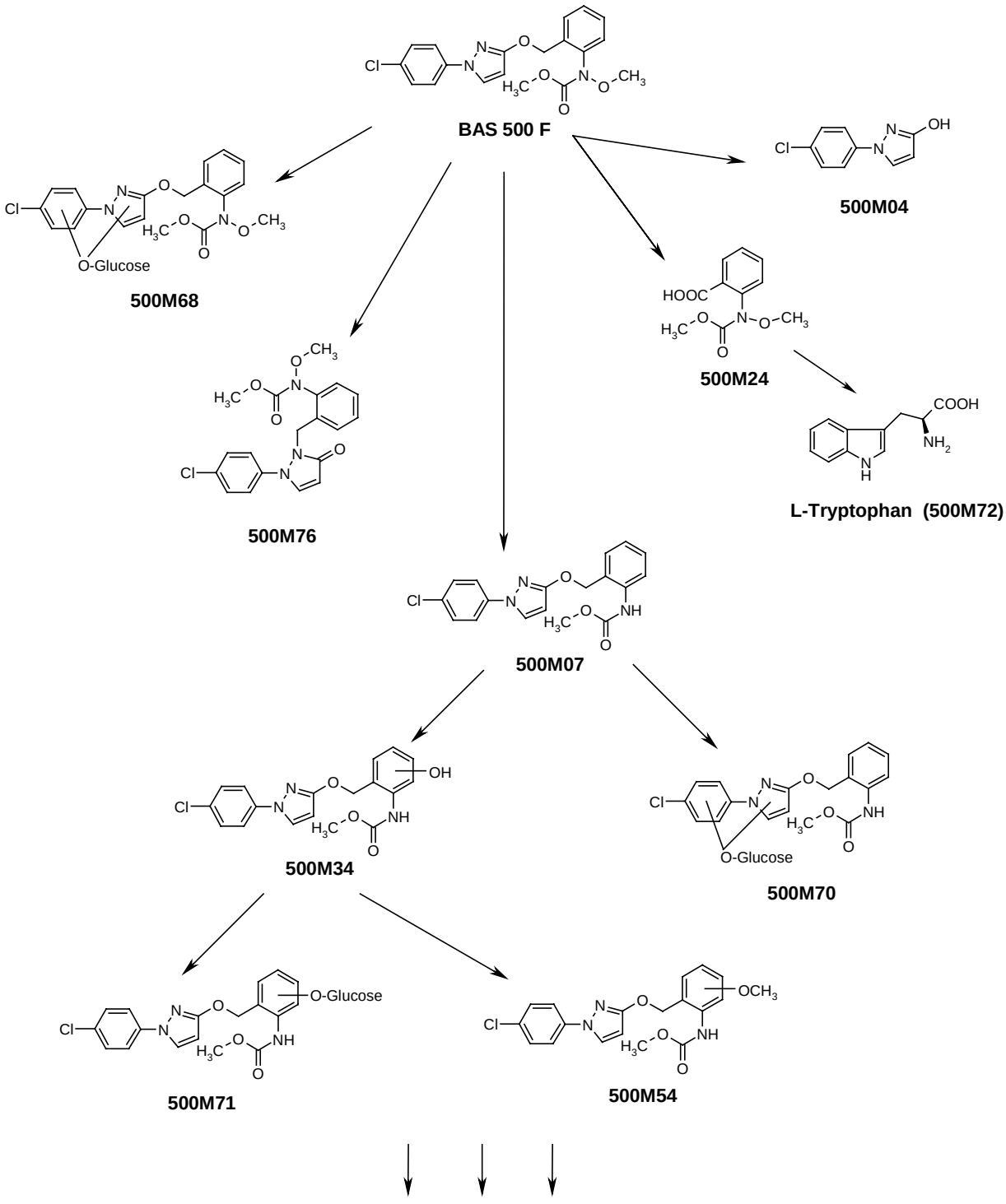


Figure 2.7-5: Metabolic pathway of pyraclostrobin in the goat

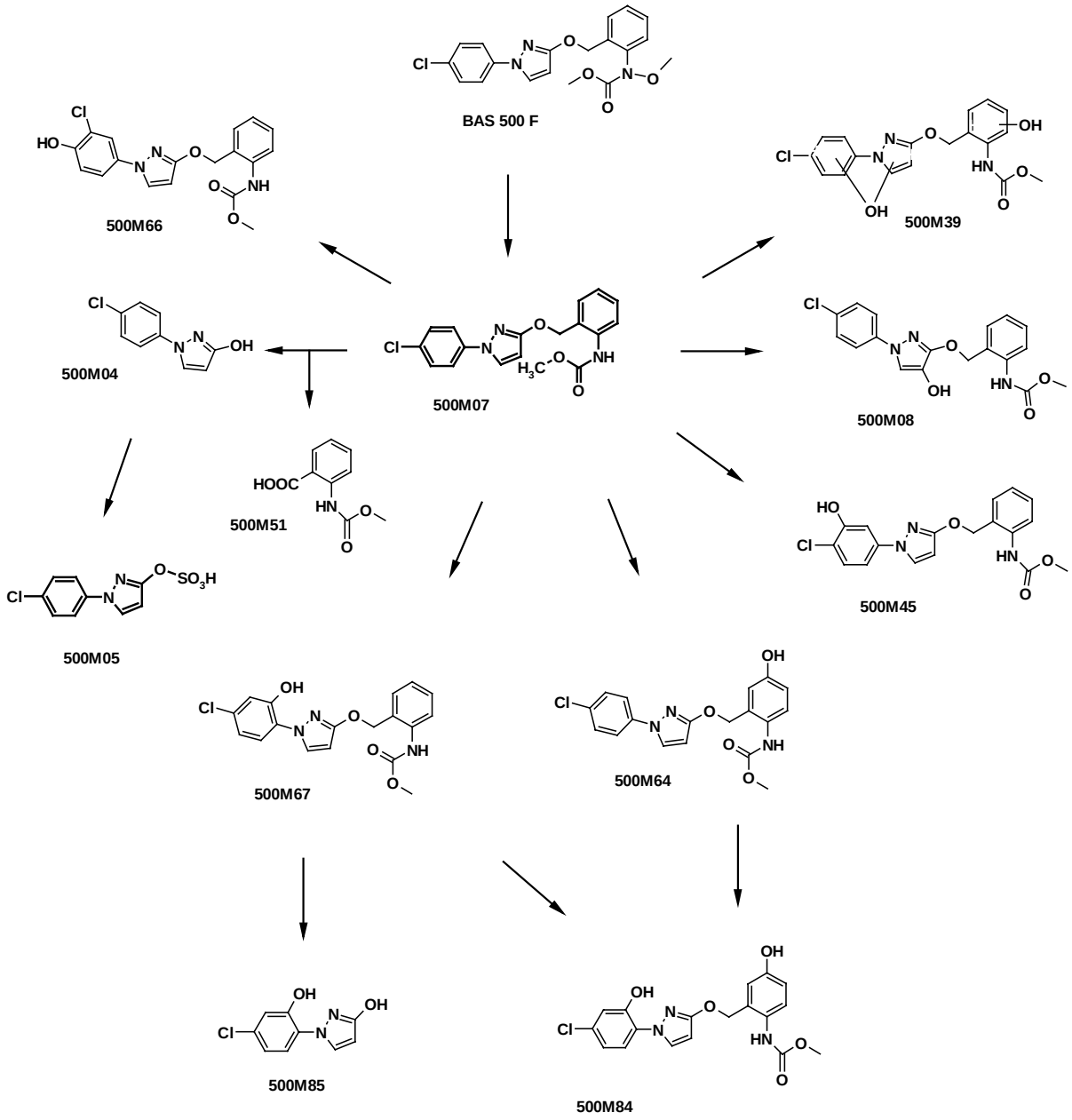
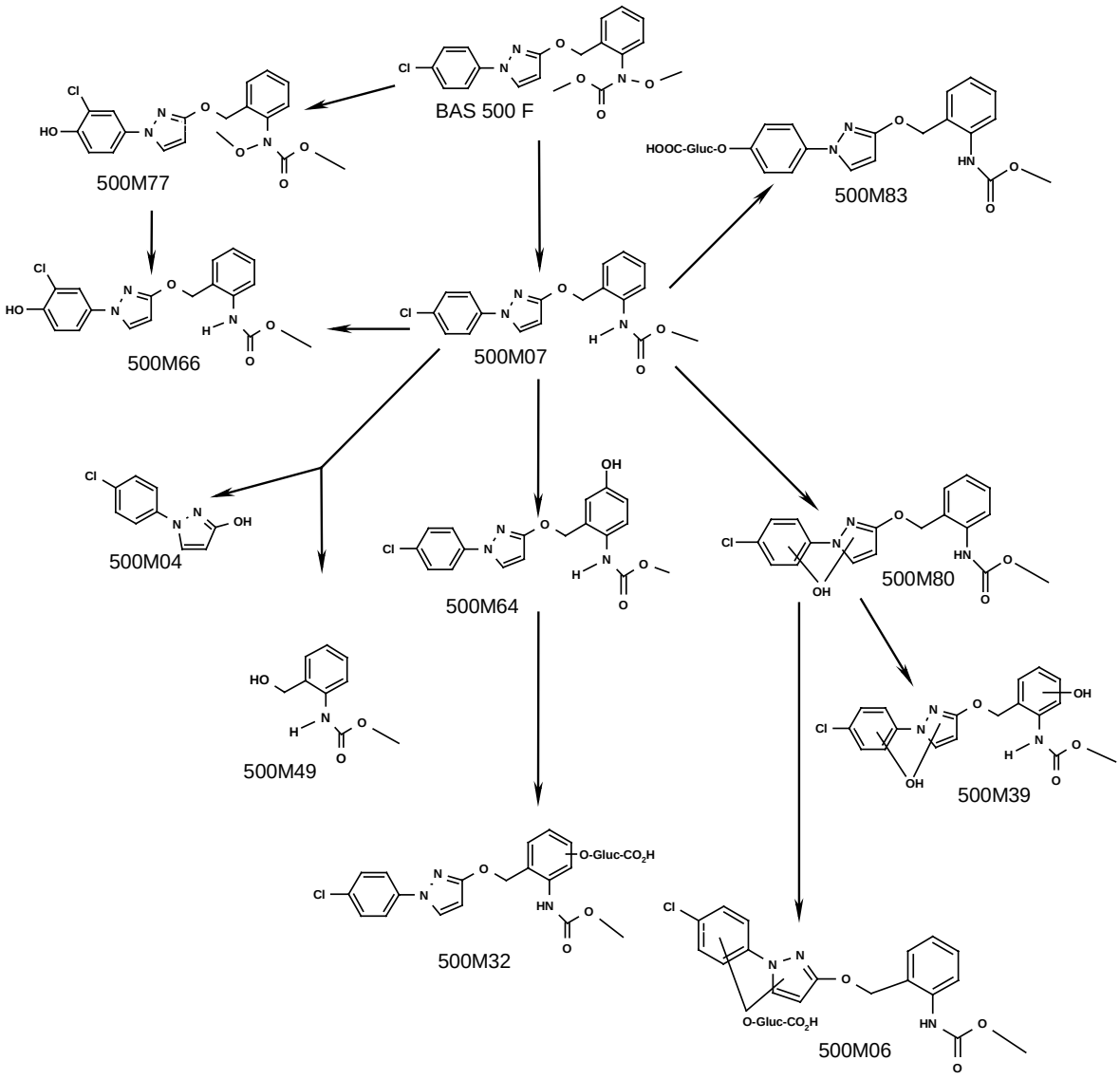


Figure 2.7-6: Metabolic pathway of ^{14}C-pyraclostrobin in laying hens



2.7.3 Fate and behaviour in the environment (soil, water, air)

Figure 2.7-7: Proposed route of degradation of pyraclostrobin in soil

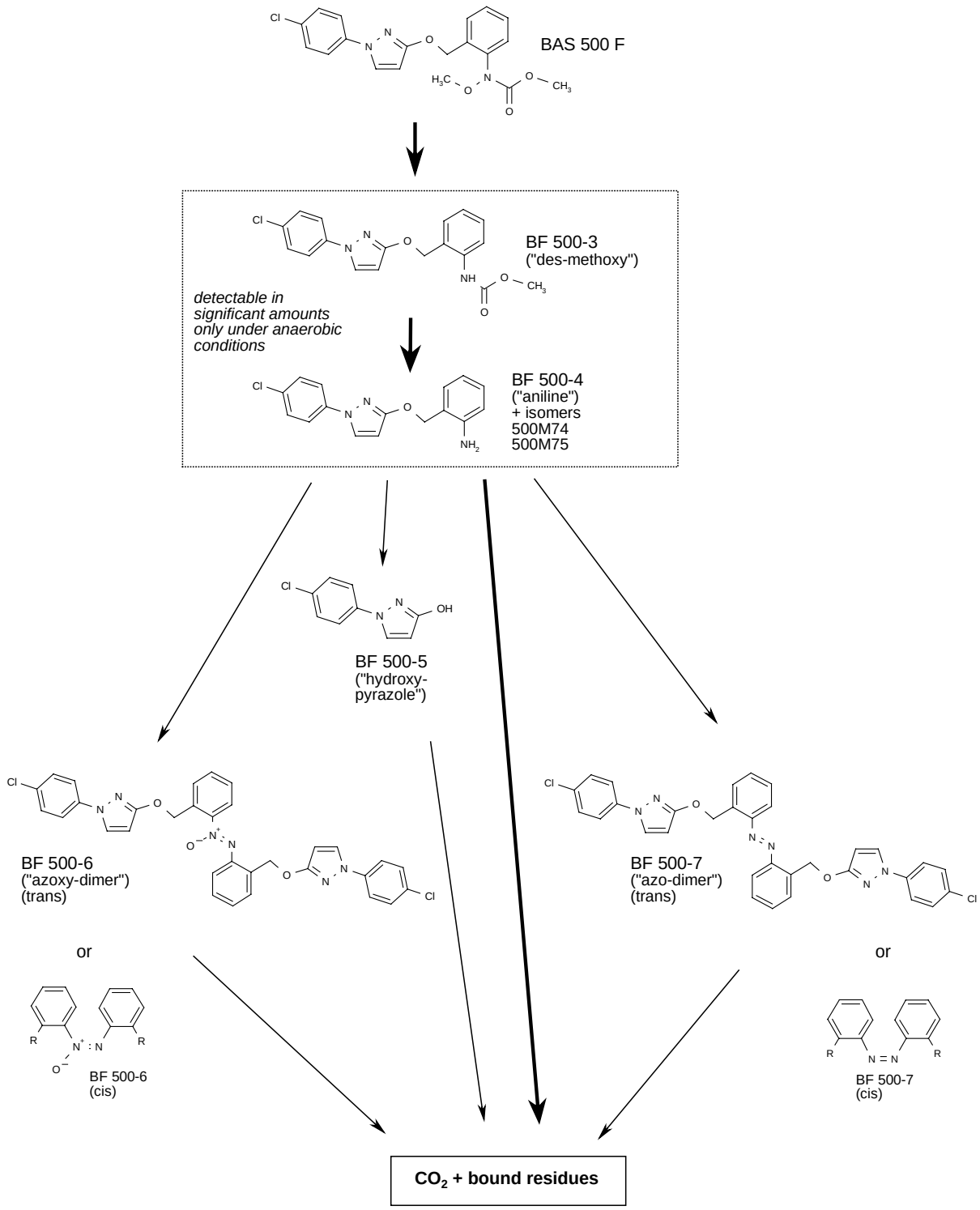


Figure 2.7-8: Proposed route of degradation of pyraclostrobin in water during aqueous photolysis

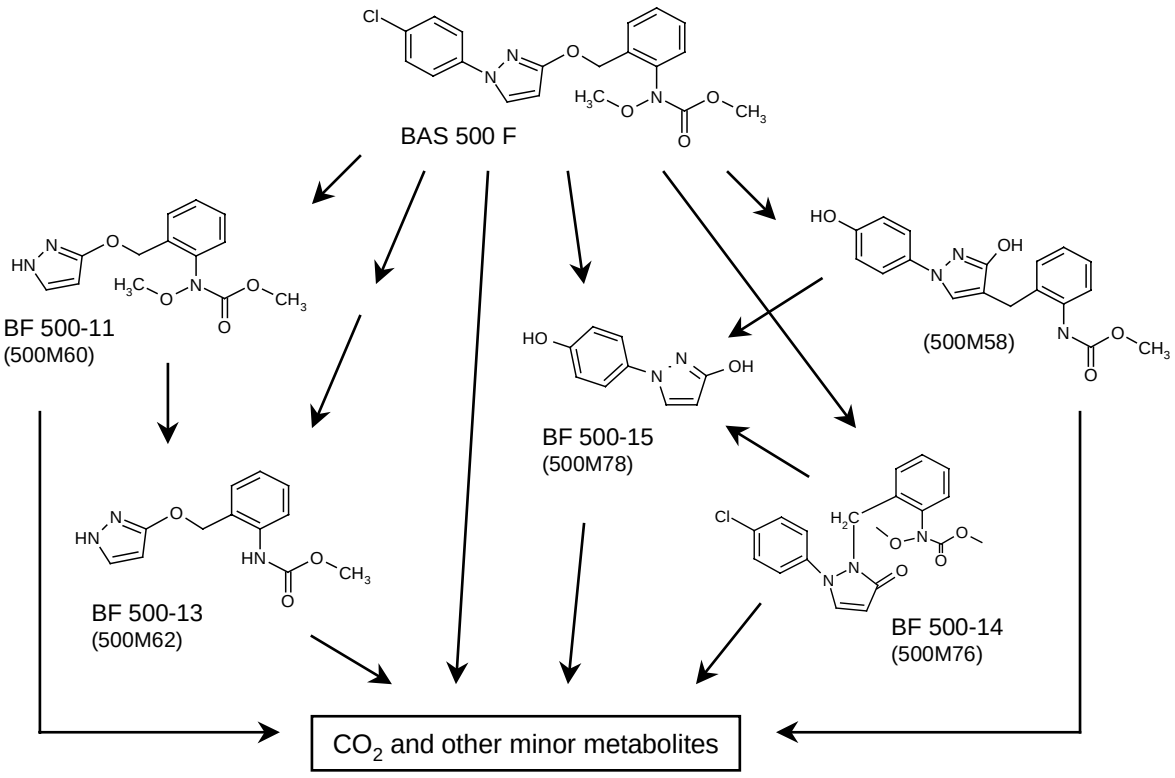


Figure 2.7-9: Proposed route of degradation of pyraclostrobin in water and sediment under realistic outdoor conditions

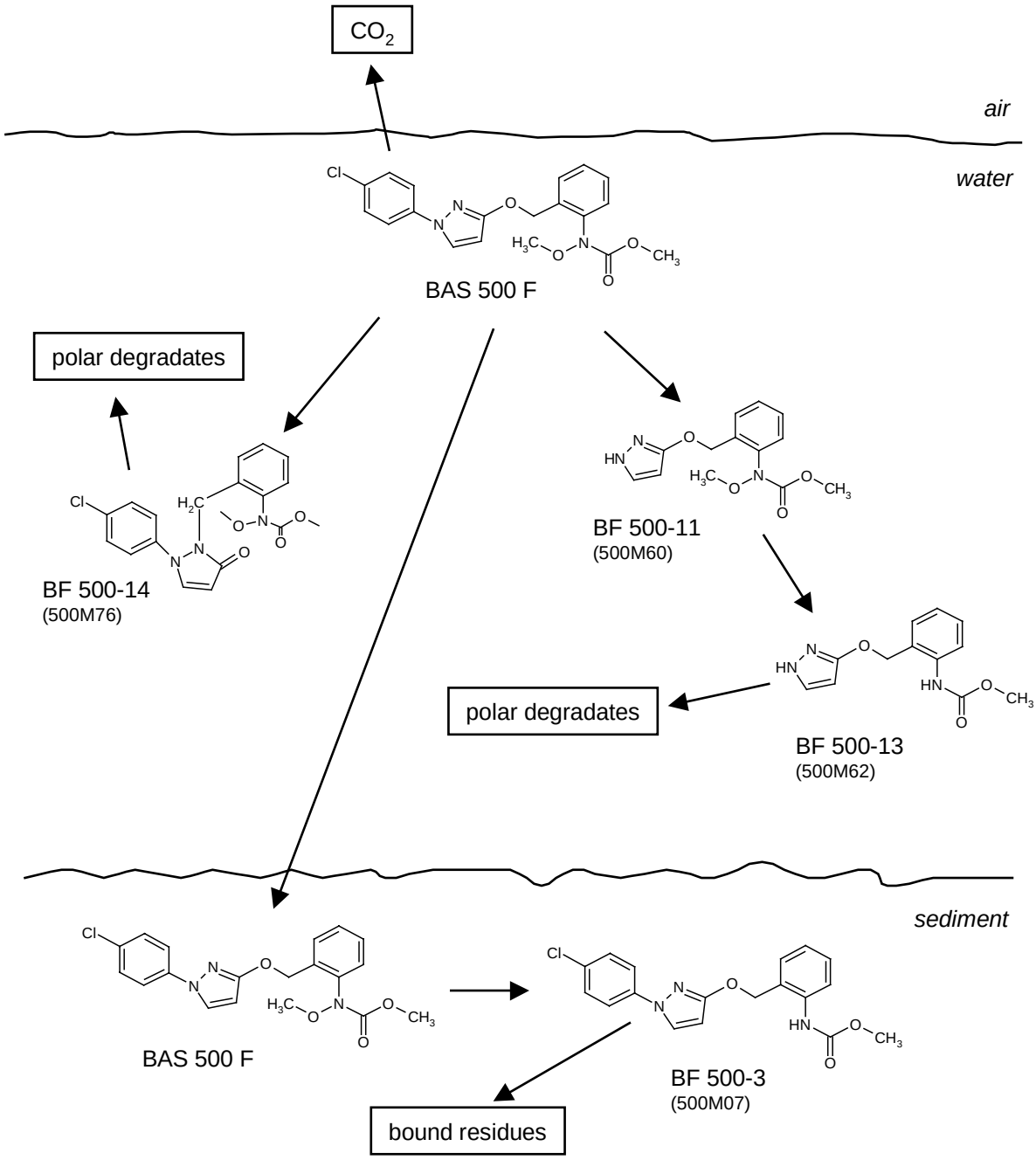
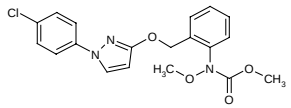
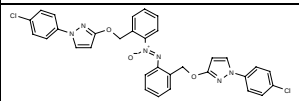
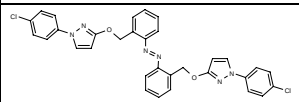
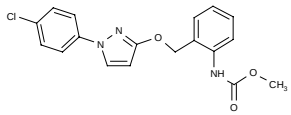
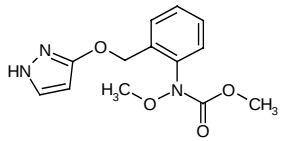
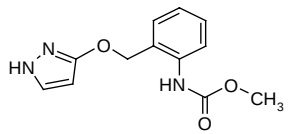
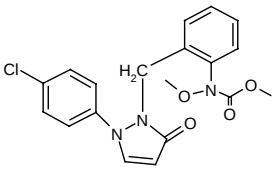
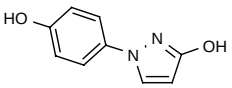
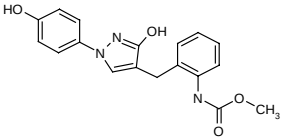


Table 2.7-1: List of major metabolites found in soil, water and sediment

Code	Active substance	Residue definition relevant to the environment			
BAS 500 F		The active substance pyraclostrobin is relevant for the compartments soil, water (including ground- and surface water), sediment and air			
Metabolites		**Occurrence in**	**Assessment of the relevance**		
Code	**Structural formula**	**Soil/Water/Sediment**	**Toxicology**	**Ecotoxicology**	**Biological activity**
BF 500-6		Soil:max. 31 % after 120 d	Not found in rats*	No unacceptable effects observed in soil micro-flora and earthworms	Not relevant
BF 500-7		Soil: max. 13 % after 62 d	Not found in rats	No unacceptable effects observed in soil micro-flora and earthworms	Not relevant
BF 500-3		Soil: max. 85 % after 14 d (tolyl-label), anaerobic conditions Sediment: 12 % after 100 d (pond system), 66 % after 14 d (river system)	Occurring in rat faeces (< 6 %) = 500M07	Detectable in significant amounts only under anaerobic conditions	Not relevant
BF 500-11		Water/photolysis study: 44.5 % after 21 d (tolyl label)	Not found in rats, no indication of point mutations *in vitro*	Considerably less toxic than parent compound to fish, daphnia and algae	Not relevant
BF 500-13		Water/photolysis study: 16.8 % after 6 d (tolyl label)	Not found in rats, no indication of point mutations *in vitro*	Considerably less toxic than parent compound to fish, daphnia and algae	Not relevant

BF 500-14		Water/photolysis study: 14.8 % after 6 h (tolyl label)	Not found in rats, no indication of point mutations *in vitro*	Considerably less toxic than parent compound to fish, daphnia and algae	Not relevant
BF 500-15		Water/photolysis study: 27 % after 1 day	Not found in rats	Not found in water/ sediment study (i.e. under more realistic conditions)	Not relevant
500 M 58		Water/photolysis study: 20.3 % after 1 d (tolyl label), 22.7 % after 6 h (chlorophenyl label)	Not found in rats	Not found in water/ sediment study (i.e. under more realistic conditions)	Not relevant

Appendix 1

Pyraclostrobin

Standard Terms and Abbreviations

2.8 Appendices

2.8.1 Appendix I: Standard terms and abbreviations

Part 1 Technical Terms

A	ampere
ACH	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosine diphosphate
AE	acid equivalent
AFID	alkali flame-ionisation detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50 %
ALT	alanine amitrotransferase (SGPT)
AMD	automatic multiple development
ANOVA	analysis of variance
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathy
BSP	bromosulfophthalein
Bt	*Bacillus thuringiensis*
Bti	*Bacillus thuringiensis israelensis*
Btk	*Bacillus thuringiensis kurstaki*
Btt	*Bacillus thuringiensis tenebrionis*
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degree Celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)

cd	candela
CDA	controlled drop(let) application
cDNA	complementary DNA
CEC	cation exchange capacity
cf	confer, compare to
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coefficient of variation
Cv	ceiling value
CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days past inoculation
DRES	dietary risk evaluation system
DT_{50}	period required for 50 percent dissipation (define method of estimation)
DT_{90}	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
EC_{50}	effective concentration
ECD	electron capture detector
ECU	European currency unit
ED_{50}	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immunosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
ERL	extraneous residue limit
F	field
F_0	parental generation
F_1	filial generation, first
F_2	filial generation, second
FIA	fluorescence immuno assay
FID	flame ionisation detector
FOB	functional observation battery

fp	freezing point
FPD	flame photometric detector
FPLC	fast protein liquid chromatography
g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography
GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulose virus
h	hour(s)
H	Henry's Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high energy electron diffraction
HID	helium ionisation detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HRGC	high resolution gas chromatography
Hs	Shannon-Weaver index
Ht	haematocrit
I	indoor
I_{50}	inhibitory dose, 50 %
IC_{50}	median immobilisation concentration

ICM	integrated crop management
ID	ionisation detector
IEDI	international estimated daily intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilisation
k	kilo
K	Kelvin or Henry's Law constant (in atmospheres per cubic meter per mole) (see also H)13
K_{ads}	adsorption constant
K_{des}	apparent desorption coefficient
K_{oc}	organic carbon adsorption coefficient
K_{om}	organic matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network
LASER	light amplification by stimulated emission
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC_{50}	lethal concentration, median
LCA	life cycle analysis
LCLo	lethal concentration low
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDLo	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometer (micron)
MC	moisture content

MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mol
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue limit or level
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration)
NAEL	no adverse effect level
nd	not detected
NEDI	no effect daily intake (mg/kg body wt/day)
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intent to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorus pesticide

Pa	Pascal
PAD	pulsed amperometric detection
2-PAM	2-pralidoxime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PEC_A	predicted environmental concentration in air
PEC_S	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
PEC_{GW}	predicted environmental concentration in ground water
PED	plasma-emissions-detector
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibition capacity
PIXE	proton induced X-ray emission
pK_a	negative logarithm (to the base 10) of the dissociation constant
PNEC	predicted no effect concentration
po	by mouth (per os)
P_{ow}	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10^{-9})
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
ppq	parts per quadrillion (10^{-24})
ppt	parts per trillion (10^{-12})
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r^2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval
R_f	ratio of fronts
RfD	reference dose
RH	relative humidity
RL_{50}	residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	reversed phase material
rRNA	ribosomal ribonucleic acid
RRT	relative retention time

RSD	relative standard deviation
s	second
SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedure
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogen free
spp	subspecies
sq	square
SSD	sulphur specific detector
SSMS	spark source mass spectrometry
STEL	short term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
$t_{1/2}$	half-life (define method of estimation)
T_3	tri-iodothyroxine
T_4	thyroxine
TADI	temporary acceptable daily intake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TCLo	toxic concentration low
TID	thermionic detector, alkali flame detector
TDLo	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ratio
TER_I	toxicity exposure ratio for initial exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake

TMRC	theoretical maximum residue contribution
TMRL	temporary maximum residue limit
TOC	total organic chlorine
Tremcard	Transport emergency card
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Part 2 Organisations and Publications

ACPA	American Crop Protection Association
ASTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
CA	Chemical Abstracts
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe
CIPAC	Collaborative International Pesticides Analytical Council Ltd
COREPER	Comité des Representants Permanents
EC	European Commission
ECB	European Chemical Bureau
ECCA	European Crop Care Association
ECDIN	Environmental Chemicals Data and Information of the European Communities

ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe
ECETOC	European Chemical Industry Ecology and Toxicology Centre
ECLO	Emergency Centre for Locust Operations
ECMWF	European Centre for Medium Range Weather Forecasting
ECPA	European Crop Protection Association
EDEXIM	European Database on Export an Import of Dangerous Chemicals
EHC (number)	Environment Health Criteria (number)
EHCD	Environmental Health Criteria Document
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organisation
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive Operator Exposure Model
FAO	Food and Agriculture Organisation of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monitoring System
GIEWS	Global Information and Early Warning System for Food and Agriculture
GIFAP	Groupement International des Association Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GRIN	Germplasm Resources Information Network
HRAC	Herbicide Resistance Action Committee
IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
IBT	Industrial Bio-Test Laboratories
ICBB	International Commission of Bee Botany
ICBP	International Council for Bird Preservation
ICES	International Council for the Exploration of the Seas
ICPBR	International Commission for Plant-Bee Relationships
ILO	International Labour Organisation
IMO	International Maritime Organisation
IOBC	International Organisation for Biological Control of noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Resistance Action Committee
IRC	International Rice Commission
ISCO	International Soil Conservation Organisation
ISO	International Organisation for Standardisation

IUPAC	International Union of Pure and Applied Chemistry
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JFCMP	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticides (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
NATO	North Atlantic Treaty Organisation
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Centre for Toxicological Research (USA)
NGO	non-governmental organisation
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Co-operation and Development
OLIS	On-line Information Service of OECD
PAN	Pesticides Action Network
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SCPH	Standing Committee on Plant Health
SETAC	Society of Environmental Toxicology and Chemistry
SI	Systeme International d'Unites
SITC	Standard International Trade Classification
TOXLINE	Toxicology Information On-line
UN	United Nations
UNEP	United Nations Environment Programme
WCDP	World Climate Data Programme
WCP	World Climate Programme
WCRP	World Climate Research Programme
WFP	World Food Programme
WHO	World Health Organisation
WTO	World Trade Organisation
WWF	World Wide Fund for Nature

Appendix 2

Pyraclostrobin

Specific Terms and Abbreviations

2.8.2 Appendix II: Specific terms and abbreviations

PAS	pure active substance
TAS	technical active substance
DFR	dislodgeable foliar residue
TAR	total applied radioactivity

Appendix 3

Pyraclostrobin

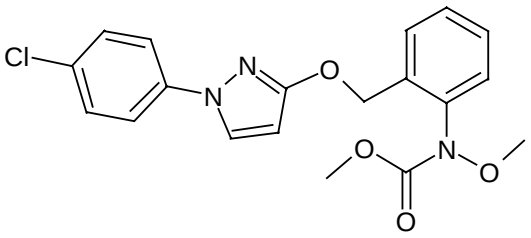
List of End Points

2.8.3 Appendix III: Listing of end points

2.8.3.1 Appendix III.1: Chapter 1 (identity, physical and chemical properties, details of uses, further information, classification and labelling)

Active substance (ISO Common Name)	Pyraclostrobin (ISO, proposed)
Function (*e.g.* fungicide)	Fungicide
Rapporteur Member State	Germany

Identity (Annex IIA, point 1)

Chemical name (IUPAC)	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate
Chemical name (CA)	carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester
CIPAC No	657
CAS No	175013-18-0
EEC No (EINECS or ELINCS)	not assigned
FAO Specification (including year of publication)	not applicable, new active substance
Minimum purity of the active substance as manufactured (g/kg)	950
Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	none
Molecular formula	$C_{19}H_{18}ClN_3O_4$
Molecular mass	387.82 g/mol
Structural formula	

Physical-chemical properties (Annex IIA, point 2)

Melting point (state purity)	63.7-65.2 °C (99.8 %)	
Boiling point (state purity)	no boiling point up to decomposition at 200°C, 99.8 %	
Temperature of decomposition	200°C, 99.8 %	
Appearance (state purity)	white to light beige cristaline solid (99.8 %)	
Relative density (state purity)	1.367 g/cm^3 (99.8 %, 20 °C)	
Surface tension	71.8 mN/m at 0.5 % (w/w) (20 °C) 71.5 mN/m at 2.0 % (w/w) (20 °C) (98.5 %)	
Vapour pressure (in Pa, state temperature)	2.6 x 10^{-8}, 20°C	
Henry's law constant (Pa m^3 mol^{-1})	5.307 x 10^{-6}	
Solubility in water (g/L , 20 °C)	19 ± 1.7 g/L at 20 °C in deionised water (pH of 5.8) There is no dissociation in water therefore pH dependence on solubility is not applicable.	
Solubility in organic solvents (in g/L 20 °C)	n-heptane	3.7
	2-propanol	30.0
	octanol	24.2
	olive oil	28.0
	methanol	100.8
	acetone	>500
	ethyl acetate	>500
	acetonitrile	>500
	dichloromethane	>500
	toluene	>500
Partition co-efficient (log P_{OW})	3.99 (20 °C, 99.8 %) Effect of pH was not investigated since there is no dissociation in water.	
Hydrolytic stability (DT_{50}) (state pH and temperature)	pH 5: stable	
	pH 7: stable	
	pH 9: stable (very slow degradation observed)	
Dissociation constant	not applicable. No indication of dissociation in water.	
UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength)	2.5 x 10^4 L mol^{-1} cm^{-1} at 205 nm 2.4 x 10^4 L mol^{-1} cm^{-1} at 275 nm (22°C, 99.8 %)	
Photostability (DT_{50}) (aqueous, sunlight, state pH)	DT50 < 2 h (mean value of tolyl- and chlorophenyl-label) at 22°C	
Quantum yield of direct phototransformation in water at λ > 290 nm	2.17 x 10^{-1}	
Flammability	not considered highly flammable	
Explosive properties	no potential for explosivity as evident from the structural formula	

List of uses supported by available data

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grapes	**France**	BAS 500 00 F	F	p+d mildew	EC	250	row, SP	09-85	3	12	0.01	1000	0.100	35	
Grapes	**Germany**	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	11-81	3	12	0.01	400-1600	0.04-0.16	35	
Grapes	**Italy**	BAS 500 00 F	F	p+d mildew	EC	250	row, SP	60-80	3	12	0.01	1000	0.100	35	
Grapes	**Portugal**	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	16-71	3	12	0.01	1000	0.100	35	
Grapes	**Spain**	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	65-81	3	12	0.01	1000	0.100	35	
Turf	**all EU MS**	BAS 500 00 F	F	mold	EC	250	overall,SP		2	14	0.025-0.05	500-1000	0.250		

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

Classification and proposed labelling (Annex IIA, point 10)

with regard to physical/chemical data	None
with regard to toxicological data	T, R 23; Xi, R 38
with regard to fate and behaviour data	None
with regard to ecotoxicological data	N R50/R53

2.8.3.2 Appendix III.2: Chapter 2 (methods of analysis)

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (principle of method)	HPLC-UV; reversed phase column
Impurities in technical as (principle of method)	HPLC-UV; reversed phase column. GC-FID
Plant protection product (principle of method)	HPLC-UV; reversed phase column

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	LC-MS-MS 0.02 mg/kg (wheat, grapes, peanut, orange) HPLC-UV
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	HPLC-UV 0.01 mg/kg (milk) 0.05 mg/kg (muscle, liver, kidney, fat, egg)
Soil (principle of method and LOQ)	LC-MS-MS 0.01 mg/kg HPLV-UV
Water (principle of method and LOQ)	LC-MS-MS 0.05 µg/L (drinking and surface water)
Air (principle of method and LOQ)	HPLC-UV 0.3 µg/m^3
Body fluids and tissues (principle of method and LOQ)	HPLC-UV 0.05 mg/kg (liver, kidney) Body fluids: no method submitted

2.8.3.3 Appendix III.3: Chapter 3 (impact on human and animal health)

Absorption, distribution, excretion and metabolism in mammals (Annex IIA, point 5.1)

Rate and extent of absorption	About 50% (based on urinary and biliary excretion within 5 d)
Distribution	Widely, highest concentrations in the liver
Potential for accumulation	None
Rate and extent of excretion	Complete within 5 d; mainly via faeces (80-90%, biliary excretion amounting to 35%), via urine 11-15%
Metabolism in animals	Extensive (>95%) with nearly 50 metabolites occurring Main metabolic pathways included N-demethoxylation, hydroxylation, cleavage of ester bond and further oxidation of the resulting molecule parts, conjugation with glucoronic acid or sulphate
Toxicologically significant compounds (animals, plants and environment)	Parent compound and metabolites

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral	> 5000 mg/kg bw (Mouse: mortality at doses ≥ 300 mg/kg bw)	
Rat LD_{50} dermal	> 2000 mg/kg bw	
Rat LC_{50} inhalation	0.69 mg/l	**T, R 23**
Skin irritation	Irritating	**Xi, R 38**
Eye irritation	Not irritating	
Skin sensitization (test method used and result)	Not sensitizing (M&K maximization test)	

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect	Reduced body weight, gastrointestinal tract, red blood cells; diarrhoea (dog); hepatocellular hypertrophy (rats); white blood cells and lymphatic organs (mice)
Lowest relevant oral NOAEL / NOEL	90 day mouse[1]: 30 ppm (4 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL	4wk rat: ≥ 250 mg/kg bw/d (systemic)
Lowest relevant inhalation NOAEL / NOEL	No data - not required (because of physical and chemical properties)

Genotoxicity (Annex IIA, point 5.4)

No genotoxic potential

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target / critical effect	Reduced body weight; liver cell necrosis (rats)
Lowest relevant NOAEL / NOEL	2yr rat / mouse: 75 / 30 ppm (4 mg/kg bw/d)
Carcinogenicity	No carcinogenic potential

[1] based on effects on body weight after 90 days in the carcinogenicity study in male mice

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction target / critical effect	Reduced pup body weight gain in the presence of parental toxicity
Lowest relevant reproductive NOAEL / NOEL	75 ppm (8.2 mg/kg bw/d)
Developmental target / critical effect	Developmental effects in rats and embryotoxicity in rabbits at maternally toxic doses
Lowest relevant developmental NOAEL / NOEL	5 mg/kg bw/d (rabbit)

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

No neurotoxic potential (rat, acute and 13wk studies)

Other toxicological studies (Annex IIA, point 5.8)

Three water metabolites (BF500-11, 500-13, 500-14) proved negative in the Ames test

Medical data (Annex IIA, point 5.9)

Limited data (new compound); no human health problems expected

Summary (Annex IIA, point 5.10)

	Value	Study	Safety factor
ADI	0.04 mg/kg bw	2yr rat / mouse	100
AOEL systemic	0.02 mg/kg bw	90 day mouse (bioavailability: 50%)	100
Drinking water limit	Not considered by ECCO	-	-
ARfD (acute reference dose)	0.04 mg/kg bw	90 day mouse	100

Dermal absorption (Annex IIIA, point 7.3)

2.6% (rat, *in vivo*); *in vitro* data suggest much lower permeability of human skin; 1% used for calculation

Acceptable exposure scenarios (including method of calculation)

Operator	Intended use acceptable (Exposure < syst. AOEL, without PPE; German model, UK-POEM)
Workers	Intended use acceptable
Bystanders	Intended use acceptable

2.8.3.4 Appendix III.4: Chapter 4 (residues)

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	wheat (cereals), grapes (fruit), potatoes (root and tuber vegetable)
Rotational crops	radish, lettuce, wheat
Plant residue definition for monitoring	Pyraclostrobin
Plant residue definition for risk assessment	Pyraclostrobin
Conversion factor (monitoring to risk assessment)	none

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	lactating goat, laying hen
Animal residue definition for monitoring	Pyraclostrobin
Animal residue definition for risk assessment	liver (except poultry liver) and milk fat only: Pyraclostrobin and its metabolites analysed as the hydroxy pyrazoles BF 500-5 and BF 500-8, sum expressed as Pyraclostrobin
Conversion factor (monitoring to risk assessment)	
Metabolism in rat and ruminant similar (yes/no)	yes
Fat soluble residue: (yes/no)	yes (Log Po/w 3.99)

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

30, 120, 365 days plant back interval after application of 0.9 kg as/ha: TRR in the edible parts for human consumption are very low (radish roots, lettuce: < 0.040 mg/kg; wheat grain: < 0.089 mg/kg). No accumulation of Pyraclostrobin or its degradation products [radish, lettuce < 0.0106 mg/kg; wheat straw < 0.0147 mg/kg; wheat grain: not detectable]

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

Food of animal origin: Pyraclostrobin stable for 8 month Metabolite BAS 500-10 (model compound) with slow degradation but stable enough to evaluate the submitted feeding study (analysed within 6 month). Plant (peanut nutmeat, peanut oil, wheat grain, wheat straw, sugarbeet tops, sugarbeet roots, tomatoes, grape juice): Pyraclostrobin, metabolite BAS 500-3 stable for 18 month

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Intakes by livestock ≥ 0.1 mg/kg diet/day:	Ruminant: yes/~~no~~	Poultry: yes/~~no~~	Pig: yes/~~no~~
Muscle	< 0.05	< 0.05	< 0.05
Liver	< 0.05	< 0.05	< 0.05
Kidney	< 0.05	< 0.05	< 0.05
Fat	< 0.05	< 0.05	< 0.05
Milk	< 0.01	not applicable	not applicable
Eggs	not applicable	< 0.05	not applicable

Summary of critical residues data (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region	Trials results relevant to the critical GAP (a)	Recommendation/comments	MRL	STMR (b)
wheat	N	17 results: 11 x < 0.02, 0.02, 0.03, 0.04, 0.04, 0.05, 0.05		0.1 mg/kg	0.02 mg/kg
	S	11 results: 11 x <0.02			
barley	N	21 results: <0.02, <0.02, <0.02, <0.02, 0.03, 0.03, 0.03, 0.03, 0.04, 0.04, 0.04, 0.05, 0.05, 0.07, 0.07, 0.07, 0.07, 0.09, 0.10, 0.12, 0.29		0.2 mg/kg	0.05 mg/kg
	S	3 results: 0.02, 0.03, 0.05			
grapes	N	8 results: 0.19, 0.25, 0.48, 0.57, 0.78, 0.82, 0.84, 0.89		2 mg/kg	0.68 mg/kg
	S	8 results: 0.18, 0.20, 0.21, 0.34, 0.38, 0.48, 0.59, 0.72			
banana	Import	12 x < 0.02	PHI in trials 0 days, fruits not covered with plastic, analysis with peel	0.02 * mg/kg	< 0.02 mg/kg

(a) Numbers of trials in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17
(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the critical GAP

Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)

ADI	0.04 mg/kg bw/d
TMDI (European Diet) (% ADI)	0.0055 mg/kg bw/d (13.8 %)
NEDI (% ADI)	not calculated
Factors included in NEDI	-
ARfD	0.04 mg/kg bw
Acute exposure NESTI (% ARfD)	grapes: UK-toddler 0.0392 mg/kg bw (98.1 %) UK-adult 0.0098 mg/kg bw (24.4 %)

Processing factors (Annex IIA, point 6.5, Annex IIIA, point 8.4)

Crop/processed crop	Number of studies	Transfer factor	% Transference *
grapes / must, juice, wine	4 trials	0.03	
grapes / wet pomace	4 trials	3.9	
grapes / rasins	1 (2 trials)	2.7	
barley/pot barley	1 trial	0.7	
barley/pearling dust	1 trial	11	
barley/malt	4 trials	1.2	
barley/malt germs	1 trial	2.3	
barley/spent grain	1 trial	10	
barley/trubs (flocs)	1 trial	0.7	
barley/beer yeast	1 trial	0.7	
barley/beer	4 trials	0.7	
wheat/flour, middlings, shorts	1	0.06	
wheat/ germ	1	0.8	

* Calculated on the basis of distribution in the different portions, parts or products as determined through balance studies

Proposed MRLs (Annex IIA, point 6.7, Annex IIIA, point 8.6)

wheat, rye, triticale	0.1 mg/kg
barley, oats	0.2 mg/kg
grapes	2 mg/kg
banana (import tolerance)	0.02* mg/kg

2.8.3.5 Appendix III.5: Chapter 5 (fate and behaviour in the environment)

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralisation after 100 days	about 5% (route study)
Non-extractable residues after 100 days	about 55% (route study)
Major metabolites - name and/or code, % of applied (range and maximum)	BF 500-6, max. 31% after 120 days (rate studies) BF 500-7, max. 13% after 62 days (rate studies)

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation	no parent after 120 days, bound residues 61% (tolyl-label) and 37% (chlorophenyl-label). Major metabolite BF 500-3: max 85 % after 14 d (tolyl-label), 80 % after 14 d (chlorophenyl-label)
Soil photolysis	after 15 days: 64-74% parent, 12% bound residues, 2% CO_2, no major metabolites (>10%)

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

Method of calculation	ModelMaker 3.0.3/3.0.4 (Cherwell Scientific Publishing Limited)
Laboratory studies (range or median, with n value, with r^2 value)	DT_{50lab} (20°C, aerobic): 12-101 days (5 soils)
	DT_{90lab} (20°C, aerobic): 143-163 days (5 soils)
	DT_{50lab} (5°C, aerobic): > 120 days
	DT_{50lab} (20°C, anaerobic): 3 days
	degradation in the saturated zone: not relevant
Field studies (state location, range or median with n value)	DT_{50f} : 2 – 37 days, 6 locations (3 Germany, 2 Spain, 1 Sweden)
	DT_{90f} : 83-230 days
Method of calculation	Timme and Frehse, 1st order kinetics
Field studies (state location, range or median with n value)	DT_{50f} : 14 - 85 days, 6 locations (3 Germany, 2 Spain, 1 Sweden). Mean of 26.1 d. DT50 of 34.4 d considered as realistic worst case and used for PEC_{soil} calculations
	DT_{90f} : 49 - 114 days

Soil adsorption/desorption (Annex IIA, point 7.1.2)

K_f /K_{oc} K_d pH dependence (yes / no) (if yes type of dependence)	soils: 3 German, 2 US, 1 Canadian Koc 6000 – 16000 Kd 30 – 368 No

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching	0% in leachate, all radioactivity in top soil layer
Aged residues leaching	0% in leachate, all radioactivity in top soil layer
Lysimeter/ field leaching studies	based on K_{oc} and DT_{50} values, no leaching expected

PEC (soil) (Annex IIIA, point 9.1.3)

Method of calculation	first order kinetics, multiple application
Application rate	3 x 100 g in **vine***, 12 d interval, DT50 of 34.4 d, plant interception 70%, 70 % and 85 %

$PEC_{(s)}$	Single application Actual [mg/kg]	Single application Time weighted average [mg/kg]	Multiple application Actual [mg/kg]	Multiple application Time weighted average [mg/kg]
Short term	n.a.	n.a.	0.075	0.075
24h			0.073	0.075
2d			0.070	0.073
4d				
Long term 7d	n.a.	n.a.	0.066	0.071
28d			0.043	0.058
50d			0.028	0.048
100d			0.010	0.033

*= worst case among supported uses concerning soil accumulation

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolysis of active substance and major metabolites (DT_{50}) (state pH and temperature)		pH 5: at 25°C, no hydrolysis through 30 days
		pH 7: at 25°C, no hydrolysis through 30 days
		pH 9: at 25°C, very slow hydrolysis through 30 days
Photolytic degradation of active substance and major metabolites		DT_{50} parent : <2 hours; CO_2: after 25 days 22% with chlorophenyl-label, about 4% with tolyl-label; 33 minor metabolites (<10%); 5 major metabolites: BF 500-11: max. 45% after 21 days, DT_{50} see higher tier study BF 500-13: max. 17% after 6 days, DT_{50} 31 days BF 500-14: max. 21% after 3 hours, DT_{50} about7 hours BF 500-15: max. 27% after 1 day, DT_{50} 5 days 500M58: max. 23% after 1 day, DT_{50} 9 days
Readily biodegradable (yes/no)		no
		Best fit
Degradation in water/sediment	- DT_{50} water	pond system: 3 days; river system: 1 day
	- DT_{90} water	pond system: 41 days; river system: 9 days
	- DT_{50} sediment	pond system: 33 days; river system: 9 days
	- DT_{90} sediment	pond system: 105 days; river system: no calc.possible
		1st-order (Timme and Frehse)
Degradation in water/sediment	- DT_{50} water	pond system: 8.7 days; river system: 1 day
	- DT_{90} water	pond system: 28.9 days; river system: not extrap.
- DT_{50} entire system		pond system: 26.8 days; river system: 29 days+
- DT_{90} entire system		pond system: 89 days; river system: 96 days+ + = low r^2 value (0.5593)
Mineralisation		about 5 % after 100 days
Non-extractable residues		pond system 62%; river system 54% after 100 days

Distribution in water / sediment systems (active substance)	pond system: sediment max. 53% after 14 days, decreasing to 7% after 100 days river system: sediment max. 62% after 2 days, decreasing to 10% after 100 days
Distribution in water / sediment systems (metabolites)	BF 500-3: in water: max. 2%, in sediment: max. 12% (pond system) after 100 days; max. 66% (river system) after 14 days, decreasing to 29% after 100 days BF 500-6: (only in pond system) in sediment max. 7% after 61 days BF 500-7: (only in pond system) in sediment max. 6% after 61 days

Degradation in water/sediment under **natural light** and temperature conditions DT_{50} water DT_{50} sediment	 8.5 days (first order) 4 days
Mineralisation	(calculated from balance difference) about 23% after 62 days (chlorophenyl-label) about 7% after 62 days (tolyl-label)
Non-extractable residues	28% after 62 days (chlorophenyl-label) 26% after 62 days (tolyl-label)
Distribution in water / sediment system (active substance)	water: <1% after 62 days sediment: max. 18% after 7 days, decreasing to 0.3% after 62 days
Distribution in water / sediment system (metabolites >10%)	in water: BF 500-11: max. 11% after 21 days, DT_{50} 20 days BF 500-13: max. 16% after 62 days, DT_{50} see aqueous photolysis study BF 500-14: max. 11% after 10 days, DT_{50} 14 days in sediment: BF 500-3: max. 16-17% after 30 days, DT_{50} 99 days

PEC (surface water) (Annex IIIA, point 9.2.3)

Method of calculation	95.percentil spray drift values, overspray and 5 m distance to sw, DT50 water: 8.7d, 1st Order calculation
Application rate	**Turf**, 2 x 250 g a.s./ha, interval of 14 d, 30 cm water layer, static water body
Main routes of entry and type of water body	Spray drift

Turf

PEC$_{(sw)}$	Multiple application Actual [µg/L]	Multiple application Time weighted average [µg/L]	Multiple application Actual [µg/L]	Multiple application Time weighted average [µg/L]
Distance to sw (m)	Overspray (0 m)		5 m buffer zone	
Initial	110.6			
Short term				
24h	102.1	106.3	0.6	0.6
2d	94.3	102.2	0.6	0.6
4d	80.4	94.7	0.5	0.56
Long term				
7d	63.3	84.8	0.4	0.51
14d	36.3	66.7	0.2	0.40
21d	20.7	53.7	0.1	0.32
28d	11.8	44.3	0.07	0.27
42d	3.9	31.9	0.02	0.19

Method of calculation	95.percentil spray drift values, overspray and 5 m distance to sw, DT50 water: 8.7 d, 1st Order calculation
Application rate	Vine, 3 x 100 g a.s./ha, interval of 12 d, 30 cm water layer, static water body
Main routes of entry and type of water body	Spray drift

Vine

PEC$_{(sw)}$	Multiple application Actual [µg/L]	Multiple application Time weighted average [µg/L]	Multiple application Actual [µg/L]	Multiple application Time weighted average [µg/L]
Distance to sw (m)	Overspray (0 m)		5 m	
Initial	51.0			
Short term 24h	47.1	49.0	2.4	2.4
2d	43.5	47.2	2.2	2.4
4d	37.1	43.7	1.9	2.2
Long term 7d	29.2	39.1	1.5	2.0
14d	16.7	30.7	0.8	1.5
21d	9.6	24.8	0.5	1.2
28d	5.5	20.4	0.3	1.0
42d	1.8	14.7	0.09	0.7

PEC (sediment)

Method of calculation	Maximum concentration of 17.9 % a.s. after 7 days in water/sediment study, $PEC_{ini\ in\ water}$ = after the last application
	Scenarios, see above PEC_{sw} 1 cm sed.-layer, bulk dens. of wet sediment:1.3 g/cm³

Distance (m)	0	5	10	20	50
turf					
$PEC_{ini.,act.}$ in water (µg/L)	110.6	0.66	0.44	0.11	-
PEC_{sed} (µg/g)	0.46	0.003	0.002	0.001	-
vine					
$PEC_{ini,\ act.}$ in water (µg/L)	51.0	2.55	0.765	0.204	0.102
PEC_{sed} (µg/g)	0.21	0.011	0.003	0.001	<0.001

PEC (ground water) (Annex IIIA, point 9.2.1)

Method of calculation and type of study (*e.g.* modelling, monitoring, lysimeter)	modelling PESTRAS 3.1; PELMO 3.00
Application rate	2 * 0.25 kg as/ha reaching soil

$PEC_{(gw)}$

Maximum concentration	< 0.001 µg/L
Average annual concentration	< 0.001 µg/L

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Direct photolysis in air	see photochemical oxidative degradation
Quantum yield of direct phototransformation	2.17×10^{-1}
Photochemical oxidative degradation in air (DT_{50})	< 2 hours
Volatilisation	from plant surfaces: about 3% in 24 hours
	from soil: <1% in 24 hours

PEC (air)

Method of calculation	not done due to low volatility and rapid photochemical oxidative degradation

$PEC_{(a)}$

Maximum concentration	not calculated

Definition of the Residue (Annex IIA, point 7.3)

Relevant to the environment	The active substance is the relevant residue in all environmental matrices. Metabolites have no biological activity, toxicity or ecotoxicity.

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	none
Surface water (indicate location and type of study)	none
Ground water (indicate location and type of study)	none
Air (indicate location and type of study)	none

2.8.3.6 Appendix III.6: Chapter 6 (effects on non-target species)

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Acute toxicity to mammals	LD50 >5000 mg/kg bw (rat)
Long-term toxicity to mammals	NOAEL 75 ppm (rat multi-generation study)
Acute toxicity to birds	LD50 >2000 mg/kg bw (bobwhite quail)
Dietary toxicity to birds	LC50 >5000 ppm (bobwhite quail and mallard duck)
Reproductive toxicity to birds	NOEL 1000 ppm (bobwhite quail and mallard duck)

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

residues: insects 7.3 mg/kg (estimated), grass 15 mg/kg (max. measured); no degradation assumed food intake rates: 40 % of body weight (insectivorous birds), 25 % (herbivorous mammals, birds)

Application rate (kg as/ha)	Crop	Category (*e.g.* insectivorous bird)	Time-scale	TER	Annex VI Trigger
0.25	Turf	Insectivorous bird	acute	>690	10
0.25	Turf	Insectivorous bird	short-term	>690	10
0.25	Turf	Insectivorous bird	long-term	136	5
0.25	Turf	Herbivorous bird	acute	>530	10
0.25	Turf	Herbivorous bird	short-term	>330	10
0.25	Turf	Herbivorous bird	long-term	67	5
0.25	Turf	Herbivorous mammal	short-term	>1300	10
0.25	Turf	Herbivorous mammal	long-term	5.4	5

Toxicity data for aquatic species (most sensitive species of each group)
(Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale	Endpoint	Toxicity (mg as/L)
Laboratory tests				
O. mykiss	BAS 500 F (pyraclostrobin)	static - 96 h	LC_{50}	0.00616[1)]
L. macrochirus		static - 96 h	LC_{50}	> 0.0196 < 0.0335[1)]
C. carpio		static - 96 h	LC_{50}	> 0.0121 < 0.0258[1)]
O. mykiss		flow-through - 28 d	NOEC	0.00464[3)]*
O. mykiss		ELS - 98 d	NOEC	0.0023[3)]
D. magna		static – 48 h	EC_{50}	0.0157*
D. magna		semi-static – 21 d	NOEC	0.0112*
C. riparius		static – 28 d	NOEC	0.040
P. subcapitata		static – 96 h	E_rC_{50}	> 0.843[4)]
O. mykiss	BAS 500 00 F (formulated product)	Static - 96 h	LC_{50}	0.0042
L. macrochirus		Static - 96 h	LC_{50}	>0.0146 <0.0299
C. carpio		Static - 96 h	LC_{50}	>0.0209 <0.0497
O. latipes		Static - 96 h	LC_{50}	>0.0325 <0.0885
P. promelas		Static - 96 h	LC_{50}	>0.012 <0.0235
B. rerio		Static - 96 h	LC_{50}	>0.0417 <0.0887
L. idus		Static - 96 h	LC_{50}	>0.0135 <0.027
D. magna		Static - 48 h	EC_{50}	0.0152[2)]
P. subcapitata		Static - 72 h	E_rC_{50}	0.788[2)]
O. mykiss	BF 500-11 (metabolite)	Static - 96 h	LC_{50}	100[1)]
D. magna		Static – 48 h	EC_{50}	> 100*
S. subspicatus		Static – 72 h	E_rC_{50}	> 100[4)] *

Group	Test substance	Time-scale	Endpoint	Toxicity (mg as/L)
Laboratory tests				
O. mykiss	BF 500-13 (metabolite)	Static - 96 h	LC_{50}	100[1)]
D. magna		Static – 48 h	EC_{50}	> 100*
S. subspicatus		Static – 72 h	E_rC_{50}	> 100[4)] *
O. mykiss	BF 500-14 (metabolite)	Static - 96 h	LC_{50}	100[1)]
D. magna		Static – 48 h	EC_{50}	> 100*
S. subspicatus		Static – 72 h	E_rC_{50}	> 100[4)] *
Microcosm or mesocosm tests				
A mesocosm study was conducted with the formulated product BAS 500 00 F. Four concentration levels ranging from 0.9 µg as/L to 24 µg as/L simulating a vineyard situation with 8 applications in 14 d intervals were investigated. Approximately 260 different taxa of aquatic invertebrates were determined in the study. In most cases only insignifcant transient effects were observed. Affected populations usually recovered until the end of the study. For the mollusc species *Bithynia tentaculata* and *Valvata* spec and the mussel species *Dreissena polymorpha* treatment related effects were observed in the highest treatment level. The EAC (ecologically acceptable concentration) was determined to be > 8 µg as/L.				

[1)] LC_{50} (1+96 h)
[2)] NOEC (1 + 98 h)
[3)] NOAEC,
[4)] = growth rate;
[5)] = E_rC_{10} ;
* measured values confirmed nominal values.

Toxicity/exposure ratios for the most sensitive aquatic organisms (Annex IIIA, point 10.2)

Application rate (kg as/ha)	Crop	Organism	Time-scale	Distance (m)	TER	Annex VI Trigger
3 x 0.1	grapevines	*O. mykiss*	acute	3	**1.6**	100
				5	**2.4**	100
				10	**8.1**	100
				15	**15.1**	100
				20	**30.2**	100
3 x 0.1	grapevines	*O. mykiss*	acute/chronic	3	**0.6**	10
				5	**0.9**	10
				10	**3.0**	10
				15	**5.6**	10
				20	11.2	10

Bioconcentration

Bioconcentration factor (BCF)	675 (whole fish, chlorophenyl label) 736 (whole fish tolyl label)
Annex VI Trigger for the bioconcentration factor	> 100 for non readily biodegradeable substances
Clearance time (CT_{50}) (CT_{90})	< 1 d 2.3 – 3.2 d
Level of residues (%) in organisms after the 14 day depuration phase	

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

Acute oral toxicity	(as)	LD_{50} = 73.1 µg/bee
Acute contact toxicity	(a.s)	LD_{50} > 100 µg/bee

Limit test

Acute oral toxicity	(formulation)	LD_{50} = 69.1 µg as/bee
Acute contact toxicity	(formulation)	$LD_{50} \geq$ 100 µg as/bee

Multiple Dose Test

Acute oral toxicity	(formulation)	LD_{50} = 79.9 µg as/ bee
Acute contact toxicity	(formulation)	LD_{50} > 100 µg as/bee

Hazard quotients for honey bees (Annex IIIA, point 10.4)

Application rate (kg as/ha)	Crop	Route	Hazard quotient	Annex VI Trigger
Laboratory tests (limit test)				
0.25	Turf	oral	3.6	50
0.25	Turf	contact	2.5	50
Laboratory tests (multiple dose test)				
0.25	Turf	oral	3.2	50
0.25	Turf	contact	2.5	50

Field or semi-field tests
Not required

Effects on other arthropod species (Annex IIA, point 8.3.2, Annex IIIA, point 10.5)

Species	Stage	Test Substance	Dose (kg as/ha)	Endpoint	Effect %	Annex VI Trigger %
Laboratory tests						
T. pyri	Protonymphs	BAS 500 00 F	0.320	Mortality Fertility	47.3 98.5	30 30
A. rhopalosiphi	Adults	BAS 500 00 F	0.320	Mortality Fertility	30 80	30 30
C. carnea	Larvae	BAS 500 00 F	0.320	Mortality Fertility	78.6 0	30 30
C. septem-punctata	Larvae	BAS 500 00 F	0.320	Mortality	100	30
P. cupreus	Adults	BAS 500 00 F	0.320	Mortality Food uptake	0 10.7	30 30
Pardosa spp	Adults	BAS 500 00 F	0.320	Mortality Food uptake	0 9.9	30 30
Extended laboratory tests						
A. rhopasiphi	Adults	BAS 500 00 F	0.320	Mortality Fertility	0 0	acceptable
C. carnea	Adult/LC	BAS 500 00 F	0.160	Mortality Fertility	27.3 79.9	acceptable
C. septem-punctata	Adults/LC	BAS 500 00 F	0.064	Mortality Fertility	0 3.1	acceptable

Field tests with BAS 500 00 F

Predatory mites

Species	Details of uses		Effects
T. pyri	8 applications	0.16-0.4 kg product/ha	2.64 kg product/ha/year 0.0 / 0.0
T. pyri	8 applications	0.16-0.6 kg product/ha	3.14 kg product/ha/year 0.0 / 12
T. pyri	8 applications	0.24-0.6 kg product/ha	3.12 kg product/ha/year 58.1/ 0.0

Summary:

Three field tests with *T. pyri* clearly demonstrated low acute effects and recovery of affected populations.

Effects on earthworms (Annex IIA, point 8.4, Annex IIIA, point 10.6)

Acute toxicity	LC50 281.8 mg form./kg (corrected 35.2 mg as/kg)
Reproductive toxicity	NOEC 1 L product/ha (corresponds to 0.443 mg as/kg)

Field tests with BAS 500 00 F and BAS 500 01 F
Two field tests were conducted with BAS 500 00 F 0.03 and 0.06 kg as/ha. In one field test there was no adverse effect on number and biomass of earthworms, on feeding activity (bait-lamina) and on overall abundance of collembola. In the second field test a slight effect with the full application rate was observed, but is regarded acceptable. One field test was conducted with BAS 500 01 F with an application rata of 2 x 0.25 kg as/ha. No long lasting effects on earthworm populations were observed.

Toxicity/exposure ratios for earthworms (Annex IIIA, point 10.6)

Application rate (kg as/ha)	Crop	Time-scale	TER	Annex VI Trigger
0.250 x 2	cereals	acute	352	10
0.250 x 2	cereals	longterm	4.4	5

Effects on soil micro-organisms (Annex IIA, point 8.5, Annex IIIA, point 10.7)

Nitrogen mineralisation	No effects up to 10 L product/ha (respective 2.5 kg as/ha)
Carbon mineralisation	No effects up to 10 L product/ha (respective 2.5 kg as/ha)

Level 3

Pyraclostrobin

Proposal for the Decision

3 Proposed decision with respect to the application for inclusion of the active substance in Annex I

3.1 Background to the proposed decision

Pyraclostrobin, chemical name (IUPAC) methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate is a new fungicidal active ingredient. It represents a modification of the structure pattern of natural fungicides called strobilurins.
The biochemical mode of action of the strobilurins is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) to the cytochrome bc_1 complex (Complex III). This leads to a reduction of energy-rich ATP that is available to support a range of essential processes in the fungal cell.

Pyraclostrobin is active against fungal development stages both on the plant surface and within the tissues. Pyraclostrobin has a protective as well as an eradicative/curative action. Pyraclostrobin is selective on a wide range of dicotyledonous and monocotyledonous crop species.

Fully validated analytical methodology is available for the determination of the active substance and impurities in the technical material as manufactured and in the formulation.

Residues of pyraclostrobin in food of plant and animal origin, soil, drinking water, surface water, air and tissues can be determined using HPLC-UV and/or LC-MS-MS. Analytical methods for metabolites are not necessary.
Analytical methods for body fluids are not submitted. Because of the classification of the active substance as T the lack of an appropriate method is considered as an essential data gap.

Pyraclostrobin (BAS 500 F) is degraded in soil under laboratory conditions with DT50 ranging from 12 to 101 days. Two main metabolites, BF 500-6 and BF 500-7, are formed in amounts exceeding 10 % of TAR. The soil metabolites have DT50 ranging from 60 to 166 days for the BF 500-6 and 38 to 159 days for the BF 500-7. The degradation in soil is characterised by a low mineralisation rate (5 % TAR in 100 d). Photolysis does not significantly influence the degradation rate, however, reduces the amounts of the main metabolites. Under anaerobic conditions the metabolite BF 500-3 is formed in high amounts reaching 96 % within 7 days.
In field studies, the DT50 values for BAS 500 F are much lower, ranging from 2 to 37 days.
Adsorption and leaching studies show that there is no risk of groundwater contamination neither for BAS 500 F nor for the metabolites.
BAS 500 F is hydrolytically stable but very UV-susceptible. In the aqueous photolysis study numerous of breakdown and rearrangement products are formed, whereas in the water/sediment study a fast binding of the active substance and metabolites to the sediment occured (DT 50 of 8.7 days in the water phase).
BAS 500 F has a very low potential to volatilisation.
According to the results presented, the parent compound is the only relevant residue for the quantification in soil, water and air.

The metabolism of pyraclostrobin in plants was investigated in grapes, wheat and potatoes. The metabolic pattern is similar in all three crop groups. Therefore the metabolism in plants is considered to be proofed.
The residue definition for plants is proposed as parent compound only.

The metabolism and distribution of radioactive labelled pyraclostrobin was investigated in lactating goats and laying hens.
The residue definition for food of animal origin is proposed as parent compound only.

The residue situation for the intended uses of pyraclostrobin in cereals and grapes is covered by a sufficient number of residue trials. On basis of these data the possible intake of residues by consumers was calculated. In the chronic risk assessment as well as in the acute risk assessment no unacceptable risk for consumers could be identified.

The available data on mammalian toxicology, mutagenicity and animal metabolism are considered sufficient to adequately support the risk evaluation of pyraclostrobin in humans and to establish the reference doses (ADI, ARfD and AOEL). All studies concerning toxicology and metabolism as required by Directive 91/414/EEC are available and were conducted according to Guideline requirements under Good Laboratory Practice regulations.

According to the recommended pattern of use risk to terrestrial vertebrates (birds and mammals), bees, other non-target arthropods, earthworms, soil micro-organisms, soil non-target macro-organisms and non-target terrestrial plants is considered to be low.
Effects on aquatic organisms, especially fish, indicate an unacceptable risk. Adequate risk mitigation measures are to be set on Member State level.

3.2 Proposed decision concerning inclusion in Annex I

It is proposed to postpone the decision on the inclusion of the active substance pyraclostrobin in Annex I of Council Directive 91/414/EEC.

3.3 Rational for the postponement of the decision to include the active substance in Annex I, or for the conditions and restrictions to be associated with a proposed inclusion in Annex I, as appropriate

Analytical methods for body fluids are not submitted. Because of the classification of the active substance as T the lack of an appropriate method is considered as an essential data gap.

Level 4

Pyraclostrobin

Demand for Further Information

4 Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex I

4.1 Data which are necessary for an unrestricted inclusion in Annex I of Council Directive 91/414/EEC

Identity of the active substance
None.

Physical and chemical properties of the active substance
None.

Data on application and further information
Data on application
None.

Further information
None.

Classification, packaging and labelling
None.

Methods of analysis
Analytical methods for formulation analysis
None.

Analytical methods for residue analysis
Analytical method for the determination of pyraclostrobin in body fluids.

Toxicology and metabolism
None.

Residue data
None.

Environmental fate and behaviour
None.

Ecotoxicology
None.

4.2 Data which should be submitted for an assessment on Member State level

Identity of the active substance
None.

Physical and chemical properties of the active substance
None.

Data on application and further information
Data on application
None.

Further information
None.

Classification, packaging and labelling
None.

Methods of analysis
Analytical methods for formulation analysis
None.

Analytical methods for residue analysis
None.

Toxicology and metabolism
None.

Residue data
Two further processing studies with wheat (follow up studies: wheat germ).

Justification:
The existing processing study indicate that an accumulation of residues may be possible in lipophilic products of wheat like wheat germ oil. These data are necessary to establish a transfer factor as defined in Directive 97/414/EC. They are therefore necessary not only in a single memberstate.

Note:
If authorisations will be sought for barley in southern Europe, then the following data would be required:
Further residue trials with barley from southern Europe

Justification:
For barley as a major crop a full data set (8 trials) is necessary for both European regions.

Environmental fate and behaviour
None.

Ecotoxicology
None.

Monograph

01 August 2001

Pyraclostrobin

Volume 2

Annex A

List of Tests and Studies

Rapporteur Member State: Germany

Contents

A.1 Identity (Annex IIA 1, 3.1 to 3.4; Annex IIIA 1, 3.1 to 3.7, 3.9 and 12.1)..... 1

A.2 Physical and chemical properties (Annex IIA 2; Annex IIIA 2) 3

A.3 Further information (Annex IIA 3; Annex IIIA 3, 4 and 6) 7

A.4 Classification, packaging and labelling (Annex IIA 10; Annex IIIA 12.3 and 12.4) 8

A.5 Methods of analysis (Annex IIA 4; Annex IIIA 5)..... 9

A.6 Toxicology and metabolism (Annex IIA 5; Annex IIIA 7)..... 14

A.7 Residue data (Annex IIA 6; Annex IIIA 8 and 12.2)..... 20

A.8 Environmental fate and behaviour (Annex IIA 7; Annex IIIA 9) 28

A.9 Ecotoxicology (Annex IIA 8; Annex IIIA 10)..... 35

A.1 Identity (Annex IIA 1, 3.1 to 3.4; Annex IIIA 1, 3.1 to 3.7, 3.9 and 12.1)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
Anonym	AIIIA-1.4	1999	Safety Data Sheet for SOLVESSO 200. BASF DocID 1999/11940 not GLP, unpublished BEI2000-156	Y	BAS
Anonym	AIIIA-1.4	1999	Safety Data Sheet for WETTOL EM 31. BASF DocID 99/10232 not GLP, unpublished BEI2000-155	Y	BAS
Anonym	AIIIA-1.4	1999	Safety Data Sheet for WETTOL EM 1. BASF DocID 1999/11544 not GLP, unpublished BEI2000-154	Y	BAS
Eisert, R.	AIIA-1.8	1999	Product Chemistry: BAS 500 F Description of the Manufacturing Process. 1999/11856 not GLP, unpublished CHE2000-459	Y	BAS
Eisert, R.	AIIA-1.9; AIIA-1.10	1999	Product Chemistry: BAS 500 F TC - Composition of the Technical Active Ingredient. 1999/11857 not GLP, unpublished CHE2000-461	Y	BAS
Eisert, R.	AIIA-1.11	1999	Characterization of six batches of technical active ingredient BAS 500 F (batches: ToxI, ToxII/Teil1, ToxII/Teil2, ToxIII/Teil1, ToxII-II/Teil2 and N2) using HPLC methods CP266 and CP337 and GC methods CP284 and M97/0028/02. 1999/11853 GLP, unpublished CHE2000-458	Y	BAS

[1] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
Eisert, R.	AIIA-1.8; AIIA-1.9; AIIA-1.10; AIIA-1.11	2000	Manufacturer of BAS 500 F - Method of manufacture of the active substance - Specification of purity of the active substance - Identity of isomers, impurities and additives - Analytical profile of batches. not GLP, unpublished CHE2000-460	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

A.2 Physical and chemical properties (Annex IIA 2; Annex IIIA 2)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
Anonymous	AIIA-2.3	2000	Henry´s Law Constant for 304428. 2000/1000171 GLP, unpublished LUF2000-248	Y	BAS
Anonymous	AIIA-2.9	2000	Physical and Chemical Properties of the Active Substance. not GLP, unpublished WAS2000-499	Y	BAS
Fries, Dr. J.	AIIIA-2.9	1999	Physical and Chemical Compatibility in A-queous Tank Mixtures of BAS 500 00 F with other products. #BASF 99/11854 not GLP, unpublished PHY2000-383	Y	BAS
Kästel, R.	AIIA-2.3	1997	Physical Properties Report. 97/10646, PCF 01721 GLP, unpublished LUF2000-247	Y	BAS
Kästel, R.	AIIA-2.1; AIIA-2.2; AIIA-2.3	1997	Physical Properties Report for 304 428. 97/10646 GLP, unpublished CHE2000-469	Y	BAS
Kästel, R.	AIIA-2.4; AIIA-2.12; AIIA-2.14	1998	Physical and Chemical Properties Report for PS 304 428. 98/10768 GLP, unpublished CHE2000-470	Y	BAS
Kästel, R.	AIIIA-2	1997	PHYSICAL AND CHEMICAL PROPERTIES REPORT for BAS 500 00 F. #BASF 97/11398 GLP, unpublished PHY2000-366	Y	BAS

[2] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
Kästel, R.	AIIIA-2.5; AIIIA-2.6	1999	Physical Properties of BAS 500 00 F. 99/10256 GLP, unpublished PHY2001-142	Y	BAS
Kästel, R.	AIIIA-2.7	1999	SHELF LIFE IN ORGINAL CONTAINER OF BAS 500 00 F Physical Properties Report (24 month-storage). #BASF 99/10561 GLP, unpublished PHY2000-384	Y	BAS
Kästel, R.	AIIIA-2.8.7	1998	PHYSICAL AND CHEMICAL PROPERTIES of BAS 500 00F. #BASF 98/11196 GLP, unpublished PHY2000-382	Y	BAS
König, W.	AIIIA-2.7	1999	Storage Stability on Orginal Container of BAS 500 00 F 24 month-storage Analytical Results. #BASF 99/10574 GLP, unpublished PHY2000-381	Y	BAS
Löffler, U.	AIIIA-2.2; AIIIA-2.3	1997	Safety characteristics of the crop protection product BAS 500 00 F. #BASF 97/11421 GLP, unpublished PHY2000-380	Y	BAS
Löffler, U.	AIIA-2.11; AIIA-2.15	1998	Safety characteristics of the crop protection product PS 304 428. 98/10734 GLP, unpublished CHE2000-464	Y	BAS
Scharf, J.	AIIA-2.9	1997	Determination of the Absorption Coefficients of BAS 500 F. 99/10257, 35889 GLP, unpublished LUF2000-251	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
Scharf., J.	AIIA-2.9	1999	Hydrolysis of BAS 500 F. 99/10060, 35884 GLP, unpublished WAS2000-352	Y	BAS
Scharf, J.	AIIA-2.9; AIIA-7.2.1.2	1999	Aqueous Photolysis of BAS 500 F. 1999/11286 GLP, unpublished LUF2000-249	Y	BAS
Scharf, J.	AIIA-2.10; AIIA-7.2.2	1999	Photochemical Oxidative Degradation Of BAS 500 F (QSAR Estimates). 99/10086, JS-99-04 GLP, unpublished LUF2000-246	Y	BAS
Türk, W.	AIIA-2.1; AIIA-2.4	1996	Determination of the appearance, the melting point and thermal conversions of Reg.-No. 304428 (PAI). 96/10327 GLP, unpublished CHE2000-471	Y	BAS
Türk, W.	AIIA-2.5	1996	Spectra of Reg.-No. 304428 (PAI). 96/10955 GLP, unpublished CHE2000-468	Y	BAS
Türk, W.	AIIA-2.6	1996	Determination of the solubility of Reg.-No. 304428 in water and buffer systems (pH4, pH7, pH9) at 20°C by column elution method and by HPLC. 96/10939 GLP, unpublished CHE2000-467	Y	BAS
Türk, W.	AIIA-2.7	1996	Determination of the solubility of Reg.-No. 304428 pure active ingredient (PAI) in organic solvents at 20°C. 96/10954 GLP, unpublished CHE2000-466	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
Türk, W.	AIIA-2.8	1996	Determination of the Octanol/Water-partition Coefficient of Reg.-No. 304428 by HPLC. 96/10383 GLP, unpublished CHE2000-465	Y	BAS
Türk, W.	AIIA-2.9	1996	Determination of the solubility of Reg.-No. 304428 in water and buffer systems (pH 4, pH 7, pH 9) at 20°C by column elution method and by HPLC. 96/10939, PCP03797 GLP, unpublished WAS2000-353	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft
BBA: Biologische Bundesanstalt für Land-und Forstwirtschaft

A.3 Further information (Annex IIA 3; Annex IIIA 3, 4 and 6)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[3]
Gerlach, H.	AIIA-3.7; AIIA-3.8	2000	Safety data sheet - BAS 500 F. 2000/1000228 not GLP, unpublished CHE2000-463	N	BAS
Gerlach, H. and Schenk, W.	AIIA-3.7; AIIA-3.8; AIIA-3.9	2000	BAS 500 F : Recommended methods and precautions concerning handling, storage, transport or fire - Procedures for destruction or decontamination - Decontamination of water in case of accident. M-II not GLP, unpublished CHE2000-472	Y	BAS
Kolb	AIIIA-4	1997	# 63 - 20 Corrosivness of BAS 500 00 F. BASF 97/11419 not GLP, unpublished PHY2000-570	Y	BAS
Schenk, W.	AIIA-3.9	1999	Possible Procedures for the Decontamination of Water from BAS 500 F. 99/10664 not GLP, unpublished CHE2000-462	N	BAS
Schreiner	AIIIA-4.1	1999	EU Performance Tests. ID1999/11733 not GLP, unpublished PHY2000-398	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

[3] Only notifier listed

A.4 Classification, packaging and labelling (Annex IIA 10; Annex IIIA 12.3 and 12.4)

No references submitted.

A.5 Methods of analysis (Annex IIA 4; Annex IIIA 5)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
Abdel-Baky, S. and Riley, M.	AIIA-4.2.1	2000	Validation of BASF analytical method D9904, Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC-UV, Study no. 63770. BASF 1999/5179 not GLP, unpublished MET2000-275	Y	BAS
Abdel-Baky, S., Riley, M	AIIA-4.2.1	2000	Validation of BASF analytical method D9904, method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC/UV. Study No. 63770; Reg. No. 1999/5179 GLP, unpublished RIP2000-1516	N	BAS
Eisert; R.	AIIA-4.1	1999	Validation of Analytical Method CP 337, Determination of impurities in technical BAS 500 F using HPLC. 1999/11852 GLP, unpublished CHE2000-786	Y	BAS
Eisert; R.	AIIA-4.1	1999	Determination of impurities in technical BAS 500 F using HPLC. 1999/11851 not GLP, unpublished CHE2000-785	Y	BAS
Grosenick, H.	AIIA-4.1	2000	Validation of the Analytical method M 97/0028/02 "Determination of dimethyl sulfate in BAS 500 F". 1999/11956 GLP, unpublished CHE2000-790	Y	BAS
Grosenick, H.	AIIA-4.1	2000	Determination of dimethyl sulfate in BAS 500 F. 1999/11896 not GLP, unpublished CHE2000-789	Y	BAS

[4] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
Jordan, J.	AIIA-4.2.1	2000	Independent method validation of BASF analytical method D9904 entitled "Method for the determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC-UV". Study No. 63832; Reg. No. 1999/5184 GLP, unpublished RIP2000-1520	N	BAS
Jordan, J.	AIIA-4.2.1	2000	Independent method validation of BASF analytical method D 9904 entitled "Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC-UV", Study no. 63832. BASF 1999/5184 not GLP, unpublished MET2000-276	Y	BAS
Kampke-Thiel, K.	AIIA-4.2.1	1999	Validation of BASF method 439/0 for the determination of BAS 500 F (as parent compound) in matrices of animal origin, Study no. 53018. BASF 99/11079 not GLP, unpublished MET2000-279	Y	BAS
Levsen, K.	AIIA-4.2.1	1999	Independent validation of BASF method 439/0 for the determination of BAS 500 F (as parent compound) in matrices of animal origin, Study No. 15 G 99015. BASF 99/11079 not GLP, unpublished MET2000-280	Y	BAS
Perez, R. and Perez, S.	AIIA-4.2.1	2000	Independent method validation of BASF method numbers D9808 (USA) and 421/0 (Germany) entitled "Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using LC/MS/MS", Study no. 63832. BASF 1999/5187 not GLP, unpublished MET2000-274	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
Reinhard, K. and Mackenroth, C.	AIIA-4.2.1	1999	Validation of BASF method no. 421/0 (Germany) / D9808 (USA): Determination of BAS 500 F and its metabolite BF 500-3 in wheat, grape, peanut and orange matrices, Study code 35509. BASF 1999/11134 not GLP, unpublished MET2000-273	Y	BAS
Reinhard, K. and Mackenroth, Ch.	AIIA-4.2.1	1999	Validation of BASF method no. 453/0: Determination of BAS 500 F and its metabolite BF 500-3 in matrices / fractions of the processing of barley, Study no. 35513, 10.12.99. BASF 1999/11135 not GLP, unpublished MET2000-277	Y	BAS
Staab, G.	AIIA-4.2.3	1998	Validation of analytical method no. 415, Determination of BAS 500 F (parent) in tap and leachate water, Study no. 35886. BASF 98/11182 not GLP, unpublished MET2000-284	Y	BAS
Tilting, N. and Lehmann, W.	AIIA-4.2.1	2000	Validation of analytical method 446 for the determination of BAS 500 F (reg. no. 304428) in sample material of animal origin, Study no. 35636. BASF 1999/11075 not GLP, unpublished MET2000-281	Y	BAS
Türk, W.	AIIA-4.1	1996	Detemination of toluene in Reg.No. 304 428. 1996/11581 not GLP, unpublished CHE2000-787	Y	BAS
Türk, W.	AIIA-4.1	1996	The determination of Reg.No. 304 428 in technical grade active ingredient by HPLC. 1996/11507 not GLP, unpublished CHE2000-783	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
Türk, W.	AIIA-4.1	1997	Validation of HPLC-method CP 266: Determination of Reg.No. 304 428 in Reg.No. 304 428 technical active ingredient (TAI). 1997/10691 GLP, unpublished CHE2000-784	Y	BAS
Türk, W.	AIIA-4.1	1998	Validation of Headspace-GC Method CP284: Determination of Toluene in RegNo. 304428. 1998/11316 GLP, unpublished CHE2000-788	Y	BAS
Weeren, R.D. and Pelz, S.	AIIA-4.2.1	1999	Examination of the applicability of DFG Method S 19 for the determination of BAS 500 F, BAS-9901V. BASF 99/10833 not GLP, unpublished MET2000-278	Y	BAS
Zangmeister, W.	AIIA-4.2.2	1999	Validation of analytical method no. 432, Determination of BAS 500 F, Reg. no. 340266, Reg. no. 369315 and Reg. no 364380 in soil, Study no. 37275. BASF 99/10076 not GLP, unpublished MET2000-283	Y	BAS
Zangmeister, W.	AIIA-4.2.3	1999	Validation of analytical method 455: Determination of BAS 500 F, BF 500-11, BF 500-12, BF 500-13, BF 500-14 and BF 500-15 residues in water (tap water and surface water), Study no. 35888. BASF 1999/10701 not GLP, unpublished MET2000-285	Y	BAS
Zangmeister, W.	AIIA-4.2.3	2000	Determination of BAS 500 F in water by HPLC/UV. BASF 2000/1000133 not GLP, unpublished MET2000-286	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
Zangmeister, W.	AIIA-4.2.4	1999	Validation of analytical method 447: Determination of BAS 500 F (Reg. no 304428) in air by HPLC/UV, Study no. 35892. BASF 1999/10694 not GLP, unpublished MET2000-287	Y	BAS
Ziegler, G.	AIIA-4.2.2	1998	Validation of analytical method no. 409, Determination of BAS 500 F (parent) in soil, Study no. 35646. BASF 98/10657 not GLP, unpublished MET2000-282	Y	BAS
Ziegler, H.	AIIIA-5.1	1997	Validation of the analytical method CF-A 535 Determination of Reg.No. 304 428 in emulsifiable concentrates (BAS 500 00 F). 1997/10709 GLP, unpublished CHE2000-782	Y	BAS
Ziegler, H.	AIIIA-5.1	1997	Determination of the content of active ingredient Reg.No. 304 428 in emulsifiable concentrates (EC) [BAS 500 00 F] using HPLC. 1997/11514 not GLP, unpublished CHE2000-781	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

A.6 Toxicology and metabolism (Annex IIA 5; Annex IIIA 7)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.1	1997	Report on the study of BAS 500 .. F (=reg. no. 304 428) (ZHT test substance no.: 96/308) in the Ames salmonella/mammalian-microsome mutagenicity test and escherichia co-li/mammalian-microsome reverse mutation assay (standard plate test and preincubation test). 40M0308/964244 ! #BASF 97/10973 GLP, unpublished TOX2000-720	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.1	1998	In vitro unscheduled DNA synthesis (UDS) assay with BAS 500 F in primary rat hepato-cytes. 81M0308/964306 ! # BASF 98/11421 GLP, unpublished TOX2000-723	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.1	1998	In vitro gene mutation test with BAS 500 F in CHO cells (HPRT locus assay). 50M0308/964303 ! 98/11422 GLP, unpublished TOX2000-721	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.1	1999	In vitro chromosome aberration assay with BAS 500 F in V79 cells. 32M0308/964304 ! 1999/11403 GLP, unpublished TOX2000-722	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.2	1998	Cytogenetic study in vivo with BAS 500 F in the mouse micronucleus test single oral admi-nistration. 26M0308/964204 ! # BASF 98/10460 GLP, unpublished TOX2000-724	Y	BAS

[5] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.8.1	1999	Salmonella typhimurium / escherichia coli reverse mutation assay (Standard Plate Test and Preincubation Test) with reg. no. 412 785. 40M0252/994127 ! 1999/12020 GLP, unpublished TOX2000-735	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.8.1	1999	Salmonella typhimurium / escherichia coli reverse mutation assay (Standard Plate Test and Preincubation Test) with reg. no. 411 847. 40M0251/994126 ! 1999/12017 GLP, unpublished TOX2000-734	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.8.1	2000	Salmonella typhimurium / escherichia coli reverse mutation assay (Standard Plate Test and Preincubation Test) with reg. no. 413 038. 40M0249/994128 ! 2000/1000005 GLP, unpublished TOX2000-736	Y	BAS
Gamer, A.O. and Hoffmann, H.D.	AIIA-5.2.3	1997	BAS 500..F - Acute inhalation toxicity study in Wistar rats 4-hour liquid aerosol exposure. 13I0308/967028 ! #BASF 97/11472 GLP, unpublished TOX2000-711	Y	BAS
Gamer, A.O. and Stadler, R.	AIIIA-7.1.7	2000	Particle size distribution of BAS 500 00 F concerning acute inhalation toxicity. APD/CA 012000 ! #BASF 99/12006 not GLP, unpublished TOX2000-744	Y	BAS
Gamer, A.O., Leibold, E. and Hoffmann, H.D.	AIIA-5.2.3	2001	BAS 500 F 40% in solvesso (technical active ingredient) - Acute inhalation toxicity study in Wistar rats 4-hour liquid aerosol exposure. 13I0283/017002 ! 2001/1010625 GLP, unpublished TOX2001-881	Y	BAS
Gamer, A.O., Leibold, E. and Hoffmann, H.D.	AIIIA-7.1.3	1998	BAS 500 00 F - Acute inhalation toxicity study in Wistar rats 4-hour liquid aerosol exposure. 13I0185/977014 ! #BASF 98/11185 GLP, unpublished TOX2000-740	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
Leibold, E. and Hoffmann, H.D.	AIIA-5.1	1999	14C-BAS 500 F - Study of the dermal absorption in rats. 01B0363/966044 ! 1999/10716 GLP, unpublished TOX2000-706	Y	BAS
Leibold, E., Hoffmann, H.D. and Hildebrand, B.	AIIA-5.1	1998	14C-BAS 500 F - Study of the biokinetics in rats. 02B0364/966007 ! #BASF 98/10997 GLP, unpublished TOX2000-705	Y	BAS
Mellert, W., Deckardt, K., Bahnemann, R. and Hildebrand, B.	AIIA-5.3.2	1999	BAS 500 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months. 50C0183/96015 ! #BASF 99/10195 GLP, unpublished TOX2000-717	Y	BAS
Mellert, W., Deckardt, K., Gembardt, C., Pappritz, G. and Hildebrand, B.	AIIA-5.5	1999	BAS 500 F - Carcinogenicity study in Wistar rats administration in the diet for 24 months. 82S0494/96086 ! 1999/11868 GLP, unpublished TOX2000-727	Y	BAS
Mellert, W., Deckardt, K., Gembardt, C., Pappritz, G. and Hildebrand, B.	AIIA-5.5	1999	BAS 500 F - Chronic toxicity study in Wistar rats administration in the diet for 24 months. 82S0494/96085 ! 1999/11672 GLP, unpublished TOX2000-726	Y	BAS
Mellert, W., Deckardt, K., Gembardt, Ch. and Hildebrand, B.	AIIA-5.3.1	1999	BAS 500 F - Repeated dose dermal toxicity study in Wistar rats administration for 4 weeks. 33S0494/96179 ! 1999/11458 GLP, unpublished TOX2000-716	Y	BAS
Mellert, W., Deckardt, K., Gembardt, Ch. and Hildebrand, B.	AIIA-5.3.1	1999	BAS 500..F - Repeated dose oral toxicity study in Wistar rats administration in the diet for 4 weeks. 30C0376/95083 ! 1999/11870 GLP, unpublished TOX2000-715	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
Mellert, W., Deckardt, K., Küttler, K. and Hildebrand, B.	AIIA-5.3.2	1998	BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice administration in the diet for 3 months. 60C0183/96016 ! #BASF 98/11345 GLP, unpublished TOX2000-718	Y	BAS
Mellert, W., Deckardt, K., Küttler, K. and Hildebrand, B.	AIIA-5.5	1999	BAS 500 F - Carcinogenicity study in B6C3F1 mice administration in the diet for 18 months. 76C0494/96101 ! 1999/11871 GLP, unpublished TOX2000-728	Y	BAS
Mellert, W., Kaufmann, W. and Hildebrand, B.	AIIA-5.7	1999	BAS 500 F - Subchronic oral neurotoxicity study in Wistar rats administration in the diet for 3 months. 50C0494/96174 ! 1999/11329 GLP, unpublished TOX2000-733	Y	BAS
Mellert, W., Kaufmann, W. and Hildebrand, B.	AIIA-5.7	1999	BAS 500 F - Acute oral neurotoxicity study in Wistar rats. 20C0494/96164 ! 1999/11111 GLP, unpublished TOX2000-732	Y	BAS
Menges, S., Schilling, K., Deckardt, K., Gembardt, Chr. and Hildebrand, B.	AIIA-5.3.2	1999	BAS 500 F - Subchronic oral toxicity study in Beagle dogs administration in the diet for 3 months. 31D0494/96089 ! 1999/11678 GLP, unpublished TOX2000-719	Y	BAS
Schilling, K., Deckardt, K., Gembardt, Chr. and Hildebrand, B.	AIIA-5.5	1999	BAS 500 F - Chronic oral toxicity study in Beagle dogs administration in the diet for 12 months. 33D0494/96144 ! 1999/11677 GLP, unpublished TOX2000-725	Y	BAS
Schilling, K., Gembardt, Chr. and Hildebrand, B.	AIIA-5.6.1	1999	BAS 500 F - Two-generation reproduction toxicity study in Wistar rats continuous dietary administration. 70R0494/96172 ! 1999/11869 GLP, unpublished TOX2000-729	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
Schilling, K., Hellwig, J. and Hildebrand, B.	AIIA-5.6.2	1999	BAS 500 F - Prenatal developmental toxicity study in Himalayan rabbits oral administration (gavage). 40R0494/96159 ! 1999/11512 GLP, unpublished TOX2000-731	Y	BAS
Schilling, K., Hellwig, J. and Hildebrand, B.	AIIA-5.6.2	1999	BAS 500 F - Prenatal developmental toxicity study in Wistar rats oral administration (gavage). 30R0494/96168 ! 1999/11511 GLP, unpublished TOX2000-730	Y	BAS
Schilling, K., Hellwig, J. and van Ravenzwaay, B.	AIIA-5.6.2	2001	BAS 500 F - Additional maternal toxicity study in Himalayan rabbits oral administration (gavage). 40R0494/96196 ! 2001/1003803 GLP, unpublished TOX2001-471	Y	BAS
Thornley, K.F. and Wood, R.A.	AIIA-5.1	1999	(14C)-BAS 500 F: Rates of penetration through human and rat skin using an in vitro system. 729/200 ! 50H0364/969131 ! 1999/11867 GLP, unpublished TOX2000-707	Y	BAS
Velic, I.	AIIA-5.1	1999	Metabolism of 14C-BAS 500F (14C-304428) in rats. 38773 ! 1999/11781 GLP, unpublished TOX2000-708	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.1	1998	Study on the acute oral toxicity of BAS 500..F in rats. 10A0183/961058 ! #BASF 98/10965 GLP, unpublished TOX2000-709	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.2	1998	Study on the acute dermal toxicity of BAS 500..F in rats. 11A0308/961120 ! #BASF 98/10966 GLP, unpublished TOX2000-710	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[5]
Wiemann, C. and Hellwig, J.	AIIA-5.2.4	1998	BAS 500..F - Acute dermal irritation / corrosion in rabbits. 14H0308/962190 ! #BASF 98/10959 GLP, unpublished TOX2000-712	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.5	1998	BAS 500..F - Acute eye irritation in rabbits. 13H0308/962191 ! #BASF 98/10963 GLP, unpublished TOX2000-713	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.6	1998	BAS 500..F - Maximization test in guinea pigs. 30H0494/962329 ! #BASF 98/10964 GLP, unpublished TOX2000-714	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.1	1998	BAS 500 00 F - Acute oral toxicity in rats. 10A0185/971108 ! #BASF 98/10804 GLP, unpublished TOX2000-738	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.2	1998	BAS 500 00 F - Acute dermal toxicity in rats. 11A0185/971109 ! #BASF 98/10646 GLP, unpublished TOX2000-739	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.4	1998	BAS 500 00 F - Acute dermal irritation / corrosion in rabbits. 14H0185/972188 ! #BASF 98/10644 GLP, unpublished TOX2000-741	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.5	1998	BAS 500 00 F - Acute eye irritation in rabbits. 13H0185/972189 ! #BASF 98/10645 GLP, unpublished TOX2000-742	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.6	1998	BAS 500 00 F - Buehler Test in guinea pigs. 32H0185/972201 ! #BASF 98/11034 GLP, unpublished TOX2000-743	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft
BBA: Biologische Bundesanstalt für Land-und Forstwirtschaft

A.7 Residue data (Annex IIA 6; Annex IIIA 8 and 12.2)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Abdel-Baky, S.	AIIA-6.0	2000	Storage Stability of BAS 500 F and BF 500-3 in Various Plant Matrices Including Processed Commodities For Up to 19 Months of Frozen Storage. Reg. Doc. No. 2000/5248 GLP, unpublished RIP2000-2043	Y	BAS
Abdel-Baky, S. and Riley, M.E.	AIIA-6.0	1999	Freezer Storage stability of BAS 500 F and BF 500-3 in Various plant matrices including processed commodities. BASF 99/5064 GLP, unpublished RIP2000-1074	Y	BAS
Beck, J.	AIIA-6.3	1999	Study on the residue behavior of BAS 500 00 F, epoxiconazole and kresoxim-methyl in cereals after treatment with BAS 500 01 F, BAS 512 00 F and BAS 513 00 F under field conditions in Belgium, France, Germany, Great Britain, Spain, Sweden and the Netherlands. BASF 99/11509 GLP, unpublished RIP2000-1031	Y	BAS
Beck, J., Benz, A. and Mackenroth, C.	AIIA-6.3	1999	Study on the residue behavior of Bas 500 F in cereals after treatment with Bas 500 01 F under field onditions in Denmark, France, Germany, Great Britain, Spain and Sweden. BASF 99/11824 GLP, unpublished RIP2000-1072	Y	BAS
Bross, M. and Mackenroth, C.	AIIA-6.1	1999	The Metabolism of 14C-BAS 500 F (14C-Reg.No.304428) in Potato. 1999/11419 GLP, unpublished RIP2000-1051	Y	BAS

[6] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Bross, M. and Mackenroth, C.	AIIA-6.1	2000	Report Amendment No. 1 to Final Report: The Metabolism of 14C-BAS 500 F (14C-Reg.No. 304428) in Potato. 2000/1000048 GLP, unpublished RIP2000-1041	Y	BAS
Bross, M. and Tilting, T.	AIIA-6.2	2000	Investigation of the Metabolism of 14C-BAS 500 F in the Goat. 2000/1000004 GLP, unpublished RIP2000-1020	Y	BAS
Hafemann, C. and Knoell, H.-E.	AIIA-6.2	1999	Metabolism of (14C) BAS 500 F in Laying Hens. BASF 99/11480 GLP, unpublished RIP2000-1022	Y	BAS
Hamm, R.T.	AIIA-6.1	1998	Metabolism of BAS 500 F in Grapes. BASF 98/10988 GLP, unpublished RIP2000-1050	Y	BAS
Hamm, R.T.	AIIA-6.1	2000	Report Amendment No. 1 to Final Report Metabolism of BAS 500 F in Grapes. BASF 2000/1000201 GLP, unpublished RIP2000-1275	Y	BAS
Haughey, D., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	The magnitude of BAS 500 F residues in pistachios. Study No. 61661; Reg. No. 1999/5150 GLP, unpublished RIP2000-1526	Y	BAS
Haughey, D., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	The magnitude of BAS 500 F residues in sugarbeets. Study No.55185; Reg. No. 1999/5157 GLP, unpublished RIP2000-1541	N	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Haughey, D., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	Magnitude of BAS 500 F residues in strawberries. Study No. 55223; Reg. No. 1999/5140 GLP, unpublished RIP2000-1528	Y	BAS
Haughey, D., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	The magnitude of BAS 500 F residues in pecans. Study No. 61660; Reg. No. 1999/5152 GLP, unpublished RIP2000-1525	Y	BAS
Haughey, D., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	The magnitude of BAS 500 F residues in almonds. Study No. 47727; Reg. No. 1999/5161 GLP, unpublished RIP2000-1523	Y	BAS
Leibold, E., Hoffmann, H.D. and Hildebrand, B.	AIIA-6.2	1998	14C-BAS 500 F - Study of the Absorption, Distribution and Excretion after repeated Oral Administration to Layin Hens. BASF 98/10637 GLP, unpublished RIP2000-1021	Y	BAS
Leibold, E., Hoffmann, H.D. and Hildebrand, B.	AIIA-6.2	1998	14C-BAS 500 F - Absorbtion, Distribution and Exkretion after Repeated Oral Adminstration in Lactating Goats. BASF 98/10636 GLP, unpublished RIP2000-1018	Y	BAS
Meumann, H.	AIIA-6.3	1999	Study on the residue behavior of BAS 500 F in grapes after treatments with BAS 500 00 F under field conditions in France, Germany and Spain. BASF 99/10981 GLP, unpublished RIP2000-1028	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Meumann, H.	AIIA-6.3	1999	Study of the residue behaviour of BAS 500 F in grapes after eight treatments with BAS 500 00 F under field conditions in France and Germany. BASF 99/10980 GLP, unpublished RIP2000-1027	Y	BAS
Meumann, H.	AIIA-6.5.2	1999	Study on the residue behavior of BAS 500 F in grapes and grape process fractions after treatments with BAS 500 00 F under field conditions in Germany. BASF 99/10982 GLP, unpublished RIP2000-1079	Y	BAS
Meumann, H., Benz, A. and Mackenroth, C.	AIIA-6.3	1999	Evaluation of the residue behavior of BAS 500 F after application of BAS 500 01 F in Cereals under field conditions in Germany, France and Sweden. BASF 99/11825 GLP, unpublished RIP2000-1073	Y	BAS
Reinhard, K.	AIIA-6.1	1999	Metabolism of 14C-BAS 500 F in Wheat. BASF 1999/11137 GLP, unpublished RIP2000-1009	Y	BAS
Reinhard, K. and Mackenroth, C.	AIIA-6.1	1999	Extractabilitiy of 14C-BAS 500 F residues from Wheat and Grape Matrices with Aqueous Methanol (according to Method No. 421/0). 35512; BASF 99/11138 GLP, unpublished RIP2001-72	N	BAS
Reinhard, K. and Mackenroth, C.	AIIA-6.1	2001	Extractabilitiy of 14C-BAS 500 F residues from Wheat and Grape Matrices with Aqueous Methanol (according to Method No. 421/0). 35512; BASF 1999/11700 GLP, unpublished RIP2001-73	N	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Scharf, J.	AIIA-6.5.1	1998	Hydrolysis of BAS 500 F at 90°C, 100°C, and 120°C. BASF 98/10840 GLP, unpublished RIP2000-1078	Y	BAS
Schat, B. and Beelen, G.M.	AIIA-6.4	1999	Feeding Study with BAS 500 F (Reg. no. 304428) in Lactating Dairy Cows. 1999/11895 GLP, unpublished RIP2000-1075	Y	BAS
Schulz, H.	AIIA-6.3	1999	Determination of the residues of BAS 500 F in Grapes following Treatment with BAS 500 00 F under field conditions in Italy 1998. BASF 99/11638 GLP, unpublished RIP2000-1029	Y	BAS
Schulz, H. and Scharm, M.	AIIA-6.5.2	2000	Determination of the residues of BAS 500 F in Barley and processed products following treatment with BAS 500 01under field conditions in Germany. BASF 99/11826 GLP, unpublished RIP2000-1082	Y	BAS
Schulz, H. and Scharm, M.	AIIA-6.5.2	2000	Determination of the residues of BAS 500 F in Barley and processed products following treatment with BAS 500 01 F under field conditions in Germany. BASF 99/11827 GLP, unpublished RIP2000-1083	Y	BAS
Tilting, N.	AIIA-6.0	2000	Investigation of the Stability of Residues of BAS 500 F (Reg. No. 304428) in Sample Materials of Animal Origin Under usual Storage Conditions. Reg. Doc.# 2000/1017116 GLP, unpublished RIP2000-2042	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Tilting, N.	AIIA-6.4	2000	Investigation of Residues of BAS 500 F (Reg. No. 304428) in tissues and Milk of Dairy Cows. 2000/1000003 GLP, unpublished RIP2000-1076	Y	BAS
Tilting, N. and Knoell, H.-E.	AIIA-6.0	2000	Investigation of the Stability of Residues of BAS 500 F (Reg. No. 304428) in Sample Materials of Animal Origin Under usual Storage Conditions. 2000/1000002 GLP, unpublished RIP2000-1077	Y	BAS
Tilting, N. and Knoell, H.-E.	AIIA-6.2	2000	14C-Validation of Method 446 for the Determination of BAS 500 F (Reg. No. 304428) and its Metabolites in Matrices of Animal Origin. 35907;2000/1000001 GLP, unpublished RIP2001-75	N	BAS
Veit, P.	AIIA-6.6	2000	Confined rotational crop study with 12C-BAS 500 F. BASF 1999/11829 GLP, unpublished RIP2000-1085	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	The magnitude of BAS 500 F residues in bell and chili peppers. Study No.61659; Reg. No. 1999/5151 GLP, unpublished RIP2000-1534	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	Magnitude of BAS 500 F residues in potatoes. Study No.46325; Reg. No. 1999/5148 GLP, unpublished RIP2000-1540	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	Magnitude of BAS 500 F residues in dry field peas. Study No.46591; Reg. No. 1999/5154 GLP, unpublished RIP2000-1537	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	Magnitude of BAS 500 F residues in lentils. Study No.46590; Reg. No. 1999/5159 GLP, unpublished RIP2000-1536	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.5.2	1999	Magnitude of BAS 500 F residues in wheat Processed fractions and aspirated grain fracti-on. 1999/5122 GLP, unpublished RIP2000-1084	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	The magnitude of BAS 500 F residues in red raspberries and highbush blueberries. Study No.46911; Reg. No. 1999/5143 GLP, unpublished RIP2000-1529	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	Magnitude of BAS 500 F residues in radishes. Study No.61658; Reg. No. 1999/5149 GLP, unpublished RIP2000-1531	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	The magnitude of BAS 500 F residues in dry bulb and green onions. Study No.46694; Reg. No. 1999/5158 GLP, unpublished RIP2000-1532	Y	BAS
Versoi, P.L., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	The magnitude of BAS 500 F residues in car-rots. Study No.46743; Reg. No. 1999/5155 GLP, unpublished RIP2000-1530	Y	BAS
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	Magnitude of BAS 500 F residues in peanuts. Study No.98026; Reg. No. 1999/5078 GLP, unpublished RIP2000-1539	Y	BAS
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	The magnitude of BAS 500 F residues in sto-nefruit. Study No. 46867; Reg. No. 1999/5146 GLP, unpublished RIP2000-1527	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[6]
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	2000	Magnitude of BAS 500 F residues in citrus. Study No. 54766; Reg. No. 1999/5144 GLP, unpublished RIP2000-1522	Y	BAS
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	Magnitude of BAS 500 F residues in peanuts. Study No.97042; Reg. No. 1999/5071 GLP, unpublished RIP2000-1538	Y	BAS
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	Magnitude of BAS 500 F residues in cucurbits. Study No.98022; Reg. No. 1999/5083 GLP, unpublished RIP2000-1535	Y	BAS
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	Magnitude of BAS 500 F residues in tomatoes. Study No.46694; Reg. No. 1999/5158 GLP, unpublished RIP2000-1533	Y	BAS
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.5.2	1999	The magnitude of BAS 500 F Residues in Grape Process Fractions. 1999/5011 GLP, unpublished RIP2000-1080	Y	BAS
Wofford, J.T., Abdel-Baky, S. and Riley, M.E.	AIIA-6.3	1999	Magnitude of BAS 500 F Residues in bananas for Import Tolerance. 1999/5095 GLP, unpublished RIP2000-1030	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

A.8 Environmental fate and behaviour (Annex IIA 7; Annex IIIA 9)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Bayer, H.	AIIA-7.1.1.2.1	1999	Storage Stability of BAS 500 F in Terrestrial Soil Samples. 1999/11288, 35881 GLP, unpublished BOD2000-650	Y	BAS
Becker, F.A., Klein, A.W., Winkler, R., Jung, B., Bleiholder, H. and Schmider, F.	AIIIA-9.2.3	1999	The degree of ground coverage by arable crops in estimating the amount of spray solution intercepted by the plants. GLP, published Nachrichtenbl. Deut. Pflanzenschutzd., 51, 9, 1999, 237-242 WAS2001-52	N	-
Ebert, D.	AIIA-7.1.1.1.1; AIIA-7.1.1.2.1	1998	The aerobicsoil metabolism of 14C-BAS 500 F. 98/11201, 35643 GLP, unpublished BOD2000-636	Y	BAS
Ebert, D.	AIIA-7.1.1.2.1	1999	The degradation Behaviour of 14C-BAS 500 F in Different Soils (DT50/DT90). 1999/11091, 35877 GLP, unpublished BOD2000-638	Y	BAS
Ebert, D.	AIIA-7.1.1.2.1	1999	Investigations on the release of soil-bound residues of 14C-BAS 500 F by earthworms. 1999/11289, 53058 GLP, unpublished BOD2000-644	Y	BAS
Ebert, D.	AIIA-7.1.1.1.1; AIIA-7.1.1.2.1	1999	The Aerobic Soil Metabolism of 14C-BAS 500 F. 1999/10090, 35644 GLP, unpublished BOD2000-637	Y	BAS

[7] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Ebert, D.	AIIA-7.2.1.3.2	1999	Degradation of BAS 500 F in Aerobic Aquatic Environment Under Irradiated Conditions. 1999/11791, 52978 GLP, unpublished WAS2000-351	Y	BAS
Gottesbueren, B.	AIIIA-9.2.3	2000	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Moving Surface Water (PECsw) for a Vineyard Scenario. 99/11798, CALC-149 not GLP, unpublished WAS2000-341	Y	BAS
Hauck, T. and Gottesbueren, B.	AIIIA-9.2.3	2000	Derivation of low flow rates for standard surface waters (slow moving water bodies). DocID 2000/1014985, CALC-189 not GLP, unpublished WAS2000-671	Y	BAS
Hollis, J.M.	AIIIA-9.2.3	2000	The Derivation of Flow Statistics for UK Rivers. DocID 2000/1004087 not GLP, unpublished WAS2000-672	Y	BAS
Kellner, O.	AIIA-7.1.1.1.2; AIIA-7.1.1.2.1	1999	The Anaerobic Soil Metabolism of BAS 500 F (14C-Chlorophenyl). 1999/11103, 35875 GLP, unpublished BOD2000-642	Y	BAS
Kellner, O.	AIIA-7.1.1.1.2; AIIA-7.1.1.2.1	1999	The Anaerobic Soil Metabolism of BAS 500 F (14C-Tolyl). 1999/10079, 35645 GLP, unpublished BOD2000-641	Y	BAS
Kellner, O. and Zangmeister, W.	AIIA-7.1.1.2.2	1999	Field soil dissipation of BAS 500 F (304428) in formulation BAS 500 01 F (1998 - 1999). 1999/11301, EU/FA/049/98 GLP, unpublished BOD2000-648	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Kellner, O. and Zangmeister, W.	AIIA-7.1.1.2.2	1999	Field soil dissipation of BAS 500 F (304428) in formulation BAS 500 01 F. 1999/11292, DE/FA/045/97 GLP, unpublished BOD2000-647	Y	BAS
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	1999	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Static Surface Water (PECsw) for a Cereal Scenario. 1999/11806, CALC-155 not GLP, unpublished WAS2000-342	Y	BAS
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	1999	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Static Surface Water (PECsw) for a Vineyard Scenario. 1999/11792, CALC-142 not GLP, unpublished WAS2000-340	Y	BAS
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	2000	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Moving Surface Water (PECsw) for a Cereal Scenario. 99/11805, CALC-154 not GLP, unpublished WAS2000-343	Y	BAS
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	1999	Calcuation of Predicted Environmental Concentrations for BF 500-13 in Surface Water (PECsw) for a Vineyard Scenario. 1999/11794, CALC-144 not GLP, unpublished WAS2000-345	Y	BAS
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	1999	Calcuation of Predicted Environmental Concentrations for BF 500-3 in Sediment (PECsed) for a Vineyard Scenario. 1999/11797, CALC-148 not GLP, unpublished WAS2000-348	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	1999	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Sediment (PEC-sed) for a Vineyard Scenario. 1999/11796, CALC-147 not GLP, unpublished WAS2000-347	Y	BAS
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	1999	Calcuation of Predicted Environmental Concentrations for BF 500-14 in Surface Water (PECsw) for a Vineyard Scenario. 1999/11795, CALC-145 not GLP, unpublished WAS2000-346	Y	BAS
Platz, K. and Gottesbueren, B.	AIIIA-9.2.3	1999	Calcuation of Predicted Environmental Concentrations for BF 500-11 in Surface Water (PECsw) for a Vineyard Scenario. 1999/11793, CALC-143 not GLP, unpublished WAS2000-344	Y	BAS
Regenstein, H.	AIIIA-9.1.3; AIIIA-11	1999	Document M III - Tier II summeries and assessments of individual tests and studies and groups of tests and studies. 1999/11982 not GLP, unpublished BOD2000-660	Y	BAS
Regenstein, H.	AIIIA-9.2.1; AIIIA-9.2.3; AIIIA-11	1999	Document M III - Tier II summeries and assessments of individual tests and studies and groups of tests and studies. 1999/11982 not GLP, unpublished WAS2000-355	Y	BAS
Regenstein, H.	AIIIA-9.3; AIIIA-11	1999	Document M III - Tier II summeries and assessments of individual tests and studies and groups of tests and studies. 1999/11982 not GLP, unpublished LUF2000-253	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Reuschenbach	AIIA-7.2.1.3.1	1999	Determination of the Biodegradability of BAS 500 F in the Manometric Respirometry Test according to GLP, EN 45001 and ISO 9002. 99/10655, 99/0164/26/1 GLP, unpublished WAS2000-349	Y	BAS
Scharf, J.	AIIA-7.1.1.1.2	1999	Soil Photolysis of BAS 500 F. 1999/11300, 35876 GLP, unpublished BOD2000-640	Y	BAS
Scharf, J.	AIIA-2.9; AIIA-7.2.1.2	1999	Aqueous Photolysis of BAS 500 F. 1999/11286 GLP, unpublished LUF2000-249	Y	BAS
Scharf, J.	AIIA-7.2.2	1999	Volatilization of BAS 500 F after Application of BAS 500 00 F on Soil and on Plant Surfaces. 1999/11093, 36008 GLP, unpublished LUF2000-250	Y	BAS
Scharf, J.	AIIA-2.10; AIIA-7.2.2	1999	Photochemical Oxidative Degradation Of BAS 500 F (QSAR Estimates). 99/10086, JS-99-04 GLP, unpublished LUF2000-246	Y	BAS
Seher, A.	AIIA-7.1.2	1999	Soil Adsorption/Desorption Study of 369315 (BF 500-7). 1999/10684, PI990009 GLP, unpublished BOD2000-654	Y	BAS
Seher, A.	AIIA-7.1.2	1999	Soil Adsorption/Desorption Study of 364380 (BF 500-6). 1999/10686, PI990002 GLP, unpublished BOD2000-653	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
Seher, A.	AIIA-7.1.2	1999	Soil Adsorption/Desorption Study of 340266 (BF 500-3) on Soils. 1999/10695, PI990019 GLP, unpublished BOD2000-652	Y	BAS
Staudenmaier, H.	AIIA-7.2.1.3.2	1999	Degradation of BAS 500 F in Aerobic Aquatic Environment. 1999/11241, 35642 GLP, unpublished WAS2000-350	Y	BAS
van de Veen, J.R.	AIIIA-9.1.3	1999	Calcuation of Predicted Environmental Concentrations for BF 500-6 and BF 500-7 in Soil (PECs) for a Vineyard and a Cereals Scenario. 1999/11802, CALC-152 not GLP, unpublished BOD2000-624	Y	BAS
van de Veen, J.R.	AIIIA-9.1.3	1999	Calcuation of Predicted Environmental Concentrations for BAS 5000 F in Soil (PECs) on a European Level in Cereals. 1999/11801, CALC-151 not GLP, unpublished BOD2000-623	Y	BAS
van de Veen, J.R.	AIIIA-9.1.3	1999	Calcuation of Predicted Environmental Concentrations for BAS 5000 F in Soil (PECs) on a European Level for a Vineyard Scenario. 1999/11800, CALC-150 not GLP, unpublished BOD2000-622	Y	BAS
van de Veen, J.R.	AIIIA-9.2.1	1999	Calcuation of Predicted Environmental Concentrations (PECgw) of BF 500-3 in Groundwater. 1999/11803, CALC-153 not GLP, unpublished WAS2000-496	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[7]
van de Veen, J.R.	AIIIA-9.2.1	1999	Calcuation of Predicted Environmental Concentrations (PECgw) of BAS 500 F and its Metabolites BF 500-6 and BF 500-7 in Groundwater on a European Level. 1999/11799, CALC-141 not GLP, unpublished WAS2000-495	Y	BAS
van de Veen, J.R.	AIIIA-9.2.3	2000	Estimation of the Drainage Entry Route Percentages of BAS 500 F and its Soil Metabolites BF 500-6 and BF 500-7 into Surface Waters. ID 2000/1014991, CALC-204 not GLP, unpublished WAS2000-669	Y	BAS
Ziegler, G.	AIIA-7.1.2	1998	Soil Adsorption/Desorption Study of 304428 (BAS 500 F). 98/10650, 35882 GLP, unpublished BOD2000-651	Y	BAS
Ziegler, G.	AIIA-7.1.3.1	1998	Leaching Behaviour of 14C-BAS 500 F in four soils under laboratory conditions. 98/11350, 42375 GLP, unpublished BOD2000-656	Y	BAS
Ziegler, G.	AIIA-7.1.3.2	1998	Leaching Behaviour of 14C-BAS 500 F after Aerobic Aging for 30 Days. 98/11202, 35648 GLP, unpublished BOD2000-657	Y	BAS

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A.9 Ecotoxicology (Annex IIA 8; Annex IIIA 10)

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Chapleo, S.	AIIA-8.2.3	1999	Bioaccumulation and metabolism of [14C]-BAS 500 F in bluegill sunfish. 391491, 17015 GLP, unpublished WAT2000-241	Y	BAS
Daum, A.	AIIA-8.2.3	1999	Determination of the octanol/water-partition coefficient of Reg.-No. 412785 (BF 500-13) by HPLC. PCP05339 GLP, unpublished WAT2000-243	Y	BAS
Daum, A.	AIIA-8.2.3	1999	Determination of the octanol/water-partition coefficient of Reg.-No. 413038 (BF 500-14) by HPLC. PCP05354 GLP, unpublished WAT2000-244	Y	BAS
Daum, A.	AIIA-8.2.3	1999	Determination of the octanol/water-partition coefficient of Reg.-No. 411847 (BF 500-11) by HPLC. PCP05338 GLP, unpublished WAT2000-242	Y	BAS
Dohmen, G-P	AIIA-8.2.1	1999	Effect of BAS 500 F on Daphnia magna Straus in a 48 hours acute toxicity test. 35806 GLP, unpublished WAT2000-247	Y	BAS
Dohmen, G-P	AIIA-8.2.5	1999	Effects of BAS 500 F on mortality and reproduction of Daphnia magna. 35811 GLP, unpublished WAT2000-251	Y	BAS

[8] Only notifier listed

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Dohmen, G-P	AIIA-8.2.6	1999	Effect of BAS 500 F on the growth of the green algae Pseudokirchneriella subcapitata. 35803 GLP, unpublished WAT2000-252	Y	BAS
Dohmen, G-P	AIIA-8.2.7	2000	Effects of BAS 500 F on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system. 35966 GLP, unpublished WAT2000-256	Y	BAS
Dohmen, G-P	AIIIA-10.2.1	1999	Effect of BAS 500 00 F on the growth of the green alga Pseudokirchneriella subcapitata. 35848 GLP, unpublished WAT2000-265	Y	BAS
Dohmen, G-P	AIIIA-10.2.1	1999	Effect of BAS 500 00 F on the immobility of Daphnia magna Straus in a 48 hour static, acute toxicity test. 35851 GLP, unpublished WAT2000-264	Y	BAS
Dohmen, G-P	AIIIA-10.2.2	2000	The effect of BAS 500 00 F on aquatic ecosystems - an outdoor mesocosm investigation. 35980 GLP, unpublished WAT2000-231	Y	BAS
Ebert, D.	AIIA-8.4.1	1999	Investigations on the release of soil-bound residues of 14C-BAS 500 F by earthworms. Std:: 53058 ! BASF 1999/11289 GLP, unpublished ARW2000-86	Y	BAS
Ehlers, H.A.	AIIIA-10.6.1.3	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms. Proj.: 6180023 ! BASF 2000/1000012 GLP, unpublished ARW2000-90	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Ehlers, H.A.	AIIIA-10.6.1.3	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms. BASF 2000/1012439 GLP, unpublished ARW2000-184	Y	BAS
Frey, L.T., Beavers, J.B. and Jaber, M.	AIIA-8.1.3	1999	BAS 500 F: A reproduction study with the northern bobwhite. 145-175 GLP, unpublished AVS2000-64	Y	BAS
Frey, L.T., Beavers, J.B. and Jaber, M.	AIIA-8.1.3	1999	BAS 500 F: A reproduction study with the mallard. 147-176 GLP, unpublished AVS2000-63	Y	BAS
Jatzek	AIIA-8.2.5	1999	Determination on the acute effect of BF 500-13 on the swimming ability of the water flea Daphnia magna Straus. 99/0518/50/1 GLP, unpublished WAT2000-249	Y	BAS
Jatzek	AIIA-8.2.5	1999	Determination on the acute effect of BF 500-14 on the swimming ability of the water flea Daphnia magna Straus. 99/0519/50/1 GLP, unpublished WAT2000-250	Y	BAS
Jatzek	AIIA-8.2.5	1999	Determination on the acute effect of BF 500-11 on the swimming ability of the water flea Daphnia magna Straus. 99/0517/50/1 GLP, unpublished WAT2000-248	Y	BAS
Krieg, W.	AIIA-8.4.1	1999	Effect of BF 500-6 on the mortality of the earthworm Eisenia foetida. Std.: 35987 ! BASF 1999/11308 GLP, unpublished ARW2000-84	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Krieg, W.	AIIA-8.4.1	1999	Effect of BF 500-7 on the mortality of the earthworm Eisenia foetida. Std:: 54484 ! BASF 1999/11309 GLP, unpublished ARW2000-85	Y	BAS
Krieg, W.	AIIA-8.4.1	1999	Effect of BAS 500 F on the mortality of the earthworm Eisenia foetida. Std.: 35801 ! BASF 1999/10708 GLP, unpublished ARW2000-83	Y	BAS
Krieg, W.	AIIA-8.5	1999	Effect of BF 500-6 and BF 500-7 on the nitrogen turnover in soil. 54482 ! BASF DocID 1999/11311 GLP, unpublished BMF2000-38	Y	BAS
Krieg, W.	AIIA-8.5	1999	Effect of BF 500-6 and BF 500-7 on soil respiration. 54483 ! BASF DocID 1999/11120 GLP, unpublished BMF2000-37	Y	BAS
Krieg, W.	AIIIA-10.6.1.1	1998	Effect of BAS 500 00 F on the mortality of the earthworm Eisenia foetida. Std.: 35846 ! BASF 1998/11395 GLP, unpublished ARW2000-87	Y	BAS
Krieg, W.	AIIIA-10.6.1.2	1999	Effect of BAS 500 00 F on growth and reproduction of the earthworm Eisenia foetida. Std.: 56987 ! BASF 1999/10650 GLP, unpublished ARW2000-88	Y	BAS
Krieg, W.	AIIIA-10.6.1.3	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms (grassland site). Std.: 60996 ! BASF 2000/1000008 GLP, unpublished ARW2000-89	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Krieg, W.	AIIIA-10.6.1.3	2000	Field study to evaluate the effects of BAS 500 01 F on earthworms. BASF 2000/1012437 GLP, unpublished ARW2000-183	Y	BAS
Krieg, W.	AIIIA-10.6.1.3	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms (grassland site). BASF 2000/1012435 GLP, unpublished ARW2000-182	Y	BAS
Krieg, W.	AIIIA-10.6.2	2000	Monitoring of Collembola populations follo-wing an exposure to BAS 500 00 F in the field (grassland). Proj.: 60997 ! BASF 2000/1000020 GLP, unpublished ARW2000-92	Y	BAS
Krieg, W.	AIIIA-10.6.2	2000	Bait-lamina test to evaluate the activity of soil organisms following an exposure to BAS 500 00 F in the field. Std.: 69959 ! BASF 2000/1000016 GLP, unpublished ARW2000-91	Y	BAS
Krieg, W.	AIIIA-10.7.1	1998	Effects of BAS 500 00 F on the nitrogen tur-nover in soil. 35845 ! BASF 98/11260 GLP, unpublished BMF2000-40	Y	BAS
Krieg, W.	AIIIA-10.7.1	1998	Effects of BAS 500 00 F on soil respiration. 35844 ! BASF 98/11252 GLP, unpublished BMF2000-39	Y	BAS
Munk, R.	AIIA-8.1.1	1997	Report BAS 500 F (Reg.No.304 428) - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus);. 11W0494/96117 /BAS 97/11136 GLP, unpublished AVS2000-60	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Munk, R.	AIIA-8.1.2	1998	Test Report BAS 500 F - Avian dietary LC50 test in chicks of the mallard duck (Anas platyrhynchos L.). 31W0494/96123/BAS 98/10933 GLP, unpublished AVS2000-62	Y	BAS
Munk, R.	AIIA-8.1.2	1998	Test Report BAS 500 F - Avian dietary LC50 test in chicks of the bobwhite quail (Colinus virginianus);. 31W0494/96126/BAS 98/10932 GLP, unpublished AVS2000-61	Y	BAS
Munk, R.	AIIA-8.2.1	1998	BAS 500 F Acute toxicity study on the common carp (Cyprinus carpio L.) in a static system (96 hours). 12F0494/965178 GLP, unpublished WAT2000-234	Y	BAS
Munk, R.	AIIA-8.2.1	1998	BAS 500 F Acute toxicity study on the bluegill (Lepomis macrochirus Raf.) in a static system (96 hours). 12F0494/965179 GLP, unpublished WAT2000-233	Y	BAS
Oberwalder, C. and Schmidt, O.	AIIA-8.6; AIIIA-10.8	2000	BAS 500 00 F: Effects on non-target plants in the greenhouse - A limit test. Std.: 67673 ! BASF 2000/1000024 GLP, unpublished PFL2000-99	Y	BAS
Reuschenbach	AIIA-8.2.6	1999	Determination of the inhibitory effect of BF 500-13 on the cell multiplication of unicellular green algae. 99/0518/60/1 GLP, unpublished WAT2000-254	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Reuschenbach	AIIA-8.2.6	1999	Determination of the inhibitory effect of BF 500-14 on the cell multiplication of unicellular green algae. 99/0519/60/1 GLP, unpublished WAT2000-255	Y	BAS
Reuschenbach	AIIA-8.2.6	1999	Determination of the inhibitory effect of BF 500-11 on the cell multiplication of unicellular green algae. 99/0517/60/1 GLP, unpublished WAT2000-253	Y	BAS
Reuschenbach	AIIA-8.7; AIIIA-10.2	1999	Determination of the inhibition of oxygen consumption by activated sludge by BAS 500 F in the activated sludge respiration inhibition test. 99/0164/08/1 GLP, unpublished WAT2000-230	Y	BAS
Sack, D.	AIIA-8.3	1999	Effects of Reg.No. 304 428 on the Honeybee (Apis mellifera L.) in Laboratory Trials. 11457 GLP, unpublished BIE2000-12	Y	BAS
Sack, D.	AIIIA-10.4	1999	Effects of BAS 500 00 F on the Honeybee (Apis mellifera L.) in Laboratory Trials. 11455 GLP, unpublished BIE2000-11	Y	BAS
Zok, S.	AIIA-8.2.1	1999	Reg.-Nr. 413038 Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0249/995035 GLP, unpublished WAT2000-237	Y	BAS
Zok, S.	AIIA-8.2.1	1999	Reg.-Nr. 412785 Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0252/995034 GLP, unpublished WAT2000-236	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Zok, S.	AIIA-8.2.1	1999	Reg.-Nr. 411847 Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0251/995037 GLP, unpublished WAT2000-235	Y	BAS
Zok, S.	AIIA-8.2.1	1999	BAS 500 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0494/965180 GLP, unpublished WAT2000-232	Y	BAS
Zok, S.	AIIA-8.2.2	1999	BAS 500 F Sublethal toxic effects on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a flow-through system (28 days). 42F0494/965177 GLP, unpublished WAT2000-238	Y	BAS
Zok, S.	AIIA-8.2.2.1	1999	BAS 500 F Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a flow through system with variable concentrations. 52F0494/965189 GLP, unpublished WAT2000-240	Y	BAS
Zok, S.	AIIA-8.2.2.1	1999	BAS 500 F Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss Walbaum 1792). 52F0494/965141 GLP, unpublished WAT2000-239	Y	BAS
Zok, S.	AIIA-8.2.2.1	2000	BAS 500 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss)after short time exposure over 0,5, 2 and 8 hours in a flow-through system followed up by a post exposure period. 12F0494/965190 GLP, unpublished WAT2001-139	N	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Zok, S.	AIIIA-10.1.1	1999	Report BAS 500 00 F - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus). 11W0185/97164 /BAS 1999/11838 GLP, unpublished AVS2000-65	Y	BAS
Zok, S.	AIIIA-10.2.1	1999	BAS 500 00 F Acute toxicity study on the fathead minnow (Pimephales promelas RAF.) in a static system (96 hours). 15F0185/975117 GLP, unpublished WAT2000-261	Y	BAS
Zok, S.	AIIIA-10.2.1	1999	BAS 500 00 F Acute toxicity study on the zebra fish (Brachidanio rerio HAM. and BUCH.) in a static system (96 hours). 17F0185/975120 GLP, unpublished WAT2000-262	Y	BAS
Zok, S.	AIIIA-10.2.1	1999	BAS 500 00 F Acute toxicity study on the golden orfe (Leuciscus idus melanotus) in a static system (96 hours). 10F0185/975121 GLP, unpublished WAT2000-263	Y	BAS
Zok, S.	AIIIA-10.2.1	1999	BAS 500 00 F Acute toxicity study on the orange red killifish (Oryzias latipes Schlegel) in a static system (96 hours). 13F0185/975122 GLP, unpublished WAT2000-260	Y	BAS
Zok, S.	AIIIA-10.2.1	1999	BAS 500 00 F Acute toxicity study on the common carp (Cyprinus carpio L.) in a static system (96 hours). 11F0185/975119 GLP, unpublished WAT2000-259	Y	BAS

Author(s)	Annex point/ reference number	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[8]
Zok, S.	AIIIA-10.2.1	1999	BAS 500 00 F Acute toxicity study on the bluegill (Lepomis macrochirus RAF.) in a static system (96 hours). 14F0185/975118 GLP, unpublished WAT2000-258	Y	BAS
Zok, S.	AIIIA-10.2.1	1999	BAS 500 00 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0185/975052 GLP, unpublished WAT2000-257	Y	BAS

Codes of owner

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Pyraclostrobin

Volume 3

Annex B

Summary, Scientific Evaluation and Assessment

Rapporteur Member State: Germany

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.1 Identity 3

B.1.1 Identity of the active substance (Annex IIA 1 and 3.1) 3
B.1.1.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1) 3
B.1.1.2 Common name and synonyms (Annex IIA 1.3) 3
B.1.1.3 Chemical name (Annex IIA 1.4) 3
B.1.1.4 Manufacturer's development code number (Annex IIA 1.5) 3
B.1.1.5 CAS, EEC and CIPAC numbers (Annex IIA 1.6) 3
B.1.1.6 Molecular and structural formulae, molecular mass (Annex IIA 1.7) 3
B.1.1.7 Manufacturer or manufacturers of the active substance (Annex IIA 1.2) 4
B.1.1.8 Method or methods of manufacture (Annex IIA 1.8) 4
B.1.1.9 Specification of purity of the active substance (Annex IIA 1. 9) 4
B.1.1.10 Identity of isomers, impurities and additives (Annex IIA 1.10) 4
B.1.1.11 Analytical profile of batches (Annex IIA 1.11) 4
B.1.2 Identity of the plant protection product (Annex IIIA 1) 5
B.1.2.1 Current, former and proposed trade names and development code numbers (Annex IIIA 1.3) 5
B.1.2.2 Manufacturer or manufacturers of the plant protection product (Annex IIIA 1.2) 5
B.1.2.3 Type of the preparation and code (Annex IIIA 1.5) 5
B.1.2.4 Function (Annex IIA 3.1; Annex IIIA 1.6) 5
B.1.2.5 Composition of the preparation (Annex IIIA 1.4) 5
B.1.3 References relied on 6

B.2 Physical and chemical properties 11

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2) 11
B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2) 17
B.2.3 References relied on 22

B.3 Data on application and further information 29

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 to 3.6) 29
B.3.1.1 Function 29
B.3.1.2 Effects on harmful organisms 29
B.3.1.3 Field of use 29
B.3.1.4 Harmful organisms 29
B.3.1.5 Mode of action 29
B.3.1.6 Information on the occurence or possible occurrence of the development of resistance and appropriate management strategies 29
B.3.2 Data on application relevant to the plant protection product (Annex IIIA 3) 30
B.3.2.1 Field of use 30
B.3.2.2 Effects on harmful organisms 30
B.3.2.3 Details of intended use 31
B.3.2.4 Application rate 31

B.3.2.5 Concentration of active substance in material used 31
B.3.2.6 Method of application 31
B.3.2.7 Number and timing of applications and duration of protection 31
B.3.2.8 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops 31
B.3.3 Summary of data on application 32
B.3.4 Further information on the active substance (Annex IIA 3.7 to 3.9) 33
B.3.4.1 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIA 3.7) 33
B.3.4.1.1 Handling 33
B.3.4.1.2 Storage 33
B.3.4.1.3 Transport 33
B.3.4.1.4 Fire-fighting measures 34
B.3.4.2 Procedures for destruction or decontamination (Annex IIA 3.8) 34
B.3.4.3 Emergency measures in the case of an accident (Annex IIA 3.9) 34
B.3.5 Further information on the plant protection product (Annex IIIA 4) 35
B.3.5.1 Description of packaging 35
B.3.5.2 Suitability of packaging 36
B.3.5.3 Resistance of the packaging material to its contents 36
B.3.5.4 Procedures for cleaning application (Annex IIIA 4.2) 37
B.3.5.5 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIA 4.3) 37
B.3.5.6 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIIA 4.4) 38
B.3.5.7 Emergency measures in case of an accident (Annex IIIA 4.5) 39
B.3.5.8 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6) 40
B.3.5.8.1 Neutralisation procedures (e.g. reaction with alkali to form less toxic compounds) for use in the event of accidental spillage 40
B.3.5.8.2 Pyrolytic behavior of the active substance under controlled conditions at 800°C and the content of polyhalogenated dibenzo-p-dioxins in the products of pyrolysis 40
B.3.5.8.3 Methods other than controlled incineration for disposal of the plant protection product, contaminated packaging and contaminated material 40
B.3.6 References relied on 41

B.4 Proposals for the classification and labelling 45

B.4.1 Proposals for the classification and labelling of the active substance (Annex IIA 10) 45
B.4.2 Proposals for the classification and labelling of preparations (Annex IIIA 12.3 and 12.4) 45
B.4.3 References relied on 46

B.5 Methods of analysis 49

B.5.1 Analytical methods for formulation analysis (Annex IIA 4.1; Annex IIIA 5.1) 49
B.5.1.1 Analytical method for the determination of pure active substance in the active substance as manufactured 49

B.5.1.2 Methods for the determination of significant and/or relevant impurities and additives (e.g stabiliser) in the actice substance as manufactured 49
B.5.1.3 Analytical methods for formulation analysis (plant protection product) (Annex IIIA 5.1) 50
B.5.1.4 Method(s) for relevant breakdown products, isomers and additives 50
B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.2.1; Annex IIIA 5.2) 51
B.5.2.1 Plant material 51
B.5.2.2 Foodstuff of animal origin 54
B.5.2.3 Additional methods 55
B.5.2.4 Structural formulae and designation of compounds 56
B.5.3 Analytical methods (residue) soil, water, air (Annex IIA 4.2. 2 to 4.2.4; Annex IIIA 5.2) 56
B.5.3.1 Soil 56
B.5.3.2 Water 58
B.5.3.3 Air 59
B.5.3.4 Structural formulae and designation of compounds 60
B.5.4 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.2.5; Annex IIIA 5.2) 61
B.5.5 Evaluation and assessment 62
B.5.5.1 Formulation analysis 62
B.5.5.2 Residue analysis 62
B.5.6 References relied on 63

B.6 Toxicology and metabolism 71

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1) 71
B.6.1.1 Absorption, distribution and excretion 72
B.6.1.2 Metabolism 76
B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2) 86
B.6.2.1 Oral 87
B.6.2.2 Percutaneous 88
B.6.2.3 Inhalation 89
B.6.2.4 Skin irritation 90
B.6.2.5 Eye irritation 92
B.6.2.6 Skin sensitisation 93
B.6.3 Short-term toxicity (Annex IIA 5.3) 94
B.6.3.1 Rat 96
B.6.3.1.1 Rat oral 28-day study 96
B.6.3.1.2 Rat oral 90-day study 98
B.6.3.1.3 Rat dermal 28-day study 101
B.6.3.2 Mouse 103
B.6.3.2.1 Mouse oral 90-day study, mouse 103
B.6.3.3 Dog 106
B.6.3.3.1 Dog oral 90-day study 106
B.6.3.3.2 Dog oral 1-year study 108
B.6.4 Genotoxicity (Annex IIA 5.4) 110
B.6.4.1 *In vitro* testing 110
B.6.4.1.1 Gene mutation in bacterial cells 110

B.6.4.1.2 Gene mutation in mammalian cells 112
B.6.4.1.3 *In vitro* cytogenetic test 113
B.6.4.1.4 DNA damage and repair 115
B.6.4.2 In vivo testing 116
B.6.4.2.1 In vivo cytogenetic test 116
B.6.4.2.2 DNA damage and repair 118
B.6.4.3 *In vivo* testing, germ cells 118
B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5) 118
B.6.5.1 Chronic toxicity, rat 119
B.6.5.2 Carcinogenicity, rat 121
B.6.5.3 Carcinogenicity, mouse 123
B.6.6 Reproductive toxicity (Annex IIA 5.6) 124
B.6.6.1 Multigeneration study in rats 126
B.6.6.2 Developmental toxicity 130
B.6.6.2.1 Rat 130
B.6.6.2.2 Rabbit 132
B.6.7 Delayed neurotoxicity (Annex IIA 5.7) 137
B.6.7.1 Neurotoxicity studies 137
B.6.7.1.1 Acute neurotoxicity 137
B.6.7.1.2 Subchronic neurotoxicity 138
B.6.7.2 Delayed Neurotoxicity 140
B.6.8 Further toxicological studies (Annex IIA 5.8) 140
B.6.9 Medical data and information (Annex IIA 5.9) 142
B.6.9.1 Medical surveillance on manufacturing plant personnel 142
B.6.9.2 Direct observation, e.g. clinical cases and poisoning incidents 142
B.6.9.3 Observations on exposure of the general population and epidemiological studies if appropriate 142
B.6.9.4 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests 142
B.6.9.5 Proposed treatment: first aid measures, antidotes, medical treatment 142
B.6.9.6 Expected effects of poisoning 143
B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.10) 143
B.6.10.1 Summary of mammalian toxicology 143
B.6.10.2 Acceptable daily intake (ADI) 147
B.6.10.3 Acute reference dose (ARfD) 148
B.6.10.4 Acceptable operator exposure level (AOEL) 148
B.6.10.5 Maximum acceptable concentration in drinking water 148
B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1) 149
B.6.11.1 Oral 149
B.6.11.2 Percutaneous 151
B.6.11.3 Inhalation 152
B.6.11.4 Skin irritation 155
B.6.11.5 Eye irritation 156
B.6.11.6 Skin sensitisation 157
B.6.11.7 Supplementary studies for combinations of plant protection products 158
B.6.12 Dermal absorption (Annex IIIA 7.3) 159
B.6.12.1 *In vivo* data 159
B.6.12.2 *In vitro* data 161

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction) ... 165
B.6.14 Exposure data (Annex IIIA 7.2) ... 165
B.6.14.1 Operator exposure ... 165
B.6.14.1.1 Estimation of operator exposure and risk assessment ... 165
B.6.14.2 Bystander exposure ... 170
B.6.14.2.1 Estimation of bystander exposure ... 170
B.6.14.2.2 Measurement of bystander exposure ... 170
B.6.14.3 Worker exposure ... 170
B.6.14.3.1 Estimation of worker exposure ... 170
B.6.14.3.2 Measurement of worker exposure ... 172
B.6.15 References relied on ... 172

B.7 Residue data ... 181

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1; Annex IIIA 8.1) ... 181
B.7.1.1 Metabolism of pyraclostrobin in grapes ... 181
B.7.1.2 Metabolism of pyraclostrobin in potatoes ... 185
B.7.1.3 Metabolism of pyraclostrobin in wheat ... 191
B.7.1.4 Extractability of residues of ^{14}C-pyraclostrobin from plant matrices ... 199
B.7.1.4.1 Grapes and wheat ... 200
B.7.1.4.2 Potato ... 201
B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2; Annex IIIA 8.1) ... 201
B.7.2.1 Lactating goats ... 201
B.7.2.1.1 Absorption, distribution and excretion ... 201
B.7.2.1.2 Investigation of the metabolism of ^{14}C pyraclostrobin in lactating goat ... 204
B.7.2.2 Laying hens ... 216
B.7.2.2.1 Absorption, distribution and exretion of ^{14}C-pyraclostrobin ... 216
B.7.2.2.2 Investigation of the Metabolism of ^{14}C-pyraclostrobin in laying hen ... 219
B.7.2.3 Pigs ... 227
B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6) ... 228
B.7.3.1 Plant matrices ... 228
B.7.3.2 Animal matrices ... 228
B.7.4 Use pattern ... 229
B.7.5 Identification of critical GAPs ... 232
B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2) ... 233
B.7.6.1 Analytical method used in the residue trials ... 233
B.7.6.2 Storage stability ... 233
B.7.6.3 Residues in grapes ... 234
B.7.6.4 Residues in cereals ... 242
B.7.6.4.1 Barley ... 243
B.7.6.4.2 Wheat ... 265
B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4) ... 291
B.7.7.1 Effects on the nature of residue ... 291
B.7.7.2 Effects on the residue level ... 292
B.7.7.2.1 Residues in grapes ... 292
B.7.7.2.2 Residues in barley ... 297

B.7.7.2.3 Residues in wheat 300
B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3) 305
B.7.8.1 Ruminants 305
B.7.8.2 Poultry 311
B.7.8.2.1 Pigs 312
B.7.8.3 Storage stability in matrices of animal origin 312
B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6; Annex IIIA 8.5) 313
B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post-harvest uses (Annex IIA 6.8; Annex IIIA 8.7) 321
B.7.11 Community MRLs and MRLs in EU Member States (Annex IIIA 12.2) 321
B.7.12 Proposed EU MRLs and justification for the acceptability of those residues (Annex IIA 6.7; Annex IIIA 8.6) 321
B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues 321
B.7.13.1 Banana 321
B.7.14 Basis for differences, if any, in conclusion reached having regard to established or proposed Codex MRLs 325
B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8) 325
B.7.15.1 Chronic Dietary Risk Assessment 325
B.7.15.2 Acute Dietary Risk Assessment 329
B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9) 330
B.7.16.1 Metabolism in plants 330
B.7.16.2 Metabolism in livestock 330
B.7.16.3 Residues in treated crops, proposed MRL's 330
B.7.16.4 Residues in food of animal origin, proposed MRL's 331
B.7.16.5 Stability of residues prior to analysis 331
B.7.16.6 Residues in succeeding crops 331
B.7.16.7 Estimates of dietary exposure to pyraclostrobin 331
B.7.17 References relied on 332

B.8 Environmental fate and behaviour 343

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1) 343
B.8.1.1 Route of degradation 343
B.8.1.1.1 Aerobic conditions 343
B.8.1.1.2 Bound residues 347
B.8.1.1.3 Supplementary studies 348
B.8.1.2 Rate of degradation 354
B.8.1.2.1 Laboratory conditions 354
B.8.1.2.2 Field conditions 357
B.8.1.2.3 Storage stability of soil residues 361
B.8.2 Adsorption, desorption and mobility in soil (Annex IIA 7.1.2, 7.1.3; Annex IIIA 9.1.2) 362
B.8.2.1 Adsorption and desorption 362
B.8.2.2 Mobility in soil 366
B.8.2.2.1 Column leaching studies 366
B.8.2.2.2 Aged residue column leaching 368
B.8.2.2.3 Lysimeter studies or field leaching studies 369

B.8.3 Predicted environmental concentrations in soil (Annex IIIA 9.1.3) 370
B.8.3.1 Notifier's calculation 370
B.8.3.2 Rapporteur's calculation 370
B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2.1, 9.2.3) 373
B.8.4.1 Rate and route of degradation in aquatic systems 373
B.8.4.1.1 Hydrolytic degradation 373
B.8.4.1.2 Photochemical degradation 374
B.8.4.1.3 Biological degradation 378
B.8.4.1.4 Degradation in the saturated zone 391
B.8.5 Impact on water treatment procedures (Annex IIIA 9.2.2) 391
B.8.6 Predicted environmental concentrations in surface water and in ground water (Annex IIIA 9.2.1, 9.2.3) 391
B.8.6.1 Predicted environmental concentration in groundwater (PEGgw) 391
B.8.6.2 Predicted environmental concentration in surface water (PECsw) 392
B.8.6.2.1 Notifiers calculation 392
B.8.6.2.2 Rapporteur's PECsw calculations 393
B.8.6.3 Predicted environmental concentrations in sediment PECsed 396
B.8.7 Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3) 398
B.8.8 Predicted environmental concentrations in air (Annex IIIA 9.3) 399
B.8.9 Definition of the residue (Annex IIA 7.3) 399
B.8.9.1 Soil 399
B.8.9.2 Water 400
B.8.10 References relied on 403

B.9 Ecotoxicology 413

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1) 413
B.9.1.1 Acute Oral Toxicity (Annex IIA 8.1, Annex IIIA 10.1.1) 413
B.9.1.2 Dietary toxicity (Annex IIA 8.1.2) 414
B.9.1.3 Effects on reproduction (Annex IIA 8.1.3) 415
B.9.1.4 Other studies (Annex IIIA 10.1.2, 10.1.3, 10.1.4) 417
B.9.1.5 Risk assessment for birds 417
B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2) 418
B.9.2.1 Acute toxicity to fish (Annex IIA 8.2.1, Annex IIIA 10.2.1) 418
B.9.2.1.1 Active Substance 418
B.9.2.1.2 Major metabolites BF 500-11, BF 500-13 and BF 500-14 420
B.9.2.1.3 Formulated product (BAS 500 00 F) 422
B.9.2.2 Chronic toxicity to fish (Annex IIA 8.2.2) 426
B.9.2.2.1 Active Substance 426
B.9.2.3 Bioconcentration in fish (Annex IIA 8.2.3) 428
B.9.2.3.1 Active substance 428
B.9.2.3.2 Metabolites 430
B.9.2.4 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4) 430
B.9.2.4.1 Active substance 430
B.9.2.4.2 Major metabolites BF 500-11, BF 500-13 and BF 500-13 431
B.9.2.4.3 Formulated product (BAS 500 00 F) 432
B.9.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5) 433
B.9.2.5.1 Active substance 433
B.9.2.6 Effects on algal growth (Annex IIA 8.2.6) 434
B.9.2.6.1 Active substance 434

B.9.2.6.2 Major metabolites BF 500-11, BF 500-13 and BF 500-14 434
B.9.2.6.3 Formulated product (BAS 500 00 F) 435
B.9.2.7 Effects on sediment-dwelling organisms (Annex IIA 8.2.7) 436
B.9.2.7.1 Active substance 436
B.9.2.8 Aquatic mesocosm (Annex III A 10.2.2) 437
B.9.2.9 Risk assessment for aquatic organisms 441
B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3) 446
B.9.3.1 Toxicity to mammals (Annex IIIA 10.3) 446
B.9.3.2 Risk assessment for mammals 447
B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.4) 448
B.9.4.1 Acute toxicity (Annex IIA 8.3.1, Annex IIIA 10.4) 448
B.9.4.1.1 Contact and oral toxicity of pyraclostrobin (Reg. No. 304428) to honeybees 448
B.9.4.1.2 Contact and oral toxicity of BAS 500 00 F 448
B.9.4.2 Bee brood feeding test (Annex IIA 8.3.1.2) 449
B.9.4.3 Residue test (Annex IIIA 10.4.2) 449
B.9.4.4 Cage test (Annex IIIA 10.4.3) 449
B.9.4.5 Field test (Annex IIIA 10.4.4) 449
B.9.4.6 Tunnel test (Annex IIIA 10.5.5) 449
B.9.4.7 Risk assessment for honeybees 450
B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5) 450
B.9.5.1 Acute toxicity (Annex IIA 8.3.2, Annex IIIA 10.5.1) 450
B.9.5.2 Field tests (Annex III 10.5.2) 457
B.9.5.3 Risk assessment for non-target terrestrial arthropods 459
B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1) 460
B.9.6.1 Acute toxicity (Annex IIA 8.4.1, Annex IIIA 10.6.1.1) 460
B.9.6.2 Other studies (Annex IIA 8.4.2, Annex IIIA 10.6.1.2, Annex IIIA 10.6.1.3) 462
B.9.6.2.1 Effect on reproduction 462
B.9.6.2.2 Effect of soil bound residues of BAS 500-F 463
B.9.6.2.3 Effects in the field 463
B.9.6.3 Risk assessment for earthworms 469
B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2) 470
B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7) 472
B.9.8.1 Carbon conversion (Annex IIA 8.5; Annex IIIA 10.7) 472
B.9.8.2 Nitrogen conversion (Annex IIA 8.5; Annex IIIA 10.7) 473
B.9.8.3 Risk assessment for soil micro-organisms 473
B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6) 474
B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7) 474
B.9.10.1 Risk assessment 475
B.9.11 References relied on 475

B.10 Appendices 487

B.10.1 Appendix I: Standard terms and abbreviations 487
B.10.2 Appendix II: Specific terms and abbreviations 499

Annex B

Pyraclostrobin

B-1: Identity

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.1 Identity 3

B.1.1 Identity of the active substance (Annex IIA 1 and 3.1) 3
B.1.1.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1) 3
B.1.1.2 Common name and synonyms (Annex IIA 1.3) 3
B.1.1.3 Chemical name (Annex IIA 1.4) 3
B.1.1.4 Manufacturer's development code number (Annex IIA 1.5) 3
B.1.1.5 CAS, EEC and CIPAC numbers (Annex IIA 1.6) 3
B.1.1.6 Molecular and structural formulae, molecular mass (Annex IIA 1.7) 3
B.1.1.7 Manufacturer or manufacturers of the active substance (Annex IIA 1.2) 4
B.1.1.8 Method or methods of manufacture (Annex IIA 1.8) 4
B.1.1.9 Specification of purity of the active substance (Annex IIA 1. 9) 4
B.1.1.10 Identity of isomers, impurities and additives (Annex IIA 1.10) 4
B.1.1.11 Analytical profile of batches (Annex IIA 1.11) 4
B.1.2 Identity of the plant protection product (Annex IIIA 1) 5
B.1.2.1 Current, former and proposed trade names and development code numbers (Annex IIIA 1.3) 5
B.1.2.2 Manufacturer or manufacturers of the plant protection product (Annex IIIA 1.2) 5
B.1.2.3 Type of the preparation and code (Annex IIIA 1.5) 5
B.1.2.4 Function (Annex IIA 3.1; Annex IIIA 1.6) 5
B.1.2.5 Composition of the preparation (Annex IIIA 1.4) 5
B.1.3 References relied on 6

B.2 Physical and chemical properties 11

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2) 11
B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2) 17
B.2.3 References relied on 22

B.3 Data on application and further information 29

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 to 3.6) 29
B.3.1.1 Function 29
B.3.1.2 Effects on harmful organisms 29
B.3.1.3 Field of use 29
B.3.1.4 Harmful organisms 29
B.3.1.5 Mode of action 29
B.3.1.6 Information on the occurence or possible occurrence of the development of resistance and appropriate management strategies 29
B.3.2 Data on application relevant to the plant protection product (Annex IIIA 3) 30
B.3.2.1 Field of use 30
B.3.2.2 Effects on harmful organisms 30
B.3.2.3 Details of intended use 31
B.3.2.4 Application rate 31

B.3.2.5 Concentration of active substance in material used 31
B.3.2.6 Method of application 31
B.3.2.7 Number and timing of applications and duration of protection 31
B.3.2.8 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops 31
B.3.3 Summary of data on application 32
B.3.4 Further information on the active substance (Annex IIA 3.7 to 3.9) 33
B.3.4.1 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIA 3.7) 33
B.3.4.1.1 Handling 33
B.3.4.1.2 Storage 33
B.3.4.1.3 Transport 33
B.3.4.1.4 Fire-fighting measures 34
B.3.4.2 Procedures for destruction or decontamination (Annex IIA 3.8) 34
B.3.4.3 Emergency measures in the case of an accident (Annex IIA 3.9) 34
B.3.5 Further information on the plant protection product (Annex IIIA 4) 35
B.3.5.1 Description of packaging 35
B.3.5.2 Suitability of packaging 36
B.3.5.3 Resistance of the packaging material to its contents 36
B.3.5.4 Procedures for cleaning application (Annex IIIA 4.2) 37
B.3.5.5 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIA 4.3) 37
B.3.5.6 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIIA 4.4) 38
B.3.5.7 Emergency measures in case of an accident (Annex IIIA 4.5) 39
B.3.5.8 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6) 40
B.3.5.8.1 Neutralisation procedures (e.g. reaction with alkali to form less toxic compounds) for use in the event of accidental spillage 40
B.3.5.8.2 Pyrolytic behavior of the active substance under controlled conditions at 800°C and the content of polyhalogenated dibenzo-p-dioxins in the products of pyrolysis 40
B.3.5.8.3 Methods other than controlled incineration for disposal of the plant protection product, contaminated packaging and contaminated material 40
B.3.6 References relied on 41

B.4 Proposals for the classification and labelling 45

B.4.1 Proposals for the classification and labelling of the active substance (Annex IIA 10) 45
B.4.2 Proposals for the classification and labelling of preparations (Annex IIIA 12.3 and 12.4) 45
B.4.3 References relied on 46

B.5 Methods of analysis 49

B.5.1 Analytical methods for formulation analysis (Annex IIA 4.1; Annex IIIA 5.1) 49
B.5.1.1 Analytical method for the determination of pure active substance in the active substance as manufactured 49

B.5.1.2 Methods for the determination of significant and/or relevant impurities and additives (e.g stabiliser) in the actice substance as manufactured 49
B.5.1.3 Analytical methods for formulation analysis (plant protection product) (Annex IIIA 5.1) 50
B.5.1.4 Method(s) for relevant breakdown products, isomers and additives 50
B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.2.1; Annex IIIA 5.2) 51
B.5.2.1 Plant material 51
B.5.2.2 Foodstuff of animal origin 54
B.5.2.3 Additional methods 55
B.5.2.4 Structural formulae and designation of compounds 56
B.5.3 Analytical methods (residue) soil, water, air (Annex IIA 4.2. 2 to 4.2.4; Annex IIIA 5.2) 56
B.5.3.1 Soil 56
B.5.3.2 Water 58
B.5.3.3 Air 59
B.5.3.4 Structural formulae and designation of compounds 60
B.5.4 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.2.5; Annex IIIA 5.2) 61
B.5.5 Evaluation and assessment 62
B.5.5.1 Formulation analysis 62
B.5.5.2 Residue analysis 62
B.5.6 References relied on 63

B.1 Identity

B.1.1 Identity of the active substance (Annex IIA 1 and 3.1)

B.1.1.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1)

BASF Aktiengesellschaft
Agricultural Center
Product Registration Management
P.O. Box 120
D-67141 Limburgerhof

Contact person: Dr. Henning Regenstein
Tel.: +49 621 60 274 13
Fax: +49 621 60 276 04

B.1.1.2 Common name and synonyms (Annex IIA 1.3)

Pyraclostrobin (ISO, proposed).

B.1.1.3 Chemical name (Annex IIA 1.4)

IUPAC: methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate

CAS: Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester

B.1.1.4 Manufacturer's development code number (Annex IIA 1.5)

BAS 500 F, LAB 304428, Reg.No. 304428, PS 304428.

B.1.1.5 CAS, EEC and CIPAC numbers (Annex IIA 1.6)

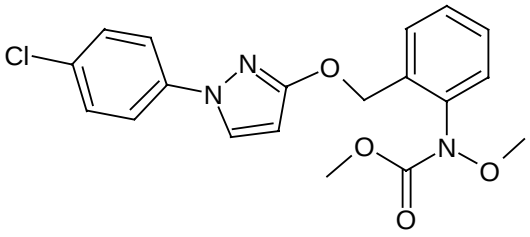
CAS: 175013-18-0
CIPAC: 657
EEC: not assigned
EINECS: not assigned

B.1.1.6 Molecular and structural formulae, molecular mass (Annex IIA 1.7)

Molecular formular: $C_{19}\ H_{18}\ Cl\ N_3\ O_4$

Molecular mass: 387.82 g/mol

Structural formula:



B.1.1.7 Manufacturer or manufacturers of the active substance (Annex IIA 1.2)

BASF Aktiengesellschaft
Agricultural Center
Product Registration Management
P.O. Box 120
D-67141 Limburgerhof

Contact person: Dr. Karl Zoller
Production Crop protection
Tel.: +49 621 60 791 46
Fax: +49 621 60 795 19

B.1.1.8 Method or methods of manufacture (Annex IIA 1.8)

Confidential information, see Annex C.

B.1.1.9 Specification of purity of the active substance (Annex IIA 1. 9)

≥ 950 g/kg (based on the analysis of material produced in a pilot plant).

B.1.1.10 Identity of isomers, impurities and additives (Annex IIA 1.10)

Confidential information, see Annex C.

B.1.1.11 Analytical profile of batches (Annex IIA 1.11)

Confidential information, see Annex C.

B.1.2 Identity of the plant protection product (Annex IIIA 1)

B.1.2.1 Current, former and proposed trade names and development code numbers (Annex IIIA 1.3)

Trade name: "BAS 500 00 F", preliminary designator

(country specific alternatives are under consideration)

Code number:	Plant protection product:	BAS 500 00 F
	Preliminary descriptor:	BAS 500 AE F
	Active Substance:	BAS 500 F
	BASF internal No.:	Reg. No. 304428

B.1.2.2 Manufacturer or manufacturers of the plant protection product (Annex IIIA 1.2)

BASF Aktiengesellschaft
Crop Protection Division
P.O. Box 1 20
67114 Limburgerhof
Germany

Contact person: Dr. Karl Zoller
Production Crop Protection
Tel. No.: (0)6 21/60-7 91 46
Fax No.: (0)6 21/60-7 95 19

B.1.2.3 Type of the preparation and code (Annex IIIA 1.5)

Emulsifiable concentrate (EC)

B.1.2.4 Function (Annex IIA 3.1; Annex IIIA 1.6)

Fungicide.

B.1.2.5 Composition of the preparation (Annex IIIA 1.4)

Confidential information, see Annex C.

B.1.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-1.8	Eisert, R.	1999	Product Chemistry: BAS 500 F Description of the Manufacturing Process. 1999/11856 not GLP, unpublished CHE2000-459	Y	BAS
AIIA-1.9; AIIA-1.10	Eisert, R.	1999	Product Chemistry: BAS 500 F TC - Composition of the Technical Active Ingredient. 1999/11857 not GLP, unpublished CHE2000-461	Y	BAS
AIIA-1.8; AIIA-1.9; AIIA-1.10; AIIA-1.11	Eisert, R.	2000	Manufacturer of BAS 500 F - Method of manufacture of the active substance - Specification of purity of the active substance - Identity of isomers, impurities and additives - Analytical profile of batches. not GLP, unpublished CHE2000-460	Y	BAS
AIIA-1.11	Eisert, R.	1999	Characterization of six batches of technical active ingredient BAS 500 F (batches: ToxI, ToxII/Teil1, ToxII/Teil2, ToxIII/Teil1, ToxIII/Teil2 and N2) using HPLC methods CP266 and CP337 and GC methods CP284 and M97/0028/02. 1999/11853 GLP, unpublished CHE2000-458	Y	BAS
AIIIA-1.4	Anonym	1999	Safety Data Sheet for SOLVESSO 200. BASF DocID 1999/11940 not GLP, unpublished BEI2000-156	Y	BAS
AIIIA-1.4	Anonym	1999	Safety Data Sheet for WETTOL EM 31. BASF DocID 99/10232 not GLP, unpublished BEI2000-155	Y	BAS

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-1.4	Anonym	1999	Safety Data Sheet for WETTOL EM 1. BASF DocID 1999/11544 not GLP, unpublished BEI2000-154	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Pyraclostrobin

B-2: Physical and chemical properties

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

Table B.2.1-1: Summary of the physical and chemical properties of the active substance pyraclostrobin

PAS: Pure active substance (purity: 99.8 %)
TAS: Technical active substance (purity: 98.5 %)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.1.1 (IIA 2.1)	Melting point, freezing point or solidification point of purified active substance	PAS	EEC A 1 DSC	Y	Melting point (range) 63.7-65.2 °C	acceptable	Türk, 1996 (CHE2000-471)
B.2.1.1.2 (IIA 2.1)	Boiling point of purified active substance		n.a.		not applicable for a solid	acceptable	
B.2.1.1.3 (IIA 2.1)	Temperature of decomposition or sublimation	PAS	DSC	Y	A melting curve was registered from 50 °C to 360 °C with a heating rate of 10 °C/min. There was the endothermic melting peak at about 65 °C detected and at about 200 °C an exothermic effect which was related to decomposition. There is no endothermic effect which is not related to the melting point, thus, sublimation of or boiling of the test substance can be excluded.	acceptable	Türk, 1996 (CHE2000-471)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.2 (IIA 2.2)	Relative density of purified active substance	PAS TAS	EEC A 3 pycnometer	Y	PAS: D_4^{20} = 1.367 g/cm^3 (at room temperature) TAS: D_4^{20} = 1.285 g/cm^3 (at 20 °C)	acceptable	Kästel, 1997 (CHE2000-469) Kästel, 1997 (CHE2000-470)
B.2.1.3.1 (IIA 2.3)	Vapour pressure of purified active substance	PAS	EEC A.4 Balance method.	Y	2.6 x 10^{-8} Pa at 20°C 6.4 x 10^{-8} Pa at 25°C	Acceptable	Kästel, 1996 (LUF2000-247)
B.2.1.3.2 (IIA 2.3)	Volatility, Henry's law constant of purified active substance		Calculation	N	5.307 x 10^{-9}kPa m³/mol (at 20°C)	Acceptable	Ohnsorge, 2000 (LUF2000-248)
B.2.1.4.1 (IIA 2.4)	Appearance: physical state	PAS TAS	Visual assessment	Y	PAS: solid (at room temperature) TAS: solid, solidified melt (at room temperature)	acceptable	Türk, 1996 (CHE2000-471) Kästel, 1997 (CHE2000-470)
B.2.1.4.2 (IIA 2.4)	Appearance: colour	PAS TAS	Visual assessment	Y	PAS: white or light beige (at room temperature) TAS: dark brown (at room temperature)	acceptable	Türk, 1996 (CHE2000-471) Kästel, 1997 (CHE2000-470)
B.2.1.4.3 (IIA 2.4)	Appearance: odour	PAS TAS	Olfactory assessment	Y	PAS: odourless (at room temperature) TAS: moderate aromatic (at room temperature)	acceptable	Türk, 1996 (CHE2000-471) Kästel, 1997 (CHE2000-470)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.5.1 (IIA 2.5)	Spectra of purified active substance	PAS	IR NMR MS UV-VIS	Y	The structure is confirmed by all spectra. IR, NMR and MS UV molecular extinction ε [L mol^{-1} cm^{-1}]: 2.5 • 10^4 at 205 nm 2.4 • 10^4 at 275 nm	acceptable acceptable	Türk, 1996 (CHE2000-468)
B.2.1.5.2 (IIA 2.5)	Spectra for impurities of toxicological, ecotoxicological or environmental concern				No impurities of toxicological or environmental significance.	acceptable	
B.2.1.6 (IIA 2.6)	Solubility in water of purified active substance	PAS	EEC A 6 (column elution method)	Y	1.9 ± 0.17 mg/L at 20 °C in deionized water at a pH of 5.8. There is no dissociation in water therefore pH dependence on solubility is not applicable	acceptable	Türk, 1996 (CHE2000-467)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.7 (IIA 2.7)	Solubility in organic solvents of the active substance as manufactured	PAS	US-EPA Subdivision D: Product Chemistry § 63-8	Y	**Solvent** / **[g/100 mL]** n-heptane 0.37 2-propanol 3.00 octanol 2.42 olive oil 2.80 methanol 10.08 acetone >50 ethyl acetate >50 acetonitrile >50 dichloromethane >50 toluene >50	acceptable	Türk, 1996 (CHE2000-466)
B.2.1.8 (IIA 2.8)	Partition coefficient of purified active substance	PAS	OECD 117	Y	The mean log P_{OW} was 3.99 and the corresponding P_{OW} was 9772. Effect of pH was not investigated since there is no dissociation in water.	acceptable	Türk, 1996 (CHE2000-465)
B.2.1.9.1 (IIA 2.9)	Hydrolysis rate of purified active substance	PAS	EEC C.7	Y	Stable within 30 d test. DT50 exceeded the period of reliable extrapolation.	Acceptable	Scharf, 1999 (WAS2000-352)
B.2.1.9.2 (IIA 2.9)	Direct photo-transformation in purified water of purified active substance	PAS	US-EPA Subdivision N, 161-2	Y	Photodegradation products in concentr. >10%: 500M78 (DT50=4.62 d), BF 500-13 (DT50=30.67 d), 500M58 (DT50=8.64 d), BF 500-14 (DT50=0.28 d) and BF 500-11.	Acceptable	Scharf, 1999, (LUF2000-249)
B.2.1.9.3 (IIA 2.9)	Quantum yield of direct photodegradation	PAS	US-EPA, Subdivision N, 161-2	Y	2.17×10^{-1}	Acceptable	Scharf, 1999 (LUF2000-249)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.9.4 (IIA 2.9)	Dissociation constant (pK_a) of purified active substance		OECD 112	Y	No indication of dissociation.	Acceptable	WAS2000-499
B.2.1.10 (IIA 2.10)	Stability in air, indirect photo-transformation		AOP Calculation	N	Half life = 1.87 h	Acceptable	Scharf, 1999 LUF2000-246
B.2.1.11.1 (IIA 2.11)	Flammability of active substance as manufactured	TAS	EEC A 10	Y	TAS is not considered highly flammable, it did not burn under test conditions.	Acceptable	Löffler, 1998 (CHE2000-464)
B.2.1.11.2 (IIA 2.11)	Auto-flammabi-lity of active substance as manufactured	TAS	EEC A 15	Y	Auto-ignition temperature = 510 °C.	Acceptable	Löffler, 1998 (CHE2000-464)
B.2.1.12 (IIA 2.12)	Flash point of the active substance as manufactured	TAS	EEC A 9 Pensky-Martens method	Y	Not required. Melting point of the TAS is >40 °C. The flash point of TAS was found to be 132 °C.	Acceptable	Kästel, 1998, (CHE2000-470)
B.2.1.13 (IIA 2.13)	Explosive properties of active substance as manufactured	TAS	EEC A 14 (Assesment based on information on active substance)	Y	TAS has no potential for explosivity. This is evident from the structural formula.	Acceptable	Löffler, 1998 (CHE2000-464)
B.2.1.14 (IIA 2.14)	Surface tension	TAS	EEC A 5	Y	71.8 mN/m at 0.5 % (w/w) (20 °C) 71.5 mN/m at 2.0 % (w/w) (20 °C)	acceptable	Kästel, 1998, (CHE2000-470)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.15 (IIA 2.15)	Oxidising properties of active substance as manufactured	TAS	EEC A 17	Y	The study of oxidizing properties indicates that the active substance is not considered to be an oxidizing agent.	acceptable	Löffler, 1998 (CHE2000-464)

B.2.1.16: Summary of data presented under points B.2.1.1 to B.2.1.15

Pyraclostrobin, chemical name (IUPAC) methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate is a new fungicidal active ingredient. It represents a modification of the structure pattern of natural fungicides called strobilurins.
The TAS is a dark brown solid (solidified melt) at room temperature with a moderate aromatic odour, the PAS is a white to light beige odourless crystalline solid with a melting point of 63.7-65.2 °C. The active substance is soluble in water (1.9 mg/L) without dissociation. The solubility in organic solvents is: slightly in n-heptane, moderately in 2-propanol, 1-octanol and olive oil, readily soluble in methanol acetone, ethyl acetate, acetonitrile, dichloromethane and toluene. BF500 F is stable in water at pH 4 - 9. During direct photolysis, a very fast degradation was observed. Five of the metabolites occured ones or several times with amounts >10% of total applied radioactivity.
There are no high flammability, explosivity, or oxidising properties of any concern.

B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2)

Product name: BAS 500 00 F (containing 250 g/l pyraclostrobin, EC)

Table B.2.2-1: Summary of the physical, chemical and technical properties of the plant protection product

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.1.1 (IIIA 2.1)	Appearance: colour	Visual assessment	Dark yellow	Acceptable	Kästel (1997) PHY2000-366
B.2.2.1.2 (IIIA 2.1)	Appearance: odour	Olfactory assessment	Moderate like naphthalene	Acceptable	Kästel (1997) PHY2000-366
B.2.2.1.3 (IIIA 2.1)	Appearance: physical state	Visual assessment	Liquid	Acceptable	Kästel (1997) PHY2000-366
B.2.2.2.1 (IIIA 2.2)	Explosive properties	EEC A 14	Test not conducted because of the chemical structure of the test substance.	Acceptable	Löffler (1997) PHY2000-380
B.2.2.2.2 (IIIA 2.2)	Oxidising properties	EEC A 17	Test not conducted because of the chemical structure of the test substance.	Acceptable	Löffler (1997) PHY2000-380
B.2.2.3.1 (IIIA 2.3)	Flash point	EEC A 9: Pensky Martens tester	98 °C	Acceptable	Kästel (1997) PHY2000-366
B.2.2.3.2 (IIIA 2.3)	Flammability		Not applicable.		
B.2.2.3.3 (IIIA 2.3)	Auto-flammability	EEC A 15	475 °C	Acceptable	Löffler (1997) PHY2000-380
B.2.2.4.1 (IIIA 2.4)	Acidity/alkalinity		Not necessary.		
B.2.2.4.2 (IIIA 2.4)	pH of a 1 % aqueous emulsion	CIPAC MT 75	6.4 at 1 % concentration in CIPAC water D; After accelerated storage for 2 weeks at 54 °C: 6.3 at 1 % concentration in CIPAC water D	Acceptable	Kästel (1997) PHY2000-366

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.5.1 (IIIA 2.5)	Kinematic viscosity		Not applicable. Ultra low volume use is not intended for BAS 500 00 F.	Acceptable	
B.2.2.5.2 (IIIA 2.5)	Dynamic viscosity	OECD 114: Rotational viscosimeter	17.5 mPa·s at 20 °C and D = 100 s^{-1} 8.8 mPa·s at 40 °C and D = 100 s^{-1}	Acceptable	Kästel (1997) PHY2000-366 Kästel (1999) PHY2001-142
B.2.2.5.3 (IIIA 2.5)	Surface tension	EEC A 5: Plate method	50.4 mN/m at 20 °C and 0.025 % concentration 35.8 mN/m at 20 °C and 0.25 % concentration 34.7 mN/m at 40 °C (undiluted)	Acceptable	Kästel (1997) PHY2000-366 Kästel (1999) PHY2001-142
B.2.2.6.1 (IIIA 2.6)	Relative density	EEC A 3: Floating hydrometer, Oscillating density meter, respectively	D_4^{20} = 1.055; D_4^{40} = 1.039; After accelerated storage for 2 weeks at 54 °C: D_4^{20} = 1.0546	Acceptable	Kästel (1997) PHY2000-366 Kästel (1999) PHY2001-142
B.2.2.6.2 (IIIA 2.6)	Bulk (tap) density		Not applicable		
B.2.2.7.1 (IIIA 2.7)	Storage stability	CIPAC MT 46	Physically and chemically stable after storage for 2 weeks at 54 °C. There is less than 0.05 % decrease in the active substance content. The alteration of the observed physical properties (pH range, density, emulsion stability) are negligible.	Acceptable	Kästel (1997) PHY2000-366
B.2.2.7.2 (IIIA 2.7)	Low temperature stability	CIPAC MT 39.1	No sediment after 7 days at 0 °C detectable.	Acceptable	Kästel (1997) PHY2000-366
B.2.2.7.3 (IIIA 2.7)	Shelf-life	GIFAP Monograph 17	Physically and chemically stable for 2 years. There is less than 2 % decrease in the active substance content. The alteration of the observed physical properties (pH range, density, emulsion stability) are negligible.	Acceptable	König (1999) PHY2000-381

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.8.1 (IIIA 2.8.1)	Wettability		Not applicable.		
B.2.2.8.2 (IIIA 2.8.2)	Persistant foaming	CIPAC MT 47.1	Foam after 1 min: 8 ml Foam after 15 min: 7 ml (at a concentration of 0.25 % at room temperature)	Acceptable	Kästel (1997) PHY2000-366
B.2.2.8.3.1 (IIIA 2.8.3)	Suspensibility		Not applicable.		
B.2.2.8.3.2 (IIIA 2.8.3)	Spontaneity of dispersion		Not applicable.		
B.2.2.8.4 (IIIA 2.8.4)	Dilution stability		Not applicable.		
B.2.2.8.5 (IIIA 2.8.5)	Dry sieve test		Not applicable.		
B.2.2.8.6.1 (IIIA 2.8.6)	Particle size distribution		Not applicable.		
B.2.2.8.6.2 (IIIA 2.8.6)	Dust content		Not applicable.		
B.2.2.8.6.3 (IIIA 2.8.6)	Friability and attrition		Not applicable.		

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.8.7.1 (IIIA 2.8.7)	Emulsifiability, emulsion stability and re-emulsifiability	CIPAC MT 36	Initial emulsification: spontaneous. After 30 min standing: no cream, no sediment. After 24 h standing: no cream, no sediment. Re-emulsification after 24 h: spontaneous, uniform emulsion. After 24.5 h: no cream, no sediment. After accelerated storage for 2 weeks at 54 °C: Initial emulsification: spontaneous. After 30 min standing: no cream, no sediment. After 24 h standing: no cream, no sediment. Re-emulsification after 24 h: spontaneous, uniform emulsion. After 24.5 h: no cream, no sediment. (Test temperature: 30 °C. Test concentration: 0.25 % (v/v) in CIPAC water D.)	Acceptable	Kästel (1997) PHY2000-366
B.2.2.8.7.2 (IIIA 2.8.7)	Stability of dilute emulsion		Not applicable		
B.2.2.8.8.1 (IIIA 2.8.8)	Flowability		Not applicable.		

Section (Annex point)	Study	Method	Results	Comment/Conclusion	Reference
B.2.2.8.8.2 (IIIA 2.8.8)	Pourability (rinsability)		Not applicable.		
B.2.2.8.8.3 (IIIA 2.8.8)	Dustability		Not applicable.		
B.2.2.9.1 (IIIA 2.9)	Physical compatibility with other products	ASTM Method E 1518-93	22 different mixtures of BAS 500 00 F with other plant protection products were tested. All of them were determined to be compatible in aqueous tank mixtures. Test substances: BAS 500 00 F and BAS 9075 1 l (Ordoval) or BAS 9116 0 l (Masai) or BAS 9042 4 l (ME 605 Spritzpulver) or BAS 9063 0 l (Insegar) or Mimic or Xen Tari or Ultrazid 40 or Decis or BAS 171 02 l (Metasystox R) or BAS 9007 0 l (Torque) or Kiron or BAS 222 28 F (Polyram WG) or BAS 175 01 F (Kumulus WG) or BAS 9125 0 F (Cuproxat flüssig) or BAS 352 40 F (Ronalin) or BAS 266 04 F (Dithane Ultra) or BAS 9185 0 F (Scala) or BAS 9126 4 F (Folicur EW) or Euparen or BAS 9196 0 F (Switch) or BAS 9272 0 F (Teldor) or BAS 9140 1 F (Botrylon)	Acceptable	Fries (1999) PHY2000-383
B.2.2.9.2 (IIIA 2.9)	Chemical compatibility with other products		There were no indications of chemical reactions between the mixed products.	Acceptable	Fries (1999) PHY2000-383
B.2.2.10 (IIIA 2.10)	Adherence and distribution to seeds		No seed dressing formulation.	Acceptable	

B.2.2.11: Summary and evaluation of data presented under points B.2.2.1 to B.2.2.10 (IIIA 2.11)

BAS 500 00 F is a dark yellow emulsifiable concentrate with a moderate naphthalene like odour. It has neither explosive nor oxidising properties. The flash point is 98 °C. Its pH value of 6.35 ± 0.5 lies within the naturally occurring range. The results of the accelerated storage test and the shelf life test confirm its stability at least for two years under practical and commercial conditions. Its technical properties indicate no particular problems when used as recommended.

B.2.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIA-2.3	Anonymous	2000	Henry´s Law Constant for 304428. 2000/1000171 GLP, unpublished LUF2000-248	Y	BAS
AIIA-2.3	Kästel, R.	1997	Physical Properties Report. 97/10646, PCF 01721 GLP, unpublished LUF2000-247	Y	BAS
AIIA-2.1; AIIA-2.2; AIIA-2.3	Kästel, R.	1997	Physical Properties Report for 304 428. 97/10646 GLP, unpublished CHE2000-469	Y	BAS
AIIA-2.1; AIIA-2.4	Türk, W.	1996	Determination of the appearance, the melting point and thermal conversions of Reg.-No. 304428 (PAI). 96/10327 GLP, unpublished CHE2000-471	Y	BAS
AIIA-2.5	Türk, W.	1996	Spectra of Reg.-No. 304428 (PAI). 96/10955 GLP, unpublished CHE2000-468	Y	BAS

[2] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIA-2.6	Türk, W.	1996	Determination of the solubility of Reg.-No. 304428 in water and buffer systems (pH4, pH7, pH9) at 20°C by column elution method and by HPLC. 96/10939 GLP, unpublished CHE2000-467	Y	BAS
AIIA-2.7	Türk, W.	1996	Determination of the solubility of Reg.-No. 304428 pure active ingredient (PAI) in organic solvents at 20°C. 96/10954 GLP, unpublished CHE2000-466	Y	BAS
AIIA-2.8	Türk, W.	1996	Determination of the Octanol/Water-partition Coefficient of Reg.-No. 304428 by HPLC. 96/10383 GLP, unpublished CHE2000-465	Y	BAS
AIIA-2.9	Anonymous	2000	Physical and Chemical Properties of the Active Substance. not GLP, unpublished WAS2000-499	Y	BAS
AIIA-2.9	Scharf., J.	1999	Hydrolysis of BAS 500 F. 99/10060, 35884 GLP, unpublished WAS2000-352	Y	BAS
AIIA-2.9	Scharf, J.	1997	Determination of the Absorption Coefficients of BAS 500 F. 99/10257, 35889 GLP, unpublished LUF2000-251	Y	BAS
AIIA-2.9; AIIA-7.2.1.2	Scharf, J.	1999	Aqueous Photolysis of BAS 500 F. 1999/11286 GLP, unpublished LUF2000-249	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIA-2.9	Türk, W.	1996	Determination of the solubility of Reg.-No. 304428 in water and buffer systems (pH 4, pH 7, pH 9) at 20°C by column elution method and by HPLC. 96/10939, PCP03797 GLP, unpublished WAS2000-353	Y	BAS
AIIA-2.10; AIIA-7.2.2	Scharf, J.	1999	Photochemical Oxidative Degradation Of BAS 500 F (QSAR Estimates). 99/10086, JS-99-04 GLP, unpublished LUF2000-246	Y	BAS
AIIA-2.4; AIIA-2.12; AIIA-2.14	Kästel, R.	1998	Physical and Chemical Properties Report for PS 304 428. 98/10768 GLP, unpublished CHE2000-470	Y	BAS
AIIA-2.11; AIIA-2.15	Löffler, U.	1998	Safety characteristics of the crop protection product PS 304 428. 98/10734 GLP, unpublished CHE2000-464	Y	BAS
AIIIA-2	Kästel, R.	1997	PHYSICAL AND CHEMICAL PROPERTIES REPORT for BAS 500 00 F. #BASF 97/11398 GLP, unpublished PHY2000-366	Y	BAS
AIIIA-2.2; AIIIA-2.3	Löffler, Dr.U.	1997	Safety characteristics of the crop protection product BAS 500 00 F. #BASF 97/11421 GLP, unpublished PHY2000-380	Y	BAS
AIIIA-2.5; AIIIA-2.6	Kästel, R.	1999	Physical Properties of BAS 500 00 F. 99/10256 GLP, unpublished PHY2001-142	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[2]
AIIIA-2.7	Kästel, R.	1999	SHELF LIFE IN ORGINAL CONTAINER OF BAS 500 00 F Physical Properties Report (24 month-storage). #BASF 99/10561 GLP, unpublished PHY2000-384	Y	BAS
AIIIA-2.7	König, W.	1999	Storage Stability on Orginal Container of BAS 500 00 F 24 month-storage Analytical Results. #BASF 99/10574 GLP, unpublished PHY2000-381	Y	BAS
AIIIA-2.8.7	Kästel, R.	1998	PHYSICAL AND CHEMICAL PROPERTIES of BAS 500 00F. #BASF 98/11196 GLP, unpublished PHY2000-382	Y	BAS
AIIIA-2.9	Fries, Dr.J.	1999	Physical and Chemical Compatibility in Aqueous Tank Mixtures of BAS 500 00 F with other products. #BASF 99/11854 not GLP, unpublished PHY2000-383	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft
BBA: Biologische Bundesanstalt für Land-und Forstwirtschaft

Annex B

Pyraclostrobin

B-3: Data on application
and further information

B.3 Data on application and further information

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 to 3.6)

B.3.1.1 Function

Pyraclostrobin will be used as fungicide.

B.3.1.2 Effects on harmful organisms

Pyraclostrobin is active against fungal development stages both on the plant surface and within the tissues. Pyraclostrobin has a protective as well as an eradicative/curative action. Pyraclostrobin is selective on a wide range of dicotyledonous and monocotyledonous crop species.

B.3.1.3 Field of use

Agriculture (viticulture) and turf

B.3.1.4 Harmful organisms

BAS 500 F affords protection against:

Downy mildew, *Plasmopara viticola* (PLASVI) of grape vine
Powdery mildew, *Unicinula nectator* (UNCINE) of grape vine
Mold, *Microdochium nivale* of turf

B.3.1.5 Mode of action

Pyraclostrobin is a fungicide belonging to the group of strobilurins.The biochemical mode of action of the strobilurins is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) to the cytochrome bc_1 complex (Complex III). This leads to a reduction of energy-rich ATP that is available to support a range of essential processes in the fungal cell.

After foliar application (spraying), pyraclostrobin is absorbed by the plant tissue and – to a limited extent- translocated acropetally.

B.3.1.6 Information on the occurence or possible occurrence of the development of resistance and appropriate management strategies

There is evidence from studies with other inhibitors of the bc_1 complex on the mechanism of resistance in baker's yeast (di Rago et al.,1989) and several none-pathogenic fungi (Kraiczy et al., 1996), that various target site mutations can lead to amino acid substitutions within the cytochrome b protein and that these changes can prevent the binding of a range of mitochondrial electron transport inhibitors to the cytochrome b protein. For plant pathogenic fungi there is not yet any published data on target site mutations that lead to strobilurin resistance, but this is presently a topic of research.

The evidence of resistance to strobilurins comes from cases of field (practical) resistance to strobilurin products shown by the plant pathogens *Blumeria graminis* f. sp. *tritici* and *Sphaerotheca fuliginea.* There is also some evidence of resistance confined to trial plots in the case of *Plasmopara viticola* and *Mycosphaerella fijiensis*. In all the above cases the pathogens have been isolated and found to be resistant to high concentrations of strobilurins indicating that in these cases a disruptive (single step) resistance is involved.

The evidence of resistance described above was found after use of the market strobilurin products kresoxim-methyl or azoxystrobin. Tests for cross resistance between these and other newer strobilurin compounds including pyraclostrobin have consistently shown that cross resistance does occur. In the case of *Plasmopara viticola* cross resistance has also been demonstrated between strobilurins and the oxazolidinedione product famoxadone (FRAC STAR Working Group).

Based on the current evidence the resistance risk assessment for strobilurin fungicides can, at least with some fungi, be moderate to high. The relatively quick appearance of field resistance in some of the above cases indicates that a single-step (also termed disruptive, qualitative or monogenic) resistance is involved.

The published use pattern for strobilurins covered by the FRAC STAR Working Group guidelines for management strategy reflects the resistance risk assessment. These guidelines for strobilurin resistance management strategy can be summarised as follows:

- a limitation of the number of applications according to the crop
- use of the full manufacturer's recommended rate
- preventative applications
- alternation with fungicides from a different cross resistance group
- use of mixtures

The limitations on the number of applications of strobilurins is independent of whether the product is a solo product or a mixture.

B.3.2 Data on application relevant to the plant protection product (Annex IIIA 3)

B.3.2.1 Field of use

Agriculture (viticulture) and turf.

B.3.2.2 Effects on harmful organisms

BAS 500 00 F is a fungicide having protective, curative and eradicative action.

BAS 500 00 F inhibits the spore germination and controls mycelium growth and sporulation.

BAS 500 00 F has a translaminar and a local-systemic action. The stage of spore germination is particularly sensitive to BAS 500 00 F .

B.3.2.3 Details of intended use

BAS 500 00 F is intended to be used in grape vines and on turf. Protection is afforded against:

Downy mildew of grape vine, *Plasmopara viticola* (PLASVI)
Powdery mildew of grape vine, *Unicinula nectator* (UNCINE)
Mold of turf, *Microdochium nivale*

B.3.2.4 Application rate

The application rate of BAS 500 00 F depends on the growth stage of the grape vines. The recommendation for Germany follows:

				Rate of BAS 500 00 F	Spray (water) volume
Growth stage (BBCH)	<	61	:	0.16 l/ha	400 l/ha
Growth stage (BBCH)		61	:	0.32 l/ha	800 l/ha
Growth stage (BBCH)		71	:	0.48 l/ha	1200 l/ha
Growth stage (BBCH)		75	:	0.64 l/ha	1600 l/ha

In steep locations, the application rate is to be increased by up to 25 %.

B.3.2.5 Concentration of active substance in material used

Considering the figures on application rate and spray volume, the concentration of the formulated product BAS 500 00 F in the spray will always be 0.04 %.

The formulation contains 250 g as/L

Accordingly, the concentration of the active substance pyraclostrobin in ready to use spray is 0.1 g as/L spray.

B.3.2.6 Method of application

The intended method of application is spraying by means of each type of spraying equipment which is normally used for applying fungicides in practical viticulture. The diluent is water.

B.3.2.7 Number and timing of applications and duration of protection

The recommended maximum number of applications is three. This is according to the FRAC (= Fungicide Resistance Action Comittee) recommandation of strobilurine fungicides.

The timing of applications is from the first risk of infection until 35 days before harvest.

B.3.2.8 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops

Not relevant, since grape vines and turf are grown in permanent culture.

B.3.3 Summary of data on application

List of uses supported by available data

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grapes	**France**	BAS 500 00 F	F	p+d mildew	EC	250	row, SP	09-85	3	12	0.01	1000	0.100	35	
Grapes	**Germany**	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	11-81	3	12	0.01	400-1600	0.04-0.16	35	
Grapes	**Italy**	BAS 500 00 F	F	p+d mildew	EC	250	row, SP	60-80	3	12	0.01	1000	0.100	35	
Grapes	**Portugal**	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	16-71	3	12	0.01	1000	0.100	35	
Grapes	**Spain**	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	65-81	3	12	0.01	1000	0.100	35	
Turf	**all EU MS**	BAS 500 00 F	F	mold	EC	250	overall,SP		2	14	0.025-0.05	500-1000	0.250		

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.3.4 Further information on the active substance (Annex IIA 3.7 to 3.9)

B.3.4.1 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIA 3.7)

All details with regard to methods and precautions concerning handling, storage, transport or fire are listed in the safety data sheet (Gerlach, 2000).
Advice on critical hazards to man and the environment: irritates the skin, very toxic to aquatic organisms.

B.3.4.1.1 Handling

Information on safe handling:
Avoid inhalation of vapour. To protect against fire and explosion, prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

Exposure controls / personal protection:
Respiratory protection: If gases/vapours are formed wear suitable breathing apparatus.
Hand protection: Chemical resistant gloves.
Body protection: Protective suit, safety shoes.
General safety and hygiene measures: The usual precautions for the handling of chemicals must be observed: no eating, drinking, smoking or snuff-taking at the work place.

B.3.4.1.2 Storage

Stow/store/load separately from food, feed and consumable items. Keep in a dry place.

B.3.4.1.3 Transport

Based on the measured physical/chemical properties and observed ecotoxicological properties standard classifications for transport and labelling requirements according to IMO, IATA and ARD/RDI were assigned for each product. Pyraclostrobin is classified as follows:

<u>Land transport</u>

ADR/RDI	Class: 9	Item number/letter: 12c
Warning panel	Hazard-no: 90	Substance no.: 3077
UN-No.: 3077		

<u>Inland waterway transport</u>

ADN/ADNR	Class: 9	Item number/letter: 12c

<u>Sea transport</u>

IMDG/GGVSee	Class: 9	UN-No: 3077	PG: III
	EMS: -	MFAG: -	

Marine pollutant: yes
Proper technical name: environmentally hazardous substance, solid, n.o.s.
Remarks: -

B.3.4.1.4 Fire-fighting measures

Use foam or dry extinguishing media. In the case of fire, carbon monoxide, oxides of nitrogen, and hydrogen chloride can be generated.
Fire-fighters should be equipped with self-contained breathing apparatus and protective fire-fighting suit.
If the product is involved in fire keep containers cool by spraying with water if exposed to fire. Dispose of fire debris and contaminated extinguishing water in accordance with local regulations. Collect separately contaminated water. Do not allow fire-fighting water to reach sewage or effluent systems.

B.3.4.2 Procedures for destruction or decontamination (Annex IIA 3.8)

Unwanted amounts of pyraclostrobin can be destroyed best by combustion in a licensed incinerator. Decontamination of equipment, packing a.s.o. is achieved by washing with water.

Controlled incineration
The halogen content of pyraclostrobin is below 60 %. Approximately 1100 °C are advised as incineration temperature. Expected combustion products are CO/CO_2, H_2O, N_2/NO_x, and HCl.

Other methods
Combustion in a licensed incinerator is the only disposal recommended, if pyraclostrobin can not be used according to its purpose: the production of fungicides.

B.3.4.3 Emergency measures in the case of an accident (Annex IIA 3.9)

Personal precautions:
Avoid contact with skin, eyes, or clothing.

First aid measures:
General advice, summon medical aid without delay.
If inhaled: Keep patient calm, remove to fresh air. Get medical help.
On skin contact: Wash thoroughly with soap and water.
On contact with eyes: Wash affected eyes for at least 15 min under running water with eyelids held open, consult eye specialist.
On ingestion: Immediately rinse mouth and then drink plenty of water, summon physician.

Environmental precautions:
Prevent product from entering water courses or the ground. Do not let product enter drains.
Incineration of the contaminated undiluted material. In case of contamination of water, the aqueous phase is to be collected and the undissolved of product has to be concentrated by phase separation or by extraction with a suitable extraction solvent. The organic phase should be incinerated too.
The remaining aqueous phase has to be treated with approximately 500 g/L activated carbon for at least 12 hours. The separated activated carbon should be incinerated too. The treated water (pH 6.5 – 9) is to be introduced into a public sewer leading to a public owned wastewater treatment works (POTW) (Gerlach, 2000, CHE2000-462).

B.3.5 Further information on the plant protection product (Annex IIIA 4)

B.3.5.1 Description of packaging

BAS 500 00 F is to be marketed in mold blown high-density polyethylene containers with an inner barrier, e.g., polyamide (PA/PE). They are sealed by foil seals, protected by screw caps of polypropylene.

1 litre bottle:	material:	PA/PE (Coex)
	shape/size:	cylindrical / approx. 88.5 mm diameter x 234 mm
	opening:	42 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
3 litre container:	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 194 mm x 112 mm x 260 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
3 litre container:	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 230 mm x 115 mm x 222 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
5 litre container	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 194 mm x 112 mm x 362 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
10 litre container	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 230 mm x 165 mm x 375 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
10 litre container:	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 226 mm x 174 mm x 368 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal

B.3.5.2 Suitability of packaging

Reference number:	III A 4.1.2 / 1
Report:	Schreiner 1999 EU performance tests: BAS 500 00 F BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep . unpublished Reference: PHY2000-398
Guidelines:	<none>
GLP:	No

The containers and outer packaging meet the ADR requirements. They are labeled individually with all the use instructions. Several bottles are packed in cardboard boxes.
These combination packs meet the requirements of UN 4G/Y.

B.3.5.3 Resistance of the packaging material to its contents

Reference number:	III A 4.1.3 / 1
Report:	Kaestel R. 1999(b) Shelf life in original container of BAS 500 00 F Physical properties report - [24 month-storage] BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. unpublished Reference: PHY2000-384
Guidelines:	Appendix 1 to § 19 a Section 1 Chemikaliengesetz of 25th July 1994
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Reference number:	III A 4.1.3 / 2
Report:	Kolb K. 1997 Corrosiveness of BAS 500 00 F BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. unpublished Reference: PHY2000-570
Guidelines:	<none>
GLP:	No

Storage test indicates no adverse interaction between the preparation and the container.

As evident from a 2-year shelf-life study including the evaluation of any interaction of BAS 500 00 F with the polyamide line polyethylene (Coex) container at 20 °C and 30 °C with 50 % relative humidity there is no potential for adverse interaction between the preparation and the Coex container. Neither the appearance of the product nor its properties are altered by the container material. There is no interaction between the formulated product and its original

container. The commercial product BAS 500 00 F is stable for at least 24 months when stored in the unopened original container under the above mentioned storage conditions.

B.3.5.4 Procedures for cleaning application (Annex IIIA 4.2)

Effectiveness of Prodecures for Cleaning Application Equipment and Protective Clothing
BAS 500 00 is a fungicide to be used for example in grapes at a max. rate of 0.64 l/ha. This amount of formulation contains 160 g Reg. No. 304 428. The product shall be applied in at least 400 litres/ha water. Regarding subsequent use of the spraying equipment, any carry-over would be insignificant according to the following estimate:

- 160 g active substance in 400 l water would result in 0.4 g as per litre as maximum concentration (recommended is a concentration of 0.1 g as per litre).
- Any surplus spray mix is to be diluted at the ratio 1:10 with water and to be sprayed onto the previously treated area, according to the use instructions. After that any remaining spray broth dilution would contain only 0.04 g as per litre water.
- If 5 l dilution should remain in the spraying equipment (an amount above usual relative volumes), it would be diluted during the preparation of the next 400 l spray broth to a remaining as concentration of 0.0005 g as per litre, - only 0.1 % of the original as concentration during crop treatment with BAS 500 00 F.

The calculation outlined above is based on ideal dilution. In reality some active substances tend to adhere preferentially to hydrophobic surfaces of seals or plastic tubing. But there is a large safety margin that plant damage can be excluded when application equipment used for crop treatment with BAS 500 00 F is used subsenquently for treatment of other crops.

Protective clothing for applicators of agrochemicals is usually made of cotton. The polar surface of the fiber presents little affinity to the unpolar active ingredient. Therefore, usual laundering with detergents will either suspend or dissolve any contamination efficiently.

Summary: Common agricultural practice implies cleaning of application equipment with water. This will remove any remainders of BAS 500 00 F so efficiently that no plant damage can be caused when the equipment is used subsequently for the treatment of different crops.
Protective clothing will be cleaned effectively when washed with usual laundry detergents.

B.3.5.5 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIA 4.3)

The following safety intervals as defined in Annex IIIA point 4.3 are adequately covered by information described in chapters mentioned below.

- pre-harvest interval for each relevant crop
  see chapters B.7.4 and B.7.10
- re-entry period for livestock to areas to be grazed
  see chapters B.7.4 and B.7.10
- re-entry period for man to crops, building or spaces treated
  see chapter B.6.14
- withholding period from animal feeding stuffs
  see chapters B.7.4 and B.7.10

- waiting period between application and handling to treated products
  see chapters B.7.4 and B.7.10
- waiting period between last application and sowing or planting succeeding crops
  see chapter B.7.9

B.3.5.6 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIIA 4.4)

Handling:

If BAS 500 00 F is handled while not enclosed:
Use full mask with filter as respiratory equipment.
Use protective gloves for chemicals as hand protection.
Keep work area clean.
Keep working clothes separate from other clothing.
Change badly soaking clothing.
Wash hands before break and at the end of work.

Storage:

No chemical hazards are inherent to BAS 500 00 F. Store out of reach of unauthorized persons. Keep away from food, feed, and consumable item. Store in original container under usual warehouse conditions, i.e. dry, frost free and avoiding temperatures above 40°C. Keep the product away from sources of ignition: no smoking. Provide good ventilation. Store BAS 500 00 F as if it were a water pollutant. Make sure that the product does not enter any drains, watercourses or the ground. In addition for warehouse storage: provide retention facilities.

Transport:

As of today BAS 500 00 F is class 9, No. 11c, UN No. 3082 (RID/ADR, IMDG, IATA) for transport.
Follow the general rules and good practices for transport. Do not stow BAS 500 00 F together with food, feed, and consumable items.

Fire:

BAS 500 00 F is an emulsifiable concentrate (EC). No exceptional fire precautions have to be taken. The main products generated in case of fire are: CO/CO_2, H_2O, N_2/NO_x, SO_2, and HCl.

In case of fire, water, foam-water mixtures, dry powder or carbon dioxide can be used as extinguishing media for BAS 500 00 F. Any packaged product close to a fire needs to be cooled by spraying it with water.
The contaminated extinguishing water is to be collected. It may not reach any sewer or effluent system. The fire debris and the contaminated extinguishing water must be disposed of in accordance with local regulations.

Procedure to minimise generation of waste:

Only purchase and store quantities of BAS 500 00 F required in the short term. Do not open containers larger than necessary for immediate requirements. Do not mix a volume of spray solution greater than is required for immediate use.

B.3.5.7 Emergency measures in case of an accident (Annex IIIA 4.5)

Containment of spillage and decontamination of areas, vehicles, and buildings:

Prevent entry into drains, water, or soil. Large spillage of BAS 500 00 F should be dammed off and pumped into containers. For small spillage, use adsorbent material. Place contaminated adsorbent in closeable containers. Use a damp cloth to clean floors and other objects after removal of the contaminated adsorbent. Adding a detergent will enhance the cleaning process. Place used cleaning materials in closeable receptacles.

Disposal of damaged packaging, adsorbents, and other materials

BAS 500 00 F as well as it's damaged packaging, contaminated adsorbents, and other materials shall be disposed of in a licensed incinerator.
Additional methods are described in the GIFAP monograph "Disposal of unwanted pesticide stocks" 1991. Unclean empty containers are to be treated in that context like full ones.

Protection of emergency workers and bystanders

For emergency workers it is a standard safety precaution that goggles, rubber gloves, mouth and nose mask, and protective clothing shall be worn during the clean-up operations.
Bystanders are requested to leave the emergency site. Only under special circumstances the personal equipment mentioned before is to be provided for bystanders.

First aid measure:

General Advice:
Remove person from danger zone.
Remove contaminated clothing.

Upon Inhalation:
Bring person to the fresh air.
Call medical help.

Following Skin Contact:
Wash skin thoroughly with soap and water.
Call medical help.

Following Eye Contact:
Wash affected eyes for at least 15 minutes under running water with eyelids held open.
Consult an eye specialist.

Upon Ingestion:
Call medical help immediately.

B.3.5.8 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6)

BAS 500 00 F as well as it's damaged packaging, contaminated adsorbents, and other materials shall be disposed of in a licensed incinerator.
Additional methods are described in the GIFAP monograph "Disposal of unwanted pesticide stocks" 1991. Unclean empty containers are to be treated in that context like full ones.

B.3.5.8.1 Neutralisation procedures (e.g. reaction with alkali to form less toxic compounds) for use in the event of accidental spillage

A neutralisation procedure cannot be proposed.

B.3.5.8.2 Pyrolytic behavior of the active substance under controlled conditions at 800°C and the content of polyhalogenated dibenzo-p-dioxins in the products of pyrolysis

Not applicable, as BAS 500 00 F contains less than 60 % halogens.

Detailed instructions for safe disposal of the plant protection products and its packaging

For purposes of disposal, combustion of BAS 500 00 F in a licensed incinerator is recommended. This method of disposal applies also to contaminated packages, which cannot be cleaned or reused.

Although it is possible to incinerate the product at lower temperatures, a combustion at approximately 1100 °C with a residence time of 2 sec. is advised. By doing so, i.e., operating the incinerator according to the conditions laid down in council directive 94/67/EC, one will achieve complete combustion and minimize the formation of undesired by-products in the off-gases.

Empty primary packages of BAS 500 00 F shall be triple rinsed as described in the ECPA "Guidelines for rinsing agrochemical containers", 1993. That reduces the amount of product to below 0.01 % of the original amount. Pressure rinsing or integrated pressure rinsing of the packaging material achieves a similar or better result. The rinsate is to be added to the spray liquid. Triple rinsed primary packages shall be rendered unusable, and then they may be disposed off according to local regulations and best available practice. For up-to-date information, a qualified adviser is to be consulted.

B.3.5.8.3 Methods other than controlled incineration for disposal of the plant protection product, contaminated packaging and contaminated material

As of today, no other methods for disposal of BAS 500 00 F are available.

B.3.6 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[3]
AIIA-3.7; AIIA-3.8	Gerlach, H.	2000	Safety data sheet - BAS 500 F. 2000/1000228 not GLP, unpublished CHE2000-463	N	BAS
AIIA-3.7; AIIA-3.8; AIIA-3.9	Gerlach, H. and Schenk, W.	2000	BAS 500 F : Recommended methods and precautions concerning handling, storage, transport or fire - Procedures for destruction or decontamination - Decontamination of water in case of accident. M-II not GLP, unpublished CHE2000-472	Y	BAS
AIIA-3.9	Schenk, W.	1999	Possible Procedures for the Decontamination of Water from BAS 500 F. 99/10664 not GLP, unpublished CHE2000-462	N	BAS
AIIIA-4	Kolb	1997	# 63 - 20 Corrosivness of BAS 500 00 F. BASF 97/11419 not GLP, unpublished PHY2000-570	Y	BAS
AIIIA-4.1	Schreiner	1999	EU Performance Tests. ID1999/11733 not GLP, unpublished PHY2000-398	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

[3] Only notifier listed

Annex B

Pyraclostrobin

B-4: Proposals for the classification and labelling

B.4 Proposals for the classification and labelling

B.4.1 Proposals for the classification and labelling of the active substance (Annex IIA 10)

The following is proposed in accordance with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

Pyraclostrobin (BAS 500 F)

Hazard symbol:	T; N	
Indication of danger:	Toxic	
Risk phrases:	R 23	Toxic by inhalation
	R 38	Irritating to skin
	R 50/53	Very toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment

Reasons for classification
For justification of R 23 see B 6.2.3 INHALATION
For justification of R 38 see B.6.2.4 SKIN IRRITATION

B.4.2 Proposals for the classification and labelling of preparations (Annex IIIA 12.3 and 12.4)

The following is proposed in accordance with Directive 78/631/EEC in combination with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

BAS 500 00 F

Hazard symbol:	Xn	
Indication of danger:	Harmful	
Risk phrases:	R 20	Harmful by inhalation
	R 22	Harmful if swallowed
	R 36	Irritating to eyes
	R 38	Irritating to skin
	R 50/53	Very toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment
	(R 65	Harmful: may cause lung damage if swallowed)

Reasons for classification

For justification of R 20 see B 6.11.3	INHALATION
For justification of R 22 see B 6.11.1	ORAL
For justification of R 36 see B 6.11.5	EYE IRRITATION

For justification of R 38 see B.6.11.6	SKIN IRRITATION
For justification of R 65 see B.6.13	TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES (The notifier stated that, based on physical properties of the product, the risk phrase R65 from Solvesso should not be transferred to the classification of the product.)

B.4.3 References relied on

No references submitted.

Annex B

Pyraclostrobin

B-5: Methods of analysis

B.5 Methods of analysis

B.5.1 Analytical methods for formulation analysis (Annex IIA 4.1; Annex IIIA 5.1)

B.5.1.1 Analytical method for the determination of pure active substance in the active substance as manufactured

The method uses reversed-phase HPLC with a Nucleosil C18 column UV-detection at 275 nm and external calibration to determine the content in the technical substance. The product is dissolved in acetonitrile/water. The solutions are directly injected into the HPLC system for separation and detection.

Ref.: Tuerk, 1996 (CHE2000-783)

Specificity, linearity, accuracy and repeatability

Specificity: Identification of the active ingredient is based on comparison of the respective HPLC retention time of the reference substance to those of the test substance. No interferences were observed.

Linearity: There is linearity in the specified measuring range. The results do not show any significant deviation from the linearity, i.e., the coefficient of correlation (r) is >0.9999. Typical results for the linear equation were: slope (m) 33925 and intercept (B) 39686.

Accuracy: Based on five replicates of a sample of technical active substance, the recovery of as was in the range 98.16 to 99.20 %.

Repeatability: Based on the analysis of five fortified samples the repeatability (% RSD) was 0.44 %. Five subsamples of a typical technical active ingredient yielded 0.42 % RSD. The acceptance of the results were confirmed by applying the modified Horwitz equation.

Ref.: Tuerk, 1997 (CHE2000-784)

CIPAC methods
To date, no CIPAC methods exist for the analysis of pyraclostrobin in either technical or formulated material.

B.5.1.2 Methods for the determination of significant and/or relevant impurities and additives (e.g stabiliser) in the actice substance as manufactured

Confidential information, see Annex C.

B.5.1.3 Analytical methods for formulation analysis (plant protection product) (Annex IIIA 5.1)

The method uses (normal-phase) HPLC with a Nucleosil CN column UV-detection and external calibration to determine pyraclostrobin in emulsifiable concentrates (BAS 500 00 F). The product is dissolved in n-heptane and small amounts of modifier tetrahydrofuran and dichloromethane. The solution are directly injected into the HPLC system for separation and detection.

Ref.: Ziegler, 1997 (CHE2000-781)

Specificity, linearity, accuracy, repeatability

Specificity: Identification of the active substance is based on comparison of the respective HPLC retention time of the reference substance to those of the test substance. No interferences were observed.

Linearity: There is linearity in the specified measuring range. The results do not show any significant deviation from the linearity, i.e., the coefficient of correlation (r) is >0.9999. Typical results for the linear equation were: slope (m) 198050 and intercept (B) 13994

Accuracy: Based on six replicates of a sample of blank formulation of BAS 500 00 F fortified with reference grade pyraclostrobin, the mean recovery has been determined to be 100.18 %.

Repeatability: Based on the analysis of six samples of typical BAS 500 00 F the repeatability (% RSD) was determined to be 0.085 %.

Ref.: Ziegler, H., 1997 (CHE2000-782)

CIPAC methods
To date, no CIPAC methods exist for the analysis of pyraclotrobin in either technical or formulated material.

B.5.1.4 Method(s) for relevant breakdown products, isomers and additives

General information
Pyraclostrobin does not contain any component of toxicological, ecotoxicological or environmental significance. As the product is stable, this holds true for the product as manufactured and after storage at 20°C for two years as well. Therefore, no respective method is required.

B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.2.1; Annex IIIA 5.2)

B.5.2.1 Plant material

Because no acceptable gas chromatographic results have been achieved (Weeren and Pelz 1999), the application of a standard multi-method seems to be not possible.
In the BASF-method No. 421/0 (Reinhard and Mackenroth, 1999a) pyraclostrobin and its metabolite BF 500-3 are extracted from wheat, grape, peanut and orange matrices with a methanol/water mixture. Peanut nutmeat and oil are extracted with acetonitrile/n-hexane and the acetonitrile layer is separated. Further clean up is carried out using a micro C18-column and a micro silicagel column. The purified residue is taken up in a methanolic buffer solution for HPLC- MS-MS determination monitoring the transition ions m/z = 388 → 194 for pyraclostrobin and m/z = 358 → 164 for metabolite BF 500-3. The independent laboratory validation of this method was performed by Perez and Perez (2000).
For validation data see Table B. 5.2-1

Table B. 5.2-1: Validation data for analytical methods for the determination of pyraclostrobin residues in food of plant origin

Reference	Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Reinhard and Mackenroth , 1999 a	wheat (forage)	Pyraclostrobin	0.02 2.00	95 91	3.0 2.2	5 5
		BF 500-3	0.02 2.00	92 90	3.9 3.5	5 5
	wheat (straw)	Pyraclostrobin	0.02 2.00	80 82	7.4 4.7	5 5
		BF 500-3	0.02 2.00	76 73	6.5 5.5	5 5
	wheat (grain)	Pyraclostrobin	0.02 0.2	75 90	1.7 4.3	4 5
		BF 500-3	0.02 0.2	76 87	3.1 4.1	5 5
	grapes	Pyraclostrobin	0.02 2.00	90 94	2.3 5.5	5 5
		BF 500-3	0.02 2.00	88 93	1.4 7.2	5 5
	peanut (nutmeat)	Pyraclostrobin	0.02 0.2	79 89	4.4 10.0	5 5
		BF 500-3	0.02 0.2	76 85	4.2 8.5	5 5
	orange	Pyraclostrobin	0.02 0.2	76 87	3.9 5.2	5 5
		BF 500-3	0.02 0.2	74 83	5.0 6.3	5 5
Perez and Perez 2000	wheat (straw)	Pyraclostrobin	0.02 6.00	91 86	11.5 4.3	5 5
		BF 500-3	0.02 6.00	81 86	21.9 2.4	5 5
	grapes	Pyraclostrobin	0.02 2.00	87 101	6.7 3.0	5 5
		BF 500-3	0.02 2.00	79 97	4.6 3.0	5 5

In the BASF-method D9904 (Abdel-Baky and Riley, 2000) plant material is extracted as described in BASF-method No. 421/0. For clean up a micro C18-column and a micro silicagel column were used. The purified residue is taken up in an acetonitrile/water mixture.The final chromatography analysis of pyraclostrobin and its metabolite BF 500-3 is performed by HPLC-UV (276 nm) using column switching on reversed phase columns.
The independent laborytory validation of this method was performed by Jordan (2000).
For validation data see Table B.5.2-2

Table B.5.2-2: Validation data for analytical methods for the determination of pyraclostrobin residues in food of plant origin

Reference	Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Abdel-Baky and Riley , 2000	Wheat (Forage)	Pyraclostrobin	0.02 2.00	101 95	4	5 5
		BF 500-3	0.02 2.00	90 87	6	5 5
	Wheat (Straw)	Pyraclostrobin	0.02 2.00	101 85	10	5 5
		BF 500-3	0.02 2.00	102 76	17	5 5
	Wheat (Grain)	Pyraclostrobin	0.02 2.00	88 101	17	5 4
		BF 500-3	0.02 2.00	77 89	19	5 4
	Grapes	Pyraclostrobin	0.02 2.00	112 98	14	5 5
		BF 500-3	0.02 2.00	84 90	15	5 5
	Peanut (nutmeat)	Pyraclostrobin	0.02 2.00	106 102	6	5 5
		BF 500-3	0.02 2.00	80 88	8	5 5
	Orange	Pyraclostrobin	0.02 2.00	99 96	12	5 5
		BF 500-3	0.02 2.00	104 87	15	5 5
Jordan , 2000	Wheat (Straw)	Pyraclostrobin	0.02 6.00	98 78	6.1 9.0	5 5
		BF 500-3	0.02 6.00	92 73	6.8 8.2	5 5
	Grapes	Pyraclostrobin	0.02 2.00	85 91	18.6 5.4	5 5
		BF 500-3	0.02 2.00	75 84	8.2 6.6	5 5

In the BASF-method No. 453/0 (Reinhard and Mackenroth, 1999b) pyraclostrobin and its metabolite BF 500-3 are determinated in marices/fractions of the processing of barley. After extraction with a mixture of methanol, water and hydrochloric acid an aliquot of the extract is centrifuged and partitioned against cyclohexane. After evaporating of cyclohexane, the purified residue is taken up in a methanolic buffer solution for HPLC-MS-MS determination monitoring the transition ions m/z = 388 $\rightarrow$ 194 for pyraclostrobin and m/z = 358 $\rightarrow$ 164 for metabolite BF 500-3. For validation data see Table B.5.2-3

Table B.5.2-3 Validation data for analytical methods for the determination of pyraclostrobin residues in food of plant origin

Reference	Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Reinhard and Mackenroth , 1999 b	beer	Pyraclostrobin	0.02 0.2	96 92	4.5 8.8	5 5
		BF 500-3	0.02 0.2	98 94	7.5 2.3	5 5
	brewer's yeast	Pyraclostrobin	0.02 0.2	102 98	5.3 9.3	4 5
		BF 500-3	0.02 0.2	100 99	1.7 10.1	5 5
	brewing malt	Pyraclostrobin	0.02 0.2	99 94	3.5 3.2	4 5
		BF 500-3	0.02 0.2	89 88	2.1 0.7	5 5
	spent, grains and flocs	Pyraclostrobin	0.02 0.2	105 105	10.6 2.2	5 5
		BF 500-3	0.02 0.2	86 102	5.2 2.0	5 5
	pot barley	Pyraclostrobin	0.02 0.2	103 110	1.4 9.0	5 5
		BF 500-3	0.02 0.2	101 103	2.1 2.9	5 5
	malt sprouts	Pyraclostrobin	0.02 0.2	105 96	5.4 2.8	5 5
		BF 500-3	0.02 0.2	95 94	3.3 2.5	5 5

B.5.2.2 Foodstuff of animal origin

In the BASF method 439/0 (Kampke-Thiel, 1999) pyraclostrobin is extracted from matrices of animal origin with acetonitrile/iso-hexane. The aqueous acetonitrile phase is partitioned into with dichlormethane, the organic layer is concentrated and subjected to a silicagel microcolumn clean up. Pyraclostrobin is then determined by HPLC using column switching on normal phase columns and UV detection at 270 nm. As confirmatory method a reversed phase HPLC-UV determination is described. The independent laboratory validation of this method was performed by Levsen and Kruppa (1999). For validation data see Table B.5.2-4.

Table B.5.2-4 Validation data for analytical methods for the determination of pyraclostrobin residues in food of animal origin

Reference	Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Kampke-Thiel, 1999	milk	Pyraclostrobin	0.01 0.1	77 87	11.5 5.9	5 5
	muscle	Pyraclostrobin	0.05 0.5	89 91	6.1 6.0	5 5
	liver	Pyraclostrobin	0.05 0.5	94 87	4.1 7.2	5 5
	kidney	Pyraclostrobin	0.05 0.5	84 90	5.1 4.2	5 5
	fat	Pyraclostrobin	0.05	86 94	3.2 7.1	4 5
	eggs	Pyraclostrobin	0.05 0.5	86 92	12.3 7.4	5 5
Levsen and Kruppa, 1999	milk	Pyraclostrobin	0.01 0.1	97 91	7.5 2.6	5 5
	meat	Pyraclostrobin	0.05 0.5	89 95	10.8 1.2	5 5

B.5.2.3 Additional methods

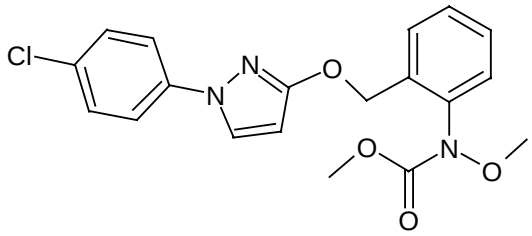
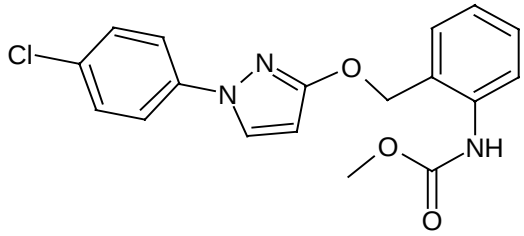
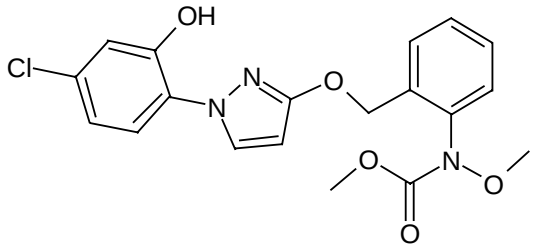
For data generation purposes the method 446 (Tilting, 1999) to determine Pyraclostrobin and its metabolites was developed. For fortification a synthetic reference compound, BF 500-10, was used as a model compound for metabolites of pyraclostrobin. A GC-MS version of this method including hydrolysis and methylation was developed and validated for milk, and a more straightforward procedure using HPLC-MS-MS for quantification was validated for milk and tissues. For validation data see Table B.5.2-5

Table B.5.2-5: Validation data for analytical methods for the determination of pyraclostrobin residues in food of animal origin

Reference	Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Tilting , 1999	milk (GC-MS)	Pyraclostrobin	0.01 0.1	74.8 68.5	3.7 8.5	5 5
	milk (GC-MS)	BF 500-10	0.01 0.1	64.8 64.5	4.6 9.0	5 5
	milk (HPLC-MS/MS)	Pyraclostrobin	0.01 0.1	96.9 66.5	11.6 5.6	5 5
	milk (HPLC-MS/MS)	BF 500-10	0.01 0.1	73.6 60.4	7.6 3.4	5 5
	meat	Pyraclostrobin	0.05 0.5	78.5 83.1	2.1 3.2	5 5
	meat	BF 500-10	0.05 0.5	56.1 54.9	4.4 1.3	5 5
	liver	Pyraclostrobin	0.05 0.5	80.0 81.7	7.6 3.2	5 5
	liver	BF 500-10	0.05 0.5	65.7 82.4	4.1 3.2	5 5
	kidney	Pyraclostrobin	0.05 0.5	85.6 67.6	7.3 5.3	5 5
	kidney	BF 500-10	0.05 0.5	66.2 60.2	4.6 7.7	5 5
	fat	Pyraclostrobin	0.05 0.5	77.4 90.6	14.9 10.5	5 5
	fat	BF 500-10	0.05 0.5	83.4 87.7	24.1 14.7	5 5

B.5.2.4 Structural formulae and designation of compounds

Table B.5.2-6: Summary of compounds used as calibration standards or for fortification

Structural formulae	Designation of compounds
	Pyraclostrobin BAS 500 F Reg. No. 304 428
	BF 500-3 Reg. No. 340266
	BF 500-10 Reg. No. 412 040

B.5.3 Analytical methods (residue) soil, water, air (Annex IIA 4.2. 2 to 4.2.4; Annex IIIA 5.2)

B.5.3.1 Soil

The method No. 409 (Ziegler,1998b) describes the extraction of the analyte pyraclostrobin with acetonitrile using a mechanical shaker. After filtration and concentration, the residue is redissolved in acetonitrile, filtered over a Baker phenyl column and cleaned up further by a gel-permeation chromatography step. For final determination, gradient HPLC-UV (270 nm) is used. For confirmatory purposes, a different HPLC procedure using API-MS at ion m/z = 388 is described. For validation data see Table B.5.3-1

Table B.5.3-1: Validation data for the analytical methods for the determination of pyraclostrobin and metabolites in soil

Reference	Sample matrix	Test substance	Fortific. level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Ziegler , 1998 b	standard soil 2.1	Pyraclostrobin	0.01 0.1 1.0	83 95 92	6.1 3.7 3.9	5 5 5
	standard soil 2.2	Pyraclostrobin	0.01 0.1 1.0	92 93 89	6.2 2.5 3.4	5 5 5

In the method 432 (Zangmeister, 1999b) the analytes pyraclostrobin and the metabolites BF 500-3, BF 500-6 and BF 500-7 are extracted with acetonitrile using a mechanical shaker. After filtration the extract is cleaned up further by sucking the filtrate through a silicagel column and eluting the column with a n-hexane/ethyl acetate mixture. For the final determination, gradient HPLC/API-MS at m/z = 358 (for BF 500-3), 388 (for pyraclostrobin), 595 (for BF 500-7) and 611 (for BF 500-6) is used. For validation data see Table B.5.3-2

Table B.5.3-2 Validation data for the analytical methods for the determination of pyraclostrobin and metabolites in soil

Reference	Sample matrix	Test substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Zangmeister, 1999 b	standard soil 2.2	Pyraclostrobin	0.01 0.1 1.0	99 91 91	2.7 3.3 8.6	5 5 4
		BF 500-3 (340 266)	0.01 0.1 1.0	110 91 86	5.0 2.0 9.1	5 5 4
		BF 500-7 (369 315)	0.01 0.1 1.0	86 91 93	6.9 6.4 4.1	5 5 4
		BF 500-6 (364 380)	0.01 0.1 1.0	83 90 100	4.9 5.4 4.9	5 5 4
	sediment	Pyraclostrobin	0.01 0.1 1.0	91 92 94	1.6 1.2 2.3	5 5 5
		BF 500-3 (340 266)	0.01 0.1 1.0	91 93 96	5.0 0.8 5.8	5 5 5
		BF 500-7 (369 315)	0.01 0.1 1.0	94 103 105	1.3 4.8 1.4	5 5 5
		BF 500-6 (364 380)	0.01 0.1 1.0	96 98 102	5.5 5.4 2.9	5 5 5
	US-soil	Pyraclostrobin	0.01 0.1 1.0	94 87 84	1.4 2.5 6.8	5 5 5
		BF 500-3 (340 266)	0.01 0.1 1.0	96 88 84	1.1 2.9 7.8	5 5 5
		BF 500-7 (369 315)	0.01 0.1 1.0	97 88 87	3.2 3.7 12.4	5 5 5
		BF 500-6 (364 380)	0.01 0.1 1.0	96 88 87	2.8 3.3 3.9	5 5 5

B.5.3.2 Water

The water sample is extracted by sucking through a C_{18} SPE (Baker) column (method No. 415/ Staab, 1998). The column is sucked dry and extracted with acetonitrile. The eluate is evaporated to dryness and dissolved in an acetonitrile/water mixture for final determination by gradient HPLC-MS (API) at m/z = 388 and 390. For validation data see Table B.5.3-3.

Table B.5.3-3: Validation data for analytical methods for the determination of pyraclostrobin residues in water

Reference	Sample matrix	Test substance	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Staab, 1998	tapwater	Pyraclostrobin	0.05	108	3.8	5
			0.5	105	2.8	5
			5.0	104	3.7	5
	leachate water	Pyraclostrobin	0.05	111	5.5	5
			0.5	110	4.5	5
			5.0	108	5.9	5

Enrichment of the active substance and all metabolites is achieved by extraction with ethylacetate in method 455 (Zangmeister, 1999c). The extract is evaporated to dryness and the residues are redissolved in acetonitrile/water. The final analysis of pyraclostrobin and all metabolites is performed by HPLC-MS-MS (pyraclostrobin: m/z = 163, 194; BF 500-11: m/z = 194, 149; BF 500-12: m/z = 278, 194; BF 500-13: m/z = 132, 216; BF 500-14: m/z = 164, 300; BF 500-15 m/z = 132, 135). The determination is considered as highly specific. An additional confirmatory method is not necessary. For validation data see Table B.5.3-4

Table B.5.3-4 Validation data for analytical methods for the determination of pyraclostrobin and metabolites in water

Reference	Sample matrix	Test substance	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Zangmeister, 1999 c	tapwater	Pyraclostrobin	0.05	93	6.1	5
			0.5	104	3.5	5
			5.0	103	4.4	5
		BF 500-11	0.05	80	5.1	5
			0.5	85	10.9	5
			5.0	93	8.2	5
		BF 500-12	0.05	98	4.7	5
			0.5	103	1.3	5
			5.0	104	2.5	5
		BF 500-13	0.05	84	5.0	5
			0.5	84	11.4	5
			5.0	90	4.6	5
		BF 500-14	0.05	103	3.4	5
			0.5	103	4.1	5
			5.0	103	4.0	5
		BF 500-15	0.05	92	9.1	5
			0.5	97	2.1	5
			5.0	93	3.5	5
	surface water	Pyraclostrobin	0.05	91	3.0	5
			0.5	109	7.9	5
			5.0	106	4.0	5
		BF 500-11	0.05	70	11.7	5
			0.5	78	15.5	5
			5.0	88	7.6	5
		BF 500-12	0.05	103	5.1	5
			0.5	109	6.8	5
			5.0	106	5.4	5
		BF 500-13	0.05	76	8.3	5
			0.5	79	16.6	5
			5.0	87	4.8	5
		BF 500-14	0.05	104	4.2	5
			0.5	106	5.5	5
			5.0	99	2.9	5
		BF 500-15	0.05	88	7.1	5
			0.5	93	4.0	5
			5.0	90	8.1	5

B.5.3.3 Air

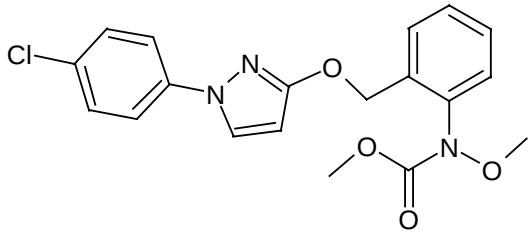
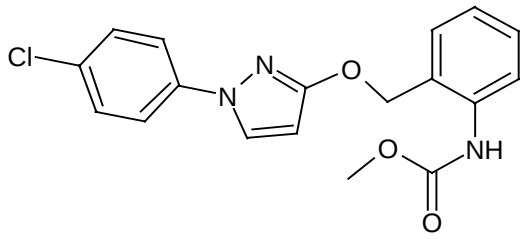
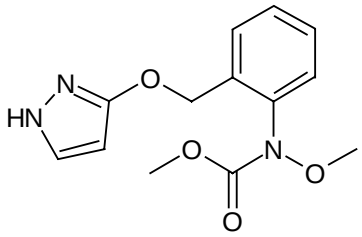
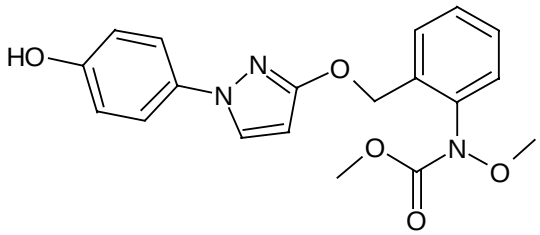
After sampling of approximately 480-600 l air by sucking air (1.5 l/min) for appr. 6 hours through a Tenax absorber tube, the tube is closed with 2 plastic caps and transported to the laboratory for analysis or stored at +6°C (storage stability for 5 days proven).
For analysis, the Tenax adsorbent is extracted with acetone. The solvent is evaporated to dryness and the residue is dissolved in acetonitrile for gradient HPLC-UV determination at 276 nm. For confirmatory purposes LC-MS can be used. For validation data see Table B.5.3-5

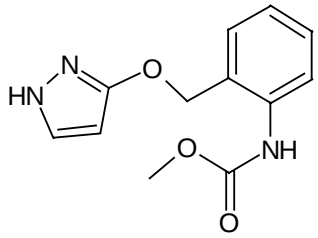
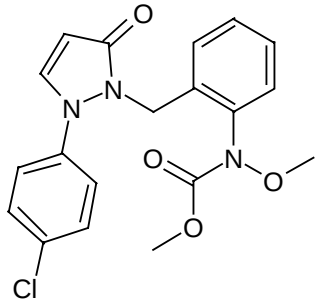
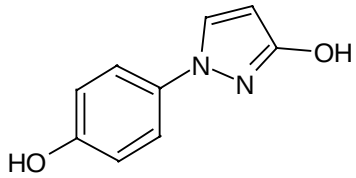
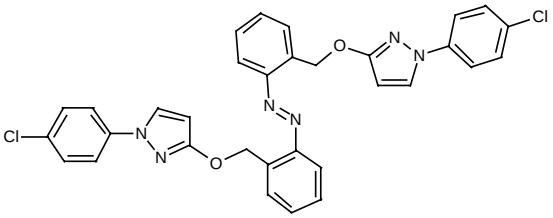
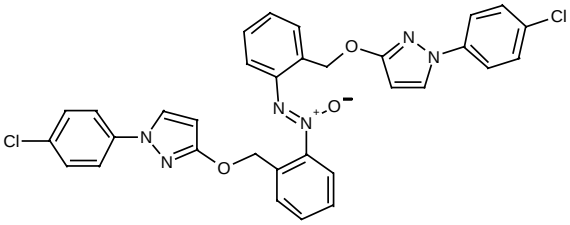
Table B.5.3-5 Validation data for the analytical method for the determination of pyraclostrobin in air

Reference	Sample matrix	Test substance	Fortification level [µg/m^3]	Average recovery [%]	RSD [%]	No. of analyses
Zangmeister , 1999	air	Pyraclostrobin	0.3 3.0	99 98	9.8 3.8	6 6

B.5.3.4 Structural formulae and designation of compounds

Table B.5.3-6: Summary of compounds used as calibration standards or for fortification

Structural formulae	Designation of compounds
	Pyraclostrobin BF 500-F Reg. No. 304 428
	BF 500-3 Reg. No. 340266
	BF 500-11 Reg. No. 411 847
	BF 500-12 Reg. No. 412 053

Structural formulae	Designation of compounds
	BF 500-13 Reg. No. 412785
	BF 500-14 Reg. No. 413 038
	BF 500-15 Reg. No. 377 613
	BF 500-7 Reg. No. 369 315
	BF 500-6 Reg. No. 364 380

B.5.4 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.2.5; Annex IIIA 5.2)

The submission of an analytical methods for the determination of residues in body fluids and tissues is necessary, because pyraclostrobin is classified as toxic.
Analytical methods for the determination of pyraclostobin in liver and kidney are described in chapter **B.5.2.2 Foodstuff ot animal origin.**
Analytical methods for the determination of pyraclostobin in body fluids are not submitted.

B.5.5 Evaluation and assessment

B.5.5.1 Formulation analysis

Analytical methodology is available for the determination of the active substanceand the impurities in the technical material and for the active substance in the EC formulation.

Pyraclostrobin in the technical active substance is determined by a HPLC external standard method on a reversed phase column with UV detection.

10 impurities in the technical active substance are determined by a HPLC method on a reversed phase column with UV detection. The residual solvent is quantified by head space gas chromatography with flame ionisation detection. Another impurity is quantified by solid-phase microextraction coupled to GC/MS.

Pyraclostrobin in the EC formulation is determined by a HPLC external standard method on a normal phase column with UV detection.

All methods are fully validated.

B.5.5.2 Residue analysis

For the assessment of the analytical methods for the determination of pyraclostrobin residues the following criteria were used:

- The submitted methods enable the enforcement of the following relevant residue limits (at the time of evaluation):

plants and plant products	0.05	mg/kg	proposed MRL for other products of plant origin
milk	0.01	mg/kg	proposed MRL
meat, fat, eggs	0.05	mg/kg	proposed MRL
soil	0.05	mg/kg	general limit
drinking water	0.1	µg/L	EU drinking water limit
surface water	3	µg/L	NOEC of Daphnia magna as most sensitive species
air	6	µg/m³	based on a proposed systemic AOEL of 0.02 mg/kg bw

- Mean recovery rates at each fortification level in the range of 70 to 110% with a relative standard deviation of ≤ 20%
- No interfering blanks (< 30% of the LOQ)
- Methods must employ the simplest approach, involve the minimum cost, and require commonly available equipment.
- The enforcement method for food must be suitable for the determination of all compounds included in the residue definition (see 2.4.1), using an additional confirmatory method if appropriate.
- The enforcement methods for environmental matrices must be able to analyse for all compounds of toxicological and/or ecotoxicological significance in soil, water and air (see 2.5.1), using an additional confirmatory method if appropriate.

Methods for the determination of metabolites are not needed, because pyraclostrobin is considered as the only relevant analyte for monitoring purposes.

According to these criteria adequate analytical methods are available for the determination of pyraclostrobin in plant material, food of animal origin, soil, drinking water, surface water and air.
Analytical methods for body fluids are not submitted. Because of the classification of the active substance as T the lack of an appropriate method is considered as an essential data gap.

B.5.6 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
AIIA-4.1	Eisert; R.	1999	Validation of Analytical Method CP 337, Determination of impurities in technical BAS 500 F using HPLC. 1999/11852 GLP, unpublished CHE2000-786	Y	BAS
AIIA-4.1	Eisert; R.	1999	Determination of impurities in technical BAS 500 F using HPLC. 1999/11851 not GLP, unpublished CHE2000-785	Y	BAS
AIIA-4.1	Grosenick, H.	2000	Validation of the Analytical method M 97/0028/02 "Determination of dimethyl sulfate in BAS 500 F". 1999/11956 GLP, unpublished CHE2000-790	Y	BAS
AIIA-4.1	Grosenick, H.	2000	Determination of dimethyl sulfate in BAS 500 F. 1999/11896 not GLP, unpublished CHE2000-789	Y	BAS
AIIA-4.1	Türk, W.	1996	Detemination of toluene in Reg.No. 304 428. 1996/11581 not GLP, unpublished CHE2000-787	Y	BAS

[4] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
AIIA-4.1	Türk, W.	1998	Validation of Headspace-GC Method CP284: Determination of Toluene in RegNo. 304428. 1998/11316 GLP, unpublished CHE2000-788	Y	BAS
AIIA-4.1	Türk, W.	1997	Validation of HPLC-method CP 266: Determination of Reg.No. 304 428 in Reg.No. 304 428 technical active ingredient (TAI). 1997/10691 GLP, unpublished CHE2000-784	Y	BAS
AIIA-4.1	Türk, W.	1996	The determination of Reg.No. 304 428 in technical grade active ingredient by HPLC. 1996/11507 not GLP, unpublished CHE2000-783	Y	BAS
AIIA-4.2.1	Abdel-Baky, S. and Riley, M.	2000	Validation of BASF analytical method D9904, Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC-UV, Study no. 63770. BASF 1999/5179 not GLP, unpublished MET2000-275	Y	BAS
AIIA-4.2.1	Abdel-Baky S., Riley M	2000	Validation of BASF analytical method D9904, method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC/UV. Study No. 63770; Reg. No. 1999/5179 GLP, unpublished RIP2000-1516	N	BAS
AIIA-4.2.1	Jordan, J.	2000	Independent method validation of BASF analytical method D9904 entitled "Method for the determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC-UV". Study No. 63832; Reg. No. 1999/5184 GLP, unpublished RIP2000-1520	N	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
AIIA-4.2.1	Jordan, J.	2000	Independent method validation of BASF analytical method D 9904 entitled "Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using HPLC-UV", Study no. 63832. BASF 1999/5184 not GLP, unpublished MET2000-276	Y	BAS
AIIA-4.2.1	Kampke-Thiel, K.	1999	Validation of BASF method 439/0 for the determination of BAS 500 F (as parent compound) in matrices of animal origin, Study no. 53018. BASF 99/11079 not GLP, unpublished MET2000-279	Y	BAS
AIIA-4.2.1	Levsen, K.	1999	Independent validation of BASF method 439/0 for the determination of BAS 500 F (as parent compound) in matrices of animal origin, Study No. 15 G 99015. BASF 99/11079 not GLP, unpublished MET2000-280	Y	BAS
AIIA-4.2.1	Perez, R. and Perez, S.	2000	Independent method validation of BASF method numbers D9808 (USA) and 421/0 (Germany) entitled "Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using LC/MS/MS", Study no. 63832. BASF 1999/5187 not GLP, unpublished MET2000-274	Y	BAS
AIIA-4.2.1	Reinhard, K. and Mackenroth, C.	1999	Validation of BASF method no. 421/0 (Germany) / D9808 (USA): Determination of BAS 500 F and its metabolite BF 500-3 in wheat, grape, peanut and orange matrices, Study code 35509. BASF 1999/11134 not GLP, unpublished MET2000-273	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
AIIA-4.2.1	Reinhard, K. and Mackenroth, Ch.	1999	Validation of BASF method no. 453/0: Determination of BAS 500 F and its metabolite BF 500-3 in matrices / fractions of the processing of barley, Study no. 35513, 10.12.99. BASF 1999/11135 not GLP, unpublished MET2000-277	Y	BAS
AIIA-4.2.1	Tilting, N. and Lehmann, W.	2000	Validation of analytical method 446 for the determination of BAS 500 F (reg. no. 304428) in sample material of animal origin, Study no. 35636. BASF 1999/11075 not GLP, unpublished MET2000-281	Y	BAS
AIIA-4.2.1	Weeren, R.D. and Pelz, S.	1999	Examination of the applicability of DFG Method S 19 for the determination of BAS 500 F, BAS-9901V. BASF 99/10833 not GLP, unpublished MET2000-278	Y	BAS
AIIA-4.2.2	Zangmeister, W.	1999	Validation of analytical method no. 432, Determination of BAS 500 F, Reg. no. 340266, Reg. no. 369315 and Reg. no 364380 in soil, Study no. 37275. BASF 99/10076 not GLP, unpublished MET2000-283	Y	BAS
AIIA-4.2.2	Ziegler, G.	1998	Validation of analytical method no. 409, Determination of BAS 500 F (parent) in soil, Study no. 35646. BASF 98/10657 not GLP, unpublished MET2000-282	Y	BAS
AIIA-4.2.3	Staab, G.	1998	Validation of analytical method no. 415, Determination of BAS 500 F (parent) in tap and leachate water, Study no. 35886. BASF 98/11182 not GLP, unpublished MET2000-284	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[4]
AIIA-4.2.3	Zangmeister, W.	2000	Determination of BAS 500 F in water by HPLC/UV. BASF 2000/1000133 not GLP, unpublished MET2000-286	Y	BAS
AIIA-4.2.3	Zangmeister, W.	1999	Validation of analytical method 455: Determination of BAS 500 F, BF 500-11, BF 500-12, BF 500-13, BF 500-14 and BF 500-15 residues in water (tap water and surface water), Study no. 35888. BASF 1999/10701 not GLP, unpublished MET2000-285	Y	BAS
AIIA-4.2.4	Zangmeister, W.	1999	Validation of analytical method 447: Determination of BAS 500 F (Reg. no 304428) in air by HPLC/UV, Study no. 35892. BASF 1999/10694 not GLP, unpublished MET2000-287	Y	BAS
AIIIA-5.1	Ziegler, H.	1997	Validation of the analytical method CF-A 535 Determination of Reg.No. 304 428 in emulsifiable concentrates (BAS 500 00 F). 1997/10709 GLP, unpublished CHE2000-782	Y	BAS
AIIIA-5.1	Ziegler, H.	1997	Determination of the content of active ingredient Reg.No. 304 428 in emulsifiable concentrates (EC) [BAS 500 00 F] using HPLC. 1997/11514 not GLP, unpublished CHE2000-781	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Pyraclostrobin

B-6: Toxicology and metabolism

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.6 Toxicology and metabolism 71

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1) 71
B.6.1.1 Absorption, distribution and excretion 72
B.6.1.2 Metabolism 76
B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2) 86
B.6.2.1 Oral 87
B.6.2.2 Percutaneous 88
B.6.2.3 Inhalation 89
B.6.2.4 Skin irritation 90
B.6.2.5 Eye irritation 92
B.6.2.6 Skin sensitisation 93
B.6.3 Short-term toxicity (Annex IIA 5.3) 94
B.6.3.1 Rat 96
B.6.3.1.1 Rat oral 28-day study 96
B.6.3.1.2 Rat oral 90-day study 98
B.6.3.1.3 Rat dermal 28-day study 101
B.6.3.2 Mouse 103
B.6.3.2.1 Mouse oral 90-day study, mouse 103
B.6.3.3 Dog 106
B.6.3.3.1 Dog oral 90-day study 106
B.6.3.3.2 Dog oral 1-year study 108
B.6.4 Genotoxicity (Annex IIA 5.4) 110
B.6.4.1 *In vitro* testing 110
B.6.4.1.1 Gene mutation in bacterial cells 110
B.6.4.1.2 Gene mutation in mammalian cells 112
B.6.4.1.3 *In vitro* cytogenetic test 113
B.6.4.1.4 DNA damage and repair 115
B.6.4.2 In vivo testing 116
B.6.4.2.1 In vivo cytogenetic test 116
B.6.4.2.2 DNA damage and repair 118
B.6.4.3 *In vivo* testing, germ cells 118
B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5) 118
B.6.5.1 Chronic toxicity, rat 119
B.6.5.2 Carcinogenicity, rat 121
B.6.5.3 Carcinogenicity, mouse 123
B.6.6 Reproductive toxicity (Annex IIA 5.6) 124
B.6.6.1 Multigeneration study in rats 126
B.6.6.2 Developmental toxicity 130
B.6.6.2.1 Rat 130
B.6.6.2.2 Rabbit 132
B.6.7 Delayed neurotoxicity (Annex IIA 5.7) 137
B.6.7.1 Neurotoxicity studies 137
B.6.7.1.1 Acute neurotoxicity 137

B.6.7.1.2 Subchronic neurotoxicity 138
B.6.7.2 Delayed Neurotoxicity 140
B.6.8 Further toxicological studies (Annex IIA 5.8) 140
B.6.9 Medical data and information (Annex IIA 5.9) 142
B.6.9.1 Medical surveillance on manufacturing plant personnel 142
B.6.9.2 Direct observation, e.g. clinical cases and poisoning incidents 142
B.6.9.3 Observations on exposure of the general population and epidemiological studies if appropriate 142
B.6.9.4 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests 142
B.6.9.5 Proposed treatment: first aid measures, antidotes, medical treatment 142
B.6.9.6 Expected effects of poisoning 143
B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.10) 143
B.6.10.1 Summary of mammalian toxicology 143
B.6.10.2 Acceptable daily intake (ADI) 147
B.6.10.3 Acute reference dose (ARfD) 148
B.6.10.4 Acceptable operator exposure level (AOEL) 148
B.6.10.5 Maximum acceptable concentration in drinking water 148
B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1) 149
B.6.11.1 Oral 149
B.6.11.2 Percutaneous 151
B.6.11.3 Inhalation 152
B.6.11.4 Skin irritation 155
B.6.11.5 Eye irritation 156
B.6.11.6 Skin sensitisation 157
B.6.11.7 Supplementary studies for combinations of plant protection products 158
B.6.12 Dermal absorption (Annex IIIA 7.3) 159
B.6.12.1 *In vivo* data 159
B.6.12.2 *In vitro* data 161
B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction) 165
B.6.14 Exposure data (Annex IIIA 7.2) 165
B.6.14.1 Operator exposure 165
B.6.14.1.1 Estimation of operator exposure and risk assessment 165
B.6.14.2 Bystander exposure 170
B.6.14.2.1 Estimation of bystander exposure 170
B.6.14.2.2 Measurement of bystander exposure 170
B.6.14.3 Worker exposure 170
B.6.14.3.1 Estimation of worker exposure 170
B.6.14.3.2 Measurement of worker exposure 172
B.6.15 References relied on 172

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Following oral administration of either a single low (5 mg/kg bw) or a high dose (50 mg/kg bw) to rats, pyraclostrobin [Methyl-N-(2-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxymethyl-phenyl)-N-methoxy carbamate; BAS 500 F] was rapidly absorbed from the gastrointestinal tract. However, oral absorption is incomplete and accounts for approximately 50% or even less of the dose. This percentage was estimated by summing up the amount of urinary and biliary excretion.
The elimination process was nearly completed after 120 hours with the major part of radioactivitiy beeing excreted within the first 48 hours irrespective of dose, dosing regimen (single versus repeated administration) or sex. About 11-15% of the applied radioactivity was eliminated via the urine while excretion via the faeces accounted for 80 – 90% of the dose. However, 35% of the radioactivity was actually eliminated from the body via the bile. Initial half-lives were approximately 10 h, terminal half-lives ranged between 20 and 37 h. A comparison of AUC values for both dose levels suggests nearly linear kinetics.
Tissue distribution determination revealed highest amounts of radioactivity in the GI tract, followed by liver. All other tissues had residues similar to or less than the concentrations in plasma. Although this compound is lipophilic, there is no evidence of accumulation. Most likely, this is due to extensive metabolism and rapid and effective excretion.

Dermal absorption rate of pyraclostrobin is poor. The highest value of approximately 2.6% was determined in an *in vivo* study in rats. It was reached when the animals were exposed to the intermediate nominal dose of 0.075 mg/cm^2 (corresponding to 4 mg/kg bw) for an 8-hour period. With regard to operator or bystander exposure, a dermal absorption rate of 2.6% is a clear overestimation since an *in vitro* comparison revealed a much lower penetration through human epidermis than through rat skin. Thus, for sufficient operator protection, a dermal penetration rate of 1% should be used in calculations for exposure scenarios. The dermal absorption studies are reported in detail under section B.6.12.

After oral administration to male and female rats, the systemically available portion of pyraclostrobin was rapidly and intensively metabolised to a large number of biotransformation products. N-demethoxylation was the quantitatively most important pathway. Phase I biotransformation is further characterised by various hydroxylations, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to the large number of observed metabolites. No major differences were observed with regard to sex and dose level.

All metabolites formed in the rat are sufficiently covered in the toxicological studies. Additional studies on isolated metabolites are not considered necessary. However, three main metabolites occurring in water but obviously not in mammals were tested for a mutagenic potential by means of the Ames test and proved all negative (see section B.6.8).

B.6.1.1 Absorption, distribution and excretion

Report: Leibold, E.; Hoffmann, H.D. and Hildebrand, B. (1998)
^{14}C-BAS 500 F - Study of the biokinetics in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 98/10997

Test material: Radiolabelled pyraclostrobin (chlorophenyl ring); batch 579-1101; chemical and radiochemical purity: > 98%.
Radiolabelled pyraclostrobin (tolyl ring); batch 566-1201; chemical and radiochemical purity: > 98%.
Non-radiolabelled pyraclostrobin; batch 00937-67; purity: 99.8%.

Test animals: Wistar rats [strain: Chbb-THOM (SPF); source: Dr. Karl Thomae, Biberach a.d. Riss, Germany].

GLP: Yes.

Test Method: OECD guideline 417 was followed.

Deviations: None. Usually, only low doses are applied when unlabelled test substance is administered on 14 consecutive days. However, no impact on the validity of results is expected when the high dose level is used. Single dose experiments have shown that there is no significant difference between dose levels with regard to absorption, distribution, metabolism and excretion.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The absorption, distribution, elimination and biokinetics of ^{14}C-pyraclostrobin in male and female Wistar rats were investigated at dose levels of 5 and 50 mg/kg bw. Stock solutions of the lipophilic test compound were prepared in toluene with subsequent evaporation of the solvent. The residue was then dissolved in Pluriol E 200 (source: BASF AG) and the solution filled up to the final volume (10 ml/kg bw) with 0.5% Tylose in aqua bidest before it was administered to the rats by oral gavage. Most trials were performed with ^{14}C-pyraclostrobin labelled in the tolyl ring since an additional experiment investigating the balance and excretion pattern of test material (high dose only) radiolabelled in the chlorophenyl ring did not reveal significant differences.
For the excretion balance studies, four male and four female rats were used for each dose level. Excreta were collected after 6, 12 and 24 hours and afterwards in 24 h-intervals until 90% of the administered radioactivity were recovered. Following sacrifice, organs and tissues were examined for remaining radioactivity. A possible impact of repeated administration on toxicokinetics was investigated by daily administration of the high dose (unlabelled material) to four male and four female animals for 14 consecutive days followed by a single low dose of ^{14}C-pyraclostrobin on day 15.
Excretion via the bile was determined using four bile duct cannulated rats per dose and sex. Bile was sampled every three hours up to termination after 48 h.

For the toxicokinetic (plasma concentration) studies, four male and four female rats per dose were used. Blood samples were drawn at ten time points ranging from 30 minutes after dosing up to 120 hours.
Tissue distribution was studied using 12 male and 12 female rats for each dose level. Three animals per sex and dose were sacrificed at four time points (see section ”Findings” below).

Findings:
The stability, homogeneity and correctness of the test substance preparation was analytically verified.

Excretion balance

The overall recovery of radioactivity after 120 hours was in the range of 91.4 - 105.0% in all experiments with the major part beeing excreted via the faeces within the first 48 hours already. Tissue residues were generally very low suggesting that there is no potential for accumulation despite the lipophilicity of this test compound.

Table B.6.1-1: Excretion balance at 48 h post dosing, including biliary excretion

Dose (mg/kg bw) Label Application site Application mode	50 Tolyl ring oral single	5 Tolyl ring oral single	50/5* Tolyl ring oral repeated	50 Chlorophenyl ring oral single
Males				
Urine 0-48	13.23	12.29	13.38	14.87
Faeces 0-48	73.72	91.20	76.34	67.67
Subtotal	86.95	103.49	89.72	82.54
Bile 0-48	36.81	37.72	---	---
Females				
Urine 0-48	10.01	10.93	11.86	10.74
Faeces 0-48	82.36	82.79	77.05	85.07
Subtotal	92.37	93.72	88.91	95.81
Bile 0-48	34.51	35.82	---	---

--- biliary excretion not determined
* repeated administration (14 d) of 50 mg unlabelled material/kg bw followed by a single dose of 5 mg radiolabelled material/kg bw

Table B.6.1-2: Excretion balance at 120 h post dosing

Dose (mg/kg bw) Label Application site Application mode	50 Tolyl ring oral single	5 Tolyl ring oral single	50/5 Tolyl ring oral repeated	50 Chlorophenyl ring oral single
Males				
Urine 0-120	14.52	12.61	13.83	16.01
Cage wash	0.67	0.13	1.30	0.63
Faeces 0-120	81.27	92.04	79.04	74.32
Carcass + organs	0.23	0.19	0.17	0.43
Total	96.68	104.96	94.35	91.38
Females				
Urine 0-120	10.78	11.32	12.29	11.54
Cage wash	0.71	0.60	0.48	1.99
Faeces 0-120	89.92	83.71	81.40	88.95
Carcass + organs	0.22	0.28	0.18	0.46
Total	101.60	95.91	94.33	102.92

During the first 48 hours after single oral dosing with 5 or 50 mg/kg bw, 10 - 13% of the administered radioactivity was excreted in urine and 74 - 91% in faeces. After 120 hours, the total amount of radioactivity excreted in urine was in the range of 11 - 15% and in faeces in the range of 81 - 92% of the administered dose.
Radioactivity remaining in tissues and organs 120 hours post dosing was less than 1 µg eq/g (1 ppm) at a dose level of 50 mg/kg bw and less than 0.1 µg eq/g (0.1 ppm) at a dose level of 5 mg/kg bw.

An excretion pattern as described above was also obtained after repeated oral administration (14 x unlabelled at 50 mg/kg bw, 1 x labelled at 5 mg/kg bw) as well as after single oral administration of 50 mg/kg bw of the chlorophenyl-labelled test substance.

After single oral administration of 50 mg/kg bw, no radioactivity was detectable in the exhaled air with either label used.

Within 48 hours after administration of 5 or 50 mg/kg bw of ^{14}C-pyraclostrobin, about 35 - 38% of the administered radioactivity was excreted via bile indicating that biliary elimination to be an important excretion route. However, as compared to the radioactivity excreted in the faeces 48 hours after single oral administration (about 74 - 91%), the 0-48 hour biliary excretion considerably lower suggesting incomplete absorption following oral intake. It can be assumed that the amount of radioactivity excreted via bile and urine represents the bioavailable amount of the administered dose. Thus, the oral absorption of pyraclostrobin is approximately 50% in male rats and somewhat lower but also at least 45-46% in females.

Pharmacokinetics
In rats exposed to a single oral dose of 50 mg/kg bw of ^{14}C-pyraclostrobin, the plasma concentration/time curve showed 2 peaks. The first plasma peak was reached 30 minutes post dosing with peak levels of 1.96 µg eq/g plasma in males and 2.62 µg eq/g in females. Following a decline, the second plasma peak occurred after 8 hours in males (2.04 µg eq/g) and after 24 hours in females (1.77 µg eq/g), respectively. Thereafter, plasma concentrations declined to levels of 0.08 µg eq/g in males and 0.05 µg eq/g in females at 120 hours post dosing. Plasma levels decreased monophasically with half-lives of 20.7 hours in males and and 19.7 hours in females.

In rats receiving the single low dose of 5 mg/kg bw, the plasma concentration/time curve also showed 2 peaks. The first one was reached at 1.0 hour in males and after half an hour in females with peak levels of 0.432 µg eq/g and 0.537 µg eq/g, respectively. At the second plasma peak occurring after 8 hours in both sexes, plasma levels were 0.458 and 0.353 µg eq/g in males and females, respectively. After this second peak, plasma concentrations declined biphasically to levels of 0.006 µg eq/g in males and 0.005 µg eq/g in females at 120 hours post dosing. The initial half life was found to be 9.0 hours in males and 10.5 hours in females. Terminal half lives in male and female rats were 37.4 and 31.6 hours, respectively.

Table B.6.1-3: Pharmacokinetic parameters

Dose (mg/kg bw)	**5**		**50**	
Sex	**Males**	**Females**	**Males**	**Females**
1st Cmax (µg eq/g)	0.432	0.537	1.96	2.62
1st Tmax (h)	1.0	0.5	0.5	0.5
2nd Cmax (µg eq/g)	0.458	0.353	2.04	1.77
2nd Tmax (h)	8	8	8	24
Initial T1/2 (h)	9.0	10.5	-	-
Terminal T1/2 (h)	37.4	31.6	20.7	19.7
AUC (µg eq x h / g)	9.46	8.74	93.97	66.41
Total clearance (g/min)	8.81	9.54	8.87	12.4

Increasing the dose level by a factor of about 10 resulted in an increase of the AUC-values by a factor of 9.9 in males and 7.6 in females.
At both dose levels, a similar course of the radioactivity with time is found for blood as for plasma. During the first 24 hours post dosing, lower concentrations of radioactivity were found in blood indicating that major parts of the radioactivity are in plasma and not bound to cellular blood constituents.

Tissue distribution
Following the single high dose of ^{14}C-pyraclostrobin, tissue radioactivity concentration was measured 0.5, 24, 36 and 72 hours after dosing. At the low dose level of 5 mg/kg bw, the corresponding radioactivity measurements were done at 0.5, 8, 20 and 42 hours after application. In general, tissue radioactivity levels in both sexes were in the same range at the respective time points and dose levels. The pattern of distribution and elimination in various organs and tissues was also similar. Throughout the time course of the experiments, by far the highest radioactivity concentrations were found in the GI tract in particular in stomach and stomach content. Among the other organs and tissues, highest values were found in the liver. Residues in most other organs and tissues were less than or similar to the plasma levels. Radioactivity concentrations were lowest in bone and brain. Due to the lipophilic properties of the pyraclostrobin, residues in body fat were of particular interest. Towards the end of the observation period, radioactivity in adipose tissue clearly exceeded the plasma concentration. However, with the exception of low dose males, residues were lower than in the liver and a decline was apparent when the actual concentration after 72 or 42 hours was compared to the initial values.

Conclusion:
The excretion balance of pyraclostrobin in rats demonstrates that only approximately 15% or even less of the applied radioactivity is excreted via the urine at 5 and 50 mg/kg bw. Excretion via the faeces accounts for 80 – 90% of the dose. However, 35% of the dose was actually eliminated from the body via the bile. Summing up the amount of urinary and biliary excretion, bioavailability is estimated to be 50% or slightly less.
Oral absorption, although incomplete, is rapid and so is elimination. Initial half-lives are approximately 10 h, terminal half-lives range between 20 and 37 hours. AUC values of both dose levels suggest nearly linear kinetics. Elimination is nearly complete after 120 hours post dosing with the major part of radioactivity being excreted during the first 48 hours.
Tissue distribution determination revealed highest amounts of radioactivity in the GI tract, followed by the liver. All other tissues had values comparable to or less than the plasma concentrations. There is no evidence of a cumulative potential of pyraclostrobin.
The described pattern of absorption, distribution and elimination is not significantly altered by differences in dose level, dosing regimen (single or multiple administration), or sex.

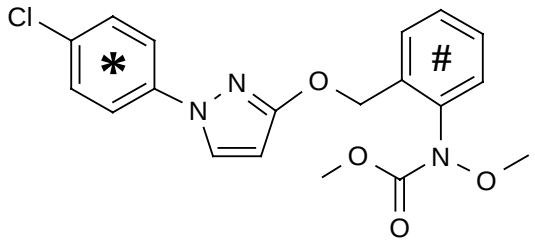
B.6.1.2 Metabolism

Report: Velic, I. (1999):
The Metabolism of ^{14}C-BAS 500F (^{14}C-304428) in Rats
BASF Aktiengesellschaft, Limburgerhof, Germany;
unpublished
BASF RegDoc.# 1999/11781

Dates of experimental work: July 1996 – February 1999

Test Material: Two labelled forms of the test compound were used either in this or in the preceding biokinetic study (see B.6.1.1):
1. [tolyl-^{14}C]-pyraclostrobin; batches 566-1047 (radiochemical purity: >99%, chemical purity: >99.5%), 566-2025 (radiochemical purity: >99%, chemical purity: >97%) and 566-1201 (chemical and radiochemical purity: > 98%).
2. [chlorophenyl-^{14}C]-pyraclostrobin; batches 579-1017 (radiochemical purity: 100%, chemical purity: 99.1%) and 579-1101 (radiochemical purity: >98%, chemical purity: > 98%).

Figure B.6.1-1: Structure and position of the ^{14}C-label for both tolyl and chlorophenyl-labelled pyraclostrobin



= [tolyl-^{14}C]-pyraclostrobin
* = [chlorophenyl-^{14}C]-pyraclostrobin

Furthermore, non-radiolabelled pyraclostrobin (batches 00937-67 and 00937-128, chemical purity: 99.8%) was applied.

Test Animals: Wistar rats (for details, see study description under B.6.1.1 above).

GLP: Yes.

Test Method: EPA/OPPTS 870.7485, EEC 87/302, Japan/MAFF

Deviations: No deviations from the EEC method were noted. The mentioned EPA or Japanese guidelines were not available to the Rapporteur.

Acceptability: The study is considered to be acceptable.

Material and Methods:
A part of the biological samples (urine and faeces; bile) used for identification and characterization of metabolites was taken during the in-life phase of the toxicokinetic study in rats described above (Leibold et al., 1998; see B.6.1.1). Material obtained from the following groups was included:

- Rats (four per sex) receiving a single low dose (5 mg/kg bw) of [^{14}C-tolyl]-pyraclostrobin;
- Rats (four per sex and labelling site) given a single high dose of either [^{14}C-tolyl]- or [^{14}C-chlorophenyl]- pyraclostrobin;
- Four male and four female treated with oral doses of 50 mg/kg bw of unlabelled test material. for 14 consecutive days followed be a single low dose of [^{14}C-tolyl]-pyraclostrobin;
- Bile-fistulated male and female rats receiving either a low (5 mg/kg bw) or high dose (50 mg/kg bw) of [^{14}C-tolyl]- pyraclostrobin.

Furthermore, additional Wistar rats were orally dosed with [^{14}C]-pyraclostrobin as part of the metabolism study. Table B.6.1.4 summarizes the dosing and sampling regimen during the in-life phase. The test material was labelled either in the tolyl- or in the chlorophenyl ring, respectively, and solubilized in 1% carboxymethylcellulose suspension in water containing ca. 10% Cremophor EL.
Ten male and ten female rats were treated at a nominal dose level of 50 mg/kg bw for isolation and identification of metabolites from urine and faeces (groups A and B). Excreta were collected once daily until 96 h after dosing.
Another four rats per sex and labelling site were administered single oral doses of 5 (groups C and D) or 50 mg pyraclostrobin/kg bw (groups E and F) for subsequent analysis of metabolite pattern in plasma, liver and kidneys. Animals were killed and blood and organs removed at 8 hours after dosing, i.e. at or near a presumed peak plasma level.

Table B.6.1-4: Summary of dose groups and analysed samples

Dose group	**A**	**B**	**E**	**F**
Nominal dose level	**50 mg/kg bw**			
^{14}C-label	Tolyl	Chlorophenyl	Tolyl	Chlorophenyl
Samples analysed	Urine, faeces	Urine, faeces	Plasma, liver, kidneys	Plasma, liver, kidneys
Dose group			**C**	**D**
Nominal dose level			**5 mg/kg bw**	
^{14}C-label			Tolyl	Chlorophenyl
Samples analysed			Plasma, liver, kidneys	Plasma, liver, kidneys

After extraction, patterns of radioactive metabolites in excreta (urine, faeces, bile), plasma and tissues were analysed chromatographically. Relevant metabolites were identified by HPLC, MS analysis (LC-MS and GC-MS) and in some cases (whenever sufficient quantities were available) by ^{1}H-NMR analysis of isolated fractions.

Findings:
The amount of radioactivity excreted in urine and faeces (recovery) during the in-life phase of this metabolis study was similar to the findings of the biokinetic study (Leibold et al., 1999). Comparison of samples generated in this study with those obtained from the biokinetic study showed that metabolite patterns were essentially the same.

After oral doses of **[tolyl-^{14}C]-pyraclostrobin** to male and female rats, several metabolites were detected in urine. They were all equivalent to between 0.15 and 2.75% of dose and there was no major metabolite in urine. Out of the eleven metabolites identified in urine eight were cleaved and eight were demethoxylated. Five were present as glucuronides, two as sulphates and four were not conjugated. No parent compound was present.

In faeces, all metabolites were demethoxylated. Little parent compound was detected which could not be separated from the demethoxylated product 500M07 (together 3 - 6% dose). The main metabolite in faeces was pyrazole hydroxylated 500M08 which accounted for 31 - 48% of the dose. Further metabolites in faeces were 500M44 (1.4 - 2.2% of dose), a double hydroxylated product and 500M45 (3.3 - 6.8% of dose), a hydroxylated product. No phase II metabolite was detected in faeces.

Metabolite patterns in bile showed no unchanged parent compound but 13 metabolites out of which 8 were glucuronides. The main metabolite in the bile was 500M46 which is a pyrazole hydroxylated and glucuronidated product (19.8 - 25.6% of dose).

After oral doses of **[chlorophenyl-^{14}C]-pyraclostrobin** to male and female rats, 8 metabolites were detected in urine. They were all equivalent to between 0.59 and 3.7% of the dose and could not be clearly separated from each other. Three metabolites were cleaved and consisted of the chlorophenylpyrazole moiety. Overall, four metabolites were glucuronidated, one sulphated and three not conjugated.

Faeces extracts showed a very similar pattern as in the other label. Besides 500M08 (43.8 - 54.8% of dose), 500M44 (1.8 - 2.9% of dose), 500M45 (ca. 4.1% of dose), 500M07 and parent (together ca. 5.6% of dose) the extracts contained 500M21 as a minor metabolite.

After dosing of both labelled forms, acetonitrile extracts of plasma removed close to the maximum ^{14}C-level in plasma, three glucuronides, namely 500M15, 500M06 and 500M46 as well as unchanged parent.
Extracts of livers showed 500M06 and 500M46 besides unchanged parent compound which was the main constituent.
Extracts of kidneys revealed only unchanged parent compound.

The overall picture is that pyraclostrobin was metabolised by N-demethoxlyation, various hydroxylations, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to the large number of observed metabolites. A summary of the identified metabolites in urine, faeces, and bile can be found in Table B.6.1-5 and B.6.1-6. The corresponding structures are depicted in Table B.6.1.7.

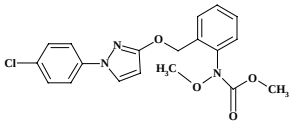
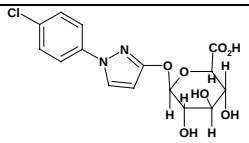
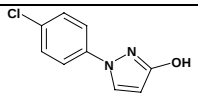
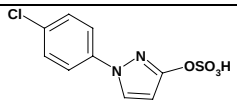
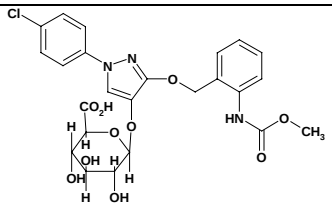
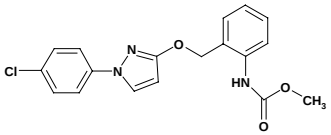
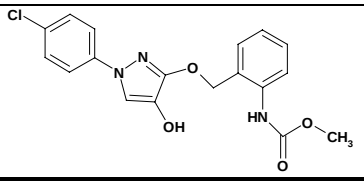
Table B.6.1-5: Identified metabolites in urine, faeces and bile after single high dose administration of [tolyl-^{14}C]-pyraclostrobin (total excretion in % of dose)

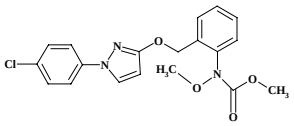
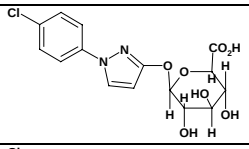
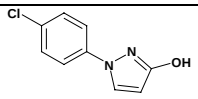
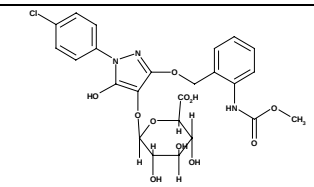
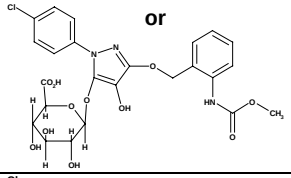
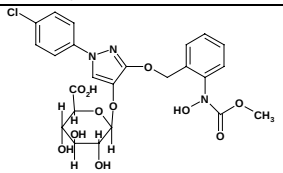
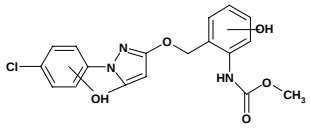
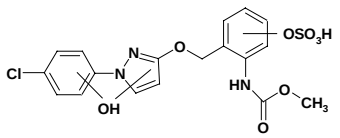
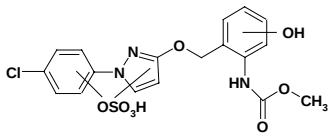
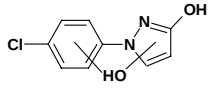
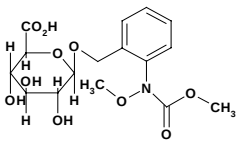
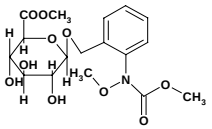
Material	**Urine (0-48 h)**		**Faeces (0-72 h)**		**Bile (0-36 h)**	
Metabolite identity	**male**	**female**	**Male**	**female**	**male**	**female**
500M00/500M07			5.77	3.13		
500M06/500M18/500M19	1.06	0.96				
500M06/500M31/500M32					2.61	2.38
500M08			31.39	47.87		
500M15					1.95	1.23
500M22/500M23	0.77	0.79				
500M24	1.06	1.16				
500M22					2.39	1.05
500M25/500M26	0.75	0.21				
500M29					0.72	0.48
500M30					2.41	2.52
500M33					0.28	
500M34					0.90	-
500M35					1.26	-
500M37/500M38/500M39					0.84	-
500M40/500M48	0.13	0.31				
500M44			1.44	2.19		
500M45			3.34	6.83		
500M46					19.84	25.55
500M51	0.35	0.44				

Table B.6.1-6: Identified metabolites in urine, bile and faeces after single high dose administration of [chlorophenyl-^{14}C]-pyraclostrobin (total excretion in % of dose)

Material	Urine (0-48 h)		Faeces (0-72 h)	
Metabolite identity	**male**	**female**	**male**	**female**
500M00/500M07			5.66	5.69
500M03/500M05	3.70	1.17		
500M04/500M52	1.12	1.22		
500M06/500M08/ 500M13/500M18	0.83	0.59		
500M08			43.82	54.76
500M21		0.54		
500M44			2.91	1.82
500M45			4.17	4.09

Table B.6.1-7: Structures of identified metabolites in rat urine, bile, feaces, plasma, liver, and kidney

Metabolite code	Structure
500M00	
500M03	
500M04	
500M05	
500M06	
500M07	
500M08	

Metabolite code	Structure
500M00	
500M03	
500M04	
500M13	 or 
500M15	
500M18	
500M19	 or 
500M21	
500M22	
500M23	

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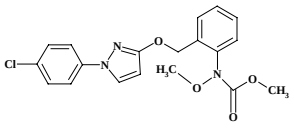
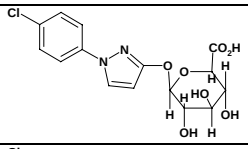
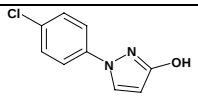
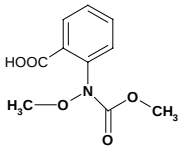
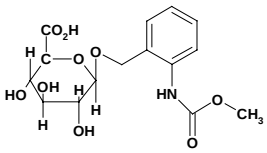
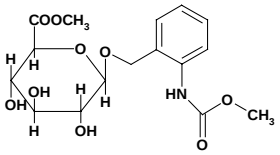
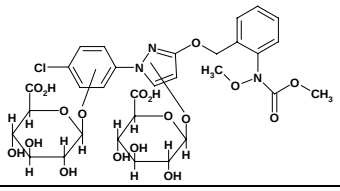
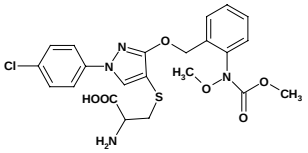
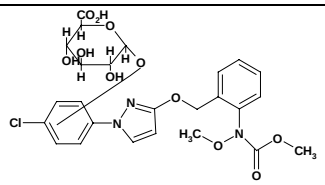
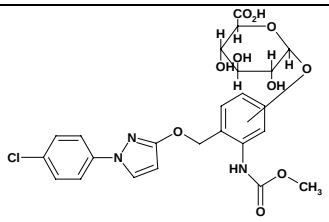
500M00	
500M03	
500M04	
500M24	
500M25	
500M26	
500M29	
500M30	
500M31	
500M32	

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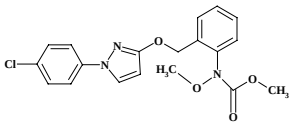
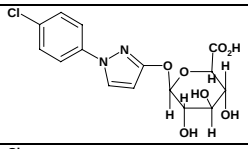
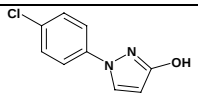
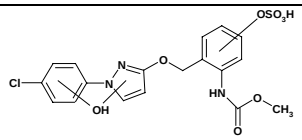
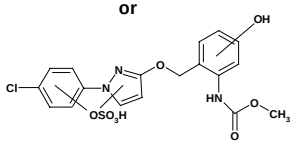
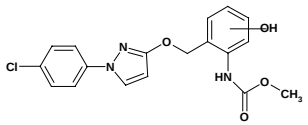
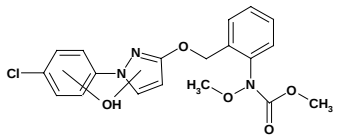
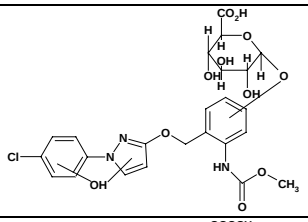
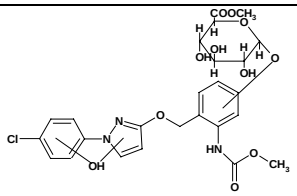
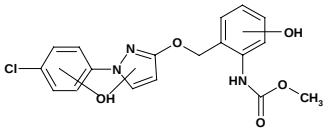
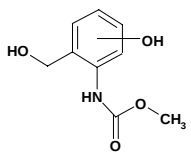
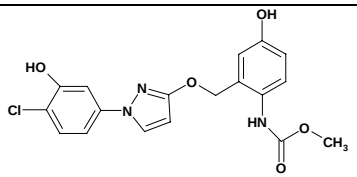
500M00	
500M03	
500M04	
500M33	 or 
500M34	
500M35	
500M37	
500M38	
500M39	
500M40	
500M44	

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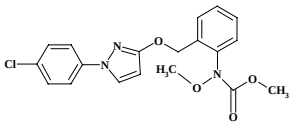
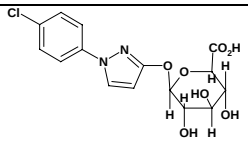
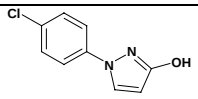
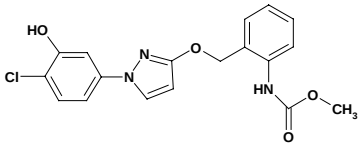
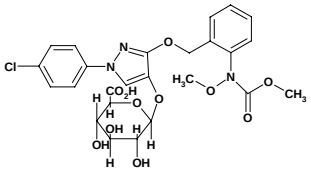
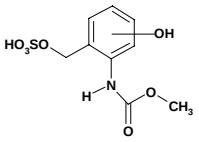
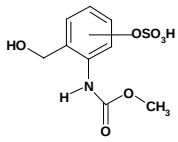
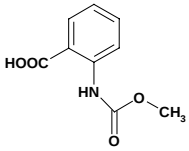
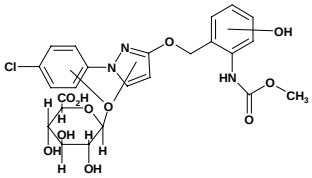
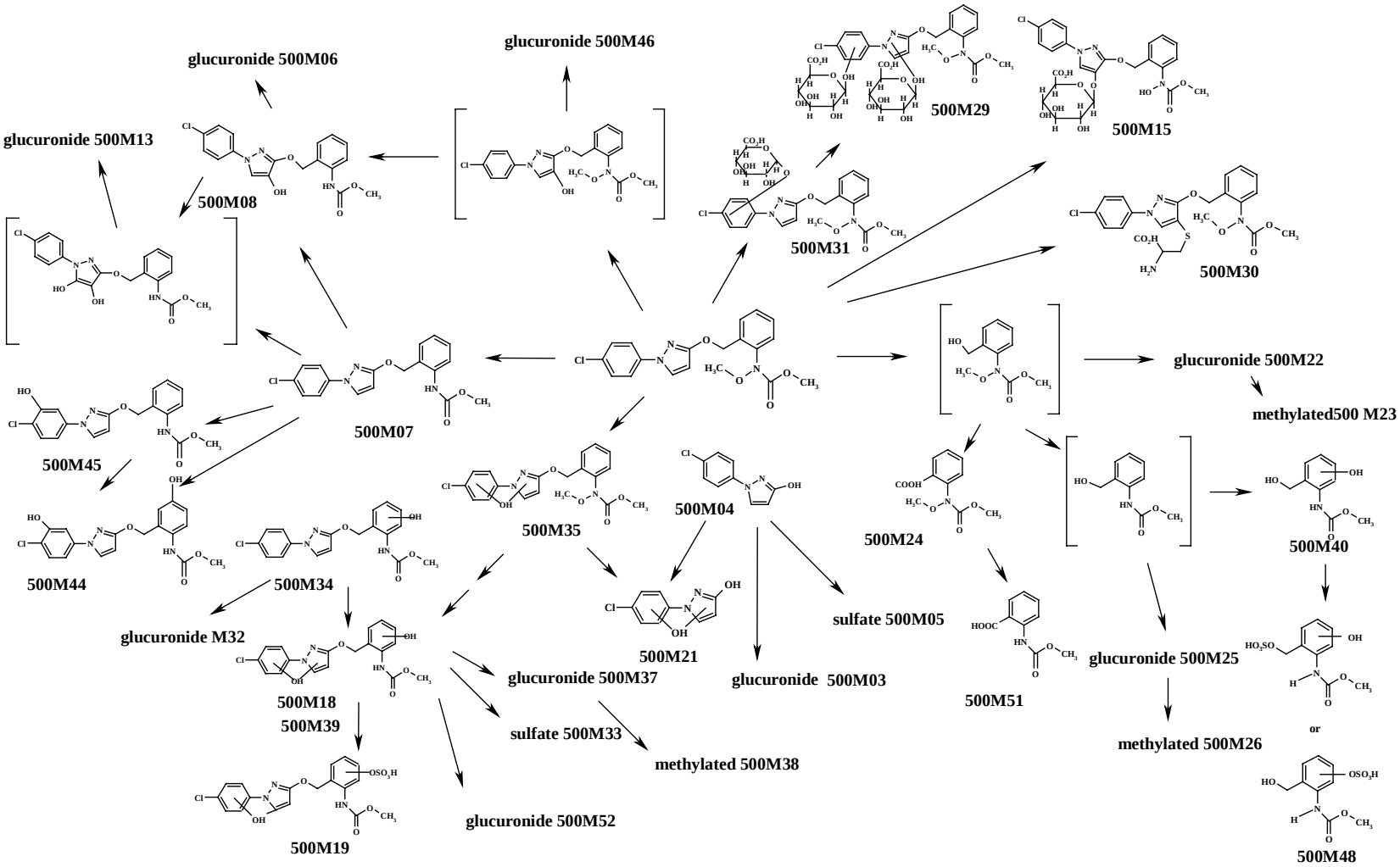
500M00	
500M03	
500M04	
500M45	
500M46	
500M48	 **or** 
500M51	
500M52	

Figure B.6.1-2: Metabolic pathway of pyraclostrobin in rats



Conclusion:
After oral administration to male and female rats, the systemically available portion of pyraclostrobin was rapidly and intensively metabolised to a large number of biotransformation products. N-demethoxylation was the quantitatively most important pathway. Phase I biotransformation is further characterised by various hydroxylations, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to the large number of observed metabolites. No major differences were observed with regard to sex and dose level.

B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2)

In rats, pyraclostrobin is characterized by a low acute oral toxicity. No mortality occurred up to the highest dose of 5000 mg/kg bw although some signs of intoxication were observed.
In mice, in contrast, there are indications of a higher acute oral toxicity coming from a micronucleus test reported under section B.6.4.2.

The acute dermal toxicity appears also low. The LD_{50} in rats was greater than 2000 mg/kg bw with no signs of systemic toxicity occurring.

Clear toxic properties of the active ingredient were noted in the inhalation study including severe symptoms of systemic poisoning and deaths. Classification and labelling (T, R23) is considered necessary, therefore. This requirement is also supported by the toxicity observed in an inhalation study with the formulation (see B.6.11).
As mentioned by the notifier, the difference between the results of the oral and inhalation studies might be based on two factors:
Systemic uptake during inhalation may be more extensive and more rapid than absorption from the GIT after oral administration which was estimated to account for nearly 50% only (see B.6.1). Thus, higher peak plasma levels could be reached after inhalation possibly resulting in the induction of toxic effects not occurring after oral dosing. This factor might have actually contributed to the high inhalative toxicity but, with regard to the whole toxicological profile of pyraclostrobin, is not considered sufficient for explanation. It appears much more likely that the obviously lower toxicity of the orally ingested compound is due to a first-pass effect in the liver where pyraclostrobin is rapidly and quantitatively metabolized. It can be assumed that this extensive biotransformation results in significant detoxification which is lacking after inhalation.
Nonetheless, the actual acute inhalative risk of the test substance is rather low. The active ingredient is a viscous fluid for which inhalation exposure is very unlikely. Its vapour pressure has been determined to be 2.6×10^{-10} hPa, i.e. negligibly low. In the inhalation study, pyraclostrobin had to be dissolved in acetone in order to make an inhalable preparation. For these reasons, the notifier in its dossier stated that classification and labelling were not warranted. However, since this decision must be based on the inherent toxic properties of a chemical, the abovementioned classification is proposed by the Rapporteur. This proposal is in line with the current EU regulation practice.

The compound produced mild skin irritation and should be labelled accordingly (Xi, R38). Pyraclostrobin was not irritant to the eyes. It was not a skin sensitiser in the Maximisation test.

The results of the acute toxicity studies with pyraclostrobin are summarised in Table 6.2-1.

Table B.6.2-1: Acute toxicity of pyraclostrobin

Study type	Species/dose level(s)/test conditions	Comments	Results
Acute oral toxicity	Wistar rat; 2000 and 5000 mg/kg bw	No mortalities, signs of toxicity disappearing within a few days	LD_{50} > 5000 mg/kg bw
Acute dermal toxicity	Wistar rat; 2000 mg/kg bw	No mortality, no convincing signs of systemic toxicity	LD_{50} > 2000 mg/kg bw
Acute inhalation toxicity	Wistar rat; 0.31, 1.07 and 5.3 mg/l air, (4 h), test substance preparation (aerosol) in acetone	100% mortality at 1.07 and 5.3 mg/l; no mortality at 0.31 mg/l but clinical signs disappearing within 7 days	LC_{50} = 0.69 mg/l air (calculation made by the Rapporteur); Toxic by inhalation (T, R 23)
Dermal irritation	New Zealand White rabbit	Mild erythema and edema. Test substance could not be fully removed after exposure period	Irritating (Xi, R38)
Eye irritation	New Zealand White rabbit	Slight to moderate reversible conjunctival irritation	Slightly irritating, classification and labelling according to EU rules not necessary
Skin sensitisation (Maximisation test)	Pirbright White guinea pig	Dermal irritation confirmed	Not sensitising

B.6.2.1 Oral

Report: Wiemann, C. and Hellwig, J. (1998d): Study on the acute oral toxicity of BAS 500 F in rats.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/10965, unpublished.

Test Material: Pyraclostrobin; batch No. CP 025 394; purity: 98.5%.

Test Animals: Young adult Wistar rats [strain: Chbb-THOM (SPF); source: Dr. Karl Thomae, Biberach a.d. Riss, Germany].

GLP: Yes.

Test Method: OECD 401, EEC 92/69, EPA/FIFRA 81-1.

Deviations: None (OECD 401 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Dose levels of 2000 and 5000 mg/kg bw of a test substance preparation in 0.5% aqueous Tylose were singly administered by gavage to five male and five female fasted Wistar rats per

dose group, respectively. The application volume was 10 and 20 ml/kg bw. The observation period lasted for up to 15 days.

Findings:
The stability of the test substance over the study period was proven. The stability of the test substance in the vehicle, the correctness of the concentrations and the homogeneity were analytically confirmed.

There was no mortality neither in male rats nor in females.

Signs of toxicity noted at 2000 and 5000 mg/kg bw included impaired or poor general state, dyspnoea, apathy, staggering, piloerection and diarrhoea in males and females. Smeared fur was observed in a single male of the 5000 mg/kg bw group. All male and female rats were affected by at least some symptoms. There was no clear difference between the dosages with regard to severity of toxic signs. Usually, onset of symptoms was more rapid following administration of the upper dose but all signs disappeared within a few hours already. In contrast, symptoms lasted longer in most animals receiving the low dose. All animals appeared normal within six days after application.

Body weight development was not affected. There were no macroscopic pathological findings at the end of the observation period.

Conclusion:
The oral LD_{50} was found to be > 5000 mg/kg bw for male and female rats. Apparent toxicity was noted in both sexes at the low and high dose levels disappearing within a few days.

B.6.2.2 Percutaneous

Report:	Wiemann, C. and Hellwig, J. (1998e): Study on the acute dermal toxicity of BAS 500 F in rats. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/10966, unpublished.
Test Material:	Pyraclostrobin; batch No. CP 026063; purity: 98.2%.
Test Animals:	Young adult Wistar rats [strain: Chbb-THOM (SPF); source: Dr. Karl Thomae, Biberach a.d. Riss, Germany].
GLP:	Yes.
Test Method:	OECD 402, EEC 92/69, EPA/FIFRA 81-2.
Deviations:	None (OECD 402 considered).
Acceptability:	The study is considered to be acceptable.

Material and Methods:
The test material dissolved in 0.5% aqueous Tylose preparation was applied dermally to five male and five female Wistar rats for 24 hours under semiocclusive dressing at a dose level of 2000 mg/kg bw. The application area was about 50 cm².

Findings:
The stability of the test substance over the study period was proven. The stability of the test substance in the vehicle and its homogeneity was confirmed by analysis.

No mortality occurred during the 14-day post-application observation period and no clinical symptoms were noted. Body weight development appeared to be normal in male rats. In female animals, however, no body weight gain was seen during the first seven days following application and only a small increase in body weight was noted during the second 7-day period thereafter. However, taking into consideration the rather low oral absorption rate (see chapter B.6.1) and the lacking effect on body weight in the acute oral study described above, this finding is rather considered an unspecific reaction to dermal application than a sign of systemic toxicity of the test substance.
One day after application, very slight to well defined erythema, mechanical skin lesion due to adhesive test substance and rests of test substance were observed in all animals.

No pathological findings were detected in the animals at necropsy.

Conclusion:
The dermal LD_{50} was found > 2000 mg/kg bw for male and female animals. Only slight local signs were observed. Possible systemic effects were confined to rather equivocal effects on body weight gain in female rats.

B.6.2.3 Inhalation

Report:	Gamer, A. O. and Hoffmann, H. D. (1997): BAS 500 F - Acute inhalation toxicity study in Wistar rats. 4-hour liquid aerosol exposure. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1997/11472, unpublished.
Test Material:	Pyraclostrobin; batch No. CP 026063; purity: 98.2%.
Test Animals:	Young adult Wistar rats [strain: Chbb-THOM (SPF); source: Dr. Karl Thomae, Biberach a.d. Riss, Germany].
GLP:	Yes.
Test Method:	OECD 403, EPA/FIFRA 81-3, EPA/TSCA 798.1150, EEC 92/69, EEC 93/21.
Deviations:	No significant deviations (OECD 403 considered). Minor deviation: Humidity of air was rather low, in particular in the low dose group. This was probably due to the need to use compressed air for aerosol generation. Because of the relative short exposure time, this deviation is considered not to have affected the validity of the study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Five male and five female Wistar rats per dose level were exposed to a liquid aerosol of the test material for four hours in a head/nose inhalation system at mean (analytical) concentrations of 0.31, 1.07 and 5.3 mg/l. Exposure of the mid and high dose animals was much shorter because all the animals died. The post-treatment observation time was 14 days. To make a preparation to which the animals could be exposed, the test substance had to be dissolved in acetone. The control group was exposed to acetone alone.

Findings:
Pyraclostrobin was demonstrated to be stable when an aerosol had been generated from a solution of the test substance in acetone. The homogenous distribution in the inhalation system has been proven. The particle size distribution revealed a mass median aerodynamic diameter (MMAD) of 1.0 (low dose) – 2.9 µm (high dose) with a geometric standard deviation of 2.5 to 3.0 which is well within the respirable range.

All animals exposed to 1.07 and 5.3 mg/l died during the exposure period. Most of these rats were dead after half an hour of treatment and the last one had died after an exposure time of 75 minutes.
No mortalities occurred in the 0.31 mg/l group. In this low concentration group, clinical examination revealed irregular respiration, bloody nose discharge, piloerection and smeared fur until day 7 p. a. In the mid and high dose level, lethargy and (in the high dose group only) intermittent respiration were additionally noticed.
Body weight development in the low concentration animals was not adversely affected.
Necropsy of the mid (1.07 mg/l) concentration animals showed agonal congestive hyperemia. No macroscopic pathologic findings were noted in animals exposed to the low concentration examined at the end of the study and in the animals being killed by the high dose.

Conclusion:
Pyraclostrobin was of high acute toxicity when an aerosol generated from this test substance was inhaled by rats. The inhalative LC_{50} was reported by the notifier to be > 0.31 and < 1.07 mg/l air (4 h) for males and females. The Rapporteur calculated an LC_{50} of 0.69 mg/l. Thus, the test substance is considered toxic by inhalation and classification and labeling (R 23, T) is required.

B.6.2.4 Skin irritation

Report: Wiemann, C. and Hellwig, J. (1998a): BAS 500 F - Acute dermal irritation/corrosion in rabbits.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/10959, unpublished.

Test Material: Pyraclostrobin; batch No. CP 026063; purity: 98.2%.

Test Animals: White New Zealand rabbits. Young adult animals (SPF) were obtained from the breeder Dr. Karl Thomae (Biberach a.d. Riss, Germany).

GLP: Yes.

Test Method: EEC 92/69, OECD 404, EPA/FIFRA 81-5.

Deviations: No significant deviations (OECD 404 considered). It is stated in the original report that the stability of the test substance over the study period will be subject to reanalysis and reported in the form of an addendum which is actually lacking. However, with regard to the proven homogeneity of the substance and to the outcome of the study, this formal data gap is considered not to alter the validity of the results in any way.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The undiluted test material (0.5 g) was applied dermally to the intact skin of three male and three female White New Zealand rabbits for 4 hours on a 2.5 x 2.5 cm test patch under a semiocclusive dressing. After the patches were removed, the treated area was rinsed with Lutrol® (BASF AG) and Lutrol®/water (1:1). Skin readings were performed at 1, 24 and 48 hours and 7 and 15 days after removal of the patch.

Findings:
The stability of the test substance over the study period was analytically confirmed.

Dermal findings are summarised in the following table:

Table B.6.2-2: Skin irritation values (scores for erythema/edema)

Animal	Time after patch removal						
Number	1 h	24 h	48 h	72 h	8 d	15 d	Mean
1	2/0	2/1	2/0	0/0	0/0	0/0	1.3/0.3
2	2/0	2/1	2/1	2/0	1/0	0/0	2.0/0.7
3	2/0	2/0	2/0	2/0	3/1	2/1	2.0/0.0
4	2/0	2/0	2/0	1/0	1/0	1/0	1.7/0.0
5	2/0	2/1	2/1	2/1	2/1	0/0	2.0/1.0
6	1/0	2/1	2/0	2/0	2/0	0/0	2.0/0.3

The average score (24 to 72 hours) for dermal irritation was calculated to be 1.8 for erythema and 0.4 for edema. In most rabbits, erythema and occasionally also edema extended beyond the area of exposure. Skin findings were not reversible within 15 days in two animals.

The observed irritation might have been, at least partly, induced by the adhesion of the test substance to the skin preventing its complete removal after the exposure period. Therefore, also mechanical irritation may have occurred.

Conclusion:
Pyraclostrobin was irritant to the skin in this study. Classification and labelling (Xi, R 38) is proposed.

B.6.2.5 Eye irritation

Report: Wiemann, C. and Hellwig, J. (1998b): BAS 500 F - Acute eye irritation in rabbits.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/10963, unpublished.

Test Material: Pyraclostrobin; batch No. CP 026063; purity: 98.2%.

Test Animals: White New Zealand rabbits. Young adult animals (SPF) were obtained from the breeder Dr. Karl Thomae (Biberach a.d. Riss, Germany).

GLP: Yes.

Test Method: EEC 92/69, OECD 405, EPA/FIFRA 81-4.

Deviations: No significant deviations (OECD 405 considered). It is stated in the original report that the stability of the test substance over the study period will be subject to reanalysis and reported in the form of an addendum which is actually lacking. However, with regard to the proven homogeneity of the substance and to the outcome of the study, this formal data gap is considered not to alter the validity of the results in any way.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The undiluted test substance was applied once to the conjunctival sac of the right eyelid of one male and five female White New Zealand rabbits. The application volume was about 0.1 ml corresponding to about 33 mg Pyraclostrobin. The test substance was washed out with tap water 24 hours after the application. Readings of the eyes were carried out at 1 hour and 1, 2, 3 and 8 days after the application of the test material. Since no signs of eye irritation were noted at last reading, the experiment was terminated.

Findings:
The stability of the test substance over the study period was analytically confirmed.

Mild eye irritation was observed. The findings (readings after 24, 48 and 72 hours, combined) are summarised in the following table:

Table B.6.2-3: Eye irritation; mean readings and symptoms

			Conjunctiva		
Animal No.	**Opacity**	**Iris**	**Redness**	**Swelling**	**Symptoms**
1	0.0	0.0	1.3	1.0	Loss of hair at margins of eyelids in all animals
2	0.0	0.0	2.0	1.0	
3	0.0	0.0	2.0	0.3	
4	0.0	0.0	2.0	0.3	
5	0.0	0.0	1.3	0.7	
6	0.0	0.0	1.3	0.3	
Mean	0.0	0.0	1.7	0.6	

These findings were reversible within 8 days after application.

Conclusion:
Signs of eye irritating properties of pyraclostrobin were confined to minor conjunctival effects which were not seen after some days any more. Thus, according to EU rules and evaluation practice, classification and labelling for slight reversible eye irritation is not necessary.

B.6.2.6 Skin sensitisation

Report: Wiemann, C. and Hellwig, J. (1998c): BAS 500 F - Maximization test in guinea pigs. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/10964, unpublished.

Test Material: Pyraclostrobin; batch No. CP 029053; purity: 99.0%.

Test Animals: Pirbright White (Dunkin-Hartley) guinea pigs. Young adult SPF animals (strain Crl:(HA)BR) were obtained from Charles River GmbH – WIGA, Kisslegg, Germany.

GLP: Yes.

Test Method: OECD 406, EEC 96/54.

Deviations: None (OECD 406 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for its skin sensitising effect in Pirbright White (Dunkin-Hartley) guinea pigs using the Maximisation test based on the method of Magnusson and Kligman. Twenty female animals were used in the test group and ten female animals in each of two control groups.

Pretest
Because even a 5% test substance preparation in 1% Tylose CB 30,000 in aqua bidest. caused discrete to moderate erythema, the 5% test substance preparation was chosen for the percutaneous induction. A 5% test substance preparation in 1% Tylose CB 30,000 / aqua

bidest. or in Freund's adjuvant / 0.9% NaCl-solution (1:1) was also used for injection. This test substance concentration did not cause systemic toxicity and local reactions were confined to swelling.
Two 24-hour percutaneous occlusive applications within 96 hours were performed. The minimum irritant concentration was found to be a 2% test substance preparation in 1% Tylose CB 30,000 in aqua bidest. The maximum non-irritant concentration was found to be a 1% test substance preparation in 1% Tylose CB 30,000 in aqua bidest. This concentration was considered suitable for challenge application.

Main study
For the main study - performed after the OECD guideline - the following concentrations were selected on the basis of the pretest results:

Intradermal induction	Test substance 5% in 1% Tylose CB 30,000 / aqua bidest. or in Freund's adjuvant / 0.9% aqueous NaCl-solution (1:1)
Percutaneous induction	Test substance 5% in 1% Tylose CB 30,000 / aqua bidest.
1st challenge	Test substance 1% in 1% Tylose CB 30,000 / aqua bidest.
2nd challenge	Test substance 1% in 1% Tylose CB 30,000 / aqua bidest.

A positive control group was not included. However, quality checks of the test procedure using Alpha-Hexylcimmanaldehyde technical 85% as a positive control substance are conducted twice a year in the laboratory.

Findings:
The stability of the test substance over the study period and in the vehicle was confirmed.
Following both intradermal and percutaneous induction, skin irritation was observed in all treated animals. Treatment with vehicle alone caused no dermal irritation. No skin findings occurred after the first and second challenges.

Conclusion:
Pyraclostrobin has no sensitising potential to the skin of the guinea pig in the Maximization Test. The skin irritating potential of this compound was confirmed once more.

B.6.3 Short-term toxicity (Annex IIA 5.3)

The oral short-term toxicity of pyraclostrobin was investigated in dietary 3-month studies in rats, mice, and dogs. Additionally, a 4-week study in rats and a 1-year dog study were performed. The short-term dermal toxicity was examined in a 28-day study in rats. The results of these studies are summarized in Table B.6.3-1.

The signs of toxicity, as observed in the three species tested, were comparable. The critical clinical effects were reduction of body weight and body weight gain in all three species. In dogs, vomitus and diarrhoea occurred additionally. The target organ in all three species was the duodenum, showing mucosal hypertrophy, which was characterised by an increased ratio of cytoplasm to the nuclei in the villi, and by hyperplastic changes in the epithelial cells.

These substance-related effects were associated with changes of several clinical-chemical parameters. The typical findings included a decrease of protein, glucose and triglycerides.

These effects might be associated with diminished vacuolization of hepatocytes as observed histologically.
The increase in serum urea values in mice might be indicative of increased protein catabolism or a slightly impaired renal function. The histopathological examination of the kidneys has shown diminished vacuolisation of proximal tubular epithelial cells.

In all three species investigated, hematological changes with correlating compensatory reactions were observed which were considered indicative of toxic effects on red blood cells. Platelet counts were increased in mice and dogs. Concerning white blood cell parameters, rats demonstrated an increase in white blood cells. In mice, adverse effects on white blood cells as well on lymphatic organs (thymus, mesenteric lymph modes) and the adrenals (decreased vacuolisation of cortical cells) were observed.

Only in the rat liver, an increase of liver cell hypertrophy occurred at high doses. The reduction in liver enzyme activity (alanine aminotransferase, alkaline phosphatase) in both sexes is considered to be of equivocal toxicological significance.

In a 4-week dermal toxicity study in rats, no substance related systemic toxicity was detected up to the highest dose tested (250 mg/kg bw/d).

For rats, a short-term NOAEL of 150 ppm (10.7 mg/kg bw/d) has been established. For dogs, the short-term NOAEL is 200 ppm, equivalent to about 6 mg/kg bw/d, based on the 3-month and 1-year feeding study in this species.
For male mice, the NOAEL in the 3-month study was lower than 50 ppm (9.2 mg/kg bw/d). Taking into consideration all available data for this species, a NOAEL of 30 ppm (ca 4 mg/kg bw/d) can be established for male mice based on the body weight data after 91 days from the carcinogenicity study. For females, the no observed adverse effect level was 50 ppm (12.9 mg/kg bw/d).

Overall, the lowest relevant NOAEL for short-term oral toxicity is 4 mg/kg bw/d, based on the body weight data after 91 days from the carcinogenicity study in mice.

Because of the high acute inhalation toxicity of the compound (see B.6.2.3), the possible need for a subchronic inhalation study could be discussed. However, since the risk for inhalative exposure is considered negligible due to physical properties of the test substance, it is not necessary to require such a study.

Table B.6.3-1: Summary of short-term toxicity studies

Study type / species / dose levels	NOAEL (mg/kg bw/d)	LOAEL / Critical effects
4-week feeding Wistar rat 0, 20, 100, 500, 1500 ppm	9/9.6 m/f [100 ppm]	500 ppm: Effects on body weight, red blood cells, duodenum and liver.
4-week dermal Wistar rat 0, 40, 100, 250 mg/kg bw/d	>250 (systemic)	250 mg/kg bw/d: No systemic toxicity. 40 mg/kg bw/d: Signs of local irritation.
3-month feeding Wistar rat 0, 50, 150, 500, 1000, 1500 ppm	10.7/12.6 m/f [150 ppm]	500 ppm: Reduced body weight and food consumption, effects on clinical-chemical parameters, liver hypertrophy, and mucosal hypertrophy of the duodenum.
3-month feeding B6C3F1 mouse 0, 50, 150, 500, 1000, 1500 ppm	<9.2/12.9 m/f [<50/50 ppm m/f] *(ca. 4; m [30 ppm], carcinogenicity study)*	50 ppm: Reduced body weight (gain) and increased urea values in males. At higher dose levels, adverse effects in the gastrointestinal tract, on red blood cells, on white blood cells and lymphatic organs, as well as on adrenals, liver and kidney.
3-month feeding Beagle dog 0, 100, 200, 450 ppm	5.8/6.2 m/f [200 ppm]	450 ppm: Body weight loss in females, vomitus, diarrhoea, clinical-chemical and hematological changes in females, hypertrophy of the duodenal mucosa.
12- month feeding Beagle dog 0, 100, 200, 400 ppm	5.4/5.4 m/f [200 ppm]	400 ppm: Reduced body weight and food consumption (females); vomitus, diarrhoea; hemoglobin and hematocrit decreased (females); increase of white blood cells and platelets (males).

B.6.3.1 Rat

B.6.3.1.1 Rat oral 28-day study

Report: Mellert, W.; Deckardt, K.; Gembardt, Ch. and Hildebrand, B. 1999(f): BAS 500 F - Repeated dose oral toxicity study in Wistar rats. Administration in the diet for 4 weeks. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11870, unpublished.

Test Material: Pyraclostrobin; batch No. 27882/37/a; purity: 94%; and batch No. 27655/160; purity: 99%.

Test Animals: Wistar rats [Chbb:THOM (SPF), supplied by Dr. Karl Thomae GmbH, Biberach/Riss, FRG].

GLP: Yes.

Test Method: OECD 407, EEC 92/69.

Deviations: None (OECD 407 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 5 male and 5 female Wistar rats at dietary concentrations of 0, 20, 100, 500 and 1500 ppm for 4 weeks.
Food consumption and body weight were determined weekly. The state of health was checked each day. During the weekly weighing, the animals were subjected to an additional comprehensive clinical examination. Clinical-chemical and hematological examinations and urinalysis were carried out towards the end of the administration period. The animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Statistical analysis of the results was based on the Fisher's exact test, the Kruskal-Wallis test (two-sided), the Mann-Whitney U-test (two-sided) and the Wilcoxon test.

Findings:
The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was analytically demonstrated.

Table B.6.3-2: Rat oral 28-day study: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	**Females**
20	1.8	2.0
100	9.0	9.6
500	42.3	46.6
1500	120	126

The clinical, clinical-chemical, hematological, and organ weight findings, which are considered to be test substance related effects are listed in the following tables:

Table B.6.3-3: Rat oral 28-day study: Clinical findings

Parameter	Sex	Dietary dose level (ppm)				
		0	**20**	**100**	**500**	**1500**
Body weight (g)	m	328.8	337.5	336.5	313.1	279.7**
	f	208.2	209.8	209.1	203.8	191.2
Body weight change (g)	m	144.7	153.1	150.8	124.9	97.8**
	f	65.2	67.0	66.3	62.0	53.0
Food consumption	m	24.3	24.4	24.5	21.5	20.1**
(g/animal/day)	f	17.8	17.9	17.5	16.8	15.0**

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02

Body weight, body weight gain and food consumption were marginally affected at 500 ppm and were statistically significantly reduced at 1500 ppm.

Table B.6.3-4: Rat oral 28-day study: Hematological and clinical-chemical findings

Parameter	Sex	Dietary dose level (ppm)				
		0	**20**	**100**	**500**	**1500**
Red blood cells (T/l)	m	8.47	8.12	8.14	8.23	8.22
	f	8.12	7.88	8.11	7.59**	7.35**
Hemoglobin (mmol/l)	m	9.6	9.4	9.6	9.3	8.9
	f	9.5	9.3	9.4	8.8**	8.8**
Prothrombin time (s)	m	28.0	28.2	26.9	28.9**	30.2**
	f	24.9	24.6	24.7	25.7	27.8*
Glucose (mmol/l)	m	8.19	8.50	8.27	7.45	7.33
	f	8.50	8.47	7.78	7.89	6.67**

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02

In females only, a reduction of serum cholinesterase activity was observed at 1500 ppm (57%) and at 500 ppm (18%). In the absence of any effect on erythrocyte and brain cholinesterase and in the absence of any effect in males, this finding is of uncertain toxicological significance but might be indicative of a reduced cholinesterase synthesis in the liver.

Table B.6.3-5: Rat oral 28-day study: Relative organ weight findings

Parameter	Sex	Dietary dose level (ppm)				
		0	**20**	**100**	**500**	**1500**
Liver (% of bw)	m	3.51	3.28	3.32	3.48	4.05*
	f	3.45	3.40	3.34	3.43	4.36**
Spleen (% of bw)	m	0.21	0.25	0.24	0.28	0.35*
	f	0.25	0.24	0.28	0.33*	0.37**

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02

Histopathology revealed the following findings:
- Mucosal hyperplasia in the duodenum at 1500 and 500 ppm.
- Hepatocellular hypertrophy in 4 males and 1 female at 1500 ppm.
- Diminished hepatocellular fat storage at 1500 and 500 ppm.
- Increased extramedullary hematopoiesis in the spleen at 1500 and 500 ppm.

Conclusion:
The no observed adverse effect level (NOAEL) in this 4-week oral rat study was 100 ppm (9 mg/kg bw/d in males and 9.6 mg/kg bw/d in females), based on effects on body weight, red blood cells, duodenum, liver and spleen at 500 ppm and above.

B.6.3.1.2 Rat oral 90-day study

Report: Mellert, W.; Deckardt, K.; Bahnemann, R. and Hildebrand, B. 1999 (a):
BAS 500 F - Subchronic oral toxicity study in Wistar rats. Administration in the diet for 3 months.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/10195, unpublished.

Mellert, W.; Bahnemann, R. and Hildebrand, B. 1999(h):
Amendment No. 1 to the report: BAS 500 F- Subchronic oral toxicity

study in Wistar rats.
Administration in the diet for 3 months.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11899, unpublished.

Test Material: Pyraclostrobin; batch No. CP 025394; purity: 98.5%.

Test Animals: Wistar rats [Chbb:THOM (SPF), supplied by Dr. Karl Thomae GmbH, Biberach/Riss, FRG].

GLP: Yes.

Test Method: EEC 87/302, OECD 408, EPA 82-1, Japan/MAFF.

Deviations: None (OECD 408 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 50, 150, 500, 1000 and 1500 ppm for 3 months.
Food consumption and body weight were determined each week. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week.
Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinical-chemical and hematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA) (two-sided), the Fisher's exact test, the Kruskal-Wallis test (two-sided), the Mann-Whitney U-test (two-sided) and the Wilcoxon test.

Findings:
The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was analytically demonstrated.

Table B.6.3-6: Rat 90-day study: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
50	3.5	4.2
150	10.7	12.6
500	35	41
1000	69	80
1500	106	119

The clinical, clinical-chemical, hematological and organ weight findings which are considered to be test substance related adverse effects are listed in the following tables:

Table B.6.3-7: Rat 90-day study: Clinical findings

Parameter	Sex	Dietary dose level (ppm)					
		0	**50**	**150**	**500**	**1000**	**1500**
Body weight (g)	m	465	470	452	432 *	392 **	344**
	f	248	261	254	242	235	226 *
Food consumption	m	22.6	22.5	22.4	20.7**	19.4**	18.1**
(g/animal/day)	f	16.1	16.8	16.8	15.7	15.5	14.9

Anova + Dunnett's test *p<0.05; **p<0.01

Table B.6.3-8: Rat 90-day study: Hematological findings

Parameter	Sex	Dietary dose level (ppm)					
		0	**50**	**150**	**500**	**1000**	**1500**
White blood cells (G/l)	m	8.41	8.97	8.05	8.92	8.93	9.59
	f	3.90	4.22	4.85	4.69	6.65***	6.55**
Red blood cells (T/l)	m	8.53	8.53	8.79	8.59	8.36	8.22
	f	7.95	7.91	7.95	7.70	7.36***	7.10***
Hemoglobin (mmol/l)	m	9.7	9.5	9.8	9.7	9.5	9.4
	f	9.2	9.3	9.3	9.3	8.7**	8.6***
Reticulocytes (‰)	m	17	17	16	19	24**	33***
	f	14	17	14	13	15	23***
Prothrombin time (s)	m	26.0	26.5	26.4	27.1	28.9***	29.4***
	f	25.6	24.7	25.5	26.1	27.5***	26.3

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002

Table B.6.3-9: Rat 90-day study: Clinical-chemical findings

Parameter	Sex	Dietary dose level (ppm)					
		0	**50**	**150**	**500**	**1000**	**1500**
Total bilirubin (µmol/l)	m	1.69	1.70	1.76	2.20	2.67***	3.29***
	f	2.17	1.93	1.94	1.93	2.69	2.94**
Alanine aminotransferase	m	1.05	1.03	0.91	0.73***	0.68***	0.79***
(µkat/l)	f	0.98	0.77**	0.89	0.78*	0.65***	0.71**
Alkaline phosphatase	m	5.55	5.65	5.94	5.29	4.28**	4.34**
(µkat/l)	f	4.45	4.06	4.38	3.82**	3.83	3.55***
Triglycerides (mmol/l)	m	3.83	3.57	4.43	2.89	1.92**	1.51***
	f	1.61	2.61	2.34	2.16	1.53	2.13
Cholesterol (mmol/l)	m	2.26	2.16	1.94	1.84*	1.67***	1.60***
	f	1.83	2.10	1.82	1.82	1.72	1.79

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002

Corresponding to the results of the 28-day rat study, in this 90-day study a reduction of serum cholinesterase activity was observed in females at 1500 ppm (49%) and at 1000 ppm (40%). In the absence of any effect on erythrocyte and brain cholinesterase and in the absence of any effect in males, this finding is of uncertain toxicological significance but might be indicative of a reduced cholinesterase synthesis in the liver.

Table B.6.3-10: Rat 90-day study: Relative organ weight findings

Parameter	Sex	Dietary dose level (ppm)					
		0	**50**	**150**	**500**	**1000**	**1500**
Liver (% of bw)	m	3.41	3.13	3.11	3.22	3.33	3.74
	f	2.94	3.01	3.01	3.09*	3.34**	3.93**
Spleen (% of bw)	m	0.20	0.20	0.20	0.21	0.26**	0.32**
	f	0.24	0.25	0.25	0.29*	0.34**	0.42**

Kruskal-Wallis + Wilcoxon-Test *p<0.05; **p<0.01

The absolute liver weight were significantly decreased in males at 500 ppm (-13%), 1000 ppm (-18%) and 1500 ppm (-20%). In contrast, there was an increase in absolute liver weights in females at the highest dose group (22%). The relative liver weights of females were increased at 500 ppm and above. Hepatocellular hypertrophy was observed histologically in males and females with incidences of 0/0/0/3/6/10 and 0/0/0/0/4, respectively. The toxicological significance of the reductions in alanine aminotransferase and alkaline phosphatase activities in both sexes is equivocal.

Histopathology revealed the following findings:
- Increased incidences of extramedullary hematopoiesis, histiocytosis and distended sinusoids in the spleen in both sexes at dose levels of 1500 and 1000 ppm.
- Mucosal hyperplasia in the duodenum in both sexes at 1500 ppm, and at 1000 and 500 ppm in males.

Conclusion:
The no observed adverse effect level (NOAEL) in this 3-month rat study was 150 ppm (10.7 mg/kg bw/d in males and 12.3 mg/kg bw/d in females), based on reduction of body weight and food consumption, effects on clinical-chemical parameters, liver hypertrophy, and mucosal hypertrophy in the duodenum at 500 ppm and above. At the two highest dose levels (1000 and 1500 ppm), the oral administration of pyraclostrobin to female rats resulted additionally in leucocytosis and effects on red blood cells with compensatory reactions.

B.6.3.1.3 Rat dermal 28-day study

Report: Mellert, W.; Deckardt, K.; Gembardt, Ch. and Hildebrand, B. 1999 (j): BAS 500 F - Repeated dose dermal toxicity study in Wistar rats. Administration for 4 weeks. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11458, unpublished.

Test Material: Pyraclostrobin; batch No. CP 029053; purity: 99%.

Test Animals: Wistar rats [Chbb:THOM (SPF), supplied by Boehringer Ingelheim Pharma KG, FRG].

GLP: Yes.

Test Method: EEC 92/69, OECD 410, EPA/OPPTS 870.3200.

Deviations: None (OECD 410 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 10 male and 10 female Wistar rats by dermal route (6 hours/day; 5 days/week; semiocclusive dressing) for 4 weeks at doses of 0 (vehicle control: 0.5% aqueous CMC solution), 40, 100 and 250 mg/kg bw/d.
Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Additionally, clinical examinations were carried out before daily treatment. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinical-chemical and hematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA, two-sided), the Fisher's exact test, the Kruskal-Wallis test (two-sided), the Mann-Whitney U-test (two-sided) and the Wilcoxon test.

Findings:
The stability of the test substance in the vehicle was verified. Homogeneity analysis of the mixtures was performed prior to the start of the study, in the first week and at the end of the administration period. The correctness of the concentrations was analytically demonstrated.

No signs of systemic toxicity were observed at any dose level.

Dose related signs of local irritation were observed at all dose levels in the area of the treated skin:

High dose (250 mg/kg bw/d):

- Focal scale formation (1 male and 3 females), multifocal scale formation (2 males and 6 females), diffuse scale formation (1 male and 9 females) and/or slight erythema (7 females).
- Epidermal thickening (all males and females) and hyperkeratosis (9 males and all females).

Mid dose (100 mg/kg bw/d):

- Multifocal scale formation (1 female) and diffuse scale formation (2 females).
- Epidermal thickening (9 males and all females) and hyperkeratosis (5 males and 4 females).

Low dose (40 mg/kg bw/d):

- Epidermal thickening in 5 females.

Thus, pathology revealed treatment related lesions in the area of the treated skin, only. Epidermal thickening was a consistent finding in all treated groups of female rats, showing dose-response relationship with respect to the number of animals affected and with respect to the graded severity of this finding. In males, epidermal thickening was noted in the control

and low dose group at a low incidence, whereas in the mid and high dose groups, incidence and graded severity were comparable to the females. Hyperkeratosis accompanied epidermal thickening in the mid and the high dose groups.
The superficial location of the lesions in the epidermis, the only minimal or slight grades of severity, the absence of necrotic cells within the epidermis and the absence of any inflammatory reaction in epidermis and/or subcutis are indicative for a transient, reversible alteration.

Conclusion:
After dermal administration of pyraclostrobin for 4 weeks to rats, the no observed adverse effect level (NOAEL) for systemic toxicity for both sexes was 250 mg/kg bw/d, the highest dose tested.
Signs of dermal irritation occurred in all treated animals, depending on dose level.

B.6.3.2 Mouse

B.6.3.2.1 Mouse oral 90-day study, mouse

Report: Mellert, W.; Deckardt, K.; Küttler, K. and Hildebrand, B. 1998: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl BR mice. Administration in the diet for 3 months. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/11345, unpublished.

Mellert, W.; Küttler, K. and Hildebrand, B. 1999(i): Amendment No. 1 to the report: BAS 500 F- Subchronic oral toxicity study in B6C3F1 Crl BR mice. Administration in the diet for 3 months. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11900, unpublished.

Test Material: Pyraclostrobin; batch No. CP 025394; purity: 98.5%.

Test Animals: B6C3F1 Crl BR mice (supplied by Charles River GmbH).

GLP: Yes.

Test Method: EEC 87/302, OECD 408, EPA 82-1, Japan/MAFF.

Deviations: Food efficiency data were not reliable because of food spilling in all groups irrespective of the dose. Therefore, uncertainties in the calculation of food intake and the test substance intake must be assumed.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 10 male and 10 female B6C3F1 Crl BR mice at concentrations of 0, 50, 150, 500, 1000 and 1500 ppm in the diet over a period of 3 months.
Food consumption and body weight were determined once a week. Water consumption was checked daily. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpation of the animals were performed once a week. Clinical-chemical and hematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examination.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA) (two-sided), the Kruskal-Wallis test (two-sided), the Mann-Whitney U-test (two-sided) and the Wilcoxon test.

Findings:
The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was analytically demonstrated.

Table B.6.3-11: Mouse oral 90-day study: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
50	9.2	12.9
150	30.4	40.4
500	119	162
1000	274	374
1500	476	635

No test substance related clinical signs or mortality occurred.

The clinical, clinical-chemical, hematological and organ weight findings which are considered to be test substance related adverse effects are listed in the following tables:

Table B.6.3-12: Mouse oral 90-day study: Clinical findings

Parameter	Sex	Dietary dose level (ppm)					
		0	50	150	500	1000	1500
Food consumption day 91 (g/animal/day)	m	5.7	5.0	5.7	6.0	5.9	7.0
	f	6.2	6.4	6.3	7.2	6.8	7.9
Body weight day 91 (g)	m	36.0	33.5	31.9**	29.0**	26.4**	23.8**
	f	26.5	26.2	25.8	23.4**	22.1**	20.0**
Body weight change day 77 (g)	m	10.7	8.2*	7.2**	4.2**	2.1**	-0.6**
	f	7.3	6.2	5.6*	3.8**	2.3**	1.3**

Anova + Dunnett's test *p<0.05; **p<0.01

During the study there was spilling of food by mice in all groups, irrespective of the dose, and therefore the food consumption values do not represent the exact food intake. But the estimate of the food consumption has shown, that there were some statistically significantly increased values for food intake in males and females at the two highest dose levels.

Body weight and body weight change at day 91 were statistically significantly decreased in males at 150, 500, 1000 and 1500 ppm and in females at 500, 1000 and 1500 ppm. At 50

ppm, there were slight, not statistically significantly decreases in terminal body weights in males (-8.1%). Decreases in body weight change in males at 50 ppm amounted to ca. -20% after 90 days and were temporally, e.g. after 77 days, statistically significant (-23.4%).

Table B.6.3-13: Mouse oral 90-day study: Hematological findings

Parameter	Sex	Dietary dose level (ppm)					
		0	50	150	500	1000	1500
Hemoglobin (mmol/l)	m	11.8	11.6	11.6	11.4	11.4	10.6***
	f	11.4	11.5	11.2	11.0	10.9**	10.4**
Hematocrit (l/l)	m	0.572	0.564	0.551*	0.542**	0.543**	0.518**
	f	0.519	0.531	0.517	0.516	0.513	0.495
White blood cells (G/l)	m	5.92	5.56	5.20	6.36	2.72***	2.67***
	f	6.04	5.15	4.10	3.72	3.25	3.17

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002

Hematological examination revealed adverse effects on red blood cells (reduction in hemoglobin and hematocrit) and white blood cells (leukopenia).

Table B.6.3-14: Mouse oral 90-day study: Clinical-chemical findings

Parameter	Sex	Dietary dose level (ppm)					
		0	50	150	500	1000	1500
Triglycerides (mmol/l)	m	1.70	1.64	1.15**	0.78***	.0.59***	0.47***
	f	1.53	1.22	0.96*	0.58***	0.59***	0.58***
Total protein (g/l)	m	63.89	67.56**	66.66**	64.88	61.42*	56.97***
	f	60.00	62.16	61.69	59.67	55.73***	55.22***
Urea (mmol/l)	m	7.25	7.93*	8.75***	10.55***	12.01***	11.97***
	f	6.09	6.73	9.07**	11.06***	10.89***	9.85***

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002

Most of the statistically significant clinical-chemical changes were not clearly dose-related. At 50 ppm, urea was increased in both sexes, revealing statistical significance in males.

Decreased absolute organ weights at the dose of 150 ppm and above were assessed as a consequence of the decreased body weight.

Histopathology revealed diminished vacuolisation of hepatocytes in the liver, proximal tubular epithelial cells in the kidney and cells of the adrenal cortex at 500 ppm and above.

Atrophy of the thymus with increased apoptotic bodies was increased with incidences of 0/0/0/3/6/8 and 0/0/6/7/8/4 in males and females, respectively. Also in mesenteric lymph nodes an increase of apoptotic bodies in follicles occurred with incidences of 0/0/0/1/1/9 and 0/0/2/4/6/7 in males and females, respectively.

In the gastrointestinal tract, an increased number of animals with erosions and ulcera in the glandular stomach was described with incidences of 1/1/2/4/5/8 and 1/3/5/7/6/6 in males and females, respectively. Thickening of the duodenal mucosa was observed in males and females at doses of 500, 1000 and 1500 ppm.

Conclusion:
The notifier assessed the lowest dose (50 ppm) as NOAEL for both sexes in this 3-month oral mouse study. However, in the opinion of the Rapporteur, the dose of 50 ppm (9.2 mg/kg bw/d) cannot be regarded as a NOAEL for male mice because body weight and body weight change at this dose level were clearly decreased during the whole study period. The urea values in the low dose male group were in the range of historical control data, but in view of the clear increase at 150 ppm and above, a substance related effect at 50 ppm cannot be ruled out. Nevertheless, taking into consideration all available data for this species, a NOAEL of 30 ppm (ca. 4 mg/kg bw/d) can be established for males, based on the body weight data after 91 days from the carcinogenicity study (dose levels: 0, 10, 30, 120 ppm).
For females, the no observed adverse effect level (NOAEL) was 50 ppm (12.9 mg/kg bw/d), based on effects on body weight, decreased hematocrit and triglyceride values and increased urea values at 150 ppm and above.
At higher dose levels, adverse effects in the gastrointestinal tract (erosions and ulcera in the glandular stomach; thickening of the duodenal mucosa), on red blood cells, on white blood cells and lymphatic organs (thymus, mesenteric lymph modes) as well as on adrenals, liver and kidney (decreased vacuolisation) have been observed.

B.6.3.3 Dog

B.6.3.3.1 Dog oral 90-day study

Report: Menges, S.; Schilling, K.; Deckardt, K.; Gembardt, Chr. and Hildebrand, B. 1999:
BAS 500 F - Subchronic oral toxicity study in Beagle dogs. Administration in the diet for 3 months.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11678, unpublished.

Test Material: Pyraclostrobin; batch No. 27882/191/c; purity: 97.09 %.

Test Animals: Beagle dogs (BASF's own breed).

GLP: Yes.

Test Method: EEC 87/302, OECD 409, EPA 82-1, Japan/MAFF.

Deviations: None (OECD 409 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of five male and five female purebred Beagle dogs at dietary concentrations of 0, 100, 200 and 450 ppm for 3 months.
Food consumption was determined daily and body weight once a week. The animals were examined at least once each working day for signs of toxicity, and a check for moribund or dead animals was made twice a day (Mondays to Fridays) and once a day (Saturdays, Sundays and on public holidays).

Clinical chemistry and hematological examinations as well as urinalysis were carried out once before and two times during the administration period.
Ophthalmological examinations were performed 8 days before the beginning of the application of the test substance, and on study day 91.
All animals were subjected to gross-pathological assessment followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA, two-sided), the Fisher's exact test, the Kruskal-Wallis test (two-sided), the Mann-Whitney U-test (two-sided) and the Wilcoxon test.

Findings:
The stability and homogeneous distribution of the test substance in the diet and dietary preparation were analytically verified. Control analysis demonstrated the correctness of the concentrations in the diet.

Table B.6.3-15: Dog oral 90-day study: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
100	2.8	3.1
200	5.8	6.2
450	12.9	13.6

At the high dose level (450 ppm), body weight loss (-0.2 kg) and reduced food consumption (about 9%) were noted in females. Food efficiency in these animals was slightly reduced.

Table B.6.3-16: Dog oral 90-day study: Clinical findings

Parameter	Sex	Dietary dose level (ppm)			
		0	100	200	450
Body weight change, day 91 (kg)	m	1.3	2.0	0.9	0.9
	f	1.2	1.5	1.7	-0.2**
Mean food consumption (%) #	m	100	100	100	100
	f	99	100	100	91

Mean food consumption: 100 % = 700 g/animal/day
Anova + Dunnett's test *p<0.05; **p<0.01

In both sexes of the high dose group (450 ppm), signs of clinical toxicity were evident, consisting of initial vomitus and diarrhoea. The rare cases of diarrhoea at 200 ppm were regarded as being of no toxicological concern, due to their isolated occurrence (week 5 only) in almost all dogs or the scattered occurrence in a single animal. Furthermore, no corresponding deviation in clinical chemistry in any of these dogs was observed, and in the 12-month dog feeding study, at the same dose level, similar findings were not observed.

Clinical-chemical and hematological examinations revealed significant decreases in serum protein and in glucose levels as well as an increases in platelets in females at 450 ppm after 90 days. The reduction in glucose levels in females of the mid dose group is not regarded as an adverse toxicological effect.

Histopathological examination revealed hypertrophy of the duodenal mucosa at the high dose level, in two males and one female. It was characterized by an increased ratio of cytoplasm to

nuclei in the villi, and hyperplastic changes in the epithelial cells. The villi seemed to be slightly elongated, and well preserved up to their tips.

Conclusion:
The no observed adverse effect level (NOAEL) in this 3-month oral dog study was 200 ppm (5.8 mg/kg bw/d in males and 6.2 mg/kg bw/d in females), based on body weight loss and clinico-chemical and hematological changes in females, and vomitus, diarrhoea and hypertrophy of the duodenal mucosa in both sexes at 450 ppm.

B.6.3.3.2 Dog oral 1-year study

Report: Schilling, K.; Deckardt, K.; Gembardt, Chr. and Hildebrand, B. 1999(c):
BAS 500 F - Chronic oral toxicity study in Beagle dogs.
Administration in the diet for 12 months.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11677, unpublished.

Test Material: Pyraclostrobin; batch No. 27882/199/b; purity: 98.7%.

Test Animals: Beagle dogs (BASF's own breed).

GLP: Yes.

Test Method: EEC 87/302, OECD 452, EPA 83-1, Japan/MAFF.

Deviations: None (OECD 452 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 5 male and 5 female purebred Beagle dogs at dietary concentrations of 0, 100, 200 and 400 ppm for about 12 months.
Food consumption was determined daily and body weight once a week. The dogs were examined at least once each working day for signs of toxicity, and a check for moribund or dead animals was made twice a day (Mondays to Fridays) and once a day (Saturdays, Sundays and on public holidays).
Clinical chemistry and hematological examinations as well as urinalysis were carried out once before and three times during the administration period.
Ophthalmological examinations were carried out 8 days before the beginning of the administration period and on study day 364.
All animals were subjected to gross-pathological assessment followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA, two-sided), the Fisher's exact test, the Kruskal-Wallis test (two-sided), the Mann-Whitney U-test (two-sided) and the Wilcoxon test.

Findings:
The stability of the test substance was verified. The homogeneous distribution, stability and correct concentration of the test substance in the diet were analytically demonstrated.

Table B.6.3-17: Dog oral 1-year study: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
100	2.7	2.7
200	5.4	5.4
400	10.8	11.2

At the highest dose level (400 ppm), vomitus was observed during the first week of administration (3 males, 4 females), whereas diarrhoea occurred in all animals during the entire administration period.

High dose males lost body weight up to study day 7, thereafter, they gained body weight comparable to the control group, and during the last weeks of the study their body weights were higher than the control values. The females lost body weight up to study day 21, and their body weight gain was reduced during the entire study period. At the end of the study, body weight gain of the high dose females was 1.1 kg compared to 2.7 kg in the control animals. In high dose females, food consumption and food efficiency was reduced. One male dog of the 400 ppm group has shown a decreased food consumption on occasional days during the whole application period.

Table B.6.3-18: Dog oral 1-year study: Clinical findings

Parameter	Sex	Dietary dose level (ppm)			
		0	100	200	400
Body weight change, day 182 (kg)	m	2.3	1.7	2.3	1.9
	f	2.2	2.6	1.7	0.3*
Body weight change, day 364 (kg)	m	2.0	1.5	2.4	2.2
	f	2.7	3.3	2.4	1.1
Mean food consumption (%) #	m	100	100	100	98
	f	97	97	95	86

Mean food consumption: 100 % = 700 g/animal/day
Anova + Dunett's test *p<0.05; **p<0.01

At 400 ppm, hematological examinations revealed transient decreased hemoglobin and hematocrit in females (day 90) and males (day 180), increased white blood cells in males (days 180, 362) and increased platelets in both sexes throughout the study.

Ophthalmoscopy, clinical-chemical investigations, urinalysis and the pathological investigations did not reveal any test substance related effects.

Conclusion:
The no observed adverse effect level (NOAEL) in this 12-month oral dog study was 200 ppm (5.4 mg/kg bw/d in males and females), based on reduced body weight gain and food consumption in females as well as clinical signs (vomitus, diarrhoea) and hematological changes in both sexes at 400 ppm.

B.6.4 Genotoxicity (Annex IIA 5.4)

The potential genotoxicity of pyraclostrobin was investigated in a series of both *in vitro* and *in vivo* studies. All regular end points for genetic damage (point mutations, chromosome damage, DNA damage and repair) were assessed. The results of these studies are summarized in Table B.6.4-1.

Pyraclostrobin was evaluated for its potential genotoxicity *in vitro* using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis (UDS) test. The results of these studies demonstrated the absence of a genotoxic effect.

In vivo, the test substance was assessed for the induction of micronuclei in mice. The result of this study showed that pyraclostrobin does not exhibit a chromosome damaging potential.

It is therefore concluded that pyraclostrobin has no mutagenic or genotoxic properties neither *in vitro* nor *in vivo*.

Table B.6.4-1: Summary of mutagenicity studies

Institute/year/reference	Study/strains/species	Dose levels and test conditions	Results
BASF (Engelhardt and Hoffmann; 1997)	Ames mutagenicity test; TA 1535, 100, 1537, 98, E. coli WP2 uvrA	Concentrations up to 5000 µg/plate; without S-9 mix with S-9 mix	 Negative Negative
BASF (Engelhardt and Hoffmann; 1998c)	CHO/HPRT mutagenicity test	Concentrations up to 20 µg/ml; without S-9 mix with S-9 mix	 Negative Negative
BASF (Engelhardt and Hoffmann; 1999a)	*In vitro* cytogenetic: chromosome aberration in Chinese hamster V79 cells	Wide range of concentrations up to 25 µg/ml; without S-9 mix with S-9 mix	 Negative Negative
BASF (Engelhardt and Hoffmann; 1998b)	*In vitro* UDS, rat hepatocytes	dose range: 0-1.0 µg/ml	Negative
BASF (Engelhardt and Hoffmann; 1998a)	*In vivo* chromosome aberration: Mouse micronucleus test	0, 75, 150 and 300 mg/kg bw; (oral gavage)	 Negative

In addition, three main water metabolites of pyraclostrobin were tested for their ability to induce gene mutations by means of the Ames test proving all negative (see section B.6.8).

B.6.4.1 *In vitro* testing

B.6.4.1.1 Gene mutation in bacterial cells

Report: Engelhardt, G. and Hoffmann, H. D. (1997):
Study of BAS 500 F (= Reg. No. 304 428) in the Ames Salmonella/mammalian-microsome mutagenicity test and Escherichia coli/mammalian-microsome reverse mutation assay (standard plate

test and preincubation test).
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.; BASF RegDoc# 1997/10973, unpublished.
[An amendment regarding the stability of the test substance over the study period was also submitted to the Rapporteur.]

Test Material: Pyraclostrobin; batch No. CP 026063; purity: 98.2%.

Test System: Salmonella typhimurium (strains TA 100, TA 1535, TA 1537 and TA 98) and Escherichia coli (strain WP2 uvrA).

GLP: Yes.

Test Method: OECD 471, OECD 472, EEC 92/69.

Deviations: None (OECD 471, 472 considered). The second (confirmatory) experiment with and without metabolic activation was a preincubation test whereas the first one was a standard plate test. This difference does not alter the validity of results and conclusions.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for its ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 and *Escherichia coli* strain WP2 uvrA were exposed to the test substance dissolved in DMSO at doses ranging from 20 to 5,000 µg/plate. The study consisted of a standard plate test and a preincubation test both with and without metabolic activation (liver S-9 mix obtained from Aroclor-1254-induced Sprague-Dawley rats). Three plates were used per dose for each strain and test condition.

For control purposes and to demonstrate the sensitivity of the test system, a negative control (DMSO) and appropriate positive controls were included. Positive control substances were 2-aminoanthracene in the activation experiments (different concentrations applied for testing of *S. typhimurium and E. coli*) and N-methyl-N-nitro-N-nitrosoguanidine, 4-nitro-o-phenylen-diamine, 9-aminoacridine or N-ethyl-N-nitro-N-nitrosoguanidine in the trials without S-9 mix.

Findings:
The stability of pyraclostrobin in the vehicle DMSO and in water has been determined analytically. The stability of the test substance throughout the study period was verified by reanalysis.

Test substance precipitation was observed at concentrations of 2,500 µg/plate and higher. However, no bacteriotoxic effect was observed as also proven by titer determination.

The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive control substances.

Conclusion:
According to the results of the study, pyraclostrobin was not mutagenic in the Ames reverse mutation assay.

B.6.4.1.2 Gene mutation in mammalian cells

Report: Engelhardt, G. and Hoffmann, H. D. (1998c): In vitro gene mutation test with BAS 500 F in CHO cells (HPRT locus assay). BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/11422, unpublished.
[An amendment regarding the stability of the test substance over the study period was also submitted to the Rapporteur.]

Test Material: Pyraclostrobin; batch No. CP026063; purity: 98.2%.

Test System: Chinese hamster ovary (CHO) cells.

GLP: Yes.

Test Method: OECD 476, EEC 87/302.

Deviations: None (OECD 476 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for its ability to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro*. Three independent experiments were carried out without exogenous metabolic activation and two with metabolic activation ((liver S-9 mix obtained from Aroclor-1254-induced Sprague-Dawley rats).
In an initial range-finding cytotoxicity test, the cloning efficiency was reduced at doses $\geq$ 5 µg/ml (without S-9 mix). With S-9 mix, a slight reduction was observed at 10 µg/ml, whereas at 50 µg/ml, the cloning efficiency was 0%. Test substance precipitation was observed at doses $\geq$ 50 µg/ml.
According to these results, the following doses were evaluated in the 1st experiment with and without S-9 mix: 0, 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 µg/ml.
Since a weak increase in mutant frequency had been observed at a concentration of 5 µg/ml without metabolic activation, a 2nd experiment without activation was conducted for clarification with the following doses: 0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 µg/ml.
In a 3rd experiment (with and without S-9 mix) for confirmation of the results, the following doses were tested: 0, 1.25, 2.5, 5.0, 10.0 and 20.0 µg/ml.

After an attachment period of 20 - 24 hours, a treatment period of 4 hours both with and without metabolic activation, an expression phase of about 7 - 9 days and a selection period of about 1 week, the colonies of each test group were fixed with methanol, stained with Giemsa and counted.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. The stability of pyraclostrobin in the vehicle DMSO and in water over a period of 4 hours has been determined analytically.

The negative controls (untreated and vehicle controls) gave mutant frequencies within the range expected for the CHO cell line.

Both of the positive control chemicals, i.e. EMS (ethyl methane sulfonate) and MCA (methylcholanthrene), led to the expected increase in the frequencies of forward mutations.

In the first experiment without S-9 mix, an increase in the mutation frequency was observed at 5 µg/ml. However, a dose response was lacking, since no increase was seen at 10 or 20 µg/ml. In a second study with concentrations closely spaced around 5 µg/ml and in the third experiment (with and without S-9 mix) no increase in the mutation frequency was observed.

Conclusion:
Under the experimental conditions of this assay, pyraclostrobin did not induce forward mutations *in vitro* in the CHO/HPRT mutation assay.

B.6.4.1.3 *In vitro* cytogenetic test

Report: Engelhardt, G. and Hoffmann, H. D. (1999a): *In vitro* chromosome aberration assay with BAS 500 F in V79 cells. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11403, unpublished.

Test Material: Pyraclostrobin; batch No. CP026063; purity: 98.2%.

Test System: V79 cells (derived from the Chinese hamster).

GLP: Yes.

Test Method: OECD 473, EEC 92/69.

Deviations: None (OECD 473 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was assessed for its potential to induce structural chromosomal aberrations in V79 cells *in vitro* both in the presence and in the absence of a metabolizing system, i.e. S-9 mix obtained from Aroclor-1254-induced Sprague-Dawley rat liver.

According to an initial range-finding cytotoxicity test, the following doses were included in the main study:

- 1st experiment: 4 hours exposure, 18 hours (after beginning of exposure) harvest time, with and without S-9 mix: 0; 6.25; 12.5; 25.0 µg/ml.
- 2nd experiment: see Table 6.4-2.

Table 6.4-2: Treatment conditions and dose levels used in the 2nd experiment

Exposure time	Harvest time	Activation conditions	Dose levels
18 h	18 h	Without S-9 mix	0.005, 0.010, 0.050 µg/ml
18 h	28 h	Without S-9 mix	0.100 µg/ml
4 h	28 h	With S-9 mix	3.125, 6.25, 12.5 µg/ml

Negative (i.e. vehicle) and appropriate positive controls were included in each experiment.

The cell cycle of the untreated V79 cells is about 13-14 hours under the selected culture conditions. Thus, the selected 1st sampling time of 18 hours was within the 1-1.5 x the normal cell cycle time, as recommended by the OECD guideline No. 473. The later sampling time of 28 hours was chosen to cover a possible cell cycle delay.

About 2-3 hours prior to harvesting the cells, colcemid was added to arrest cells in a metaphase-like stage of mitosis (c-metaphase). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture in the case of the test substance and vehicle controls, or 50 cells for each culture in the case of the concurrent positive controls, were analysed for chromosomal aberrations. For each experiment two cultures were used.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. The stability of Pyraclostrobin in the vehicle DMSO and in water over a period of 4 hours has been determined analytically.

According to the results of the determination of the mitotic index, no suppression of the mitotic activity was observed under any of the experimental conditions. The cell count indicated occasionally that growth inhibition occurred. Cell attachment was slightly reduced (i.e. few cells rounded) from about 12.5 µg/ml onward (without S-9 mix, 1st experiment) and at 0.1 µg/ml (without S-9 mix, 2nd experiment).

Osmolality and pH-values were not altered by the test substance.
The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line.
Both of the positive control chemicals, i.e. EMS (ethyl methanesulfonate) and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations.

The test substance did not cause any biologically relevant and dose-dependent increase in the number of structurally aberrant metaphases including and excluding gaps at both sampling times neither without S-9 mix nor with S-9 mix in two independent experiments. No increase in the frequency of cells containing numerical aberrations was demonstrated either.

Conclusion:
Under the experimental conditions of this assay, pyraclostrobin is considered not to be a chromosome-damaging (clastogenic) agent.

B.6.4.1.4 DNA damage and repair

Report: Engelhardt, G. and Hoffmann, H. D. (1998b): *In vitro* unscheduled DNA synthesis (UDS) assay with BAS 500 F in primary rat hepatocytes.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/11421, unpublished.
[An amendment regarding the stability of the test substance over the study period was also submitted to the Rapporteur.]

Test Material: Pyraclostrobin; batch No. CP026063; purity: 98.2%.

Test System: Primary hepatocytes of Wistar rats.

GLP: Yes.

Test Method: OECD 482, EEC 87/302.

Deviations: None (OECD 482 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for its ability to induce DNA-repair synthesis (unscheduled DNA synthesis, UDS) in primary hepatocytes of Wistar rats *in vitro*. Two independent experiments were carried out. The quantification of UDS was performed microscopically using 2 or 3 slides per test group. 25 - 50 cells in good morphological condition were randomly selected per slide and examined to achieve a total number of 100 cells/dose group. Both test substance treatment and labelling with ^{3}H-thymidine lasted for about 18 - 20 hours.

For each cell, the following counts were performed with an automatic image analyzer (ARTEK):
- the nuclear grain (NG) count (= number of silver grains overlying the nucleus).
- the cytoplasmic grain (CG) count (= number of grains in two or three nucleus-equivalent areas adjacent to the nucleus).

In an initial range-finding cytotoxicity test, the LDH release was increased at doses $\geq$ 0.5 µg/ml (> 2 fold). According to these results, the following doses were evaluated in the 1st experiment: 0, 0.01, 0.03, 0.1, 0.3, 1.0 µg/ml.
In a 2nd experiment for confirmation of the results of the 1st experiment, the following doses were tested: 0, 0.004, 0.02, 0.1, 0.5 µg/ml.

An untreated control, a vehicle (DMSO) control and a positive control (2-acetylaminofluorene, 2-AAF) were included in the experiments.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. The stability of pyraclostrobin in the vehicle DMSO and in water over a period of 4 hours has been determined analytically.

Cytotoxicity was observed from about 0.3 µg/ml onward (LDH release).

The negative controls (untreated and vehicle controls) gave UDS activities within the range expected for rat hepatocytes.
The positive control chemical 2-acetylaminofluorene (2-AAF) revealed a distinct increase in the mean number of nuclear and net grain counts.

The test substance did not cause an increase in the mean number of net nuclear grain counts at any dose level in isolated rat hepatocytes in two experiments performed independently of each other.

Conclusion:
Under the experimental conditions of this assay, the test article pyraclostrobin is considered to be negative in causing unscheduled DNA synthesis.

B.6.4.2 In vivo testing

B.6.4.2.1 In vivo cytogenetic test

Report: Engelhardt, G. and Hoffmann, H. D. (1998a): Cytogenetic study *in vivo* with BAS 500 F in the mouse micronucleus test. Single oral administration.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/10460, unpublished.

Test Material: Pyraclostrobin; batch No. CP026063; purity: 98.2%.

Test Animals: NMRI mice (source: Charles River GmbH - WIGA, Sulzfeld, Germany).

GLP: Yes.

Test Method: EEC 92/69, OECD 474.

Deviations: None (OECD 474 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for clastogenicity and for the ability to have spindle poison effects in NMRI mice using the micronucleus test method. The test substance, dissolved in olive oil, was administered once orally by gavage to five male and five female animals per group at dose levels of 75, 150 and 300 mg/kg bw. The dosing volume was 10 ml/kg body weight in

each case. The dose levels were selected on the basis of a pretest revealing a rather high acute toxicity in mice as compared to rats (see section B.6.2) since deaths occurred down to the dose of 400 mg/kg bw. As a negative control, five male and female mice were administered the vehicle, olive oil, by the same route. Cyclophosphamide and vincristine sulphate were given to groups of five mice per sex serving as positive control substances for either clastogenic or spindle poison effects.
The animals were sacrificed and the bone marrow of the two femora was prepared 24 and 48 hours after administration in the highest dose group of 300 mg/kg bw and in the vehicle controls. In the low and medium dose groups as well as in the positive control groups, the 24-hour sacrifice interval was investigated only. After staining of the preparations, 2,000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2,000 polychromatic erythrocytes were also registered.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. The stability of pyraclostrobin in the vehicle olive oil and the correctness of the concentrations in the vehicle was analytically confirmed.

Animals which were administered the vehicle or the positive control substances cyclophosphamide or vincristine did not show any clinical signs of toxicity.

The administration of the test substance led to piloerection and squatting posture in all dose groups after about 30 minutes. The day after treatment, signs of toxicity were not observed any longer. One of the top dose males died on the second day following dosing.

The negative control gave frequencies of micronucleated polychromatic erythrocytes within the historical control range.
Both of the positive control chemicals, i.e. cyclophosphamide for clastogenicity and vincristine for spindle poison effects, led to the expected increase in the rate of polychromatic erythrocytes containing small or large micronuclei.

No inhibition of erythropoiesis induced by the treatment of mice with Pyraclostrobin was detected; the ratio of polychromatic to normochromatic erythrocytes was always in the same range as that of the control values.
No increase in the number of polychromatic erythrocytes containing either small or large micronuclei was detected. The percentage of micronucleated cells was always in the same range as in the negative control group.

In the lack of any bone marrow toxicity, the question might arise whether this tissue was actually reached by the test compound. However, this has been proven in the rat toxicokinetic study in which radioactivity was detected in the bone marrow after administration of 50 mg/kg bw (B.6.1). Thus, the bone marrow micronucleus test is suitable for testing the clastogenic or aneugenic potential of pyraclostrobin.

Conclusion:
Under the experimental conditions chosen, the test substance pyraclostrobin does not have any chromosome damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis.

B.6.4.2.2 DNA damage and repair

Because of the unambiguously negative outcome of the *in vitro* UDS assay with pyraclostrobin, no *in vivo* study is necessary.

B.6.4.3 *In vivo* testing, germ cells

The results of the *in vitro* as well as the *in vivo* studies demonstrated that pyraclostrobin has no mutagenic or genotoxic potential. Therefore, there is no necessity to evaluate the test substance in an *in vivo* study using germ cells.

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

One chronic toxicity study in rats and two carcinogenicity studies in rats and mice were performed with pyraclostrobin.

Restricted to the high doses, body weight and body weight gain were decreased in all studies, mostly accompanied by a diminished food consumption.

In the carcinogenicity study in rats, liver cell necrosis occurred in males. Additionally, in the chronic toxicity study in rats, alanine aminotransferase and alkaline phosphatase values were decreased at the high dose. A reduction in the activity of these enzymes is not known to be associated with an adverse effect.

There was no evidence of a carcinogenic effect of pyraclostrobin.

The overall NOAEL, obtained from the chronic/carcinogenicity studies in rats (males and females combined) and the carcinogenicity study in mice, is about 4 mg/kg bw/d (see Table B.6.5-1).
This value is the basis for deriving an ADI.

Table B.6.5-1: Summary of long-term toxicity studies

Study type / species / dose levels	NOAEL (mg/kg bw/d)	LOAEL / Critical effects
24-month chronic toxicity Wistar rats 0, 25, 75, 200 ppm	3.4/4.6 m/f [75 ppm]	200 ppm: Reduced body weight. No increase in tumour incidences.
24-month carcinogenicity Wistar rats 0, 25, 75, 200 ppm	3.4/4.7 m/f [75 ppm]	200 ppm: Reduced body weight, reduced food consumption (m), liver cell necrosis (m). Not carcinogenic.
18-month carcinogenicity B6C3F1 mice 0, 10, 30, 120 ppm (m, f), 180 ppm (f)	4.1/4.8 m/f [30 ppm]	120 ppm (m, f) & 180 ppm (f): Reduced body weight. Not carcinogenic.

B.6.5.1 Chronic toxicity, rat

Report: Mellert, W.; Deckardt, K.; Gembardt, C.; Pappritz, G. and Hildebrand, B. 1999(d):
BAS 500 F - Chronic toxicity study in Wistar rats.
Administration in the diet for 24 months.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11672, unpublished.

Test Material: Pyraclostrobin; batch No. 27882/191/c; purity: 97.09%.

Test Animals: Wistar rats [Chbb:THOM (SPF), supplied by Dr. Karl Thomae GmbH, Biberach/Riss, FRG].

GLP: Yes.

Test Method: EEC 87/302, OECD 452, EPA 83-1, Japan/MAFF.

Deviations: None (OECD 452 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 20 male and 20 female Wistar rats at dietary concentrations of 0, 25, 75 and 200 ppm for 24 months.
Food consumption and body weight were determined once a week during the first 13 weeks,, thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinico-chemical and hematological examinations were carried out 3, 6, 12, 18 and 24 months after start of the administration period. The animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA, two-sided), the Fisher's exact test, the Kruskal-Wallis test (two-sided), the Mann-Whitney U-test (two-sided) and the Wilcoxon test.

Findings:
The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

Table B.6.5-2: Chronic toxicity rat: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
25	1.1	1.5
75	3.4	4.6
200	9.0	12.3

There was no test substance related increase in mortality or clinical signs of toxicity in this study. Food consumption was not affected.

Table B.6.5-3: Chronic toxicity rat: Clinical findings

Parameter	Sex	Dietary dose level (ppm)			
		0	**25**	**75**	**200**
Body weight, day 728 (g)	m	706	700	706	675
	f	374	376	368	352
Total body weight gain, day 728 (g)	m	518	515	521	489
	f	230	231	226	207

The following findings were obtained at 200 ppm:
In both sexes, the terminal body weight was somewhat lower than in the control group. During the course of the study, the maximum differences between the control and the high dose group achieved values of 10% (males) and 9% (females). Body weight gain was also reduced in top dose males and females, mainly in the second part of the study. Maximum differences in body weight change between the control and high dose group achieved 11% (males) or 14% (females). However, statistical significance (ANOVA and Dunnett´s test) was reached only in females and only occasionally (i.e., on study days 7 and 539).

Clinico-chemical investigations demonstrated, at the high dose level only, a statistically significant decrease in alkaline phosphatase activity in both sexes, and alanine aminotransferase activity in the males. Also restricted to the high dose, there was a slight, not statistically significant decrease in protein and globulins in males.

Hematological examinations, ophthalmoscopy and urinalysis did not reveal test substance related effects.

The pathological investigations did not demonstrate any test substance related adverse effect. A slight, not statistically significant increase of absolute and relative testis weights, and kidney weights in females at 200 ppm were regarded as toxicologically not significant.
In males, there were higher, but not dose-related incidences of tubular degeneration (1/7/7/6) and tubular mineralization (1/4/6/4) in the testes of all treated groups. However, these findings were often associated with tubular atrophy (9/5/5/6) and Leydig cell tumours (9/12/11/8), and they are hence not regarded as treatment-related findings, but being secondary events to atrophy and/or Leydig cell tumours.

There were no test substance related effects at 75 and 25 ppm pyraclostrobin in the diet.

There was no indication of a carcinogenic response.

Conclusion:
The no observed adverse effect level (NOAEL) in this 24-month chronic toxicity study in rats was 75 ppm (3.4 mg/kg bw/d in males and 4.6 mg/kg bw/d in females), based on a slight decrease in body weight at 200 ppm.
There was no indication of a carcinogenic potential in rats.

B.6.5.2 Carcinogenicity, rat

Report: Mellert, W.; Deckardt, K.; Gembardt, C.; Pappritz, G. and Hildebrand, B. 1999(e):
BAS 500 F – Carcinogenicity study in Wistar rats.
Administration in the diet for 24 months.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11868, unpublished.

Test Material: Pyraclostrobin; batch No. 27882/191/c; purity: 97.09%.

Test Animals: Wistar rats [Chbb:THOM (SPF), supplied by Dr. Karl Thomae GmbH, Biberach/Riss, FRG].

GLP: Yes.

Test Method: EEC 87/302, OECD 451, EPA 83-2, Japan/MAFF.

Deviations: None (OECD 451 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 50 male and 50 female Wistar rats at dietary concentrations of 0, 25, 75 and 200 ppm for 24 months.

Food consumption and body weight were determined once a week during the first 13 weeks, thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Differential blood counts were determined for all surviving animals at the end of the study and also from all animals killed in extremis during the study. After about 24 months, the animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA, two-sided), the Kruskal-Wallis test (two-sided) and the Wilcoxon test.

Findings:
The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

Table B.6.5-4: Carcinogenicity rat: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
25	1.2	1.5
75	3.4	4.7
200	9.2	12.6

There was no test substance related increase in mortality or clinical signs of toxicity in this study.

Table B.6.5-5: Carcinogenicity rat: Clinical findings

Parameter	Sex	Dietary dose level (ppm)			
		0	**25**	**75**	**200**
Body weight, day 91 (g)	m	497	487	494	466**
	f	286	294	291	279
Body weight, day 371 (g)	m	659	651	663	611**
	f	354	348	347	324**
Body weight, day 728 (g)	m	684	689	699	656
	f	417	396	390	360**
Food consumption, day 91 (g/animal/day)	m	26.1	25.8	26.6	25.0*
	f	19.4	19.7	20.0	19.5

Anova + Dunnett's test *p<0.05; **p<0.01

The following findings were obtained at the high (200 ppm) dose level:
Body weight was 7% (males) and 14% (females) lower than in the control group towards the end of the study. Body weight gain was reduced by 10% and 22% in males and females, respectively. The effects on body weight were statistically significant during the first year in males and during the second year in females.

Food consumption was statistically significantly decreased in males at the high dose level from day 7 to day 91.

Relative kidney weights were increased in males at 200 ppm and in females at the high (200 ppm) and the mid (75 ppm) dose level. Restricted to the high dose group, histopathological investigation of the kidneys revealed higher incidences of tubular casts and tubular atrophy in males and tubular atrophy in females. However, these findings show high spontaneous incidences. Additionally, lower incidences in kidney findings were observed, concerning pyelitis and chronic nephropathy in high dose males.

Absolute liver weights were decreased in females at the high dose level. Histopathological investigations revealed an increased incidence of liver cell necrosis (1/2/2/10) in males.

Furthermore, at 200 ppm, lower incidences of adrenal cortex hyperplasia and ovarial pigment deposition were found.

There was no evidence of a carcinogenic response.

Conclusion:
The no observed adverse effect level (NOAEL) in this 24-month carcinogenicity study in rats was 75 ppm (3.4 mg/kg bw/d for males and 4.7 mg/kg bw/d for females), based on decreased body weights in both sexes and increased incidence of liver cell necrosis in males at 200 ppm. Again, there was no indication of carcinogenic potential in rats.

B.6.5.3 Carcinogenicity, mouse

Report: Mellert, W.; Deckardt, K.; Küttler, K. and Hildebrand, B. 1999(g): BAS 500 F - Carcinogenicity study in B6C3F1 mice. Administration in the diet for 18 months. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11871, unpublished.

Test Material: Pyraclostrobin; batch No. 27882/191/c; purity: 97.09%.

Test Animals: B6C3F1/CrlBR mice (supplied from Charles River Lab., USA).

GLP: Yes.

Test Method: EEC 87/302, OECD 451, EPA 83-2, Japan/MAFF.

Deviations: None (OECD 451 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 50 male and 50 female B6C3F1 mice at dietary concentrations of 0, 10, 30 and 120 ppm and additionally 180 ppm (females only) for 18 months.

Food consumption and body weight were determined once a week during the first 13 weeks, thereafter at 4-week intervals. A check of the general state of health of the animals was made at least daily. Additionally, the animals were examined in detail and palpated once a week. Blood smears were prepared after 12 months and 18 months, and from all animals killed in extremis. After 18 months of treatment, the animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA, two-sided), the Kruskal-Wallis test (two-sided) and the Wilcoxon test.

Findings:
The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

Table B.6.5-6: Carcinogenicity mouse: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
10	1.4	1.6
30	4.1	4.8
120	17.2	20.5
180	-	32.8

There was no test substance related increase in mortality or clinical signs of toxicity in this study.

Table B.6.5-7: Carcinogenicity mouse: Clinical findings

Parameter	Sex	Dietary dose level (ppm)				
		0	**10**	**30**	**120**	**180**
Body weight, day 91 (g)	m	34.2	33.4	33.2	32.1**	
	f	27.3	26.9	28.4	27.1	26.8
Body weight, day 371 (g)	m	43.1	41.6	42.5	40.3**	
	f	38.7	38.2	39.9	38.1	35.8*
Body weight, day 546 (g)	m	43.1	38.9**	39.7**	37.6**	
	f	39.0	35.7**	36.7	35.3**	34.0**
Food consumption, day 546 (g/animal/day)	m	5.8	4.8**	5.1	4.8**	
	f	6.2	4.6**	4.6**	5.0**	5.2**

Anova + Dunnett's test *p<0.05; **p<0.01

After 90 days, body weight and body weight change were significantly reduced at 120 ppm in males only. After one year, this effect was observed at 120 ppm in males and 180 ppm in females.
In the second year, body weight and body weight change were significantly reduced at all dose levels in males on days 455-546 and at 10 ppm, 120 ppm and 180 ppm in females on days 539 and 546. At the high dose levels, body weight was 13% lower than controls in both sexes and body weight development was reduced by 27% and 29% in males and females, respectively. However, the reduced body weights at the end of the treatment period were without any dose-response relationship.

Occasionally, food consumption was decreased in the treated groups, in particular in females, without showing any dose-response relationship.

Blood smears did not indicate any substance-related effect.

Histopathological investigations did not reveal any test substance related adverse effect.

There was no evidence of a carcinogenic response.

Conclusion:
The no observed adverse effect level (NOAEL) in this 18-month carcinogenicity study in mice was 30 ppm (4.1 mg/kg bw/d for males), based on reduction of body weights in males at 120 ppm during the first year of the study.
During the last 6 months of the study, food consumption, body weight and body weight change were significantly reduced at all dose levels. However, these effects at the end of the treatment period were without any dose-response relationship and not regarded as treatment-related.
There was no indication of a carcinogenic potential of pyraclostrobin in mice.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

The reproduction toxicity of pyraclostrobin was investigated in a two-generation reproduction study as well as in teratogenicity studies in rats and rabbits.

Fertility was not affected up to the highest dose level of 300 ppm (ca 32.6 mg/kg bw/d) in the two-generation study. The NOAEL concerning parental toxicity in this study was 75 ppm

(approximately 8.2 mg/kg bw/d) for F0 and F1 animals, and this was also the NOAEL for reproductive toxicity in the F1 and F2 litters. Offspring effects were confined to reduced body weight gain and associated changes of organ weights. A single developmental landmark (vaginal opening) was delayed in F1 pups only at 300 ppm suggesting a possible (slight) retardation in female pup development.

In the prenatal toxicity study in rats, developmental toxicity was observed at the highest dose tested (50 mg/kg bw/d), based on increased incidences of several soft tissue and skeletal variations inside the range of the historical control values. This dose level was clearly toxic to the dams, as demonstrated by a 16% reduction in body weight gain. Thus, the NOAEL for maternal toxicity was established at 10 mg/kg bw/d, and the developmental NOAEL was 25 mg/kg bw/d.
In the rabbit developmental toxicity study, maternal toxicity was proven by clear reduction of body weight gain and a lower food consumption at 5 mg/kg bw/d and above. Thus, the NOAEL for maternal toxicity was <5 mg/kg bw/d suggesting a higher susceptibility of this species as compared to the rat at least when the test compound is administered during gestation. Prenatal toxicity was substantiated by embryolethality resulting in elevated postimplantation losses and a reduction in the mean number of live fetuses/doe at 20 mg/kg bw/d. At 10 and 20 mg/kg bw/d, increased incidences of skeletal malformations outside the historical control range of this laboratory were observed. However, when compared to the control group, the difference did not attain statistical significance. It must be emphasised that developmental toxicity was observed only in the presence of severe maternal toxicity. The NOAEL for developmental toxicity was 5 mg/kg bw/d. A further rabbit study was required by the Rapporteur to establish a clear NO(A)EL for maternal toxicity.
In this second study, a reduction in food consumption at the highest dose of 5 mg/kg bw/d as observed in the 1st experiment was confirmed. Again, body weight gain was reduced at this dose level, however, as compared to the control group, the difference reached statistical significance during the first three days of the exposure period only. In the mid dose group receiving a daily dose of 3 mg/kg bw from gestation days 7 through 28, food consumption was still decreased due to a significant lower food intake on days 7 and 8. In contrast to the top dose level, this effect was not accompanied by an impaired food utilisation and there was no statistically significant impact on the body weight. Thus, 3 mg/kg bw/d is considered the NOAEL for maternal toxicity. No evidence of developmental toxicity was obtained in this additional study but the range of parameters investigated was rather limited since the fetuses were counted and weighed only.

The results of the reproduction and developmental toxicity studies are summarised in Table B.6.6-1.

Table B.6.6-1: Summary of reproduction toxicity studies

Study type / species / dose levels	NOAEL	LOAEL / Critical effects
2-generation study Wistar rats 0, 25, 75, 300 ppm	Parental toxicity: ca 8.2 mg/kg bw/d [75 ppm] Reproductive toxicity: ca 8.2 mg/kg bw/d [75 ppm]	300 ppm: Parental toxicity: reduced food consumption and body weight gain; Reproductive toxicity: reduced pup body weight gain, organ weight changes and a delay in vaginal opening (F1 females only). No adverse effects on fertility.
Developmental toxicity Wistar rats 0, 10, 25 and 50 mg/kg bw/d; days 6-19	Maternal toxicity: 10 mg/kg bw/d Developmental toxicity: 25 mg/kg bw/d	25 mg/kg bw/d: Maternal toxicity: reduced food consumption and body weight (gain). 50 mg/kg bw/d: Developmental toxicity: increased variations.
Developmental toxicity Himalayan rabbits (1st study) 0, 5, 10 and 20 mg/kg bw/d; days 7-28	Maternal toxicity: <5 mg/kg bw/d Developmental toxicity: 5 mg/kg bw/d	5 mg/kg bw/d: Maternal toxicity: reduced food consumption, reduced body weight gain. 10 mg/kg bw/d: Developmental toxicity: increased skeletal malformations; at 20 mg/kg bw/d increased resorptions and postimplantation losses; reduced number of live fetuses.
Developmental toxicity Himalayan rabbits (2nd study with special regard to maternal effects) 0, 1, 3, 5 mg/kg bw/d; days 7-28	Maternal toxicity: 3 mg/kg bw/d Developmental toxicity: 5 mg/kg bw/d	5 mg/kg bw/d: Maternal toxicity: reduced food consumption, reduced body weight gain. No evidence of developmental toxicity (limited range of parameters investigated).

B.6.6.1 Multigeneration study in rats

Report: Schilling, K.; Gembardt, C. and Hildebrand, B. (1999d): BAS 500 F - Two-generation reproduction toxicity study in Wistar rats. Continuous dietary administration.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11869, unpublished.

Test Material: Pyraclostrobin; batch No. 27882/199/b; purity: 98.7%.

Test Animals: Wistar rats [Chbb:THOM (SPF), provided by Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany].

GLP: Yes.

Test Method: EEC 87/302, OECD 416, EPA/OPPTS 870.3800, Japan/MAFF

Deviations: None (OECD 416 considered). In addition to OECD guideline requirements, some special investigations (spermatology, estrous cycle determination, ovarian follicle count) were performed. After parturition, food consumption was determined less frequently than recommended in the guideline, however, these deviations were

sufficiently justified by the study authors and do not alter the validity of the results obtained.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 25 male and 25 female sexually immature rats (F0 parental generation) via their diet in concentrations of 0, 25, 75 or 300 ppm. The test substance was dissolved in acetone. At least 74 days after the beginning of treatment, F0 animals were mated to produce a litter (F1). Mating pairs were from the same dose group and F1 animals selected for breeding were continued in the same dosing group as their parents. Groups of 25 males and 25 females selected from F1 pups as F1 parental generation were offered diets containing 0, 25, 75 or 300 ppm of the test substance post weaning, and the breeding program was repeated to produce F2 litter. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 adult animals. Test diets containing Pyraclostrobin were offered continuously throughout the study.

The parents' and the pups' state of health was checked each day, and parental animals were examined for their mating and reproductive performances.

Food consumption of the F0 and F1 parents was determined regularly during premating (once weekly over a period of 7 days each), and weekly during gestation (days 0, 7, 14, 20) and lactation periods (days 1, 4, 7, 14).

In general, body weights of F0 and F1 parents were determined once weekly. However, during gestation and lactation F0/F1 females were weighed on days 0, 7, 14 and 20 of gestation, and on days 1, 4, 7, 14 and 21 after birth.

Estrus cycle data were evaluated for F0 and F1 generation females over a three week period prior to mating until evidence of mating occurred. Moreover, the estrus stage of each female was determined on the day of scheduled sacrifice.

Different sperm parameters (sperm head count, morphology) were assessed in all control and high dose males from the F0 and F1 generations at scheduled sacrifice or shortly thereafter. Sperm motility was even evaluated in all groups.

The F1 and F2 pups were sexed and weighed on the day after birth and on days 4, 7, 14 and 21 post partum. Their viability was recorded. All pups were examined macroscopically at necropsy (including weight determinations of brain, spleen and thymus in one pup/sex/litter). Sexual maturation (day of preputial separation/vaginal opening) of all pups selected to become F1 parental generation animals was determined.

All F0 and F1 parental animals were assessed by gross pathology (including weight determinations of several organs). All control and high dose rats as well as those low and mid dose animals with suspected impaired fertility were subjected to an extensive histopathological examination with special attention being paid to the organs of the reproductive system. Histology of the kidneys was performed on all adult animals on study. Quantitative assessment of primordial follicles, growing follicle and antral follicles in the ovaries was made for all control and high dose F0 and F1 parental females.

Methods used for statistical analysis of the results included the two-sided Dunnett test, Fisher´s exact test, the Kruskal-Wallis test and the one- or two-sided Wilcoxon test and were considered appropriate.

Findings:
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the dietary test substance preparation was analytically verified. The correctness of the concentrations was analytically demonstrated.

Table B.6.6-2: Multigeneration study: Mean test substance intake

Dietary dose level (ppm)	Mean test substance intake (mg/kg bw/d)
25	2.7
75	8.2
300	32.6

F0 and F1 parental animals
There were no overt clinical signs of toxicity. One high dose F0 dam died on the first day of lactation following complete delivery. In the absence of any gross or histopathological findings which could explain this premature death, and taking into account that there were no further deaths neither in this nor in the F1 high dose group and since there were no signs of toxicity any more, this isolated case may not be attributed to treatment.

The following effects on food consumption and body weight were confined to the highest dose level:

- Significantly decreased food consumption was observed in the male rats during the first weeks of the premating phase (F0 up to 5%, F1 up to 12%) and in the females during the premating (F0 up to 8%, F1 up to 9%) and gestation period (F1 only, up to 7%).
- Significantly reduced mean body weights were noted in F0 females during gestation (up to 5%) and lactation (up to 4%) in several weeks. In the F1 generation, a lower body weight than in the control group was determined in the male animals during certain phases of treatment period (weeks 4-11, week 14; up to 7%) and in the females during the first weeks after weaning (up to 9%).
- A reduction in body weight gain attaining statistical significance was observed during the premating period in high dose F0 females (up to 12% 1st week after initiation of treatment) as well as occasionally in F0 males (weeks 9 –10, about 25%).

Organ weight determination as well as gross or histopathology did not reveal any evidence of compound-related changes.

There were no adverse effects on reproductive performance including sperm and ovarian parameters as well as the estrus cycle of the rats of both generations.

Table B.6.6-3: Multigeneration study: Reproductive findings (F0 --> F1)

Parameter	Dose level (ppm)			
	0	**25**	**75**	**300**
Implantation sites (total / mean)	417 / 16.7	404 / 16.2	348 / 14.5*	361 / 14.4*
Postimplantation loss (total / mean)	54 / 2.2	53 / 2.1	47 / 2.0	33 / 1.3
Pups delivered (total / mean)	363 / 14.5	351 / 14.0	301 / 12.5	328 / 13.1
Pups liveborn (total / mean)	356 / 14.2	336 / 13.4	295 / 12.3	322 / 12.9

Dunnett-test *p<0.05

A lower mean number of implantation sites in mid and high dose F0 dams, although statistically significant, did not show a clear dose response. Mainly because of a lower postimplantation loss in the high dose group, this difference did not result in a significant decrease in the mean number of pups delivered or the number of liveborn pups and, thus, did not affect the reproductive success. In the F1 generation, no changes in mean implantation site number were to be seen. Therefore, this finding is not considered to indicate an effect of compound administration.

Table B.6.6-4: Multigeneration study: Pup findings

Parameter	Dose level (ppm)			
	0	**25**	**75**	**300**
F1 - Body weight, day 21, m / f (g)	52.8 / 51.4	53.7 / 51.3	52.8 / 50.3	47.4**/ 45.2**
F1 - Vaginal opening (days)	31.7	32.1	32.4	33.3**
F2 - Body weight, day 7, m / f (g)	15.2 / 14.5	14.9 / 14.6	14.7 / 14.2	13.5**/ 13.1**
F2 - Body weight, day 21, m / f (g)	52.0 / 49.8	52.6 / 50.2	51.4 / 48.9	45.0**/ 43.5**

Dunnett-test *p<0.05; **p< 0.01

Pup birth weight and pup viability were not affected by treatment.

Reduced mean body weights and body weight gains became apparent in F1 and F2 pups from day 4 *post partum* up to weaning at the highest dose levels.

Pup organ weight determination demonstrated the following changes:

- Reduced mean absolute weights of: thymus (F1 about -18%; F2 about -17%), spleen (F1 about -16%; F2 about -17%) and brain (F2 about -4%).
- Increased mean relative brain weight (F1 about 13%; F2 about 11%).

These findings were confined to the group receiving 300 ppm and might be attributed to the reduction in pup body weight (gain).

A delay in vaginal opening in the F1 female pups was observed at the highest dose level confirming the suspected delay in physical development. This is rather related to the reduced body weight development than providing evidence of a selective effect of the test substance.

Conclusion:
The NOAEL for parental toxicity of pyraclostrobin was 75 ppm (8.2 mg/kg bw/d), based on signs of systemic toxicity in male and female animals occurring in both parental generations at 300 ppm. Toxicity was characterised by decreased food consumption and impairments in body weight and body weight gain.

The NOAEL for reproductive toxicity was 75 ppm (8.2 mg/kg bw/d), based on impairments in body weight/body weight gain in F1 and F2 pups and by a delay in vaginal opening in F1 female rats at 300 ppm.
There were no treatment-related effects on fertility.

B.6.6.2 Developmental toxicity

B.6.6.2.1 Rat

Report: Schilling, K.; Hellwig, J. and Hildebrand, B. (1999a): BAS 500 F - Prenatal developmental toxicity study in Wistar rats. Oral administration (gavage). BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11511, unpublished.

Test Material: Pyraclostrobin; batch No. CP028719; purity: 98.9%.

Test Animals: Wistar rats [Chbb:THOM (SPF), provided by Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany].

GLP: Yes.

Test Method: EEC 87/302, OECD 414, EPA/OPPTS 870.3700

Deviations: None (OECD 414 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for its prenatal developmental toxicity in Wistar rats. The test substance was administered once daily as an aqueous (0.5 % Tylose CB 30.000 in doubly distilled water) suspension to 25 mated female Wistar rats/group by stomach tube at doses of 0, 10; 25 and 50 mg/kg body weight from day 6 through day 19 *post coitum* (p.c.). The dose levels had been chosen on the basis of a preliminary maternal toxicity dose-finding study which was not reported in detail. A standard dose volume of 10 ml/kg body weight was used for each group. The control group was dosed with the vehicle only.

Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health was checked at least once a day.

On day 20 p.c., all females were sacrificed and assessed by gross pathology. The weight of the unopened uterus was determined. For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as early and late resorptions, live and dead fetuses) determined. The fetuses were removed from the uterus, sexed, weighed and examined for any external malformations or variations. Viability of the fetuses and the condition of the placentae, umbilical cords, fetal membranes and fluids were examined. Individual placental weights were recorded. Subsequently, approximately one half of the fetuses was processed to

become examined for visceral changes and the other half was used for skeletal including cartilage examination.

Statistical analysis of data was performed by means of two-sided Dunnett's test, one-sided Fisher's exact test and one-sided Wilcoxon test.

Findings:
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparations and correctness of the concentrations were analytically verified.

In all groups, at least 20 females became pregnant. Actual numbers of non-pregnant animals were 3, 5, 4 and 0 rats in the control, low, mid and high dose groups, respectively.

There were no deaths attributable to compound administration in any of the groups and no treatment-related clinical signs were observed.
The following findings were obtained in the dams and considered as test substance related [see Table B.6.6-5]:

Table B.6.6-5: Developmental toxicity rat: Maternal findings

Parameter	Dose level (mg/kg bw/d)			
	0	**10**	**25**	**50**
Food consumption; day 6-8 (g/animal/day)	24.6	23.6	21.2**	17,9**
Body weight; day 20 (g)	369	373	354	350*
Body weight change; day 6-19 (g)	104	107	96	88**
Gravid uterus (g)	79.4	83.0	77.4	78.0
Carcass weight (g)	290	290	277	272**
Corrected body weight gain; from day 6 (g)	40.7	40.9	31.9*	22.3**

Dunnett-test *p<0.05; **p<0.01

The oral administration of Pyraclostrobin to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 19 p.c.) elicited overt maternal toxicity at 50 mg/kg bw/d and was still toxic to the dams at 25 mg/kg bw/d. Maternal toxicity was substantiated by reduced food consumption, impairments in body weight gain (high dose only) and reductions in corrected body weight gain.
A slightly higher rate of late resorptions in the mid dose group was not confirmed at the highest dose level and, thus, is not considered to indicate an effect attributable to compound administration.

There was no evidence of an increase in the incidence of malformations up to and including the highest dose level of 50 mg/kg bw/d.

However, the incidence of several soft tissue and skeletal variations was increased at 50 mg/kg bw/d. Although all increased incidences were inside the range of the historical control values, a treatment related increase at the highest dose level can not be completely excluded. At the lowest dose level, the slight increased incidence of dilated ureter, closely related to the finding dilated renal pelvis, appeared without a clear relation to dosing. It should be also noticed that dilatation of the renal pelvis is a very common finding in the offspring of this rat strain.

Table B.6.6-6: Developmental toxicity rat: Developmental findings

Parameter (Affected fetuses/Litter mean %)	Historical control range (%)	Dose level (mg/kg bw/d)			
		0	10	25	50
Total fetal soft tissue variations	8.9 – 29.8	5.0	12.1*	17.9	19.0**
Dilated renal pelvis	8.3 – 29.8	5.0	10.6	17.1	19.0**
Dilated ureter	0.5 – 6.2	0.0	2.7*	0.6	3.0*
Total fetal skeletal variations	90 – 100	82.0	89.4	81.4	90.8*
Incomplete ossification of sternebrae	0 – 33.9	17.9	24.4	28.5	32.7*
Cervical rib with no cartilage	0.5 – 5.8	1.1	1.2	1.3	4.4*

Wilcoxon test *p<0.05; **p<0.01

Conclusion:
The NOAEL for maternal toxicity was 10 mg/kg bw/d, based on reduced food consumption and reductions in corrected body weight gain.
Under the conditions of this prenatal developmental toxicity study, pyraclostrobin was not teratogenic in rats. The NOAEL for developmental toxicity was 25 mg/kg bw/d, based on increased incidences of several soft tissue and skeletal variations inside the range of the historical control values.

B.6.6.2.2 Rabbit

Report: Schilling, K.; Hellwig, J. and Hildebrand, B. (1999b): BAS 500 F - Prenatal developmental toxicity study in Himalayan rabbits. Oral administration (gavage).
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11512, unpublished.

Test Material: Pyraclostrobin; batch No. CP028719; purity: 98.9%.

Test Animals: Himalayan rabbits [Chbb:HM (outbred strain), provided by Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany].

GLP: Yes.

Test Method: EEC 87/302, OECD 414, EPA/OPPTS 870.3700

Deviations: None (OECD 414 considered).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for its prenatal developmental toxicity in Himalayan rabbits. The test substance was administered as an aqueous suspension to 24 or 25 inseminated females/group by stomach tube at doses of 0, 5, 10 and 20 mg/kg bw/d on day 7 through day 28 post insemination (p.i.). A standard dose volume of 10 ml/kg bw/d was used. The control group was dosed with the vehicle only (i.e., 0.5% Tylose CB 30.000 in doubly distilled water).

Food consumption and body weights were recorded regularly throughout the study period. The state of health of the animals was checked each day.

On day 29 post insemination, all does were sacrificed and assessed by gross pathology (including weight determination of the unopened uterus and the placentae). For each doe, corpora lutea were counted and number and distribution of implantation sites (differentiated as early and late resorptions, live and dead fetuses) were determined. Viability of the fetuses and the condition of the placentae, umbilical cords, fetal membranes and fluids were examined. Individual placental weights were recorded. The fetuses were removed from the uterus, sexed, weighed and further investigated for external, soft tissue and skeletal findings.
The abdomen and thorax were opened to become examined for visceral changes. The heart and the kidneys were removed and sectioned in order to assess their internal structure. Thereafter, nearly one half of the fetuses of each litter was examined for skull findings by severing the heads from the trunk, fixation and processing followed by about 10 transverse sections per head. In the remaining fetuses, the brain was examined after cross section in the parietal bone area. All fetuses (however, partly without heads) were examined for skeletal findings using a modified method according to Dawson.

Statistical analysis of data was performed by means of two-sided Dunnett's test, one-sided Fisher´s exact test and one-sided Wilcoxon test.

Findings:
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparation was analytically verified. The correctness of the concentrations was analytically demonstrated.

Two accidental deaths occurred in the control and mid dose group, respectively. Furthermore, two mid dose females were not pregnant at termination. Thus, 23 - 25 does/group became pregnant and were included in calculations.

The following test substance related findings were obtained [see Table B.6.6-8]:

Table B.6.6-7: Developmental toxicity rabbit: Maternal findings

Parameter	Dose level (mg/kg bw/d)			
	0	**5**	**10**	**20**
Food consumption; day 7-8 (g/animal/day)	98.1	35.7**	20.4**	10.5**
Body weight; day 29 (g)	2961	2807	2851	2748**
Body weight change; day 7-9 (g)	-3.8	-43.8**	-85.5**	-146.3**
Gravid uterus (g)	352.6	302.6	271.2*	209.6**
Corrected body weight gain; from day 7 (g)	-135.7	-142.4	-132.9	-146.8

Dunnett-test *p<0.05; **p<0.01

The oral administration of pyraclostrobin to pregnant Himalayan rabbits elicited pronounced maternal toxicity at 20 mg/kg bw/d and was still toxic to the does at 10 and 5 mg/kg bw/d. Maternal toxicity was substantiated by dose-related reduced food consumption with subsequent impairments in body weight gain at all dose levels and decreased defecation occurring in 10 does, days 10-14 p.i and 1 doe, day 10 p.i. at 50 and 25 mg/kg bw/d, respectively. The corrected body weight gain (i.e., minus gravid uterus weight) was not affected, but additional adverse clinical findings (blood in bedding, observed in 4 does, days

16-29 p.i. and 2 does, days 16-24 p.i. at 50 and 25 mg/kg bw/d, respectively) and reductions in mean gravid uterus weight were observed which are well in line with the embryotoxic or fetotoxic effects of the test compound at maternally toxic doses [see Table B.6.6-8]. Necropsy of the does did not reveal indications of a treatment-related effect.

There was a dose-related impact on some gestational parameters reaching statistical significance at the the top dose level. Postimplantation losses were elevated mainly due to an increase in early resorptions and the mean number of live fetuses/doe was reduced. These are not unusual findings in prenatal developmental toxicity studies in rabbits at dose levels which are clearly toxic to the does.

Table B.6.6-8: Developmental toxicity rabbit: Reproductive findings

Parameter	Dose level (mg/kg bw/d)			
	0	**5**	**10**	**20**
Implantation sites (mean)	7.4	6.6	6.9	7.0
Total resorptions (mean / mean %)	0.5 / 6.2	0.6 / 10.2	1.3 / 17.8	2.7**/ 38.6**
Early resorptions (mean / mean %)	0.4 / 5.5	0.5 / 9.6	1.2 / 15.9	2.6**/ 37.6**
Dams with viable fetuses / live fetuses (no.)	24 / 166	24 / 145	20 / 123	22 / 107
Live fetuses (mean / mean %)	6.9 / 93.8	6.0 / 89.8	6.2 / 90.4	4.9**/ 69.8**

Dunnett-test *p<0.05; **p<0.01

The incidence of external or soft tissue malformations or variations of any type was not affected by treatment but there was an apparent increase in the occurrence of skeletal malformations. The absolute number of affected fetuses was increased. In the intermediate and high dose groups, the percentage of fetuses with skeletal malformation was obviously higher than in the control group. Statistical significance was not attained, but the percentage of affected fetuses was just outside the historical control range of this laboratory and strain.

Table B.6.6-9: Developmental toxicity rabbit: Developmental findings

Parameter (Affected fetuses/Litter mean %)	Historical Control	Dose level (mg/kg bw/d)			
		0	**5**	**10**	**20**
Total fetal skeletal malformations	0.0 – 4.8	3.8	2.2	5.3#	12.1#
Fetuses with multiple malformations	0.0 – 0.7	0.0	0.6	2.9#	6.1#
Absent thoracic vertebrae	0.0 – 0.8	0.0	0.0	0.0	0.9#
Absent lumbar vertebrae	0.0 – 0.8	0.6	0.6	0.6	3.2#
Misshapen lumbar vertebrae	0.0 – 2.4	0.5	0.6	0.0	1.9

Wilcoxon test *p<0.05; **p<0.01; # > historical control values.

Conclusion:
Under the conditions of this prenatal developmental toxicity study, pyraclostrobin was embryotoxic in rabbits at dose levels with severe maternal toxicity.
The NOAEL for maternal toxicity was <5 mg/kg bw/d, suggesting a higher susceptibility of this species as compared to the rat at least when the test compound is administered during gestation. The NOAEL for developmental toxicity was 5 mg/kg bw/d, based on increased incidences of skeletal malformations outside the historical control range of this laboratory at 10 and 20 mg/kg bw/d. At the highest dose level of 20 mg/kg bw/d, elevated postimplantation losses (mainly due to increased early resorptions) and a reduction in the mean number of live fetuses/doe were observed.

On request of the Rapporteur, a second prenatal developmental toxicity study in rabbits was performed with the main objective to establish a clear NOAEL for maternal toxicity.

Report:	Schilling, K., Hellwig, J. and van Ravenzwaay, B. (2001): BAS 500 F – Additional maternal toxicity study in Himalayan rabbits. Oral administration (gavage). BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 2001/1003803, unpublished.
Test Material:	Pyraclostrobin; batch No. CP028719; purity: 98.9%.
Test Animals:	Himalayan rabbits [Chbb:HM (outbred strain), provided by Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany].
GLP:	Yes.
Test Method:	EEC 87/302, OECD 414 [i.e., "Proposals for updating Guideline 414: Prenatal developmental toxicity study (Draft June 2000)"], EPA/OPPTS 870.3700
Deviations:	With regard to examination for fetal effects, the range of parameters investigated was limited since the focus of this additional study was on maternal toxicity.
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was tested for its maternal toxicity in Himalayan rabbits. The test substance was administered as an aqueous suspension to 25 inseminated females/group by stomach tube at doses of 0, 1, 3 and 5 mg/kg bw/d on day 7 through day 28 post insemination (p.i.). A standard dose volume of 10 ml/kg bw/d was used. The control group was dosed with the vehicle only (i.e., 0.5% Tylose CB 30.000 in doubly distilled water).

Food consumption and body weights were recorded regularly throughout the study period. The state of health of the animals was checked each day.

On day 29 post insemination, all does were sacrificed and assessed by gross pathology (including weight determination of the unopened uterus and the placentae). For each doe, corpora lutea were counted and number and distribution of implantation sites (differentiated as early and late resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus and weighed. Individual placental weights were also recorded.

Statistical analysis of data was performed by means of two-sided Dunnett's test and one-sided Fisher´s exact test.

Findings:
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparation was analytically verified. The correctness of the concentrations was analytically demonstrated.

There was no unscheduled mortality in this study. No abnormal clinical signs occurred in any of the groups. 24 (low dose group only) or 25 does/group became pregnant.

The following test substance related findings were obtained [see Table :6.6-10]:

Table B.6.6-10: Developmental toxicity rabbit: Maternal findings

Parameter	Dose level (mg/kg bw/d)			
	0	**1**	**3**	**5**
Food consumption, day 7 – 28 (g/animal/day)	99.0	93.9	84.0	80.0
Food consumption; day 7-8 (g/animal/day)	113.2	107.8	84.2**	66.7**
Body weight; day 29 (g)	2880	2834	2806	2806
Body weight change; day 7-9 (g)	12.8	7.5	-3.8	-14.2**
Gravid uterus (g)	321.8	320.5	306.2	354.4
Corrected body weight gain; from day 7 (g)	-82.9	-102.7	-109.4	-136.8

Dunnett-test *p<0.05; **p<0.01

A dose-related decrease in food consumption was observed at the medium and upper dose levels of 3 and 5 mg/kg bw/d throughout the administration period. As compared to the control group, the difference remained statistically significant up to study day 17 at least in the high dose group but was apparently more pronounced during the first days of treatment. Food utilisation was impaired at the highest dose level only. The lower food consumption did not result in a significant reduction in body weight. Initially, body weight gain was affected but this effect reached statistical significance for the first days of exposure and in the group receiving 5 mg/kg bw/d only.

Necropsy of the does did not reveal treatment-related findings.

The gestational parameters were not altered. There was no impact of test substance administration up to the highest dose of 5 mg/kg bw/d on the number or the mean body weight of the fetuses. The marginally lower mean placental weight in the highest dose group (4.2 g as compared 4.6 g in all the other groups) is considered a consequence of the incidentally higher number of live fetuses/doe in this group (7.3 live fetuses/doe at 5 mg/kg bw/d versus 6.8 in the control).

Conclusion:
In this additional prenatal developmental toxicity study in rabbits, a weak adverse effect on maternal food consumption and body weight gain at a dose level of 5 mg/kg bw/d was confirmed. The next lower dose of 3 mg/kg bw/d is considered the NOAEL for maternal toxicity since the occasionally reduced food intake at this dose level was not accompanied by an impaired food utilisation or a statistically significantly compromised body weight gain. In contrast, the notifier had argued that the minor effects at the highest dose level were not related to compound administration and because of this assessment established a maternal NOAEL of 5 mg/kg bw/d.

The gestational parameters were not affected and no evidence of developmental toxicity was obtained up to the highest dose of 5 mg/kg bw/d although the range of fetal parameters investigated was rather limited.

B.6.7 Delayed neurotoxicity (Annex IIA 5.7)

B.6.7.1 Neurotoxicity studies

Pyraclostrobin was tested in an acute and in a short-term neurotoxicity study in rats. These studies included extensive functional observation batteries as well as neurohistopathological investigations. No indications of a specific neurotoxic potential of pyraclostrobin were observed confirming the lack of toxic properties of this kind as suggested by the other toxicological studies.

B.6.7.1.1 Acute neurotoxicity

Report: Mellert, W.; Kaufmann, W. and Hildebrand, B. 1999(b): BAS 500 F - Acute oral neurotoxicity study in Wistar rats. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11111, unpublished.

Test Material: Pyraclostrobin; batch No. CP029053; purity: 99.0%.

Test Animals: Wistar rats [Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, location of breeding facility not reported].

GLP: Yes.

Test Method: EPA/OPP 81-8 (Neurotoxicity Screening Battery). This test procedure is considered to be in accordance with OECD guideline 424 (Neurotoxicity Study in Rodents).

Deviations: None.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was administered to groups of 10 male and 10 female Wistar rats as a single oral administration by gavage at dose levels of 0, 100, 300 and 1000 mg/kg bw. The vehicle was a 0.5% aqueous solution of carboxymethyl-cellulose, and the administration volume was 20 ml/kg bw.

The animals were observed up to 2 weeks after dosing. The general state of health of the rats was examined daily. Body weight was determined on day -7 (prior to dosing), day 0 (test substance administration), day 7 and day 14 thereafter. Functional observational batteries and motor activity measurements were carried out in all animals on day -7, on day 0 (within few hours after dosing), as well as on days 7 and 14. At termination on day 14, five animals per sex and dose were fixed by *in situ* perfusion and subjected to neuropathological examinations.

The remaining animals were sacrificed under CO_2-anesthesia without any further investigations.

For statistical analysis of body weight data, clinical and special neurotoxicity parameters, the following methods were applied: ANOVA, Dunnett´s test, two-sided Kruskal-Wallis test and Mann-Whitney U-test.

Findings:
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparation was analytically verified. The correctness of the concentrations was analytically demonstrated.

At the high dose level (1000 mg/kg bw), piloerection was observed in 4 females. Impaired body weight change in males on day 7 was also evident. At this dose level, also diarrhoea was observed in 5 males and 4 females. In animals dosed with 300 mg/kg bw, diarrhoea was observed in 2 males and 1 female.
Furthermore, soft faeces were observed in 1 male rat of the 1000 mg/kg bw group, 5 males of the 300 mg/kg bw group, and in 3 males and 1 female of the 100 mg/kg bw group. All clinical findings were observed on day 0 (day of administration), only, and were reversible by day 7 post exposure.

Functional observational batteries and motor activity measurements did not reveal evidence of treatment-related differences between the groups. The histological examination of the central and peripheral nervous system did not reveal any substance-dependent changes in the organs examined.

Conclusion:
Pyraclostrobin did not exhibit neurotoxicity following a single oral administration to rats. The unspecific signs of toxicity recorded in the present study were rather due to acute systemic toxicity and/or local effects on the digestive tract than indicative of a specific neurotoxic potential. The NOEL for neurotoxicity under the conditions of this study was 1000 mg/kg bw in both sexes, i.e. the highest dose tested.

B.6.7.1.2 Subchronic neurotoxicity

Report:	Mellert, W.; Kaufmann, W. and Hildebrand, B. 1999(c): Pyraclostrobin - Subchronic oral neurotoxicity study in Wistar rats. Administration in the diet for 3 months. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/11329, unpublished.
Test Material:	Pyraclostrobin; batch No. 27882/191/c; purity: 97.09%.
Test Animals:	Wistar rats [Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, location of breeding facility not reported].
GLP:	Yes.

Test Method: EPA/OPP 82-7 (Neurotoxicity Screening Battery).
This test procedure is considered to be in accordance with OECD guideline 424 (Neurotoxicity Study in Rodents).

Deviations: None.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Pyraclostrobin was dissolved in acetone and administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 50, 250 and 750 (males only) or 1500 ppm (females) for 3 months.

Food and water consumption were determined once a week. Body weight was determined once a week and on the days when functional observational batteries were performed. A check of the general state of health was made at least daily. Furthermore, the animals were thoroughly examined and palpated once a week. Functional observational batteries and motor activity measurements were carried out in all animals on days –7 (prior to start of administration), 22, 50 and 85. Five animals per sex and dose were fixed by *in situ* perfusion and subjected to neuropathological examinations. The remaining animals were sacrificed under CO_2-anesthesia without any further examinations.

Statistical analysis of the results were: ANOVA, Dunnett´s test, two-sided Kruskal-Wallis test, Mann-Whitney U-test and Wilcoxon test.

Findings:
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the dietary test substance preparation was analytically verified. The correctness of the concentrations was analytically demonstrated.

Table B.6.7-1: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	Males	Females
1500	-	111.9
750	49.9	-
250	16.9	20.4
50	3.5	4.0

Reduced food and water consumption was observed in animals of the high (1500 / 750 ppm) dose level as well as in males of the mid (250 ppm) dose level.

At the high dose level, body weight was reduced in males (11.9%) and females (8.9%). Body weigh gain at this dose level was reduced by 17.4% (males) and 18.1% (females).

An impairment of grip strength of forelimbs at the end of the study was noted in high dose animals but was attributed to the lower body weight.

There were no test substance related effects at 50 ppm.

Neurohistopathological investigations did not show any test substance related changes at any dose level.

Conclusion:
Pyraclostrobin caused signs of general toxicity at 1500 ppm in females and 750 and 250 ppm in males. The no observed effect level (NOEL) for systemic effects was 250 ppm (20.4 mg/kg bw/d) in female rats and 50 ppm (3.5 mg/kg bw/d) in males. Thus, the results obtained in the regulatory subchronic study in this species (see chapter B.6.3) were confirmed. In contrast, no signs of selective neurotoxicity were detected under the conditions of this specially designed study. The NOELs for neurotoxicity were 1500 ppm (111.9 mg/kg bw/d) in females and 750 ppm (49.9 mg/kg bw/d) in males, the highest dosages applied.

B.6.7.2 Delayed Neurotoxicity

Specific testing for delayed neurotoxicity in hen was not considered necessary, since pyraclostrobin does not belong to the substance class of organophosphates and since there was no evidence of primary neurotoxic effects in the acute, subchronic or long-term studies.

B.6.8 Further toxicological studies (Annex IIA 5.8)

The additional toxicological studies adressed in this section are confined to the examination of three metabolites of pyraclostrobin occurring in water for their potential to cause gene (point) mutations in two bacterial systems. According to the results of these studies, the metabolites BF500-11, BF500-13 and BF500-14 were not mutagenic in the Ames reverse mutation assay neither in *S. typhimurium* strains nor in *E. coli*. Because of the unequivocal findings, statistical analysis of the data (apart from determination of mean and standard deviation) was not necessary. Since all three studies were very similar, the study design and the results obtained are not reported separately in this monograph.

Reports:

Engelhardt, G. and Hoffmann, H. D. (1999b): Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg. No. 411 847. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/12017, unpublished.

Engelhardt, G. and Hoffmann, H. D. (1999c): Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg. No. 412 785. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/12020, unpublished.

Engelhardt, G. and Hoffmann, H. D. (2000): Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg. No. 413 038. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 2000/1000005, unpublished.

Test Material: Reg. No. 411 847 (Metabolite BF500-11); batch No. 01183-172; purity: 98.9%;
Reg. No. 412 785 (Metabolite BF500-13); batch No. 01586-60; purity: 99.6%;
Reg. No. 413 038 (Metabolite BF500-14); batch No. 01586-236; purity: 94.5%.

Test System: Salmonella typhimurium (strains TA 100, TA 1535, TA 1537 and TA 98) and Escherichia coli (strain WP2 uvrA).

GLP: Yes.

Test Method: OECD 471, 472.

Deviations: None.

Acceptability: All these studies are considered to be acceptable.

Material and Methods:
The abovementioned aquatic metabolites of pyraclostrobin were tested for their potential to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. Each of the studies consisted of a standard plate test and a preincubation test both with and without metabolic activation (liver S-9 mix obtained from Aroclor-1254-induced Sprague-Dawley rats). Three plates were used per dose for each strain and test condition.
The *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 and *Escherichia coli* strain WP2 uvrA were exposed to the test substances dissolved in DMSO. The concentrations tested ranged from 20 to 5,000 µg/plate (BF500-11, standard plate test and a preincubation test; BF500-13, standard plate test); 4 to 2,500 µg/plate (BF500-13, preincubation test) or 22 to 5,500 µg/plate (BF500-14, standard plate test and preincubation test).
For control purposes and to demonstrate the sensitivity of the test system, a negative (vehicle) control and positive controls (2-aminoanthracene with S-9 mix and either N-methyl-N-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine or 4-nitroquinoline-N-oxide without metabolic activation) were tested. Furthermore, a sterility control was included.

Findings:
The stability of the test substances throughout the study period, in DMSO and in water was verified.
Test substance precipitation was not observed. With the metabolite BF500-11, slight bacteriotoxic effects were occasionally observed at concentrations of 2,500 µg/plate and higher. With BF500-13, a similar bacteriotoxic effect was noted at concentrations of 500 µg/plate and higher in the preincubation test and at 2,500 µg/plate and, much more pronounced, 5000 µg/plate in the standard plate test. Also with BF500-14, a weak bacteriotoxic effect was observed at concentrations of 2,750 µg/plate and above in the preincubation test and at the highest concentration of 5,500 µg/plate in the standard plate test.

In all three studies, the mean number of revertant colonies was not increased in any bacterial strain neither with nor without S-9 activation. In contrast, expected increases in revertant colonies were obtained with the positive control substances.

Conclusion:
According to the results of these independent studies, the metabolites BF500-11, BF500-13 and BF500-14 were not mutagenic in the Ames reverse mutation assay.

B.6.9 Medical data and information (Annex IIA 5.9)

Since pyraclostrobin is a new compound, no data and medical experience regarding possible health effects in humans are available to the Rapporteur. Therefore, the information provided by the sole notifier is presented here.

B.6.9.1 Medical surveillance on manufacturing plant personnel

Since industrial production of this active ingredient has not yet commenced, no data on medical surveillance on manufacturing plant personnel are available. The personnel which is handling developmental compounds is surveyed by regular medical examinations. However, this surveillance programme is not aimed to specifically detect pyraclostrobin-related symptoms or diseases. Upt to now, there are no indications of a causal association between the compound and any specific medical effect.

B.6.9.2 Direct observation, e.g. clinical cases and poisoning incidents

No clinical cases or poisoning incidents have been reported so far.

B.6.9.3 Observations on exposure of the general population and epidemiological studies if appropriate

No observations regarding health effects after exposure of the general public are available.

B.6.9.4 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Methods for determination of active substance or metabolites in biological fluids are not established. Specific signs of poisoning or clinical tests are not known.

B.6.9.5 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet/precautions. In case of an poisoning incident, symptomatic and supportive treatment is recommended. No specific antidote is known.

B.6.9.6 Expected effects of poisoning

Effects of poisoning are not known. It is concluded from the acute toxicity studies that the acute hazard of pyraclostrobin is low.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.10)

B.6.10.1 Summary of mammalian toxicology

Toxicokinetics and metabolism

Following oral administration of either a single low (5 mg/kg bw) or a high dose (50 mg/kg bw) to rats, pyraclostrobin [Methyl-N-(2-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxymethyl-phenyl)-N-methoxy carbamate; BAS 500 F] was rapidly absorbed from the gastrointestinal tract. However, oral absorption is incomplete and accounts for approximately 50% or even less of the dose. This percentage was estimated by summing up the amount of urinary and biliary excretion.

The elimination process was nearly completed after 120 hours with the major part of radioactivitiy beeing excreted within the first 48 hours irrespective of dose, dosing regimen (single versus repeated administration) or sex. About 11-15% of the applied radioactivity was eliminated via the urine while excretion via the faeces accounted for 80 – 90% of the dose. However, 35% of the radioactivity was actually eliminated from the body via the bile. Initial half-lives were approximately 10 h, terminal half-lives ranged between 20 and 37 h. A comparison of AUC values for both dose levels suggests nearly linear kinetics.

Tissue distribution determination revealed highest amounts of radioactivity in the GI tract, followed by liver. All other tissues had residues similar to or less than the concentrations in plasma. Although this compound is lipophilic, there is no evidence of accumulation. Most likely, this is due to extensive metabolism and rapid and effective excretion.

The systemically available portion was rapidly and intensively metabolised with N-demethoxylation being the quantitatively most important pathway. Phase I biotransformation was further characterised by various hydroxylations, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to a large number of observed metabolites. No major differences were observed with regard to sex and dose level.

Dermal absorption of pyraclostrobin is poor. A skin penetration rate of 1% in humans should be used for calculations but is still considered an overestimation.

Acute toxicity including irritation and sensitisation

In rats, pyraclostrobin was of low acute oral and dermal toxicity. No mortality occurred up to the highest oral dose of 5,000 mg/kg bw although some signs of intoxication were observed.

The dermal LD_{50} was greater than 2,000 mg/kg bw with no signs of systemic toxicity occurring. In contrast, clear toxic properties of the active ingredient were noted in the inhalation study in rats including severe symptoms of systemic poisoning and deaths. Classification and labelling (T, R23) is considered necessary although the actual inhalative risk of this active ingredient for humans is considered rather low.

The compound produced mild skin irritation and should be labelled accordingly (Xi, R38). Pyraclostrobin was not irritant to the eyes and was not a skin sensitiser in the Maximisation test.

Short-term toxicity

The oral short-term toxicity of pyraclostrobin was investigated in rats, mice, and dogs. Additionally, subacute dermal toxicity was examined in rats. The results of these studies are summarised in Table B.6.10-1.

Table B.6.10-1: Summary of short-term toxicity studies

Study type / species / dose levels	NOAEL (mg/kg bw/d)	LOAEL / Critical effects
4-week feeding Wistar rat 0, 20, 100, 500, 1500 ppm	9/9.6 m/f [100 ppm]	500 ppm: Effects on body weight, red blood cells, duodenum and liver.
4-week dermal Wistar rat 0, 40, 100, 250 mg/kg bw/d	>250 (systemic)	250 mg/kg bw/d: No systemic toxicity. 40 mg/kg bw/d: Signs of local irritation.
3-month feeding Wistar rat 0, 50, 150, 500, 1000, 1500 ppm	10.7/12.6 m/f [150 ppm]	500 ppm: Reduced body weight and food consumption, effects on clinical-chemical parameters, liver hypertrophy, and mucosal hypertrophy of the duodenum.
3-month feeding B6C3F1 mouse 0, 50, 150, 500, 1000, 1500 ppm	<9.2/12.9 m/f [<50/50 ppm m/f] (*ca. 4; m [30 ppm], carcinogenicity study)*	50 ppm: Reduced body weight (gain) and increased urea values in males. At higher dose levels, adverse effects in the gastrointestinal tract, on red blood cells, on white blood cells and lymphatic organs, as well as on adrenals, liver and kidney.
3-month feeding Beagle dog 0, 100, 200, 450 ppm	5.8/6.2 m/f [200 ppm]	450 ppm: Body weight loss in females, vomitus, diarrhoea, clinical-chemical and hematological changes in females, hypertrophy of the duodenal mucosa.
12- month feeding Beagle dog 0, 100, 200, 400 ppm	5.4/5.4 m/f [200 ppm]	400 ppm: Reduced body weight and food consumption (females); vomitus, diarrhoea; hemoglobin and hematocrit decreased (females); increase of white blood cells and platelets (males).

The signs of toxicity, as observed in the three species tested, were comparable. The critical clinical effects were reduction of body weight and body weight gain in all three species. In dogs, vomitus and diarrhoea occurred additionally. The target organ in all three species was the duodenum, showing mucosal hypertrophy, which was characterized by an increased ratio of cytoplasm to the nuclei in the villi, and by hyperplastic changes in the epithelial cells. Furthermore, some clinical chemical and haematological parameters were affected suggesting, together with pathological findings, minor adverse effects on the blood and the liver.

In a 4-week dermal toxicity study in rats, no substance related systemic toxicity was detected up to the highest dose tested (250 mg/kg bw/d) whereas local effects were seen at all dose levels.

For rats, a short-term NOAEL of 150 ppm (10.7 mg/kg bw/d) has been established. For dogs, the short-term NOAEL is 200 ppm, equivalent to about 6 mg/kg bw/d, based on the 3-month and 1-year feeding study in this species.
For female mice, the no observed adverse effect level was 50 ppm (12.9 mg/kg bw/d).
For males, the NOAEL in the 3-month mouse study was lower than 50 ppm (9.2 mg/kg bw/d). Taking into consideration all available data for this species, a NOAEL of 30 ppm (ca 4 mg/kg bw/d) can be established for male mice based on the body weight data after 91 days from the carcinogenicity study. This dose is also considered the lowest relevant NOAEL for overall short-term oral toxicity.

Mutagenicity
Pyraclostrobin was evaluated for its potential genotoxicity *in vitro* using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis (UDS) test. *In vivo*, the test substance was assessed for the induction of micronuclei in mice. The results of these studies unequivocally demonstrated the absence of a genotoxic effect.

Long-term toxicity and cancerogenicity
A chronic toxicity study in rats and two carcinogenicity studies in rats and mice were performed with pyraclostrobin and failed to provide evidence of a carcinogenic effect.
Restricted to the high doses, body weight and body weight gain were decreased in all studies, mostly accompanied by a diminished food consumption.
In the carcinogenicity study in rats, liver cell necrosis occurred in males. Additionally, in the chronic toxicity study in rats, alanine aminotransferase and alkaline phosphatase values were decreased at the high dose. The toxicological significance of these reduced enzyme activities is equivocal.
The overall NOAEL, obtained from the chronic/carcinogenicity studies in rats (males and females combined) and the carcinogenicity study in mice, is about 4 mg/kg bw/d [see Table B.6.10-2].

Table B.6.10-2: Summary of long-term toxicity studies

Study type / species / dose levels	NOAEL (mg/kg bw/d)	LOAEL / Critical effects
24-month chronic toxicity Wistar rats 0, 25, 75, 200 ppm	3.4/4.6 m/f [75 ppm]	200 ppm: Reduced body weight. No increase in tumour incidences.
24-month carcinogenicity Wistar rats 0, 25, 75, 200 ppm	3.4/4.7 m/f [75 ppm]	200 ppm: Reduced body weight, reduced food consumption (m), liver cell necrosis (m). Not carcinogenic.
18-month carcinogenicity B6C3F1 mice 0, 10, 30, 120 ppm (m, f), 180 ppm (f)	4.1/4.8 m/f [30 ppm]	120 ppm (m, f) & 180 ppm (f): Reduced body weight. Not carcinogenic.

Reproduction toxicity and teratogenicity
The reproduction toxicity of pyraclostrobin was investigated in a two-generation reproduction study in rats as well as in teratogenicity studies in rats and rabbits.
Fertility was not affected up to the highest dose level of 300 ppm (ca 32.6 mg/kg bw/d) in the two-generation study. The NOAEL concerning parental toxicity in this study was 75 ppm

(approximately 8.2 mg/kg bw/d) for F0 and F1 animals, and this was also the NOAEL for reproductive toxicity in the F1 and F2 litters. Offspring effects were confined to reduced body weight gain and associated changes of organ weights. A single developmental landmark (vaginal opening) was delayed in F1 pups only at 300 ppm.

Pyraclostrobin was not teratogenic neither in rats nor in rabbits.
In the prenatal toxicity study in rats, developmental toxicity was observed at the highest dose tested (50 mg/kg bw/d), based on increased incidences of several soft tissue and skeletal variations inside the range of the historical control values. The high and intermediate dose levels were clearly toxic to the dams, as demonstrated by a marked reduction in body weight gain. Thus, the NOAEL for maternal toxicity was established at 10 mg/kg bw/d, and the developmental NOAEL was 25 mg/kg bw/d.
In the first rabbit prenatal toxicity study, developmental toxicity was observed in the presence of severe maternal toxicity suggesting a higher vulnerability of this species. Maternal toxicity was proven by clear reduction of body weight gain and a lower food consumption at 5 mg/kg bw/d and above. Prenatal toxicity was substantiated by embryolethality resulting in elevated postimplantation losses and a reduction in the mean number of live fetuses/doe at 20 mg/kg bw/d. At 10 and 20 mg/kg bw/d, increased incidences of skeletal malformations outside the historical control range of this laboratory were observed. The NOAEL for developmental toxicity was 5 mg/kg bw/d. A clear NOAEL for maternal toxicity could not be established and, therefore, a further rabbit study was performed by the notifier on request of the Rapporteur. In this second study, a lower food consumption and an initial impact on body weight gain was confirmed in the does receiving the highest dose of 5 mg/kg bw/d. The medium dose of 3 mg/kg bw/d was considered the maternal NOAEL. There were no indications of fetal effects in this study, however, the range of parameters investigated was rather limited since the focus of this additional study was on maternal toxicity.
The results of the reproduction toxicity studies are summarised in Table B.6.6-1.

Table B.6.10-3: Summary of reproduction toxicity studies

Study type / species / dose levels	NOAEL	LOAEL / Critical effects
2-generation study Wistar rats 0, 25, 75, 300 ppm	Parental toxicity: ca 8.2 mg/kg bw/d [75 ppm] Reproductive toxicity: ca 8.2 mg/kg bw/d [75 ppm]	300 ppm: Parental toxicity: reduced food consumption and body weight gain; Reproductive toxicity: reduced pup body weight gain, organ weight changes and a delay in vaginal opening (F1 females only). No adverse effects on fertility.
Developmental toxicity Wistar rats 0, 10, 25 and 50 mg/kg bw/d; days 6-19	Maternal toxicity: 10 mg/kg bw/d Developmental toxicity: 25 mg/kg bw/d	25 mg/kg bw/d: Maternal toxicity: reduced food consumption and body weight (gain). 50 mg/kg bw/d: Developmental toxicity: increased variations.
Developmental toxicity Himalayan rabbits (1st study) 0, 5, 10 and 20 mg/kg bw/d; days 7-28	Maternal toxicity: <5 mg/kg bw/d Developmental toxicity: 5 mg/kg bw/d	5 mg/kg bw/d: Maternal toxicity: reduced food consumption, reduced body weight gain. 10 mg/kg bw/d: Developmental toxicity: increased skeletal malformations; at 20 mg/kg bw/d increased resorptions and postimplantation losses; reduced number of live fetuses.
Developmental toxicity Himalayan rabbits (2nd study with special regard to maternal effects) 0, 1, 3, 5 mg/kg bw/d; days 7-28	Maternal toxicity: 3 mg/kg bw/d Developmental toxicity: 5 mg/kg bw/d	5 mg/kg bw/d: Maternal toxicity: reduced food consumption, reduced body weight gain. No evidence of developmental toxicity (limited range of parameters investigated).

Neurotoxicity

No indications of a specific neurotoxic potential of pyraclostrobin were observed in rats neither in an acute nor in a subchronic neurotoxicity study. There was also no evidence of neurotoxicity coming from any other of the toxicological studies.

Further toxicological studies

Three groundwater metabolites of pyraclostrobin were tested for their ability to cause gene mutations and proved all negative in the Ames test.

Medical data

Since pyraclostrobin is a new compound, human data and experience are very limited.
No poisoning incidents have been reported so far. With regard to the toxicological profile of pyraclostrobin, serious health problems are not anticipated.

B.6.10.2 Acceptable daily intake (ADI)

The ADI should be based on the overall chronic NOAEL of 4 mg/kg bw/day as established in the long-term studies in rats and mice. According to the comprehensive toxicological database, the compound has no genotoxic or carcinogenic potential, is not teratogenic and does not affect fertility. Therefore, the standard assessment factor of 100 is considered appropriate.

The calculation results in a proposed ADI of:

0.04 mg/kg bw.

This ADI value is in agreement with the proposal of the notifier.

B.6.10.3 Acute reference dose (ARfD)

The long-term as well as oral short-term toxicity studies with pyraclostrobin suggest an overall NOAEL of 4 mg/kg bw/day. This is also supported by the outcome of the teratogenicity studies in rabbits since the LOEL for maternal toxicity was 5 mg/kg bw/day. Thus, it is considered most appropriate to derive also the ARfD on this basis resulting in a proposed value of

0.04 mg/kg bw.

In contrast, the notifier had proposed to derive the ARfD from the 4-week study in rats resulting in a numerical value of of 0.09 mg/kg bw. However, the range of parameters investigated in this study was rather limited. Moreover, the evidence of maternal effects in two rabbit developmental toxicity studies at a dose level of 5 mg/kg bw/day does not allow to accept this proposal.

B.6.10.4 Acceptable operator exposure level (AOEL)

The AOEL is usually derived on the basis of so-called mid-term toxicity studies, i.e. the subacute/subchronic or teratogenicity studies. For pyraclostrobin, the lowest relevant oral NOAEL established in studies of these types was 4 mg/kg bw/day which is mainly based on body weight data in male mice but also supported by results obtained in the other subchronic studies as well as by the maternal findings in the developmental toxicity studies in rabbits.
For establishing the systemic AOEL, the oral absorption rate of approximately 50% must be taken into account. Because of the toxicological profile of pyraclostrobin and in accordance with current EU assessment practice, the standard assessment factor of 100 should be applied resulting in a **systemic AOEL of 0.02 mg/kg bw/day.**
The notifier had proposed a systemic AOEL of 0.08 mg/kg bw/day using a lower assessement factor of 25.

B.6.10.5 Maximum acceptable concentration in drinking water

The determination of a MAC value is not necessary, because according to Directive 91/414/EC only the ADI and AOEL values have to be determined. Therefore, the establishment of a maximum admissible concentration for drinking water from an ADI value is not yet confirmed by a harmonised EU proposal. In addition to that, the maximum admissible concentration of an active substance is 0.1 µg/l, as established by the Directive 89/778/EEC.

B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1)

BAS 500 00 F is an emulsifiable concentrate (EC) with a relative density of 1.06 containing 250 g/l active ingredient pyraclostrobin (= BAS 500 F). BAS 500 00 F is considered as harmful based on its acute oral and inhalation toxicity. The formulation has a low acute toxicity after dermal exposure. It is irritating to skin and eyes. Because with BAS 500 00 F there were no skin reactions in the Buehler test and the active ingredient was proved to be non sensitizing in a maximisation test, the formulation BAS 500 00 F is considered to have no skin sensitising properties.

Table B.6.11-1: Acute toxicity of BAS 500 00 F

Study type	Results	Proposed classification and labelling
Acute oral LD_{50} rat	LD_{50} >500 mg/kg bw (males); 260 mg/kg bw (females); about 500 mg/kg bw (m + f)	R 22
Acute dermal LD_{50} rat	LD_{50} > 4000 mg/kg bw	None
Acute inhalation LC_{50} rat	LC_{50} = 3.51 mg/l air (4 h); aerosol	R 20
Skin irritation	Irritating	R 38
Eye irritation	Irritating	R 36
Skin sensitisation (Buehler test)	Not sensitising	None

Additionally based on the toxicological data on the co-formulants, a labelling with R65 ”Harmful: may cause lung damage if swallowed” should be discussed (Dir. 98/98/EEC). The notifier, however, stated that, based on physical investigations of the product, the risk phrase R65 from Solvesso should not be used for the classification of the product.

B.6.11.1 Oral

Report: Wiemann, C. and Hellwig, J. 1998(d): BAS 500 00 F - Acute oral toxicity in rats.
BASF AG, Ludwigshafen/Rhein, Germany;
BASF RegDoc# 1998/10804, unpublished.

Test Material: BAS 500 00 F; formulated product; batch No. 97-2; a.i.: 247.83 g/l.

Test Animals: Young adult Wistar rats [strain: Chbb-THOM (SPF); source: Dr. K. Thomae GmbH, Biberach, Germany].

GLP: Yes

Test Method: EEC 92/69; OECD 401; EPA/FIFRA 81-1

Deviations: None.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Single administration of a test substance preparation in aqua bidest. by gavage to five male and five female fasted Wistar rats at a dose levels of 50, 200, 500 and 2,000 mg/kg bw, using an application volume of 10 ml/kg bw. The observation period lasted for up to 14 days.

Findings:
The test substance formulation was demonstrated to be stable. The correctness of the concentration and its homogeneity were analytically confirmed.

Mortality observed in this study is shown below:

Table B.6.11-2: Cumulative mortality

Dose level (mg/kg bw)	**50**	**200**	**500**	**2,000**
No of males	**5**	**5**	**5**	**5**
1 h	0	0	0	2
2 h	0	1	0	4
4 h	0	1	0	5
14 d	0	1	0	-
No of females	**5**	**5**	**5**	**5**
0 h	0	0	4	0
2 h	0	2	4	4
3 h	0	2	4	5
14 d	0	2	4	-
Mortality (m + f)	0 %	30 %	40 %	100 %

Signs of toxicity noted at 2,000, 500 and 200 mg/kg bw included impaired or poor general state, dyspnea, gasping, apathy, lateral position, staggering, ataxia, paresis, twitching, saltatory, flexion and extension spasm, rolling convulsions, opisthotonus, spasm of the jaws, diarrhea, salivation, eyelid closure and exophthalmus. 4 female animals of the 50 mg/kg dose group exhibited diarrhea. No symptoms were noted in the male animals of the 50 mg/kg dose group. All surviving animals appeared normal one day after the application.

Body weight development appeared to be normal. Necropsy findings of animals that died consisted of discoloured contents of stomach and intestines, discolouration of lung lobes and congestive hyperemia. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion:
The oral LD_{50} was found to be about 500 mg/kg bw for male and female rats (LD_{50} for males: >500 mg/kg bw; LD_{50} for females: 260 mg/kg bw; all animals of the 2000 mg/kg dose group died within 4 hours after application).
Risk phrase: R22 (harmful if swallowed).

B.6.11.2 Percutaneous

Report: Wiemann, C. and Hellwig, J. 1998(c): BAS 500 00 F - Acute dermal toxicity in rats.
BASF AG, Ludwigshafen/Rhein, Germany;
BASF RegDoc# 1998/10646, unpublished

Test Material: BAS 500 00 F; formulated product; batch No. 97-2; a.i.: 247.83 g/l.

Test Animals: Young adult Wistar rats [strain: Chbb-THOM (SPF); source: Dr. K. Thomae GmbH, Biberach, Germany].

GLP: Yes

Test Method: EEC 92/69; OECD 402; EPA/FIFRA 81-2

Deviations: None.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The undiluted test material was applied dermally to five male and five female Wistar rats for 24 hours under semiocclusive dressing at a dose level of 4,000 mg/kg bw. The application area was about 50 cm² (at least 10% of the body surface).

Findings:
The test substance formulation was demonstrated to be stable. The homogeneity was analytically confirmed.

No mortality occurred during the 14-day post-application observation period.

Signs of systemic toxicity consisted of impaired or poor general state of health, dyspnea and apathy. The animals appeared normal three days after the application.

Body weight development appeared to be normal, with the exception of a stagnation in 4 females at day 7 only.

The following local signs were detected: very slight erythema in 1 male up to day 7 and in 3 females from day 1 - 7, a well defined erythema in 1 male on day 1 and in 3 females on day 1 – 7, moderate to severe erythema in 4 males in day 1 and in1 female on day 1.

A very slight edema was noted in 3 males from day 1 to 7 and in 2 females on day 1, slight edema in 3 males on day 1 and 2 females on day 1 to 7.

Petechiae, severe scaling, superficial scabbing and bleeding was observed in single males and females on day 1 and day 7. No skin reactions were noted by the end of the 14-day observation period.

No pathological findings were detected in the animals.

Conclusion:
The dermal LD_{50} was found to be > 4,000 mg/kg bw for male and female animals.

B.6.11.3 Inhalation

Report:	Gamer, A.O. et al. (1998): BAS 500 00 F - Acute inhalation toxicity study in Wistar rats; 4-hour liquid aerosol exposure. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/11185, unpublished.
Test Material:	BAS 500 00 F; formulated product; batch No. 97-2; a.i.: 247.83 g/l.
Test Animals:	Young adult Wistar rats [strain: Chbb-THOM (SPF); source: Dr. K. Thomae GmbH, Biberach, Germany].
GLP:	Yes
Test Method:	EEC 92/69; EEC 93/21; OECD 403; EPA/FIFRA 81-3, EPA/TSCA 798.1150
Deviations:	None.
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Five male and five female Wistar rats per dose level were exposed to a liquid aerosol of the test material for four hours in a head/nose inhalation system at mean analytical concentrations of 1.06, 2.72 and 5.2 mg/l air. The observation time was 14 days.

Findings:
The test substance is a formulation demonstrated to be stable. The homogeneity of the test substance was confirmed by analysis. The homogenous distribution of atmospheres in this inhalation system has been proven in technical tests with model aerosols.

The particle size distribution revealed a mass median aerodynamic diameter (MMAD) of 0.9 – 1.3 µm, which is within the respirable range.

On the day of exposure (day 0), 4 out of 5 males and 4 out of 5 females died at 5.2 mg/l air. At a concentration of 2.72, 1 out of 5 males and 2 out of 5 females died on day 0. There were no mortalities in the 1.06 mg/l group. There were no additional mortalities in any group after day 0.

Table B.6.11-3: Cumulative mortality

Dose level (mg/l air)	1.06	2.72	5.2
No of males	**5**	**5**	**5**
day 0	0	1	4
day 1 - 14	0	1	4
No of females	**5**	**5**	**5**
day 0	0	2	4
day 1 - 14	0	2	4
Mortality (m + f)	0 %	30 %	80 %

The LC_{50} for male and female animals was calculated to be 3.51 mg/l air.
All concentrations led to clinical signs of toxicity indicative for respiratory stress, local irritant action and systemic toxicity. No abnormalities were observed in the surviving animals from post exposure day 6 (low dose), 7 (mid dose) and 9 (high dose) onward.

Body weight development in surviving animals was reduced in the first week after exposure, but recovered in the second week, with the exception of the surviving high dose female which did not gain weight throughout the observation period.

Necropsy of animals which died on study revealed a stinging odor, diffuse dark red coloration and edema of the lung and a clear nasal discharge. No macroscopic pathological findings were noted in the surviving animals at the end of the study.

Conclusion:
The inhalation LC_{50} was found to be 3.51 mg/l air (4 h) for male and female rats.
Risk phrase: R20 (harmful by inhalation).

The notifier stated that, based on the particle size distribution of the product BAS 500 00 F, if determined under realistic scenarios, a classification is not necessary. A supplementary study was submitted, respectively:

Report: Gamer A.O. and Stadler, R. (2000): Particle size distribution of BAS 500 00 F concerning acute inhalation toxicity.
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany;
BASF RegDoc# 1999/12006, unpublished.

Test Material: BAS 500 00 F; formulated product.

Test Method: Not applicable.

GLP: Not applicable.

Material and methods:
The purpose of this study was to compare particle size distribution of BAS 500 00 F as generated in an acute inhalation study in Wistar rats (see above) with the particle size distribution prevailing in agricultural practice when applied with common sprayers. For this evaluation the BAS 500 00 F spray was generated by a flat jet nozzle with an application angle of 110 degrees using a pressure of 10 bar simulating practical worst case condition.

Particle size measurements were made by means of laser scattering in a Malvern Master Sizer SX .The principle of this technique is based on laser ensemble light scattering. This system can be used for measurements of sprays, aerosols, dispersible powders or multiphase fluids (ISO/FDIS 13320-1, 1999 “Particle size analysis – Laser diffraction methods”).
At least 2000 – 5000 droplets were measured for evaluating the particle size distribution.
The Master Sizer SX has an optical range of 0.5 µm to 600 µm with a 300 mm lens.
The temperature during the measurement was between 20 – 26 °C, the relative air humidity was between 70 – 80%.
The measurement took place in a system, in which the nozzle was positioned in a vertical distance of 0.4 m to the laser beam.

Findings:
The aerosol generated with BAS 500 00 F had a mass median aerodynamic diameter of 125 µm.
Particles with an aerodynamic diameter of 50 µm or less contributed to 6.5% of the total mass.
The fraction of particles of toxicological relevance, i.e. particles which would deposit in the thoracic region of the respiratory tract was calculated to be 0.13% of the total aerosol mass.
Opposite to this result the mass median aerodynamic diameter in the acute inhalation study was between 0.9 and 1.3 µm, indicating that the majority of the particles was within the respirable range.

Conclusion:
The particle size spectrum generated with BAS 500 00 F under conditions typical for the agricultural practice and evaluated by means of a laser ensemble light scattering indicate that only a minor part of the aerosol is available for inhalation by operators (about 6.5% of the particles with a aerodynamic diameter of 50 µm and less).
Those particles being prone to enter the thoracic region contribute to 0.13% of the total of the aerosol only. Opposite to this finding in the inhalation study almost 100% of the particles generated are in the respirable range.

These results indicate that under typical agricultural conditions the particle size spectrum generated is different to that generated in acute inhalation studies as being requested by the corresponding guidelines.

On this basis, the notifier stated that there is no need for a specific classification with regard to the acute inhalation toxicity of BAS 500 00 F.

Regarding the practical relevance of the requested particle sizes in the acute inhalation studies as being requested by the corresponding guidelines, the opinion of the notifier can be agreed with. Nevertheless, based on the inherent toxic properties of the formulation as shown in the inhalation study, classification/labelling of BAS 500 00 F is considered necessary by the Rapporteur.

B.6.11.4 Skin irritation

Report: Wiemann, C. and Hellwig, J. 1998(a): BAS 500 00 F - Acute dermal irritation/corrosion in rabbits.
BASF AG, Ludwigshafen/Rhein, Germany;
BASF RegDoc# 1998/10644, unpublished.

Test Material: BAS 500 00 F; formulated product; batch No. 97-2; a.i.: 247.83 g/l.

Test Animals: Young adult White New Zealand rabbits (SPF); [source: Dr. K. Thomae GmbH, Biberach, Germany].

GLP: Yes

Test Method: EEC 92/69; OECD 404; EPA/FIFRA 81-5

Deviations: None.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The undiluted test material (0.5 ml) was applied dermally to the intact skin of two male and four female White New Zealand rabbits for 4 hours on a 2.5 cm x 2.5 cm test patch under a semiocclusive dressing. After the patches were removed the treated area was rinsed with Lutrol and Lutrol/water (1 : 1). The animals were observed for skin irritation for 14 days after test material application. Skin readings were performed at 1h, 24h, 48h, 72h, 7 days and 14 days after removal of the patch.

Findings:
The test substance is a formulation demonstrated to be stable. The homogeneity of the test substance was analytically confirmed.

Skin findings are summarised in the following table:

Table B.6.11-4: Skin irritation values (erythema/edema)

Animal Number	Time after patch removal						Mean (24-72 h)
	1 h	24 h	48 h	72 h	7 d	14 d	
1	2/0	2/1	3/1	3/1	2/1	1/0	2.7/1.0
2	3/1	3/1	3/1	3/0	2/0	2/0	3.0/0.7
3	3/1	3/2	3/2	3/1	2/1	2/1	3.0/1.7
4	3/1	3/1	3/0	3/0	2/0	2/0	3.0/0.3
5	3/1	3/2	3/2	2/1	2/0	2/0	2.7/1.7
6	3/1	3/2	3/1	3/1	2/0	2/0	3.0/1.3

The mean score (24-72 h) for all animals was determined to be 2.9 for erythema and 1.1 for edema.
Erythema (up to day 14) and edema (up to 72 h) extending beyond the area of exposure was noted in most animals. Scaling and severe scaling was seen on day 7 and day 14 in all animals.

Conclusion:
BAS 500 00 F is irritant to the skin. The effects were irreversible within the the observation period of 14 days.
Risk phrase: R 38 (irritating to skin).

B.6.11.5 Eye irritation

Report:	Wiemann, C. and Hellwig, J. 1998(b): BAS 500 00 F - Acute eye irritation in rabbits. BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1998/10645, unpublished.
Test Material:	BAS 500 00 F; formulated product; batch No. 97-2; a.i.: 247.83 g/l.
Test Animals:	Young adult White New Zealand rabbits (SPF); [source: Dr. K. Thomae GmbH, Biberach, Germany].
GLP:	Yes
Test Method:	EEC 92/69; OECD 405; EPA/FIFRA 81-4
Deviations:	None.
Acceptability:	The study is considered to be acceptable.

Material and Methods:
The undiluted test substance was applied once to the conjunctival sac of two male and four female White New Zealand rabbits. The application volume was about 0.1 ml. The test substance was washed out with tap water 24 hours after the application. Readings of the eyes were carried out at 1 hour and 1, 2, 3, 7 and 14 days after the application of the test material.

Findings:
The test substance is an aqueous formulation demonstrated to be stable. The correctness of the concentration and its homogeneity were analytically confirmed.

Opacities (grade 1 scattered or diffuse areas, details of iris clearly visible) were observed in all animals at 24 and 48 hours. At the 72 hour reading 3 animals were found with grade 1 and three animals with grade 2. At day 7 there were no effects on the cornea anymore.

Iridial effects were confined to grade 1 in all animals at 24 and 48 h, to grade 1 in 3 animals at the 72 h reading and to grade 1 in 1 animal at 7 days after application.

Redness and swelling of the conjuctiva were noted in all animals up to day 7: at 1 hour grades 1 or 2, at 24 h grades 2 or 3 for redness and mostly grade 4 for swelling, at 48 h and 72 h always grades 2 or 3 and after 7 days grade 1. On day 14 there were no findings for any parameter.

The mean values (readings of 24, 48 and 72 hours) for the individual animals, the mean values for all animals and additional findings are given in the following table:

Table B.6.11-5: Eye irritation; mean readings and symptoms

Animal No.	Opacity	Iris	Conjunctiva		**Symptoms** (always 24 – 72 h)
			Redness	**Swelling**	
1	1.0	0.7	2.0	2.7	pupil contracted, discharge of blood
2	1.3	1.0	2.7	2.7	suppuration, pupil contracted, discharge of blood
3	1.0	1.0	3.0	3.3	suppuration, pupil contracted, discharge of blood
4	1.0	1.0	3.0	3.0	suppuration, pupil contracted, discharge of blood
5	1.0	0.7	2.0	2.7	suppuration, pupil contracted, discharge of blood
6	1.3	0.7	2.7	3.0	suppuration, pupil contracted, discharge of blood, slight loss of corneal tissue
Mean	**1.1**	**0.8**	**2.6**	**2.9**	

Conclusion:
BAS 500 00 F is irritant to the eye. The effects were reversible within the the observation period of 14 days.
Risk phrase: R 36 (irritating to eyes).

B.6.11.6 Skin sensitisation

Report: Wiemann, C. and Hellwig, J. 1998(e): BAS 500 00 F - Buehler Test in guinea pigs.
BASF AG, Ludwigshafen/Rhein, Germany;
BASF RegDoc# 1998/11034, unpublished.

Test Material: BAS 500 00 F; formulated product; batch No. 97-2; a.i.: 247.83 g/l.

Test Animals: Pirbright White (Dunkin-Hartley) guinea pigs. Young adult SPF animals (strain Crl:(HA)BR) were obtained from Charles River GmbH – WIGA, Kisslegg, Germany.

GLP: Yes

Test Method: EEC 96/54; OECD 406; EPA/FIFRA 81-6

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
BAS 500 00 F was tested for its sensitising effect on the skin of the guinea pig by means of the Buehler test.
The concentrations used in this study were selected based on the results of a pre-test for skin irritation.The minimum irritant concentration was found to be a 10% test substance preparation in aqua bidest. The maximum non-irritant concentration was found to be a 5% test

substance preparation in aqua bidest. The 25% test substance preparation only caused slight erythema in 1 animal. Therefore this concentration was chosen for the inductions.
For the main study the following concentrations were selected on the basis of the pretest results:

1st, 2nd and 3rd induction	Test substance 25% in aqua bidest.
Challenge	Test substance 5% in aqua bidest.

A total of 20 animals were used for the treatment group and 10 animals served as control group 1. All inductions were performed with a 25% test substance preparation in aqua bidest. For the induction, 2 x 2 cm gauze patches containing the test substance formulation were applied to the skin of the flank under an occlusive dressing. A volume of 0.5 ml of the test substance formulation was applied to each animal. The control animals were not treated since the distilled water used as formulating agent was not expected to influence the result of the study.

The duration of exposure was 6 hours, the material was applied on the anterior left flank. Reading of the skin was performed at 24 h after the beginning of application.

A challenge was performed 14 days after the last induction. A volume of 0.5 ml of the test substance formulation (as a 5% aqueous preparation) was applied to each animal. The test group and control group 1 were treated with the test substance formulation (control group 2 remained untreated). The duration of exposure was 6 hours, the test substance was applied on the right flank. Readings were performed at 24 and 48 h after the removal of the patch.

A positive control (reliability check) with a known sensitiser is performed twice a year in the laboratory. The positive control with Alpha-Hexylcinnamaldehyde techn. 85% showed that the chosen guinea pig strain was able to detect sensitising compounds under the laboratory conditions chosen.

Findings:
The test substance is a formulation demonstrated to be stable. The homogeneity was analytically verified. The stability of the test substance preparations were analytically confirmed.

During the inductions there were no sign of skin irritation. However, after the second and third induction in two and seven animals, respectively scaling was observed.
There were no test group animals with skin findings after the challenge.

Conclusion:
Based on the results of this study it is concluded that BAS 500 00 F does not have a sensitising potential to the skin of the guinea pig in the Buehler test.

B.6.11.7 Supplementary studies for combinations of plant protection products

The notifier informed that pyraclostrobin (BAS 500 F) is a new active substance to be used in a great variety of crops. It will be applied as well as a solo product as well as in combination with other fungicidal active substances. The EC formulation can be used as such or as a tank mix with other plant protection products. According to that, BAS 500 00 F is compatible with a variety of commercial crop protection products according to model experiments.
Corresponding toxicological studies are not available.

B.6.12 Dermal absorption (Annex IIIA 7.3)

The dermal absorption of pyraclostrobin is generally poor. The highest value of approximately 2.6% was determined in an *in vivo* study in rats. It was reached when the animals were exposed to the intermediate nominal dose of 0.075 mg/cm^2 (corresponding to 4 mg/kg bw) for an 8-hour period. With regard to operator or bystander exposure, this dermal absorption rate of 2.6% appears to be still an overestimation since an *in vitro* comparison revealed a much lower penetration rate through human epidermis than through rat skin.

The differences in the rate of penetration of BAS 500 F through human skin and rat skin as elucidated in the *in vitro* dermal penetration study, are shown in Table B.6.12-1

Table B.6.12-1: Rat versus human skin: differences in penetration rates

Concentration (mg/cm^2)	0.015	0.075	0.375
Difference between rat and human skin	42 fold higher in rats	12 fold higher in rats	9 fold higher in rats

The best estimate for human dermal penetration can be calculated by using the rat *in vivo* dermal resorption values at the different dose levels and combine these values with the relative difference in skin penetration between rat and human skin based on the results of the *in vitro* study. This calculation results in the following values:

Table B.6.12-2: Human skin penetration values

Concentration (mg/cm^2)	0.015	0.075	0.375
Calculated human skin penetration	0.04%	0.22%	0.18%

Taking into account the difference between rat and human skin permeability, human skin penetration being 9-fold lower than rat skin penetration as demonstrated by *in vitro* investigations, the dermal absorption of the compound in humans is negligible.
Therefore, the proposal of the notifier is supported to use a value of 1% as a standard value for a compound which does not significanly penetrate human skin keeping in mind that the actual rate is probably less than 0.2%.

B.6.12.1 *In vivo* data

Report: Leibold, E. and Hoffmann, H. D. (1999): ^{14}C-BAS 500 F - Study of the dermal absorption in rats.
BASF AG, Ludwigshafen/Rhein, Germany; BASF RegDoc# 1999/10716, unpublished.

Test Material: Radiolabelled pyraclostrobin (tolyl-ring); batch 566-2101; radiochemical purity: > 95%.
Non radiolabelled pyraclostrobin; batch CP 025394; purity: 98.5

Test Animals: Wistar rats [strain: Chbb-THOM (SPF); source: Dr. Karl Thomae, Biberach a.d. Riss, Germany].

GLP: Yes

Test Method: EEC 87/302, OECD 417, EPA/OPPTS 870.7600

Deviations: None.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The absorption, distribution and excretion of radioactivity was studied in male Wistar rats following a single dermal administration of ^{14}C-pyraclostrobin diluted in the neat solvent of a commercial formulation (BAS 500 01 F, 250 g/l, solvent: Solvesso) at nominal dose levels of 0.015, 0.075 and 0.375 mg/cm^2 corresponding to 0.15, 0.75 and 3.75 mg/animal and about 0.8, 4 and 18 mg/kg body weight. Animals were exposed for 4 or 8 hours and sacrificed 4, 8, 24 or 72 hours after beginning of exposure.
Twenty-four hours prior to dosing the back shoulders of the rats were clipped free of hair and the area (about 10 cm^2) was washed with acetone. A silicone ring was glued to the skin and the test substance preparation (about 10 µl/cm^2) administered with a syringe which was weighed before and after application. A nylon mesh was then glued to the surface of the silicone ring and a porous bandage used to encircle the trunk of the animal.
After dosing, the animals were placed in metabolism cages in order to collect excreta up to 72 hours. For each dose level 16 animals were used. The test group design for each dose level was as follows:

Table B. 6.12-3: Study design of the *in vivo* dermal penetration experiment

Duration of exposure [h]	4	8		
Sacrifice after [h]	4	8	24	72
number of animals	4	4	4	4

After the exposure period, respectively the protective cover was removed and the exposed skin washed with a mild soap solution. At the end of the various collection periods, animals were sacrificed and the following specimens/tissues were checked for remaining radioactivity: excreta, blood cells, plasma, liver, kidneys, carcass, treated skin (application site) and non-treated areas (surrounding skin).
For balance estimates, the cage wash and skin wash as well as the protective cover (including the silicone ring) were also checked for radioactivity.

Findings:
The stability, homogeneity and correctness of the test substance preparation were analytically verified. Mean recovery of radioactivity from all dose groups was in the range of 99 – 110% of the total dose administered with the major portion being recovered from the dressing and skin wash. The total amount of radioactivity absorbed (including excreta, cage wash, tissues/organs and carcass) increased with growing exposure period and delaying sacrifice time. However, dermal absorption was generally very limited and rather similar at all dose levels. About 2.6% of the radioactivity applied was the maximum amount absorbed. This value was reached after administration of the intermediate dose of 0.075 mg/cm^2.
These results are summarised in the following table:

Table B.6.12-4: Percentage and total amount of radioactive material absorbed

Exposure time [h]	Sacrifice time [h]	0.375 mg/cm^2		0.075 mg/cm^2		0.015 mg/cm^2	
		% abs.	mg/animal	% abs.	mg/animal	% abs.	mg/animal
4	4	0.51	0.0205	0.43	0.0035	0.55	0.0010
8	8	0.51	0.0224	0.85	0.0076	0.64	0.0010
8	24	1.19	0.0511	2.56	0.0216	1.49	0.0024
8	72	1.58	0.0682	2.59	0.0241	1.57	0.0025

The radioactivity absorbed was excreted mainly via the feces. Due to the very limited skin penetration, concentrations of radioactivity in organs and tissues analyzed were very low.
Some of the radioactivity remained in the skin at the application site after end of exposure. However, at two dose levels, this material did not decrease during the post-observation period suggesting that this material did no serve as a reservoir for delayed absorption.

Conclusion:
The *in vivo* dermal absorption of pyraclostrobin in rats was poor reaching a maximum amount of approximately 2.6% or even less depending on the concentration applied and the duration of exposure.

B.6.12.2 *In vitro* data

Report: Thornley, K. F. and Wood, R. A. (1999): (^{14}C)-BAS 500 F: Rates of penetration through human and rat skin using an in vitro system. Covance Laboratories (formerly Corning Hazleton), Harrogate, North Yorkshire HG3 1PY, United Kingdom; BASF RegDoc# 1999/11867, unpublished.

Test Material: Radiolabelled pyraclostrobin (chlorophenyl-ring); batch 579-1201; radiochemical purity: > 98%, chemical purity: > 97%.
Non radiolabelled pyraclostrobin; batch 27882/199/b.

Test System: Rat and human epidermal membranes.

GLP: Yes

Test Method: Draft OECD Guideline *in vitro* dermal penetration

Deviations: None.

Acceptability: The study is considered to be acceptable.

Material and Methods:
The *in vitro* dermal absorption of (^{14}C)-BAS 500 F, from a commercial formulation (BAS 500 00 F) was determined at three dose levels (375, 75 and 15 µg active ingredient/cm^2), through rat and human epidermal membranes. The epidermal membranes were left un-occluded throughout the 24 h exposure period.

To determine the integrity of the skin, on the day prior to dose application, tritiated water was applied to the epidermal surface of the skin and penetration was measured.
At termination of the membrane integrity check and prior to dose application, the receptor chamber was refilled with ethanol:water (1:1 v/v). The receptor fluid was chosen on the basis that the test substance is readily soluble in ethanol.
The dose formulation was applied to the upper surface of the epidermal membranes using a positive displacement pipette. The amount of dose solution applied to each membrane was calculated by weight difference of the positive displacement pipette before and after dose administration. Duplicate aliquots (0.1 ml) of receptor fluid were taken at 0 (i.e. pre-dose), 1, 2, 4, 6, 10 and 24 h after application of the formulation to the skin. An equal volume of fresh receptor fluid was added to the receptor chamber after each sampling occasion, excluding the final sample time, in order to maintain a constant volume of receptor fluid in the receptor chamber of the diffusion cell.
At 24 h post-application (after the last receptor fluid sampling) the receptor fluid was removed from the receptor chamber of all cells and retained. Any residual formulation was washed from the surface of the skin with a solution of Liquid Ivory™ soap (approximately 10% w/v) containing no organic solvent and rinsed with deionised water. The washings were retained for analysis. The skin preparations were removed from the cells and solubilised. All parts of the cell (excluding the metal clamp) were placed in a suitable container and covered with approximately 60 ml ethanol. The apparatus was removed from the container and the washings retained.
Radioactivity was determined in the receptor fluid, skin section, skin washings and apparatus washings to determine the overall mass balance of radioactivity. The percentage of the applied dose in each sample and the rate of penetration (µg equivalents /cm^2/h) was determined.

Findings:
The stability, homogeneity and correctness of the test substance preparation was analytically verified.
A material balance of between 91 and 106% of the applied dose recovered for all dose groups at study termination (24 h).

Rat skin:
Absorption through rat skin was rapid, with up to 51% of the applied dose present in the receptor fluid at 24 h. However, this value underestimates absorption through rat skin, since there was little or no absorption of radioactivity from this formulation after the 6 h time point irrespective of dose level.

Human skin:
For human epidermal membranes, a maximum of 8% of the applied dose was absorbed over 24 h. Unlike rat skin, absorption remained constant throughout the duration of the study.

The relevant parameters for the assessment of skin penetration are shown in the following table:

Table B.6.12-5: Comparison of the absorption of radioactivity through rat and human epidermal membranes following a single application of (^{14}C)-BAS 500 F formulated at three nominal dose levels of 375, 175 and 15 µg/cm^2

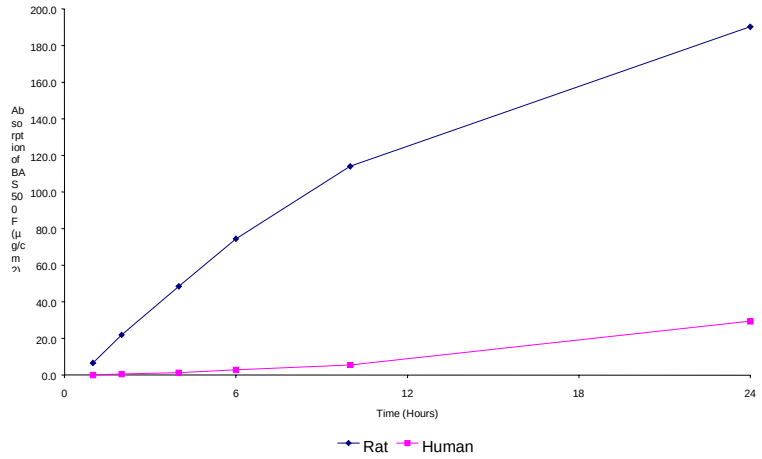
	Mean cumulative absorption of (^{14}C)-BAS 500F (µg/cm^2 skin)					
Administered dose	375 µg/cm^2		75 µg/cm^2		15 µg/cm^2	
Time (hours)	Rat skin (Group A)	Human skin (Group D)	Rat skin (Group B)	Human skin (Group E)	Rat skin (Group C)	Human skin (Group F)
1	6.555	ND	0.922	ND	0.339	ND
2	21.82	0.238	4.596	0.046	1.339	ND
4	48.41	0.862	7.626	0.158	2.740	0.019
6	74.40	1.962	13.43	0.461	5.591	0.038
10	114.0	5.478	17.87	1.270	8.127	0.108
24	190.3	29.40	14.56	5.471	7.657	0.521
Lag Time (hours)	0.513	5.398	0.545	3.853	0.782	3.662
Mean rate of penetration (µg/cm^2/h)	13.87	1.542	2.475	0.210	1.099	0.026
Permeability coefficient (x 10^{-4} cm/h)	3.688	0.410	3.421	0.290	6.976	0.163
ND – Not detected						

Difference in skin penetration through rat and human skin:

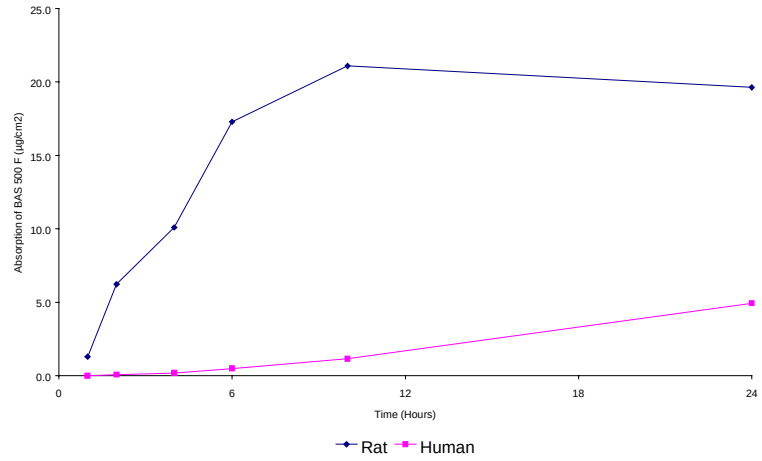
The differences in the skin penetration between human and rat skin at the different dose levels are shown in Figure B.6.12-1:

Figure B.6.12-1: Comparison of the absorption of radioactivity through rat and human skin sections following a single application of (^{14}C)-BAS 500 F formulated at three nominal dose levels of 375, 175 and 15 µg/cm^2

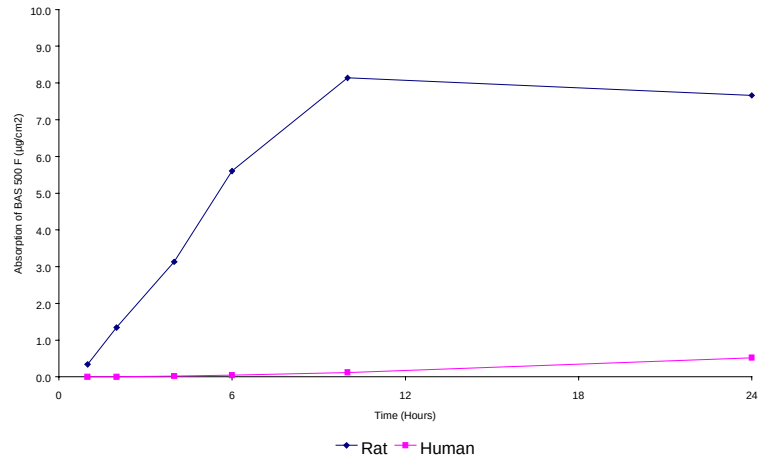
Group A and D: 37.5 mg active ingredient/mL (375 µg/cm^2)



Group B and E: 7.5 mg active ingredient/mL (75 µg/cm^2)



Group C and F: 1.5 mg active ingredient/mL (15 µg/cm^2)



Conclusion:

At the high dose level, absorption through rat epidermal membranes was 9 times greater than that for human epidermis. This difference was 12 and 42 times greater at the intermediate and low dose levels, respectively.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

Besides its active ingredient pyraclostrobin, the emulsifiable concentrate BAS 500 00 F contains different co-formulants. The data respectively are given in Safety Data Sheets. The possibly toxic properties of all co-formulants are covered by the studies with the preparation. Because of the presence of one of the solvents in the product the indication of danger ”Harmful: may cause lung damage if swallowed (R 65)” should be discussed (Dir. 98/98/EEC) for BAS 500 00 F. The notifier stated that, based on physical properties of the product, the risk phrase R65 from Solvesso should not be transferred to the classification of the product.

B.6.14 Exposure data (Annex IIIA 7.2)

Considering the results of the risk assessments based on the German BBA-Model as well as the UK-POEM it is concluded that BAS 500 00 F can be handled safely under the recommended conditions of use.

Information on product and use

BAS 500 00 F is formulated as an emulsifiable concentrate (EC) containing nominal 250 g/l of the active ingredient (a.i.) pyraclostrobin. According to the information by the notifier, at time, the only intended use is as a fungicide in vine crops. Its recommended application is in different growing stages of the crop. For all use situations water will be the diluent/carrier. The spray solution as applied will contain 0.01% of the a.i. and the maximum spray volume will be 1,600 liter per hectar. Therefore, the maximum application rate is 0.64 l product/ha and 0.160 kg a.i./ha. Applications of BAS 500 00 F will be carried out by using vehicle-mounted or drawn boom sprayers with hydraulic nozzles.
Additionally, the notifier informed that pyraclostrobin is developed to be used in a great variety of crops. It will be applied as well as a solo product as well as in combination with other fungicidal active substances.

B.6.14.1 Operator exposure

B.6.14.1.1 Estimation of operator exposure and risk assessment

The operator exposure estimates are calculated using both the German model and the UK-POEM:

- Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protections); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirschaft, Berlin-Dahlem, n° 277;

- Scientific Subcommittee on Pesticides and British Agrochemicals Joint Medical Panel., Estimation of Exposure and Absorption of Pesticides by Spray Operators (UK MAFF) 1986 and the Predictive Operator Exposure Model (POEM) (UK MAFF) 1992.

In Table B.6.14-1 the assessed scenarios are summarised.

Table B.6.14-1: Scenarios/use conditions in high crops - for the exposure calculation

Technique	Treated surface per working day	Max. use rate		max. in-use concentration (mg a.i./ml)	Models used
		kg a.i./ha	product: l /ha		
Vehicle mounted sprayer	8 ha	0.16	(0.64)	–	German model
	30 ha	(0.16)	0.64	0.1	UK-POEM

Estimated operator exposures using the German model

The following assumptions are made for the estimation of operator exposure:
Formulation type: EC
Application technique: tractor mounted equipment
Maximum application rate: 0.160 kg a.i./ha
Area treated per day: 8 ha
Dermal absorption rate: 1%
Body weight of an operator: 70 kg
Penetration gloves: 1%; penetration standard protective garment: 5%

Using the input parameters and the scheme of the calculation model, the estimated operator exposure can be calculated for mixing/loading (m/l) and application (appl.). The results for the estimated dermal and inhalation exposures are given in Table B.6.14-2. The calculations were carried out for different conditions, as recommended by the notifier:

Scenario 1:	No PPE, disregarding the recommendations on the label, no protective equipment used when handling the undiluted product and during application
Scenario 2:	PPE: gloves, standard protective garment and sturdy footwear used when handling the undiluted product (handling of product during mixing/loading)
Scenario 3:	PPE: gloves, standard protective garment and sturdy footwear used when handling the undiluted and the diluted product (handling of product during mixing/loading and application)

Table B.6.14-2 Estimated operator exposures using the German model

Exposure route and type of work	Estimated operator exposures (mg/person/day)		
	Scenario 1 (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl. garment: appl.)
Dermal exposure			
– Mixing/loading	3.072	0.031	0.031
– Application	14.720	14.720	2.159
Total, dermal	17.792	14.751	2.190
Inhalation exposure			
– Mixing/loading	0.001	0.001	0.001
– Application	0.023	0.023	0.023
Total, inhalation	0.024	0.024	0.024
Total: Dermal + inhalation	**17.816**	**14.775**	**2.214**
Systemic exposure (absorbed dose)*	0.202	0.172	0.046

* dermal absorption rate: 1%; inhalation absorption rate: 100%

Using the German model without PPE, the estimated exposure is calculated to be 17.815 mg/person/day. Then, calculating with 1% dermal absorption, the systemic exposure/absorbed dose is 0.202 mg/person/day. By wearing of PPE, this values can be reduced to 0.172 and 0.046 mg/person/day, respectively.

Estimated operator exposures using the UK model

The following assumptions are made for the estimation of operator exposure:

Formulation type: EC
Application technique: orchard sprayer air assisted
Maximum application rate: 0.640 kg product/ha (0.160 kg a.i./ha);
Application volume: 1600 l/ha;
Maximum in use concentration: 0.01% of the a.i.
Area treated per day: 30 ha
Packaging: 1, 5 or 10 litres with wide necks (calculation with 1 l / worst case)
Dermal absorption rate: 1%
Body weight of an operator: 60 kg
Hand contamination: 0.01 ml/operation
Penetration gloves: 10%

Using the input parameters and the scheme of the calculation model, the estimated operator exposure can be calculated for mixing/loading and application. The results for the estimated dermal and inhalation exposures are given in Table B.6.14-3. The calculations were carried out for different conditions, as recommended by the notifier:

Scenario 1:	No PPE, disregarding the recommendations on the label, no protective equipment used when handling the undiluted product and during application
Scenario 2:	PPE: gloves only during mixing/loading
Scenario 3:	PPE: gloves during mixing/loading and during spray application

Using the UK-POEM without PPE, the estimated exposure is calculated to be 62.15 mg/person/day. Then, calculating with 1% dermal absorption, the systemic exposure/absorbed dose is 0.65 mg/person/day. By wearing of PPE, this value can be reduced to 0.20 and 0.17 mg/person/day, respectively.

Table B.6.14-3: Estimated operator exposures using the UK-POEM

Exposure route and type of work	**Estimated operator exposure (mg/person/day)**		
	Scenario 1 (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl.)
Dermal exposure			
– Mixing/loading	50.00	5.00	5.00
– Application	12.12	12.12	8.52
Total, dermal	62.12	17.12	13.52
Inhalation exposure			
– Mixing/loading	-	-	-
– Application	0.03	0.03	0.03
Total, inhalation	0.03	0.03	0.03
Total exposure: (dermal + inhalation)	**62.15**	**17.15**	**13.55**
Total systemic exposure (absorbed dose)*	0.6512	0.2012	0.1652
* dermal absorption rate: 1%; inhalation absorption rate: 100%			

Determination of the tolerable exposures

To assess the estimated exposures, a comparison with tolerable exposure values has to be done. In the German model, the different parts of estimated exposures should be compared with the route specific AOELs (dermal or inhalation) to see whether there are problems and if so take specific PPEs into consideration in order to reduce the risk for the critical route of exposure. In the UK-POEM, the total estimated systemic exposures are to be compared with the systemic AOEL. In cases where no route specific AOELs can be derived, the estimated exposures of both models are to be assess via the absorption rates on the basis of the systemic AOEL derived for the active ingredient.

To derive the oral AOEL, a NOAEL of 4 mg/kg bw/d should be used. This value based on effects on body weight after 90 days in the carcinogenicity study in male mice (see B.6.10).
The oral AOEL can be calculated by using an assessment factor of 100. Due to the level of gastro-intestinal absorption of about 50%, the application of an additional correction factor of 0.5 is required.

The calculation results in a **systemic AOEL of 0.02 mg/kg bw/d.**

The NOAEL of the oral toxicity studies (4 mg/kg bw/d) corrected to the systemic NOAEL (2 mg/kg bw/d) should be used to derive both the tolerable dermal and inhalation exposure. The "degree of exposure" in the German model is then comparable to the calculation of the total absorbed dose as percentage of the AOEL, oral/systemic, if the same value for body weight is used.
Assuming 1% for dermal absorption (see B.6.12) and 100% for inhalation absorption, a body weight of 70 kg, and an assessment factor of 100, the tolerable dermal (D_{tol} = AOEL, dermal) and inhalation (I_{tol} = AOEL, inhalation) exposures are calculated to be:

AOEL, dermal (D_{tol}) = 2 mg/kg bw/d : 1% x 70 kg : 100 = 140 mg/person/d (= 2 mg/kg bw/d)

AOEL, inhal. (I_{tol}) = 2 mg/kg bw/d x 70 kg : 100 = 1.4 mg/person/d (= 0.02 mg/kg bw/d)

On the basis of a dermal 4wk-rat study with no effects at the highest dose tested of 250 mg/kg bw/d, the notifier derives a specific dermal AOEL of 10 mg/kg bw/d (SF 25). Applying an SF of 100, this value would be in the same range as derived from the systemic AOEL.

Comparison of the estimated and tolerable exposures
The calculated systemic exposures are compared with the proposed AOEL of 0.02 mg/kg bw/d. The values are given in Table B.6.14-4.

Table B.6.14-4: Results of the model calculations and a comparison with the proposed systemic AOEL

Model used	**Treated area**	**Protective clothing** (relevant for calculation)	**Systemic exposure*** (mg/kg bw/d)	**Amount of AOEL** (0.02 mg/kg bw/d)
German model	8 ha/d	none	0.00288	14.4%
		m/l: gloves	0.00245	12.2%
		m/l: gloves; appl.: overall, gloves	0.00065	3.3%
UK-POEM	30 ha/d	none	0.01085	54.3%
		m/l: gloves	0.00335	16.8%
		m/l: gloves; appl.: gloves	0.00275	13.8%
*See tables B.6.14-2 and B.6.14-3; in the calculations a body weight of 70 kg (German model) or 60 kg (UK-POEM) and a dermal absorption rate of 1% is used.				

Assessment:
The estimated exposure of the unprotected operator is about 14% (German model) or about 54% (UK-POEM) of the systemic AOEL.
Thus, based on the results of this risk assessment, no additional protective measures are needed. Nevertheless, taking into account the toxicological properties of the plant protection product BAS 500 00 F, reflected by the corresponding classification and labelling, personal

protective equipment must be used. This protection measure lowers the estimated exposure by the corresponding reduction coefficient.

The notifier proposed a route to route comparison of the dermal and inhalation exposures derived in both models. Using a specific dermal AOEL (10 mg/kg bw/d) on the basis of the dermal 4wk-rat study (SF 25), a higher dermal exposure was accepted and higher margins of safety, respectively were derived. Additionally the notifier emphasised that the classification of the formulation has no relevance for the spray solution since the content of the formulated product in this solution is 0.04 % only (content of the a.i. pyraclostrobin is just 0.01%).

B.6.14.2 Bystander exposure

BAS 500 00 F with its active ingredient pyraclostrobin is a fungicide applied in vine crops. The usual form of application is by tractor-mounted sprayers without bystanders. The spray solution as applied will contain only 0.04% of the formulated product or 0.01% of the active ingredient pyraclostrobin.

In view of the recommended application technique in combination with Good Agricultural Practice (GAP) bystanders may be exposed only incidental, i.e. briefly and to relatively low quantities of spray compared to an operator.
A comparison with the estimated operator exposures (estimated operator exposures for mixing/loading + application: < systemic AOEL, without PPE) shows that for a bystander it is excluded that exposure levels exceeding the AOEL would be achieved.

B.6.14.2.1 Estimation of bystander exposure

Due to considerations as indicated above estimation of bystander exposure was considered not necessary and therefore not performed.

B.6.14.2.2 Measurement of bystander exposure

Measurement of bystander exposure was considered not necessary and therefore not performed.

B.6.14.3 Worker exposure

B.6.14.3.1 Estimation of worker exposure

BAS 500 00 F containing the fungicidal active ingredient pyraclostrobin will be applied in vine with a maximum of 8 applications. No re-entry for maintenance of the vine may be anticipated after the 7^{th} application. At this and the preceding treatment application rates will be 0.120 and 0.160 kg a.i./ha respectively. It is conservatively assumed that during this late re-entry residues from these two treatments will be found on the leaves and, therefore, the combined rate of 0.280 kg a.i./ha will be considered for the estimations.

The estimation will be based on the model as developed by the German BBA (Biologische Bundesanstalt) [Hoernicke E. et al.; 1998; Hinweise in der Gebrauchsanleitung zum Schutz von Personen bei Nachfolgearbeiten in mit Pflanzenschutzmitteln behandelten Kulturen (worker re-entry); Nachrichtenbl. Deut. Pflanzenschutzd. 50, Berlin] and the US EPA [EPA, Science Advisory Council for Exposure; 1998; Agricultural Default Transfer Coefficients, Policy #: 003; #98/11675].

The re-entry/dermal exposure (D) is calculated by the formula:

D (dermal exposure) = DFR x TF x A x (P) x R

Assumptions of the re-entry model / by the notifier

FDR:	Foliar dislodgeable residues	1	µg/cm^2/kg ai
TF:	Transfer factor	15000*	cm^2/person x h
A:	Work rate/d	8	h/d
P:	Penetration through clothing (PPE)	5	%
R:	Application rate (0.120 + 0.160 kg ai/ha)	0.280	kg ai/ha

(*EPA: Agricultural Default Transfer Coefficients)

D = 1 µg/cm^2/kg ai x 15000 cm^2/person x 8h (x 5%) x 0.280 kg ai/ha

Resulting exposures:

D:	Dermal exposure, not wearing PPE	33.6	mg/person/d
	Dermal exposure, wearing PPE	1.68	mg/person/d

Systemic Exposure = D (mg/person/d) x AF (%) : bw (kg)

AF:	Dermal absorption / absorption factor	1	%
bw:	Worker body weight	70	kg

Systemic Exposure, not wearing PPE = 33.6 mg/person x 1% : 70 kg bw
Systemic Exposure, wearing PPE = 1.68 mg/person x 1% : 70 kg bw

Resulting systemic exposures

Systemic exposure, not wearing PPE	0.00480	mg/kg bw/d
Systemic exposure, wearing PPE	0.00024	mg/kg bw/d

Based on the given assumption, the systemic worker exposure would be 0.0048 mg/kg bw/d for the person not wearing PPE and 0.00024 mg/kg bw/d for the person wearing PPE. This corresponds to 24% and 1.2% of the proposed systemic AOEL of 0.02 mg/kg bw/d, respectively.
Therefore, the estimated exposure to pyraclostrobin during re-entry operations does not present an undue risk to the worker also if no PPE is worn. But it should be noted that a re-entry of treated areas/crops should not be performed before the spray deposit is completely dry.

B.6.14.3.2 Measurement of worker exposure

The validity of the used model, in particular the assessment of the Dislodgeable Foliar Residue (DFR), is supported by the DFR study on BAS 500 00 F in grapes [Clark J.A.; 1999; #1999/5090] carried out in the U.S.A., based on 6 applications of 0.168 kg a.i./ha pyraclostrobin each, at spray intervals of approximately 14 days. The average DT_{50} of the dislodgeable residue was found to be 7.4 days. The average dislodgeable residue at day 0 after the last application is ranging between 0.08 and 0.47 µg/cm^2 which is the result of the above calculation, when the rates applied at the two latest applications are taken into consideration.

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.1	Leibold, E. and Hoffmann, H.D.	1999	14C-BAS 500 F - Study of the dermal absorption in rats. 01B0363/966044 ! 1999/10716 GLP, unpublished TOX2000-706	Y	BAS
AIIA-5.1	Leibold, E., Hoffmann, H.D. and Hildebrand, B.	1998	14C-BAS 500 F - Study of the biokinetics in rats. 02B0364/966007 ! #BASF 98/10997 GLP, unpublished TOX2000-705	Y	BAS
AIIA-5.1	Thornley, K.F. and Wood, R.A.	1999	(14C)-BAS 500 F: Rates of penetration through human and rat skin using an in vitro system. 729/200 ! 50H0364/969131 ! 1999/11867 GLP, unpublished TOX2000-707	Y	BAS
AIIA-5.1	Velic, I.	1999	Metabolism of 14C-BAS 500F (14C-304428) in rats. 38773 ! 1999/11781 GLP, unpublished TOX2000-708	Y	BAS
AIIA-5.2.1	Wiemann, C. and Hellwig, J.	1998	Study on the acute oral toxicity of BAS 500..F in rats. 10A0183/961058 ! #BASF 98/10965 GLP, unpublished TOX2000-709	Y	BAS

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.2.2	Wiemann, C. and Hellwig, J.	1998	Study on the acute dermal toxicity of BAS 500..F in rats. 11A0308/961120 ! #BASF 98/10966 GLP, unpublished TOX2000-710	Y	BAS
AIIA-5.2.3	Gamer, A.O. and Hoffmann, H.D.	1997	BAS 500..F - Acute inhalation toxicity study in Wistar rats 4-hour liquid aerosol exposure. 13I0308/967028 ! #BASF 97/11472 GLP, unpublished TOX2000-711	Y	BAS
AIIA-5.2.3	Gamer, A.O., Leibold, E. and Hoffmann, H.D.	2001	BAS 500 F 40% in solvesso (technical active ingredient) - Acute inhalation toxicity study in Wistar rats 4-hour liquid aerosol exposure. 13I0283/017002 ! 2001/1010625 GLP, unpublished TOX2001-881	Y	BAS
AIIA-5.2.4	Wiemann, C. and Hellwig, J.	1998	BAS 500..F - Acute dermal irritation / corrosion in rabbits. 14H0308/962190 ! #BASF 98/10959 GLP, unpublished TOX2000-712	Y	BAS
AIIA-5.2.5	Wiemann, C. and Hellwig, J.	1998	BAS 500..F - Acute eye irritation in rabbits. 13H0308/962191 ! #BASF 98/10963 GLP, unpublished TOX2000-713	Y	BAS
AIIA-5.2.6	Wiemann, C. and Hellwig, J.	1998	BAS 500..F - Maximization test in guinea pigs. 30H0494/962329 ! #BASF 98/10964 GLP, unpublished TOX2000-714	Y	BAS
AIIA-5.3.1	Mellert, W., Deckardt, K., Gembardt, Ch. and Hildebrand, B.	1999	BAS 500 F - Repeated dose dermal toxicity study in Wistar rats administration for 4 weeks. 33S0494/96179 ! 1999/11458 GLP, unpublished TOX2000-716	Y	BAS
AIIA-5.3.1	Mellert, W., Deckardt, K., Gembardt, Ch. and Hildebrand, B.	1999	BAS 500..F - Repeated dose oral toxicity study in Wistar rats administration in the diet for 4 weeks. 30C0376/95083 ! 1999/11870 GLP, unpublished TOX2000-715	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.3.2	Mellert, W., Deckardt, K., Bahnemann, R. and Hildebrand, B.	1999	BAS 500 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months. 50C0183/96015 ! #BASF 99/10195 GLP, unpublished TOX2000-717	Y	BAS
AIIA-5.3.2	Mellert, W., Deckardt, K., Küttler, K. and Hildebrand, B.	1998	BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice administration in the diet for 3 months. 60C0183/96016 ! #BASF 98/11345 GLP, unpublished TOX2000-718	Y	BAS
AIIA-5.3.2	Menges, S., Schilling, K., Deckardt, K., Gembardt, Chr. and Hildebrand, B.	1999	BAS 500 F - Subchronic oral toxicity study in Beagle dogs administration in the diet for 3 months. 31D0494/96089 ! 1999/11678 GLP, unpublished TOX2000-719	Y	BAS
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	1998	In vitro unscheduled DNA synthesis (UDS) assay with BAS 500 F in primary rat hepatocytes. 81M0308/964306 ! # BASF 98/11421 GLP, unpublished TOX2000-723	Y	BAS
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	1999	In vitro chromosome aberration assay with BAS 500 F in V79 cells. 32M0308/964304 ! 1999/11403 GLP, unpublished TOX2000-722	Y	BAS
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	1998	In vitro gene mutation test with BAS 500 F in CHO cells (HPRT locus assay). 50M0308/964303 ! 98/11422 GLP, unpublished TOX2000-721	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	1997	Report on the study of BAS 500 .. F (=reg. no. 304 428) (ZHT test substance no.: 96/308) in the Ames salmonella/mammalian-microsome mutagenicity test and escherichia coli/mammalian-microsome reverse mutation assay (standard plate test and preincubation test). 40M0308/964244 ! #BASF 97/10973 GLP, unpublished TOX2000-720	Y	BAS
AIIA-5.4.2	Engelhardt, G. and Hoffmann, H.D.	1998	Cytogenetic study in vivo with BAS 500 F in the mouse micronucleus test single oral administration. 26M0308/964204 ! # BASF 98/10460 GLP, unpublished TOX2000-724	Y	BAS
AIIA-5.5	Mellert, W., Deckardt, K., Gembardt, C., Pappritz, G. and Hildebrand, B.	1999	BAS 500 F - Carcinogenicity study in Wistar rats administration in the diet for 24 months. 82S0494/96086 ! 1999/11868 GLP, unpublished TOX2000-727	Y	BAS
AIIA-5.5	Mellert, W., Deckardt, K., Gembardt, C., Pappritz, G. and Hildebrand, B.	1999	BAS 500 F - Chronic toxicity study in Wistar rats administration in the diet for 24 months. 82S0494/96085 ! 1999/11672 GLP, unpublished TOX2000-726	Y	BAS
AIIA-5.5	Mellert, W., Deckardt, K., Küttler, K. and Hildebrand, B.	1999	BAS 500 F - Carcinogenicity study in B6C3F1 mice administration in the diet for 18 months. 76C0494/96101 ! 1999/11871 GLP, unpublished TOX2000-728	Y	BAS
AIIA-5.5	Schilling, K., Deckardt, K., Gembardt, Chr. and Hildebrand, B.	1999	BAS 500 F - Chronic oral toxicity study in Beagle dogs administration in the diet for 12 months. 33D0494/96144 ! 1999/11677 GLP, unpublished TOX2000-725	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.6.1	Schilling, K., Gembardt, Chr. and Hildebrand, B.	1999	BAS 500 F - Two-generation reproduction toxicity study in Wistar rats continuous dietary administration. 70R0494/96172 ! 1999/11869 GLP, unpublished TOX2000-729	Y	BAS
AIIA-5.6.2	Schilling, K., Hellwig, J. and Hildebrand, B.	1999	BAS 500 F - Prenatal developmental toxicity study in Himalayan rabbits oral administration (gavage). 40R0494/96159 ! 1999/11512 GLP, unpublished TOX2000-731	Y	BAS
AIIA-5.6.2	Schilling, K., Hellwig, J. and Hildebrand, B.	1999	BAS 500 F - Prenatal developmental toxicity study in Wistar rats oral administration (gavage). 30R0494/96168 ! 1999/11511 GLP, unpublished TOX2000-730	Y	BAS
AIIA-5.6.2	Schilling, K., Hellwig, J. and van Ravenzwaay, B.	2001	BAS 500 F - Additional maternal toxicity study in Himalayan rabbits oral administration (gavage). 40R0494/96196 ! 2001/1003803 GLP, unpublished TOX2001-471	Y	BAS
AIIA-5.7	Mellert, W., Kaufmann, W. and Hildebrand, B.	1999	BAS 500 F - Subchronic oral neurotoxicity study in Wistar rats administration in the diet for 3 months. 50C0494/96174 ! 1999/11329 GLP, unpublished TOX2000-733	Y	BAS
AIIA-5.7	Mellert, W., Kaufmann, W. and Hildebrand, B.	1999	BAS 500 F - Acute oral neurotoxicity study in Wistar rats. 20C0494/96164 ! 1999/11111 GLP, unpublished TOX2000-732	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	2000	Salmonella typhimurium / escherichia coli reverse mutation assay (Standard Plate Test and Preincubation Test) with reg. no. 413 038. 40M0249/994128 ! 2000/1000005 GLP, unpublished TOX2000-736	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	1999	Salmonella typhimurium / escherichia coli reverse mutation assay (Standard Plate Test and Preincubation Test) with reg. no. 412 785. 40M0252/994127 ! 1999/12020 GLP, unpublished TOX2000-735	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	1999	Salmonella typhimurium / escherichia coli reverse mutation assay (Standard Plate Test and Preincubation Test) with reg. no. 411 847. 40M0251/994126 ! 1999/12017 GLP, unpublished TOX2000-734	Y	BAS
AIIIA-7.1.1	Wiemann, C. and Hellwig, J.	1998	BAS 500 00 F - Acute oral toxicity in rats. 10A0185/971108 ! #BASF 98/10804 GLP, unpublished TOX2000-738	Y	BAS
AIIIA-7.1.2	Wiemann, C. and Hellwig, J.	1998	BAS 500 00 F - Acute dermal toxicity in rats. 11A0185/971109 ! #BASF 98/10646 GLP, unpublished TOX2000-739	Y	BAS
AIIIA-7.1.3	Gamer, A.O., Leibold, E. and Hoffmann, H.D.	1998	BAS 500 00 F - Acute inhalation toxicity study in Wistar rats 4-hour liquid aerosol exposure. 13I0185/977014 ! #BASF 98/11185 GLP, unpublished TOX2000-740	Y	BAS
AIIIA-7.1.4	Wiemann, C. and Hellwig, J.	1998	BAS 500 00 F - Acute dermal irritation / corrosion in rabbits. 14H0185/972188 ! #BASF 98/10644 GLP, unpublished TOX2000-741	Y	BAS
AIIIA-7.1.5	Wiemann, C. and Hellwig, J.	1998	BAS 500 00 F - Acute eye irritation in rabbits. 13H0185/972189 ! #BASF 98/10645 GLP, unpublished TOX2000-742	Y	BAS
AIIIA-7.1.6	Wiemann, C. and Hellwig, J.	1998	BAS 500 00 F - Buehler Test in guinea pigs. 32H0185/972201 ! #BASF 98/11034 GLP, unpublished TOX2000-743	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-7.1.7	Gamer, A.O. and Stadler, R.	2000	Particle size distribution of BAS 500 00 F concerning acute inhalation toxicity. APD/CA 012000 ! #BASF 99/12006 not GLP, unpublished TOX2000-744	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft
BBA: Biologische Bundesanstalt für Land-und Forstwirtschaft

Annex B

Pyraclostrobin

B-7: Residue data

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.7 Residue data 181

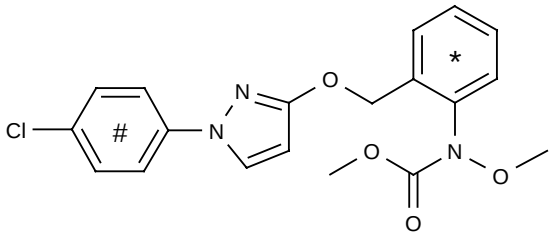
B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1; Annex IIIA 8.1) 181
B.7.1.1 Metabolism of pyraclostrobin in grapes 181
B.7.1.2 Metabolism of pyraclostrobin in potatoes 185
B.7.1.3 Metabolism of pyraclostrobin in wheat 191
B.7.1.4 Extractability of residues of ^{14}C-pyraclostrobin from plant matrices 199
B.7.1.4.1 Grapes and wheat 200
B.7.1.4.2 Potato 201
B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2; Annex IIIA 8.1) 201
B.7.2.1 Lactating goats 201
B.7.2.1.1 Absorption, distribution and excretion 201
B.7.2.1.2 Investigation of the metabolism of ^{14}C pyraclostrobin in lactating goat 204
B.7.2.2 Laying hens 216
B.7.2.2.1 Absorption, distribution and exretion of ^{14}C-pyraclostrobin 216
B.7.2.2.2 Investigation of the Metabolism of ^{14}C-pyraclostrobin in laying hen 219
B.7.2.3 Pigs 227
B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6) 228
B.7.3.1 Plant matrices 228
B.7.3.2 Animal matrices 228
B.7.4 Use pattern 229
B.7.5 Identification of critical GAPs 232
B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2) 233
B.7.6.1 Analytical method used in the residue trials 233
B.7.6.2 Storage stability 233
B.7.6.3 Residues in grapes 234
B.7.6.4 Residues in cereals 242
B.7.6.4.1 Barley 243
B.7.6.4.2 Wheat 265
B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4) 291
B.7.7.1 Effects on the nature of residue 291
B.7.7.2 Effects on the residue level 292
B.7.7.2.1 Residues in grapes 292
B.7.7.2.2 Residues in barley 297
B.7.7.2.3 Residues in wheat 300
B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3) 305
B.7.8.1 Ruminants 305
B.7.8.2 Poultry 311
B.7.8.2.1 Pigs 312
B.7.8.3 Storage stability in matrices of animal origin 312
B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6; Annex IIIA 8.5) 313

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post-harvest uses (Annex IIA 6.8; Annex IIIA 8.7) 321
B.7.11 Community MRLs and MRLs in EU Member States (Annex IIIA 12.2) 321
B.7.12 Proposed EU MRLs and justification for the acceptability of those residues (Annex IIA 6.7; Annex IIIA 8.6) 321
B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues 321
B.7.13.1 Banana 321
B.7.14 Basis for differences, if any, in conclusion reached having regard to established or proposed Codex MRLs 325
B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8) 325
B.7.15.1 Chronic Dietary Risk Assessment 325
B.7.15.2 Acute Dietary Risk Assessment 329
B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9) 330
B.7.16.1 Metabolism in plants 330
B.7.16.2 Metabolism in livestock 330
B.7.16.3 Residues in treated crops, proposed MRL's 330
B.7.16.4 Residues in food of animal origin, proposed MRL's 331
B.7.16.5 Stability of residues prior to analysis 331
B.7.16.6 Residues in succeeding crops 331
B.7.16.7 Estimates of dietary exposure to pyraclostrobin 331
B.7.17 References relied on 332

B.7 Residue data

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

The metabolism and distribution of pyraclostrobin (BAS 500 F) in plants was investigated using [tolyl-U-^{14}C]-pyraclostrobin and [chlorophenyl-U-^{14}C]- pyraclostrobin.



* tolyl label
\# chlorophenyl label

The studies were conducted in the following crops / crop groups

grapes	fruit
potato	root and tuber vegetable
wheat	cereals

B.7.1.1 Metabolism of pyraclostrobin in grapes

Report: Hamm, 1998, RIP 2000-1050 and RIP 2000-1275

^{14}C-Pyraclostrobin was applied in the form of an EC-formulation to grapevines (variety: Mueller-Thurgau). In total, six applications were performed. The first application was performed at growing stage BBCH 53 – 55 (inflorescences visible to fully developed). The last application was done at growing stage 81 (beginning of ripening), 40 days before harvest.

Table B.7.1-1: Data on application

No. of application	time interval [days]	amount ^{14}C-pyraclostrobin/ha [kg/ha]
1	-	0.48
2	16	0.24
3	19	0.18
4	17	0.13
5	18	0.24
6	17	0.24
sum		1.5

Samples were taken for analysis only at harvest, 40 days after the last application (= PHI). The samples were separated into leaves and grapes.

Grape and leaf samples were mainly extracted with methanol. The extractable radioactivity was characterised and quantified by radio HPLC. In addition, liquid/liquid partitioning experiments using cyclohexane and ethyl acetate were carried out. The metabolites were identified by comparison with reference substances. Where possible, they were isolated by HPLC and their structures elucidated by LC/MS/MS.

For characterisation of the non-extractable radioactivity in grapes, the radioactive residue that was not extractable with methanol was further extracted with ammonia or water. In order to get information on the storage stability of the grape samples, the only relevant raw agricultural commodity (RAC), the extractability and the HPLC metabolite profiles were investigated at the beginning and at the end of the study.

Findings:
In grape samples, the solvent extractability was high; it ranged from 84 to 88% of the TRR. To increase the extractability, ammonia/water extraction was applied. The Total Radioactive Residues (TRR) determined by combustion analyses and the extraction behaviour are summarised in Table B.7.1-2

Table B.7.1-2: Total radioactive residues (TRR) and extraction behaviour in work-up procedure [mg/kg (% TRR)]

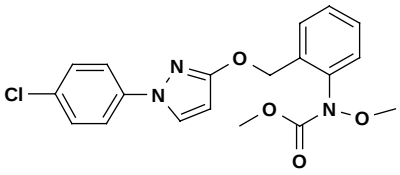
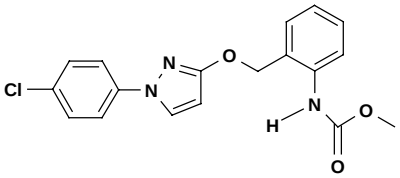
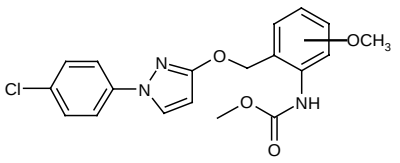
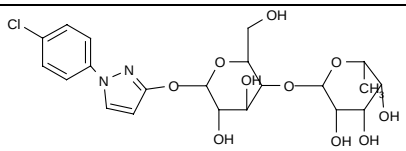
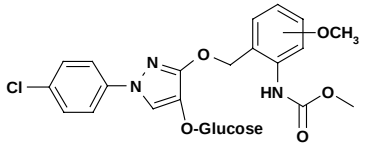
	Tolyl label	**Chlorophenyl label**
Grapes (at harvest)	1.559	0.951
extractable with methanol	1.314 (84.3)	0.835 (87.8)
extraction cyclohexane	1.011 (65.5)	0.663 (69.7)
+ extraction ethylacetate	0.119 (7.7)	0.077 (8.1)
Organosoluble (sum)	1.13 (73.2)	0.74 (77.8)
watersoluble	0.096 (6.2)	0.075 (7.8)
Not extractable with methanol	0.245 (15.7)	0.116 (12.2)
extractable with NH_4OH/H_2O	0.023 (1.5)	0.006 (0.6)
+ Lignin, raw	0.071 (4.6)	0.039 (4.1)
+ Lignin of lower weight (supernant)	0.111 (7.1)	0.027 (2.8)
+ not soluble raw cellulose	0.044 (2.8)	0.017 (1.8)
Leaves (at harvest) (no extraction results reported)	39.2	40.0

In order to classify the metabolites into organosoluble and water-soluble ones, liquid/liquid partition experiments were carried out. In grape samples treated with the tolyl label or the chlorophenyl label most of the radioactivity was found in the organic phases.

The leave samples were used to identify minor metabolites only. There are no details of the investigation of leaves reported except in the dossier.

In grapes, ^{14}C-pyraclostrobin is metabolised by three key transformation steps:
(1) desmethoxlation at the oxime ether bond, (2) hydroxylation of the tolyl and the chlorophenyl ring systems followed by glucosylation or methylation, and (3) cleavage between both ring systems and subsequent transformation of the resulting intermediates by glucosylation.
The quantification of the individual metabolites present in grapes is summarised in Table B.7.1-3

Table B.7.1-3: Summary of identified components in grape samples after treatment with ^{14}C-pyraclostrobin

Metabolite code	Metabolite identity	Grapes	
		Tolyl label	Chlorophenyl label
		mg/kg (% TRR)	mg/kg (% TRR)
Pyraclostrobin (BAS 500 F)		0.860 (55.7)	0.688 (66.1)
500M07 (BF 500-3)		0.170 (11.2)	0.159 (15.3)
500M54		0.045 (2.9)	0.011 (1.1)
500M55		n.d.	0.029 (2.7)
500M56		0.048 (3.11)	0.016 (1.5)
further metabolites detected		3 (0.034 - 0.072 mg/kg, 2.1 - 4.7 % TRR,)	3 (0.015 – 0.038 mg/kg, 1.7 - 4 % TRR,)

n.d. not detected

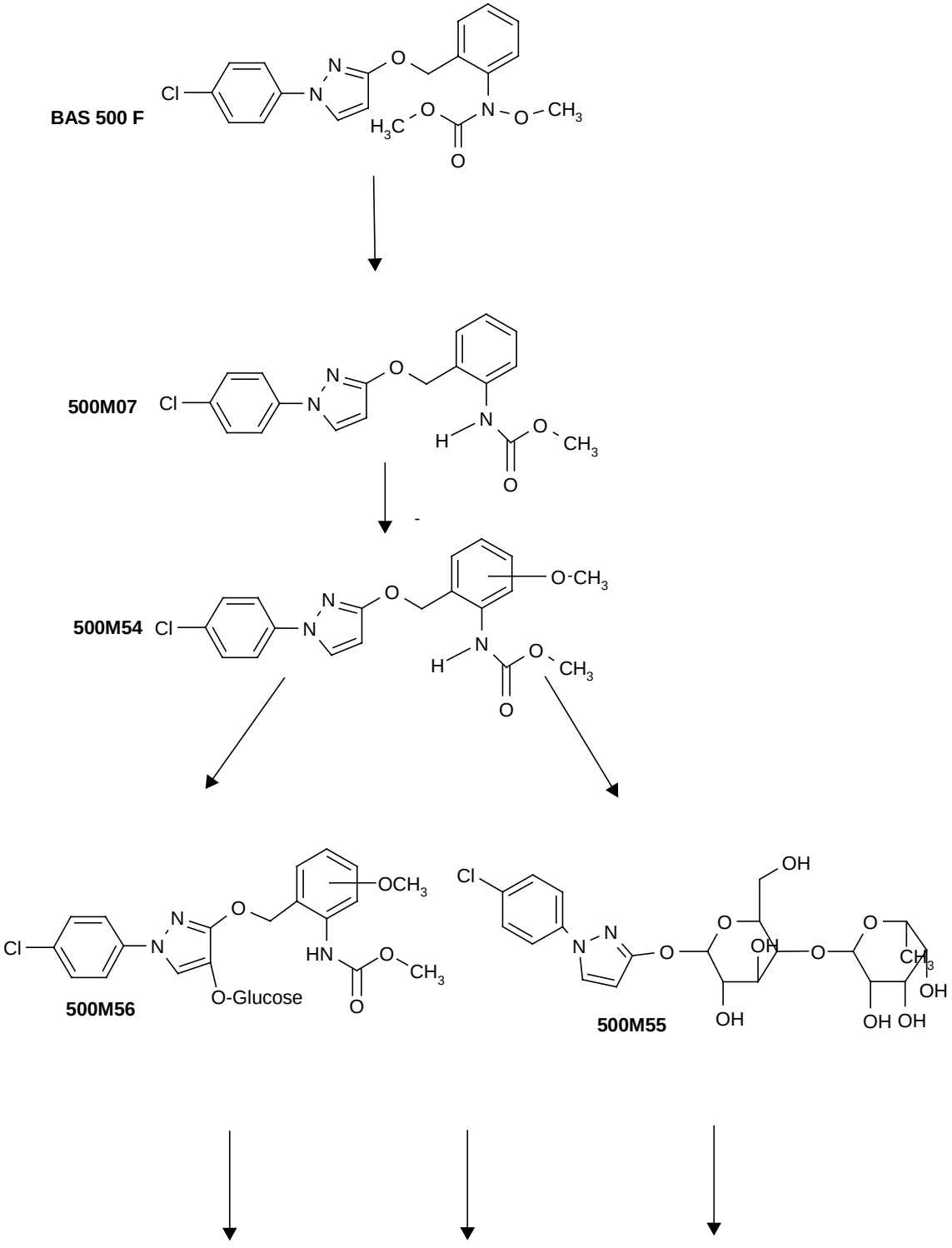
A comparison of the extractability and the HPLC metabolite patterns showed that there was no noticeable change in the nature of radioactive residues during sample storage over a period of more than 6 months.

Conclusion:

The relevant residue in grapes consists of the parent compound pyraclostrobin (BAS 500 F) and its desmethoxy metabolite 500M07 (BF 500-3).

Some other compounds identified as products formed by cleavage of the molecule, O-glucosylation or methoxylation turned out to be of minor importance because their respective amount is far below 10% of the TRR and the absolute amounts are low (< 0.05 mg/kg).

Figure B.7.1-1: Metabolic pathway of pyraclostrobin in grapes



B.7.1.2 Metabolism of pyraclostrobin in potatoes

Report: Bross, Mackenroth, 1999, RIP 2000-1051 and RIP 2000-1041

^{14}C-Pyraclostrobin was applied in the form of an EC-formulation to potato plants (variety: Quarta). Six applications at an intended use rate of 300 g as/ha were performed. The first application was performed about 8 weeks after sowing at growth stage BBCH 31 (main stem elongation). The application was repeated 5 times approximately every 9 days thereafter.

Table B.7.1-4: Data on application

No. of application	time interval [days]	amount ^{14}C-pyraclostrobin/ha [kg/ha]
1	-	0.3
2	9 / 8	0.3
3	9	0.3
4	10	0.4
5	9	0.3
6	6	0.3

The potato plants were grown in pots filled with Limburgerhof sand type soil under natural conditions.

Samples were taken for analysis seven days after the third application and at full maturity (seven days after last application). The samples were separated into green matter, tubers and roots.

Green matter and tuber samples of both labels were mainly extracted with methanol. The extractable radioactivity was characterised and quantified by radio HPLC. In addition, liquid/liquid partitioning experiments using *iso*-hexane, dichloromethane and ethyl acetate were carried out. The metabolites were identified by comparison with reference substances. Where possible, they were isolated by HPLC and their structures elucidated by LC/MS/MS.

For characterisation of the non-extractable radioactivity in tuber (tolyl label), the radioactive residue that was not extractable with methanol was further extracted with ammonia. In order to get information on the storage stability of the green matter and tuber samples, the extractability and the HPLC metabolite profiles were investigated at the beginning and at the end of the study.

Findings:
The Total Radioactive Residues (TRR) are summarised in Table B.7.1-5.

Table B.7.1-5: Total radioactive residues (TRR) after treatment of potatoes with ^{14}C-pyraclostrobin

Total radioactive residues, TRR [mg/kg]		
Label position	**Tolyl label**	**Chlorophenyl label**
Green matter*	9.860	19.636
Tuber*	0.014	0.009
Roots*	0.208**	0.450**
Green matter	47.785	69.846
Tuber	0.048	0.036
Roots	0.678**	0.986**

* harvested after three applications
** determined by direct combustion analysis

In all green matter samples, the solvent extractability was high; it ranged from 91 to 95% of the TRR. In tubers, a slight difference between the extractabilities of the two labels could be observed. In the case of the tolyl label, 39% and 42% of the TRR could be extracted by methanol whereas the extractable portions from the tubers treated with the chlorophenyl label were higher ranging between 49% and 68% of the TRR. Due to this lower extractability, ammonia extraction was applied. After ammonia extraction, the non-extractable residue was significantly below 0.050 mg/kg. The extraction behaviour is summarised in Table B.7.1-6.

Table B.7.1-6: Extraction of radioactivity after treatment of potatoes with ^{14}C-pyraclostrobin

Extraction of radioactivity [mg/kg] (% TRR)				
Solvent	**Green matter***	**Tuber***	**Green matter**	**Tuber**
Tolyl label				
TRR	9.860	0.014	47.785	0.048
Methanolic extracts	9.337 (94.7)	0.006 (39.1)	45.198 (94.6)	0.020 (41.6)
Not extractable (MeOH)	0.523 (5.3)	0.009 (61.0)	2.588 (5.4)	0.025 (51.8)
Ammonia extract	n.a.	n.a.	n.a.	0.012 (23.4)
Chlorophenyl label				
TRR	19.636	0.009	69.846	0.036
Methanolic extracts	18.531 (94.4)	0.005 (48.7)	63.413 (90.8)	0.022 (61.9)
Aqueous extracts	n.a.	n.a.	n.a.	0.001 (5.6)
Not extractable	1.105 (5.6)	0.005 (51.3)	6.433 (9.2)	0.012 (32.6)

* harvested after three applications
n.a. not applicable

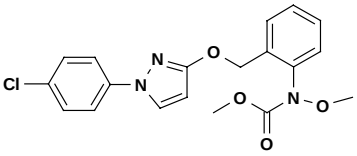
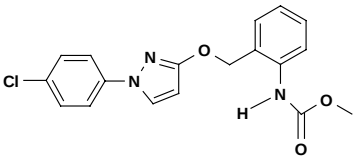
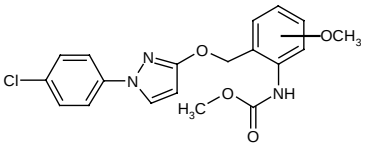
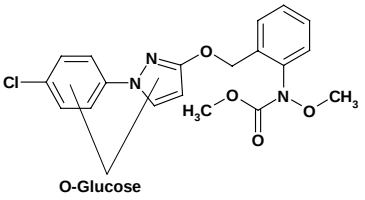
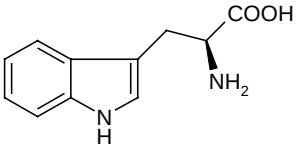
In order to classify the metabolites into organosoluble and water-soluble ones, liquid/liquid partition experiments were carried out. In the case of the samples treated with the chlorophenyl label most of the radioactivity was found in the organic phases. In the case of the tolyl labelled samples, the behaviour of tubers and green matter was different. For tubers, most of the radioactivity was detected in the aqueous phase whereas in green matter the organosoluble metabolites are predominant.

In potatoes, pyraclostrobin is metabolised by three key transformation steps: (1) desmethoxlation at the oxime ether bond, (2) hydroxylation of the tolyl and the chlorophenyl ring systems followed by glucosylation or methylation, and (3) cleavage between both ring systems and subsequent transformation of the resulting intermediates by glucosylation or the shikimate pathway which led to the natural amino acid tryptophan.

The formation of tryptophan and the subsequent incorporation of the natural amino acid into the protein structures of the potato tubers is an explanation for the different extraction behaviour described in Table B.7.1-6.

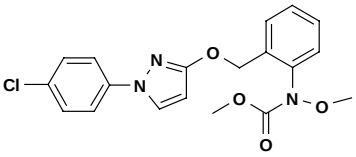
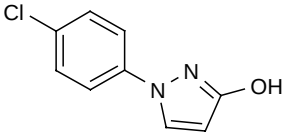
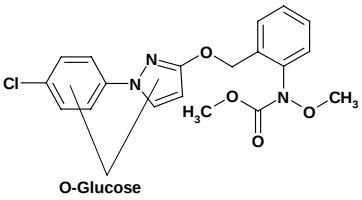
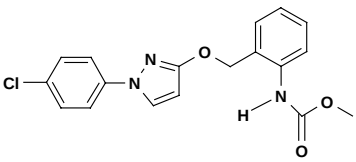
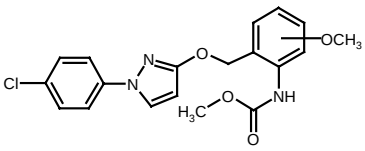
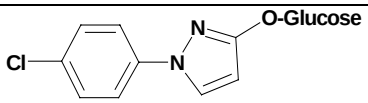
The quantification of the individual metabolites present in the different plant matrices is summarised in Table B.7.1-7 and Table B.7.1-8.

Table B.7.1-7: Summary of identified components in potato samples after treatment with ^{14}C-tolyl-pyraclostrobin

Metabolite code	Metabolite identity	Green matter		Tuber	
		GS 70	GS 85-89	GS 70	GS 85-89
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Pyraclostrobin (BAS 500 F)		6.427 (65.2)	30.888 (64.6)	< 0.001 (2.5)	n.d.
500M07 (BF 500-3)		1.600 (6.2)	10.231 (21.4)	< 0.001 (0.6)	n.d.
500M54		0.177 (1.8)	n.d.	n.d.	n.d.
500M68		0.058 (0.6)	n.d.	n.d.	n.d.
500M72		n.d.	n.d.	0.001 (10.0)	0.014 (29.2)
number of peaks		20	3	17	many
identified (sum)		8.262 (83.8)	41.119 (86.0)	0.002 (13.1)	0.014 (29.2)
characterised (sum)		1.212 (12.3)	2.342 (4.9)	0.003 (26.6)	0.009 (18.0)

n.a. not applicable
n.d. not detected

Table B.7.1-8: Summary of identified components in potato samples after treatment with ^{14}C-chlorophenyl-pyraclostrobin

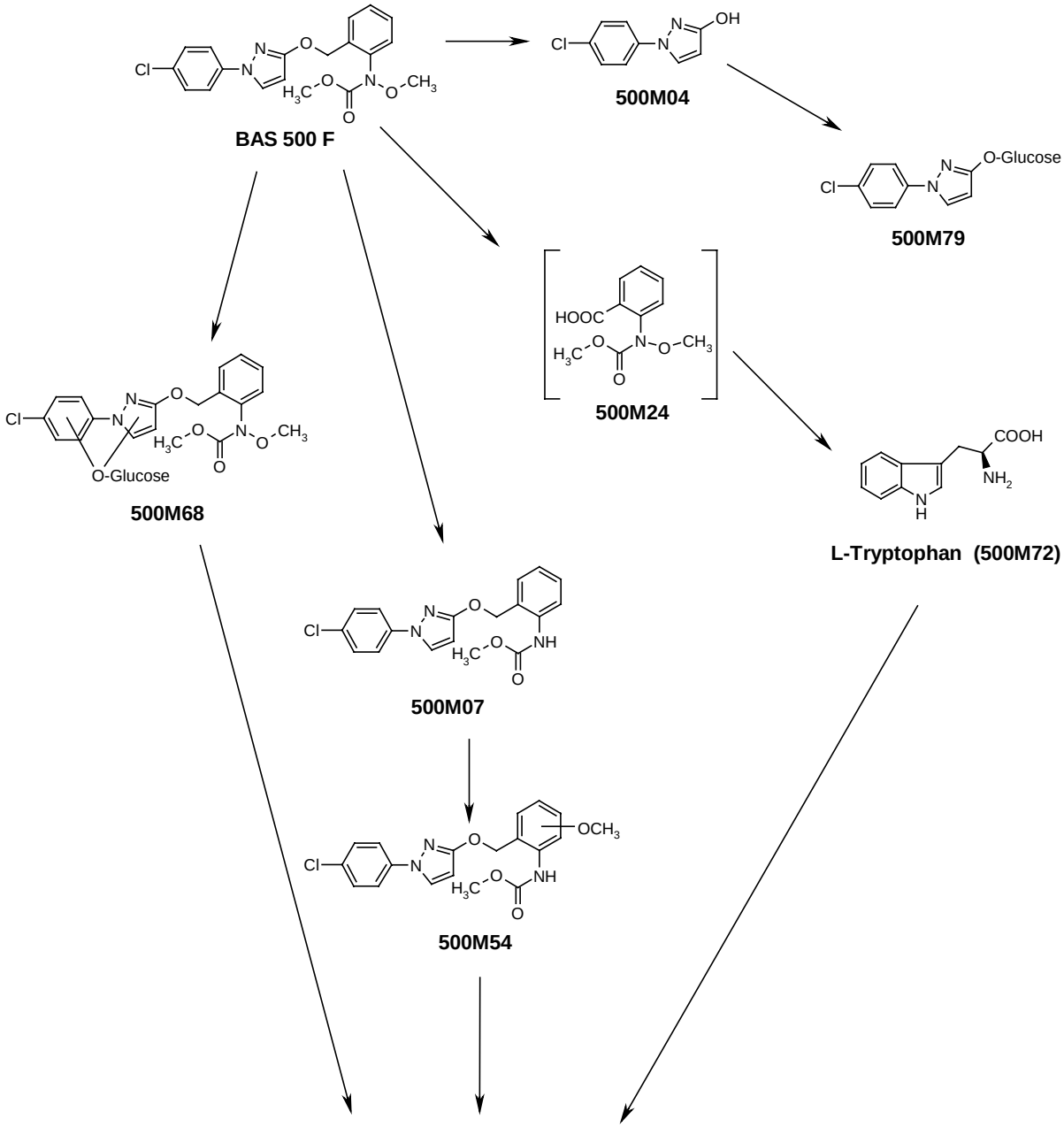
Metabolite Code	Metabolite identity	Green matter		Tuber	
		GS 70	GS 85-89	GS 70	GS 85-89
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Pyraclostrobin		11.098 (56.6)	38.515 (55.1)	0.002 (21.0)	0.012 (29.4)
500M04		0.570 (2.9)	3.068 (4.4)	0.000 (1.5)	0.001 (1.7)
500M68					
500M07 (BF 500-3)		3.157 (16.1)	14.502 (20.8)	0.001 (5.8)	0.003 (6.6)
500M54		0.271 (1.4)	1.782 (2.6)	0.001 (6.2)	0.001 (2.6)
500M79		0.072 (0.4)	0.072 (0.1)	0.000 (0.4)	0.001 (3.3)
number of peaks		29	5	13-22	6
identified (sum)		15.168 (77.2)	57.939 (83.0)	0.003 (34.9)	0.018 (43.8)
characterised (sum)		3.282 (16.7)	4.451 (6.3)	0.001 (14.8)	0.003 (8.5)

n.a. not applicable
n.d. not detected

A comparison of the extractability and the HPLC metabolite patterns showed that there was no noticeable change in the nature of radioactive residues during sample storage over a period of more than 2 years.

Conclusion:
The residue situation after application of pyraclostrobin to potatoes is significant different in leaves and tubers. In leaves, which are in direct contact with the formulation applied, the metabolites are approximately the same as in grapes. The parent compound and its methoxy metabolite 500M07 (BF 500-3) are the main components of the residue. No other metabolite above 10 % TRR was found.
The total residues in the edible portion (potato tubers) are very low. Only the parent compound and the naturally occurring amino acid L-tryptophan were determined in amounts above 0.01 mg/kg.
Some other compounds which were identified as products formed by cleavage of the molecule, O-glucosylation or methoxylation turned out to be of minor importance.

Figure B.7.1-2: Metabolic pathway of pyraclostrobin in potatoes



B.7.1.3 Metabolism of pyraclostrobin in wheat

Report: Reinhard,1999, RIP 2000-1009

Summer wheat plants at growth stage BBCH 31 - 32 were dug up in an outdoor test site and planted in plastic pots which were kept under natural climatical conditions in a glass-roofed vegetation hall. The wheat plants were separately treated with EC formulations of [chlorophenyl-U-^{14}C]-pyraclostrobin and [tolyl-U-^{14}C]-pyraclostrobin. According to agricultural practice, two applications of the test substance were performed in total, each at a slightly increased rate (1.2 x) of 300 g as/ha. The first application took place at growth stage BBCH 32 followed by the second at growth stage BBCH 61.

Table B.7.1-9 Data on application

No. of application	time interval [days]	amount ^{14}C-pyraclostrobin/ha [kg/ha]
1	-	0.3
2	24/25	0.3

Wheat samples were collected 0, 31 and 41 days after the last treatment. The harvest samples (41 DALT) were separated into straw, grain and chaff. Forage (31 DALT) as well as straw and grain represent the raw agricultural commodities and were therefore analysed in full detail.

The samples of both labels were sequentially extracted with methanol and water. The non-extractable radioactive residue was analysed by combustion. The total radioactive residues were calculated as the sum of extractable and non-extractable radioactivity. For a rough classification of the extracted radioactivity into organo-soluble and water-soluble portions, liquid/liquid partitions between cyclohexane / water followed by ethyl acetate / water were carried out. For a more detailed analysis, the extractable radioactivity was characterised and quantified by radio HPLC analysis. Metabolite identification was performed by comparison with reference substances or - where possible - by LC/MS/MS after isolation by HPLC.

For characterisation of the non-extractable radioactivity, the residue after MeOH and water extraction was first treated with ammonia. Thus, alkali-soluble radioactivity and radioactivity associated with grain protein could be released under mild conditions. Grain protein (tolyl label) that precipitated from the ammonia extract after acidification was further treated with a protease and the resulting amino acid solution analysed by HPLC. In the case of grain, the residue after ammonia extraction was subjected to DMSO/water treatment in order to isolate starch. The corresponding residues from forage and straw were examined for radioactivity associated with cellulose and lignin. Different methods were applied to isolate these biopolymers, e.g. cellulase treatment (for cellulose), refluxing with NaOH 10% (for separation of cellulose and lignin) and treatment with H_2SO_4 (for lignin).

Findings:
The total radioactive residues (TRR) are summarised in Table B.7.1-10. Except for grain, no significant differences in the TRR levels between the two labels occurred.
By far the lowest TRR were found in grains varying between 0.098 mg/kg in the chlorophenyl labelled and 0.441 mg/kg in the tolyl labeled matrix. The difference by a factor of 4.5 in the TRR between chlorophenyl and tolyl labeled grain originates from the cleavage of the test substance and the subsequent translocation of one of the fragments (amino acid tryptophan).

Table B.7.1-10: Total radioactive residues after treatment of wheat with ^{14}C-pyraclostrobin

Total radioactive residues [mg/kg]		
Label position	**Chlorophenyl**	**Tolyl**
Forage 31 DALT	6.527	6.793
Straw 41 DALT	37.768	40.461
Grain 41 DALT	0.098	0.441
Chaff 41 DALT	24.251	30.617

The extraction of forage and straw with MeOH followed by water was very effective releasing about 85% of the total radioactive residues. No significant difference between the labels was observed.
Extraction of radioactivity from grain was less effective with values ranging from 51% (tolyl label) to 71% (chlorophenyl label). The label dependent difference in the total extractability as well as in the relative portions extracted with MeOH (25.6% versus 55.8%) and water (25.6% versus 15.2%) was caused by the different composition of radioactivity in the tolyl and chlorophenyl labeled grain extracts. The extraction results are shown in Table B.7.1-11.

Except for grain / tolyl label, most of the extractable radioactivity was organosoluble when partitioned between water / cyclohexane and water / ethylacetate.

Table B.7.1-11: Extraction of radioactivity after treatment of wheat with ^{14}C-pyraclostrobin

Matrix	TRR mg/kg (% TRR)	MeOH mg/kg (% TRR)	H_2O mg/kg (% TRR)	ERR[1)] mg/kg (% TRR)	RRR[2)] mg/kg (% TRR)
Chlorophenyl label					
Forage	6.527 (100)	5.282 (80.9)	0.274 (4.2)	5.556 (85.1)	0.970 (14.9)
Straw	37.768 (100)	28.191 (74.6)	3.755 (9.9)	31.946 (84.5)	5.822 (15.4)
Grain	0.098 (100)	0.055 (55.8)	0.015 (15.2)	0.070 (71.0)	0.028 (29.0)
Chaff	24.251 (100)	13.257 (54.7)	3.421 (14.1)	16.678 (68.8)	7.573 (31.2)
Tolyl label					
Forage	6.793 (100)	5.436 (80.0)	0.281 (4.1)	5.717 (84.1)	1.076 (15.8)
Straw	40.461 (100)	31.777 (78.5)	2.915 (7.2)	34.692 (85.7)	5.769 (14.3)
Grain	0.441 (100)	0.113 (25.6)	0.113 (25.6)	0.226 (51.2)	0.216 (48.8)
Chaff	30.617 (100)	18.183 (59.4)	3.639 (11.9)	21.822 (71.3)	8.795 (28.7)

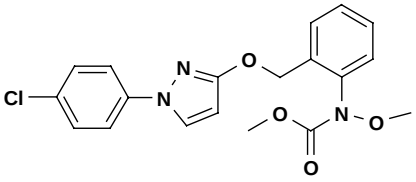
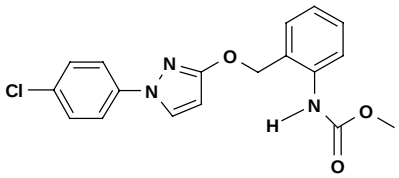
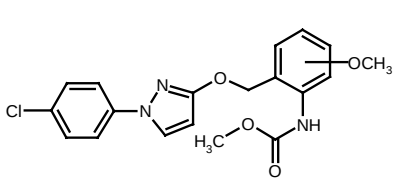
[1)]ERR = Extractable Radioactive Residue (sum of MeOH and water)
[2)]RRR = Residual Radioactive Residue

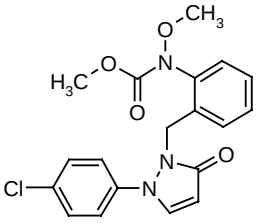
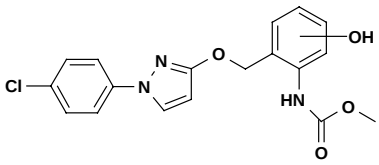
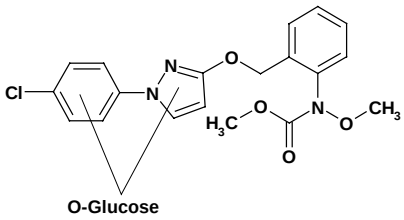
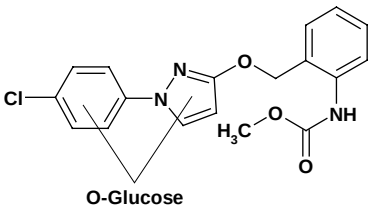
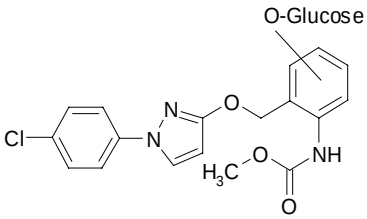
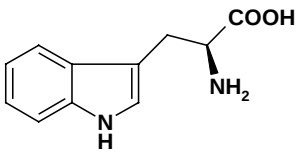
The metabolic pathway of pyraclostrobin in wheat is outlined in Figure B.7.1-3; detailed data on which metabolite was found in which wheat matrix at which concentration are provided in Table B.7.1-12.

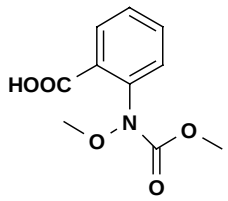
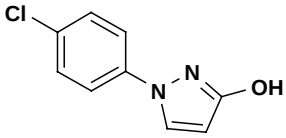
In forage and straw, the metabolite patterns looked almost identical with unchanged pyraclostrobin being the most prominent compound of the residue (53 – 58% TRR).
In grain, smaller amounts of pyraclostrobin were found that differed between the tolyl and the chlorophenyl labeled matrix (8 and 36% TRR).
Overall, the most important step for metabolising pyraclostrobin is the N-desmethoxylation to 500M07 (BF 500-3). The ratio of pyraclostrobin to its main metabolite varied between 4 : 1 in forage and straw and 3 : 1 in grain. To a minor extent, pyraclostrobin and 500M07 (BF 500-3) were hydroxylated at the chlorophenyl or tolyl ring. Some of the hydroxy compounds were conjugated with glucose or methylated. Mainly in grain, an additional degradation route for pyraclostrobin was observed involving the oxidative cleavage at the ether bridge and the subsequent transformation of the tolyl fragment 500M24 to the natural amino acid L-tryptophan (23% TRR) via the shikimate pathway. Besides, the pyraclostrobin isomer 500M76 occurred in small amounts obviously formed under the influence of light.

Storage stability investigations demonstrated that the metabolite patterns did not significantly change during the experimental period of about 20 months.

Table B.7.1-12: Summary of metabolites after treatment of wheat with ^{14}C-pyraclostrobin

Metabolite code	Structure	Forage (31 DALT) mg/kg (% TRR)	Straw mg/kg (% TRR)	Grain mg/kg (% TRR)
Pyraclostrobin		**Chlorophenyl label**		
		3.724	21.155	0.036
		(57.0)	(56.0)	(36.1)
		Tolyl label		
		3.598	23.295	0.034
		(52.9)	(57.5)	(7.8)
500M07 (BF 500-3)		**Chlorophenyl label**		
		0.782	4.885	0.010
		(12.0)	(12.9)	(10.5)
		Tolyl label		
		0.892	6.159	0.014
		(13.1)	(15.2)	(3.2)
500M54		**Chlorophenyl label**		
		0.136	0.462	n.d.
		(2.1)	(1.2)	n.d.
		Tolyl label		
		0.260	0.617	n.d.
		(3.8)	(1.6)	n.d.

Metabolite code	Structure	Forage (31 DALT) mg/kg (% TRR)	Straw mg/kg (% TRR)	Grain mg/kg (% TRR)
500M76		**Chlorophenyl label**		
		0.052	0.439	<0.004
		(0.8)	(1.2)	(<4.3)
		Tolyl label		
		0.054	0.607	n.d.
		(0.8)	(1.5)	n.d.
500M34		**Chlorophenyl label**		
		n.d.	0.462	n.d.
		n.d.	(1.3)	n.d.
		Tolyl label		
		0.083	0.289	n.d.
		(1.2)	(0.7)	n.d.
500M68		**SUM OF GLUCOSIDES (500M68, 500M70, 500M71)**		
500M70		**Chlorophenyl label**		
		0.163	1.640	<0.004
		(2.5)	(4.3)	(<4.3)
		Tolyl label		
		0.170	1.619	n.d.
		(2.5)	(4.0)	n.d.
500M71				
500M72 (L-Tryptophan)		**Tolyl label**		
		not detected		0.101
				(23.0)

Metabolite code	Structure	Forage (31 DALT) mg/kg (% TRR)	Straw mg/kg (% TRR)	Grain mg/kg (% TRR)
500M24		**Tolyl label**		
		not detected		0.030
				6.7
500M04		**Chlorophenyl label**		
		0.111	0.415	<0.004
		(1.7)	(1.1)	(<4.3)
Sum of components identified		**Tolyl label**		
		5.06 (74.3)	33.23 (82.1)	0.18 (41.2)
		Chlorophenyl label		
		4.97 (76.1)	31.36 (83.0)	0.05 (50.9)
Sum of other extractable components (each below 5 % TRR)		**Tolyl label**		
		0.66 (9.7)	1.47 (3.6)	0.05 (10.1)
		Chlorophenyl label		
		0.59 (9.0)	0.45 (1.2)	0.02 (20.2)

The non-extractable residues accounted for about 15% TRR in forage and straw and for up to 49% TRR in grain. Therefore, they were characterised in detail (compare Table B.7.1-13 and Table B.7.1-14).

In forage and straw, extraction with ammonia as the initial step released about 3% TRR. The residue after ammonia extraction was further analysed for radioactivity associated with the biopolymers cellulose and lignin using different methods for isolation. The mean values of radioactivity associated with cellulose from forage and straw ranged from 1.0 to 2.6% TRR. Comparably more radioactivity (4.4 – 7.9% TRR) was found to be associated with lignin. When adding the amounts of radioactivity associated with cellulose and lignin, approx. 40% - 50% of the non-extractable radioactive residue (RRR) could be assigned to these biopolymers. About 14 – 20% RRR was extractable with ammonia (as mentioned above). The remainder of about 30 – 40% RRR could be characterised as alkali-soluble radioactivity that did not precipitate with HCl. This fraction may contain hemicelluloses or fragments of the lignin-polysaccharide complex that could not be classified by the methods used in this study.

Table B.7.1-13: Summary of released non-extractable radioactivity from wheat forage and straw

Fraction name	Forage		Straw	
	Cl-phenyl label mg/kg (%TRR)	Tolyl label mg/kg (%TRR)	Cl-phenyl label mg/kg (%TRR)	Tolyl label mg/kg (%TRR)
RRR[1)]	0.885 (15.4)	1.111 (17.9)	8.449 (18.0)	8.777 (19.7)
Ammonia extract of RRR[1)]	0.173 (3.0) = 19.5% RRR[1)]	0.159 (2.6) = 14.3% RRR[1)]	1.582 (3.4) = 18.7% RRR[1)]	1.261 (2.8) = 14.4% RRR[1)]
Residue after ammonia extraction	0.712 (12.4)	0.952 (15.3)	6.867 (14.6)	7.516 (16.9)
Cellulose	0.103 (1.8) = 11.6% RRR[1)]	0.112 (1.8) = 10.1% RRR[1)]	0.470 (1.0) = 5.6% RRR[1)]	1.157 (2.6) = 13.2% RRR[1)]
Lignin	0.252 (4.4) = 28.5% RRR[1)]	0.423 (6.8) = 38.1% RRR[1)]	3.197 (6.8) = 37.8% RRR[1)]	3.514 (7.9) = 40.0% RRR[1)]
Alkali-solubles not precipitating with HCl	0.306 (5.3) = 34.5% RRR[1)]	0.373 (6.0) = 33.6% RRR[1)]	3.137 (6.7) = 37.1% RRR[1)]	2.327 (5.4) = 26.5% RRR[1)]

[1)]RRR = Residual (= non-extractable) radioactive residue

In grain, distinct differences of 8:1 in the absolute amounts of non-extractables between tolyl and chlorophenyl labels were seen, pointing to the cleavage of the test substance and to a different metabolic fate of the fragments.

As shown with the extractable radioactivity, the fragments of pyraclostrobin in tolyl labelled grain were 500M24 and tryptophan. Additional amounts (about 7% TRR) of these metabolites were released from the non-extractables by ammonia. However, a more significant portion of the ammonia extractable radioactivity (about 11% TRR) was obviously incorporated into grain protein as ^{14}C-tryptophan which was released after protease treatment.
The radioactivity in the residue after ammonia extraction (22% TRR) could roughly be classified into portions associated with starch, cellulose and alkali-soluble material, e.g. hemicelluloses.
The non-extractables in chlorophenyl labelled grain accounted for only 0.027 mg/kg (33.7% TRR). About 2/3 of the radioactivity were found to be ammonia-soluble or associated with starch.

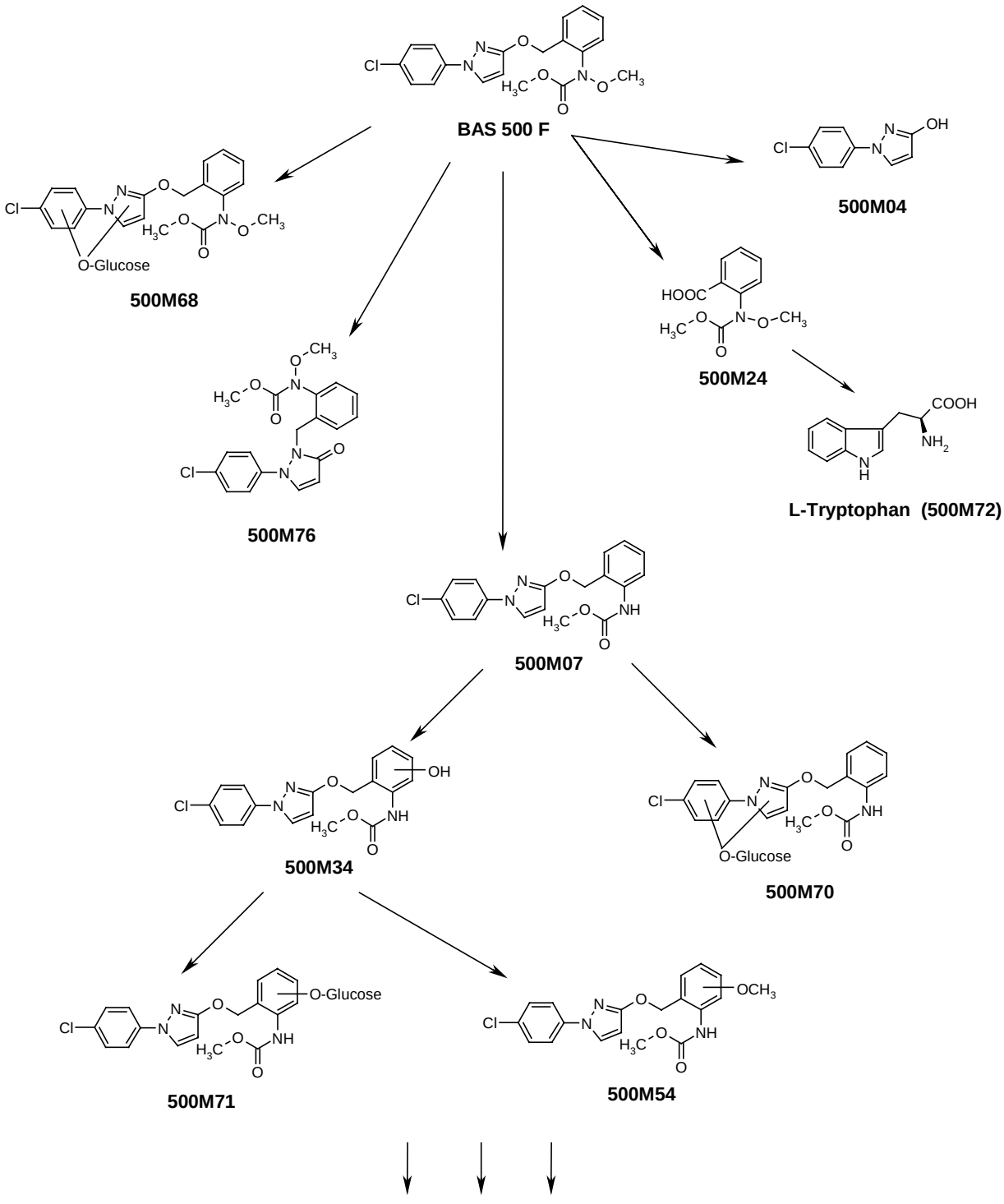
Table B.7.1-14: Summary of released non-extractable radioactivity from wheat grain

Fraction name	Grain	
	Chlorophenyl label mg/kg (%TRR)	**Tolyl label mg/kg (%TRR)**
RRR[1)]	0.027 (33.7)	0.216 (48.8)
Ammonia extract of RRR[1)]	0.009 (11.3)	0.113 (25.5)
Amino acid fraction after pronase treatment of the precipitated protein from the ammonia extract	n. d.	0.047 (10.7)
Supernatant from protein precipitation	n. d.	0.089 (20.1)
Residue after ammonia extraction	0.018 (22.4)	0.098 (22.1)
Starch precipitate from the DMSO / water extract	0.009 (10.5)	0.022 (5.0)
Supernatant of starch precipitation from the DMSO / water extract	0.004 (5.1)	0.012 (2.7)
Residue after DMSO / water extraction	0.008 (9.9)	0.065 (14.9)
Hot water extract of the DMSO / water residue	n. d.	0.015 (3.4)
NaOH extract of the residue after hot water extraction	n. d.	0.043 (9.8)
Residue after NaOH extraction (cellulose)	n. d.	0.022 (5.0)

[1)]RRR = Residual (= non-extractable) radioactive residue
n. d. = not determined

Conclusion:
The relevant residue of ^{14}C-pyraclostrobin in wheat consists of the unchanged parent compound and its desmethoxy metabolite 500M07 (BF 500-3). Tryptophan that is formed in considerable amounts from pyraclostrobin in grain is a natural ingredient and therefore of no toxicological concern. All other metabolites identified are clearly below 10% TRR and thus of minor importance. The low non-extractable residues in forage and straw demonstrate that pyraclostrobin and its metabolites do not tend to be firmly associated with cell wall polymers to a larger extent. Somewhat higher amounts of non-extractables were found in grain since portions of the radioactivity were incorporated into or associated with grain protein and starch.

Figure B.7.1-3: Metabolic pathway of pyraclostrobin in wheat



B.7.1.4 Extractability of residues of ^{14}C-pyraclostrobin from plant matrices

Extraction procedures used in analytical methods for residue trials or for monitoring often differ from those used in metabolism studies. By comparison of the different methods using

radiolabled material originating from metabolism studies it is possible to get information about the efficiency of analytical methods for grown material.

The samples were analysed with the BASF Analytical Method Number 421/0 (Reinhard and Mackenroth, 1999, MET 2000-273; Perez and Perez, 2000, MET 2000-274) which is based on the determination of pyraclostrobin and its metabolite BF 500-3 using LC/MS/MS. For details see chapter B.5.
All recoveries (fortification level 0.02 ,1.0, 5.0 mg/kg) reported in the residue studies were acceptable (No results outside the range 70 – 110 %).

B.7.1.4.1 Grapes and wheat

Report: Reinhard, Mackenroth, 1999, RIP 2001-72

Samples of wheat forage, grain and straw and of grapes which had been treated with ^{14}C-chlorophenyl-pyraclostrobin were used in this study. The samples originated from metabolism studies.
The samples were extracted using the analytical method No. 421/0 (Reinhard and Mackenroth, 1999, MET 2000-273; Perez and Perez, 2000, MET 2000-274; for details see chapter B.5.) and the results were compared to the results of the relevant metabolism study.

Extraction principles:
Wheat metabolism 1. methanol 2. water
Grape metabolism methanol
Method No. 421/0 methanol/water mixture (7/3, v/v)

Findings
Table B.7.1-15 clearly indicates that the extraction procedures used in the metabolism studies and in method No. 421/0 are equivalent.

Table B.7.1-15: Results of extraction

Matrix		Method No. 421/0 [% (mg/kg)]	Metabolism study [% (mg/kg)]
wheat forage	TRR	100 (5.56)	100 (6.53)
	ERR	83.3 (4.63)	85.1 (5.56)
	RRR	16.7 (0.93)	14.9 (0.97)
wheat grain	TRR	100 (0.10)	100 (0.10)
	ERR	61.6 (0.06)	71 (0.07)
	RRR	38.4 (0.04)	29 (0.03)
wheat straw	TRR	100 (50)	100 (37.8)
	ERR	85.9 (43)	84.5 (31.99
	RRR	14.1 (7)	15.4 (5.8)
grapes	TRR	100 (1.08)	100 (0.95)
	ERR	86.3 (0.93)	87.8 (0.84)
	RRR	13.7 (0.15)	12.2 (0.12)

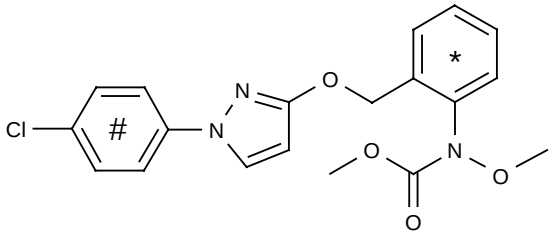
TRR = Total Radioactive Residue
ERR = Extractable Radioactive Residue
RRR = Residual Radioactive Residue

B.7.1.4.2 Potato

The extraction behaviour of treated potatoes is included in the metabolism investigations (Bross, Mackenroth, 1999, RIP 2000-1051 and RIP 2000-1041).
Potatoes treated with both lables were extracted with methanol/water in addition to pure methanol used in the metabolism study.
In the case of tolyl label the extractions led to different results. Methanol: 41.6 % TRR, methanol/water: 57.3 % TRR. This behaviour can be explained by the higher water solubility of tryptophan and related compounds in water.
With the chlorophenyl label the results are equivalent. Methanol: 67.5 % TRR, methanol/water: 66.5 % TRR.

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

The metabolism and distribution in livestock of pyraclostrobin was investigated using [tolyl-U-^{14}C]-pyraclostrobin and [chlorophenyl-U-^{14}C]-pyraclostrobin in lactating goats and laying hens.



* tolyl label
\# chlorophenyl label

B.7.2.1 Lactating goats

B.7.2.1.1 Absorption, distribution and excretion

Report: RIP 2000-1018
Leibold, E. et al. 1998(a)
^{14}C-BAS 500 F - Absorption, distribution and excretion after repeated oral administration in lactating goats
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.
unpublished
BASF RegDoc# 1998/10636

Test Material: [Chlorophenyl-UL-^{14}C]BAS 500 F (pyraclostrobin) and
[Tolyl-UL-^{14}C]BAS 500 F (pyraclostrobin)

Guidelines: US, EPA Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue – Plants, Livestock (August 1996)

GLP: Yes (laboratory certified)

Acceptability: The study is considered to be acceptable.

The objectives of this study were :

- To determine rates and routes of excretion and distribution of ^{14}C-pyraclostrobin in excreta, blood, plasma, milk and tissues of lactating goats.
- To generate samples of biological material following administration of ^{14}C-pyraclostrobin (excreta, milk, organs/tissues) which were transferred to an investigation on the metabolism performed as a separate study.

Material and Methods :

The metabolism and distribution of ^{14}C-pyraclostrobin was investigated in lactating goats ("Bunte deutsche Edelziege, age about 15 - 18 month) following repeated oral administration of chlorophenyl-^{14}C-pyraclostrobin and tolyl-^{14}C-pyraclostrobin at two dose levels. The test compound was administered daily on 5 consecutive days at nominal dose levels of 12 mg/kg and 50 mg/kg feed (dry matter). For the low dose two animals per label were used, the test substance was administered orally via gavage in a capsule. In case of the high dose only one goat per label was used , the test substance (dissolved in Pluriol E and 0.5% tylose) was orally applied by gavage with a syringe connected to an intubation catheter through the mouth of the goats.

The low dose level was chosen to reflect the expected residue level in plant parts to be used as feed items. The additional high dose level was added in order to facilitate metabolite identification and characterisation.

Table B.7.2-1: Dosing of lactating goats with ^{14}C-pyraclostrobin

Dose group (*label*)	Number of animals	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time [hour]
			[mg/kg feed intake]	[mg/kg feed intake]	[mg/kg b.w.]	
Low dose (*chlorophenyl*)	2	5	12	12.6 11.8	0.9 1.0	23
Low dose *(tolyl*)	2	5	12	11.9 12.5	0.65 0.75	23
High dose (*chlorophenyl*)	1	5	50	78.1	2.72	23
High dose (*toly*l)	1	5	50	69.7	1.37	23

Sampling and sample storage :

Urine and feces were collected daily. Milk was sampled in the morning (before administration of the substance) and in the afternoon.

During the application period, aliquots were taken for determination of radioactivity immediately after collection. In order to get some information on the blood and plasma concentration of radioactivity, blood samples were taken during the 1 hour before and 1 hour after administration and on the last day additionally at 2, 3, 4, 6, 8 and 23 hours post dosing.

23 hours after the last administration, animals were sacrificed and liver, kidney, blood, kidney fat, intraperitoneal fat, back muscles, leg muscles, bile, urine in bladder, gastrointestinal tract and contents were taken for determination of radioactivity. All samples were stored at -18^{o}C.

Measurements of radioactivity :
Aliquots of liquid samples were mixed with scintillation cocktail and analysed for radioactivity without any additional treatment. Other samples were lyophilisized and/or homogenized and solubilized before counting.

Findings:
No behavioral or physical abnormalities were observed at the low dose level. Body weights increased slightly, the milk production was constant. At the high dose level the animals showed a slight decrease in the feed intake and body weight during the application period resulting in a slightly reduced milk production.
Macroscopic examination at sacrifice revealed no abnormalities for the low and high dose animals.
The radioactivity was rapidly and almost completely excreted (see Table B.7.2-2 and Table B.7.2-3). Excretion mainly occurred via the feces which contained 39 to 64% of the dose. 9 to 23% of the dose were found in urine. Radioactivity recovered from urine and feces together with cage wash ranged from 73 to 92% of the total radioactivity recovered. Concentration of radioactivity in milk increased during day 1 - 3 of the application period and remained virtually constant thereafter, except in the tolyl label high dose, where the concentration did not increase.
At sacrifice, the highest tissue concentration of radioactivity were found in the GI-tract.
The concentration of radioactivity in blood were comparable to those observed in plasma indicating that the radioactivity was equally distributed among blood cells and plasma.

The total recoveries of radioactivity were in the range of 71 to 91 % of dose for both labels.

Table B.7.2-2: Material balance after administration of [*chlorophenyl*-^{14}C]-pyraclostrobin to lactating goats in % of total administered dose

Material	Low dose (Animal 3/Animal 4)	High dose
Organs and Tissues		
Liver	0.11/0.12	0.23
Kidney	0.00/0.00	0.01
Muscle	0.05/0.04	0.09
Fat	0.06/0.02	0.24
Milk	0.21/0.31	0.54
Gut and Gut content	5.33/4.0	10.11
Bile, Urine of Bladder, Lung, Heart, Blood, Cage wash, Skin, Stomach	4.15/3.04	2.14
Urine	12.38/11.43	22.93
Feces	48.35/61.16	39.28
Total	70.63/80.09	75.58

Table B.7.2-3: Material balance after administration of [*tolyl*-^{14}C]-pyraclostrobin to lactating goats in % of total administered dose

Material	Low dose (Animal 1/Animal 2)	High dose
Organs and Tissues		
Liver	0.25/0.19	0.24
Kidney	0.01/0.01	0.01
Muscle	0.09/0.07	0.09
Fat	0.11/0.05	0.06
Milk	0.11/0.11	0.20
Gut and Gut content	3.88/6.13	15.74
Bile, Urine of Bladder, Lung, Heart, Blood, Cage wash, Skin, Stomach	2.67/4.63	8.68
Urine	10.98/9.07	13.70
Feces	63.67/54.09	52.63
Total	81.87/74.34	91.32

Conclusion:
After 5 consecutive daily oral administrations of ^{14}C-pyraclostrobin at a nominal dose level of either 12 mg/kg or 50 mg/kg feed, there was a rapid absorption from the gastrointestinal tract. Excretion of radioactivity mainly occurred via the feces. Radioactivity in milk amounted only to 0.1 to 0.5% of the total radioactivity applied.
Except for the animal which received the chlorophenyl labelled test substance at the high dose level and which showed a higher bioavailability of the test substance, the excretion pattern of the tolyl- and chlorophenyl - labelled test substance were very similar. Plasma kinetics indicate an increased terminal half-life of the tolyl-label as compared to the chlorophenyl-label.
There was no indication of accumulation of ^{14}C-pyraclostrobin in goat tissues, organs and milk.

B.7.2.1.2 Investigation of the metabolism of ^{14}C pyraclostrobin in lactating goat

Report: RIP 2000-1020
Bross, M.;Tilting, N.; 2000
Investigation of the metabolism of ^{14}C BAS 500 F in the goat
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof
Germany Fed.Rep.
unpublished
BASF RegDoc# 2000/1000004

Test Material: [Chlorophenyl-UL-^{14}C]BAS 500 F (pyraclostrobin)
[Tolyl-UL-^{14}C]BAS 500 F (pyraclostrobin)

Guidelines: US, EPA Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue – Plants, Livestock (August 1996)

GLP: Yes (laboratory certified)

Acceptability: The study is considered to be acceptable.

This study was designed to investigate the nature of the residue of pyraclostrobin fed to goats at a nominal concentration corresponding to 12 mg/kg feed (dry matter) in the low dose group and 50 mg/kg feed (dry matter) in the high dose group.

Material and Methods :
The in-life and biokinetical part of the study is reported under B.7.2.1.1.

Sample storage and preparation for analysis
All samples were stored at -18°C (seeB.7.2.1.1). The extracts were stored in a refrigerator or, for longer periods, in a freezer.
In order to demonstrate the storage stability of the residues in milk and kidney, the extraction and/or HPLC investigation were carried out at the beginning and the end of the study.
Prior to analysis, the tissue samples of both goats in each low dose group were pooled. In the case of milk, pooling was performed for both dose groups.

Analysis
The total radioactive residues of milk, muscle, fat, liver and kidney were determined by combustion analysis (see also B.7.2.1.1). The samples were extracted with methanol or acetonitrile and water. In order to defat the extracts the acetonitrile extracts of milk and fat were partitioned against hexane prior to analysis. The extractable radioactivity was identified/characterised and quantified using radio HPLC. Major metabolites present in the methanol or acetonitrile extracts were identified by cochromatography with compounds isolated from urine or feces of the test animals. The identification of these metabolites is based on MS and NMR experiments. For the characterization of the non-released radioactivity in liver and kidney, the radioactive residue that was not extractable with organic solvents and water was further treated with pronase and/or subjected to acid hydrolysis.

Findings:
The total radioactive residues (TRR) of both dose groups and both labels are summarized in Table B.7.2-4 and Table B.7.2-5. In the low dose group, the TRRs in the milk and the tissues destined for human consumption were low. The values ranged from 0.018 mg/kg to 0.383 mg/kg. The TRR values for both labels were comparable for all samples under investigation.

In the exaggerated dose group which was used for identification and characterization purposes, the TRRs were higher; they ranged from 0.063 mg/kg to 1.505 mg/kg.

All results are calculated as parent equivalent.

Table B.7.2-4: Total radioactive residues in edible matrices after dosing of lactating goats with ^{14}C-[*chlorophenyl*]-pyraclostrobin

Matrix	Dose group: 12 mg/kg feed [mg/kg]	Dose group: 50 mg/kg feed [mg/kg]
Milk	0.038	0.382
Muscle	0.018	0.117
Fat	0.094	0.928
Liver	0.241	1.505
Kidney	0.054	0.335

Table B.7.2-5: Total radioactive residues in edible matrices after dosing of lactating goats with ^{14}C-[*tolyl*]-pyraclostrobin

Matrix	Dose group: 12 mg/kg feed [mg/kg]	Dose group: 50 mg/kg feed [mg/kg]
Milk	0.026	0.127
Muscle	0.022	0.063
Fat	0.082	0.380
Liver	0.383	0.828
Kidney	0.085	0.316

Extractability:
Milk and fat were extracted with acetonitrile and hexane; muscle, liver and kidney with methanol and water.
For most of the samples, the extractability was good: About 80% to 100% of the TRR could be extracted in case of milk, muscle and fat. In the case of kidney, the extractability was lower; it ranged from 56% to 85% of the TRR. For liver, the extractability was worse; less than 40% were extractable. A summary of the extraction behaviour is given in Table B.7.2-6 and Table B.7.2-7.

Table B.7.2-6: Extractability of goat matrices after dosing of lactating goats with ^{14}C-[*chlorophenyl*]-pyraclostrobin

Matrix	TRR	Methanol or acetonitrile	Hexane	Water	ERR	RRR	Recovery
	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Dose group: 12 mg/kg feed							
Milk	0.038 (100%)	0.034 (90.1%)	0.002 (4.7%)	n.a.	0.036 (94.7 %)	0.002 (5.8%)	0.038 (100.5%)
Muscle	0.018 (100%)	0.016 (88.1%)	n.a.	0.001 (4.9%)	0.017 (93.0 %)	0.001 (7.2%)	0.018 (100.2%)
Fat	0.094 (100%)	0.089 (95.1%)	0.006 (5.7%)	n.a.	0.095 (100.8%)	0.008 (8.2%)	0.103 (109.0%)
Liver	0.241 (100%)	0.081 (33.6%)	n.a.	0.003 (1.4%)	0.084 (35.0 %)	0.163 (67.5%)	0.247 (102.5%)
Kidney	0.054 (100%)	0.043 (79.2%)	n.a.	0.003 (5.1%)	0.046 (84.3%)	0.007 (13.7%)	0.053 (98.0%)
Dose group: 50 mg/kg feed							
Milk	0.382 (100%)	0.363 (95.0%)	0.003 (0.9%)	n.a.	0.366 (95.9 %)	0.017 (4.4%)	0.383 (100.3%)
Muscle	0.117 (100%)	0.099 (84.3%)	n.a.	n.a.	0.099 (84.3 %)	0.013 (11.1%)	0.112 (95.4%)
Fat	0.928 (100%)	0.901 (97.0%)	0.014 (1.5%)	n.a.	0.915 (98.5 %)	0.012 (1.3%)	0.927 (99.8%)
Liver	1.505 (100%)	0.434 (28.9%)	n.a.	0.051 (3.4%)	0.485 (32.3%)	0.997 (66.3%)	1.482 (98.6%)
Kidney	0.335 (100%)	0.278 (83.0%)	n.a.	0.007 (2.2%)	0.285 (85.2%)	0.055 (16.3%)	0.340 (101.5%)

Table B.7.2-7: Extractability of goat matrices after dosing of lactating goats with ^{14}C-*[tolyl]*-pyraclostrobin

Matrix	TRR	Methanol or Acetonitrile	Hexane	Water	ERR	RRR	Recovery
	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Dose group: 12 mg/kg feed							
Milk	0.026 (100%)	0.020 (75.8%)	0.001 (4.5%)	n.a.	0.021 (80.3%)	0.005 (17.5%)	0.025 (97.8%)
Muscle	0.022 (100%)	0.017 (77.6%)	n.a.	0.001 (4.7%)	0.018 (82.3%)	0.003 (15.3%)	0.021 (97.6%)
Fat	0.082 (100%)	0.077 (94.2%)	0.005 (5.8%)	n.a.	0.082 (100%)	0.009 (10.5%)	0.091 (110.5%)
Liver	0.383 (100%)	0.088 (23.1%)	n.a.	0.010 (2.5%)	0.098 (25.6 %)	0.286 (74.6%)	0.384 (100.2%)
Kidney	0.085 (100%)	0.040 (47.0%)	n.a.	0.008 (9.1%)	0.048 (56.1 %)	0.031 (36.7%)	0.079 (92.8%)
Dose group: 50 mg/kg feed							
Milk [1])	0.127 (100%)	0.116 (91.5%)	0.003 (2.3%)	n.a.	0.119 (93.8 %)	0.014 (10.7)	0.133 (104.5%)
Muscle	0.063 (100%)	0.053 (83.4%)	n.a.	n.a.	0.053 (83.4%)	0.012 (18.9%)	0.059 (102.3%)
Fat	0.380 (100%)	0.376 (98.8%)	0.007 (1.9%)	n.a.	0.383 (100.7 %)	0.010 (2.7%)	0.393 (103.4%)
Liver	1.013 (100%)	0.349 (34.4%)	n.a.	0.030 (2.9%)	0.379 (37.3 %)	0.681 (67.3%)	1.060 (104.6%)
Kidney	0.316 (100%)	0.226 (71.6%)	n.a.	0.019 (6.0%)	0.245 (77.6%)	0.079 (24.8%)	0.324 (102.4%)

[1]) organic extract (1. acetonitrile, 2. solvent change to methanol)

TRR: Total radioactive residue
ERR: Extractable radioactive residue
RRR: residual (= non-released) radioactive residue
n.a. not applicable

Identification, characterisation and quantification of the extractable radioactivity :

For the identification of polar metabolites, pooled urine samples were worked-up and for the identification of unpolar metabolites, feces samples were used.

Metabolites isolated from the chlorophenyl label in urine were 500M04, 500M05, 500M85 and from the tolyl label 500M39 and 500M51.
Metabolites isolated from the chlorophenyl label in feces were 500M04, 500M07 (BF 500-3), 500M08, 500M45, 500M64, 500M66, 500M67, 500M84 and parent pyraclostrobin. Results from the tolyl label were not reported "due to the comparable pattern" in the HPLC chromatogram of the methanol extract.

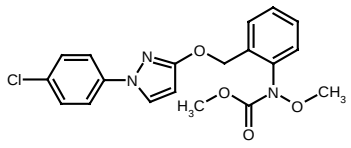
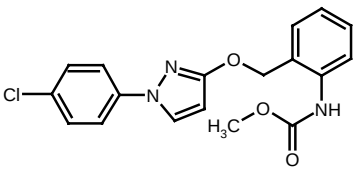
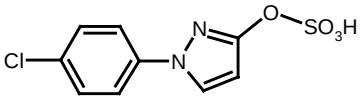
In milk the metabolic patterns of the low and high dose goats were comparable for the individual labels. The metabolites 500M04, 500M05 and 500M85, formed by cleavage reaction, were identified by retention time comparison with metabolites from urine. The metabolites 500M07 (BF 500-3), 500M08, 500M45, 500M67 and the parent compound were found in both labels. The metabolites 500M64 and 500M66 were only quantified in the chlorophenyl label because of different amounts of radioactivity injected for analysis.
Less polar metabolites were identified by comparison with metabolites isolated from feces.

In muscle and fat only metabolite 500M07 (BF 500-3) and parent compound could be identified.

In liver and kidney the metabolite profiles of the individual labels were slightly different. In the case of chlorophenyl label, the same metabolites as in milk were detected in liver and kidney : 500M04, 500M05, 500M85. The metabolites 500M07 (BF 500-3), 500M39, 500M66 and 500M67 as well as the parent molecule were identified in liver in both labels, the metabolites 500M08 and 500M45 only in the chlorophenyl label.
In the methanolic extract of the tolyl labelled kidney sample 500M51, an anthranilic acid derivative, was found.
In kidney the metabolites 500M07 (BF 500-3), 500M67 and the parent compound were found in both labels, the metabolits 500M64 and 500M66 only in the chlorophenyl label.

The summary of all identified metabolites present in the edible portions is shown in Table B.7.2-8 and Table B.7.2-9 for the low dose group (12 mg/kg based on feed intake). The data of the exaggerated dose group (50 mg/kg based on feed intake) can be found in Table B.7.2-10 and Table B.7.2-11.

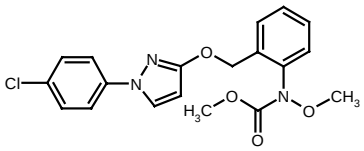
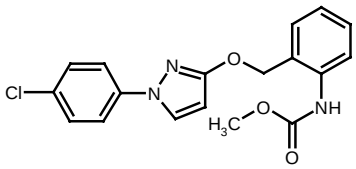
Table B.7.2-8: Summary of identified and quantified metabolites in edible matrices of goat tissues and milk after dosing with ^{14}C-pyraclostrobin *chlorophenyl label* (nominal dose level: 12 mg/kg feed)

Metabolite code	Metabolite Identity	Milk	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Pyraclo-strobin		0.012 (31.6)	0.010 (57.9)	0.069 (73.4)	0.008 (3.1)	0.010 (19.6)
500M07 (BF 500-3)			0.003 (14.2)	0.020 (21.7)		
500M05		0.012 (31.1)	n.d.	n.d.	n.d.	n.d.

In the case of milk, liver and kidney, pyraclostrobin and the desmethoxy metabolite 500M07 (BF 500-3) were quantified as sum.
n.d. not detected

Characterised residues in milk : 0.012 mg/kg (32.1 % of TRR), sum of 5 minor HPLC peaks 0.01 mg/kg (27.3 % of TRR), each not more than 0.005 mg/kg.

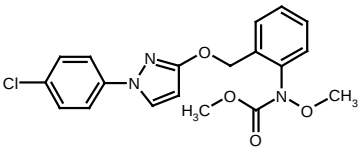
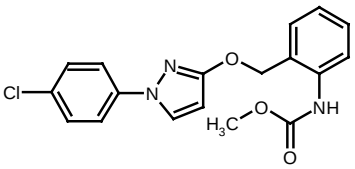
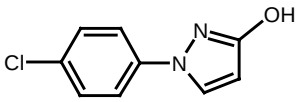
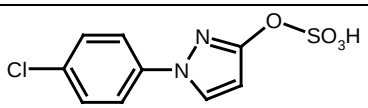
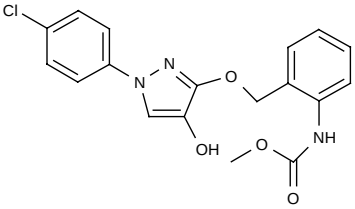
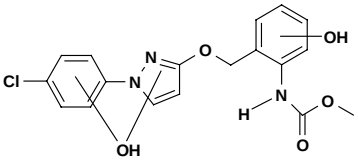
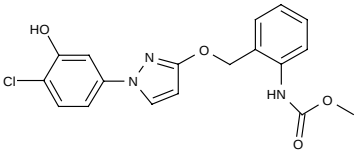
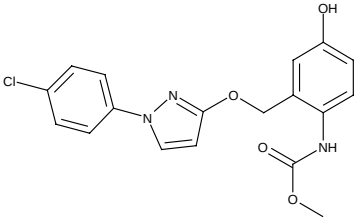
Table B.7.2-9: Summary of identified and quantified metabolites in edible matrices of goat tissues and milk after dosing with ^{14}C-pyraclostrobin *tolyl label* (nominal dose level: 12 mg/kg feed)

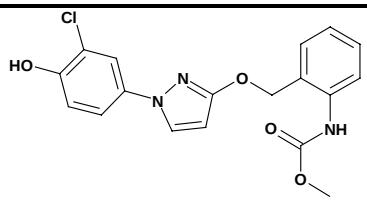
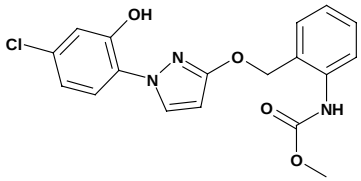
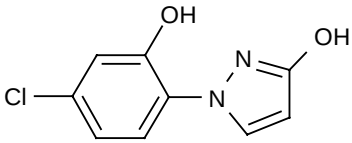
Metabolite code	Metabolite Identity	Milk	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Pyraclo-strobin		0.010 (37.4)	0.011 (53.6)	0.061 (74.2)	0.006 (1.4)	0.007 (8.8)
500M07 (BF 500-3)			0.003 (12.0)	0.016 (20.0)		

In the case of milk, liver and kidney, pyraclostrobin and the desmethoxy metabolite 500M07 (BF 500-3) were quantified as sum.

Characterised residues in milk**:** 0.011 mg/kg (42.9 % of TRR), sum of 3 minor peaks 0.01 mg/kg (38.4 % of TRR) : 0.001/0.001/0.008 mg/kg.

Table B.7.2-10: Summary of identified and quantified metabolites in edible matrices of goat tissues and milk after dosing with ^{14}C-pyraclostrobin *chlorophenyl label* (nominal dose level: 50 mg/kg feed)

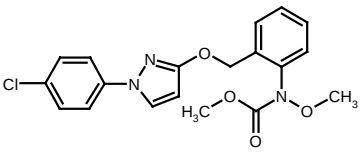
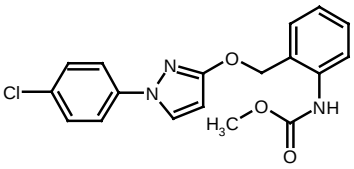
Metabolite code	Metabolite identity	Milk	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Pyraclo-strobin		0.067 (17.4)	0.089 (76.2)	0.819 (88.2)	0.021 (1.4)	0.074 (22.1)
500M07 (BF 500-3)			0.010 (8.1)	0.082 (8.8)	0.022 (1.5)	
500M04		0.062 (16.3)	n.d.	n.d.	0.013 (0.9)	0.015 (4.4)
500M05		0.054 (14.1)	n.d.	n.d.	0.002 (0.1)	0.045 (13.4)
500M08		0.004 (1.0)	n.d.	n.d.	0.004 (0.3)	n.d.
500M39		n.d.	n.d.	n.d.	0.015 (1.0)	n.d.
500M45		0.006 (1.6)	n.d.	n.d.	0.003 (0.2)	n.d.
500M64		0.010 (2.6)	n.d.	n.d.	n.d.	0.003 (1.0)

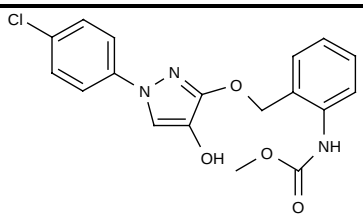
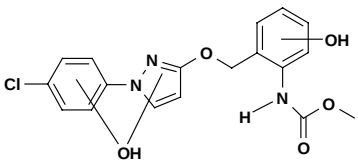
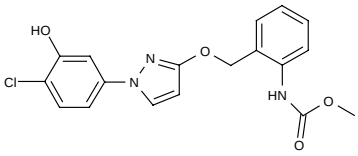
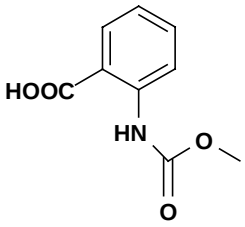
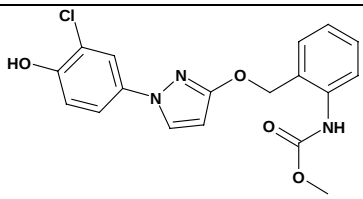
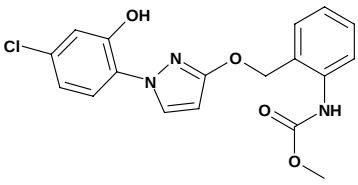
Metabolite code	Metabolite identity	Milk	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
500M66		0.006 (1.5)	n.d.	n.d.	0.020 (1.3)	0.004 (1.2)
500M67		0.008 (2.1)	n.d.	n.d.	0.069 (4.6)	0.043 (13.0)
500M85		0.021 (5.5)	n.d.	n.d.	0.025 (1.6)	0.022 (6.5)
	Total identified in the acetonitrile or methanol extract	0.238 (62.1)	0.099 (84.3)	0.9 (97)	0.194 (12.9)	0.206 (61.6)

In case of milk and kidney, pyraclostrobin and the desmethoxy metabolite 500M07 (BF 500-3)were quantified as sum.
n.d. not detected

Characterised residues in milk**:** 0.129 mg/kg (33.8 % of TRR); sum of 11 minor HPLC peaks 0.126 mg/kg (32.8 % of TRR), each less than 0.05 mg/kg.

Table B.7.2-11: Summary of identified and quantified metabolites in edible matrices of goat tissues and milk after dosing with ^{14}C-pyraclostrobin *tolyl label* (nominal dose level: 50 mg/kg feed)

Metabolite code	Metabolite identity	Milk	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Pyraclo-strobin		0.027 (21.4)	0.048 (76.3)	0.318 (83.4)	0.070 (8.4)	0.073 (23.2)
500M07 (BF 500-3)			0.005 (7.1)	0.058 (15.4)	0.024 (2.9)	

Metabolite code	Metabolite identity	Milk	Muscle	Fat	Liver	Kidney
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
500M08		0.001 (0.8)	n.d.	n.d.	n.d.	n.d.
500M39		n.d.	n.d.	n.d.	0.007 (0.8)	n.d.
500M45		0.001 (1.1)	n.d.	n.d.	n.d.	n.d.
500M51		n.d.	n.d.	n.d.	n.d.	0.039 (12.4)
500M66		n.d.	n.d.	n.d.	0.021 (2.5)	n.d.
500M67		0.004 (2.8)	n.d.	n.d.	0.024 (2.8)	0.025 (7.8)
	Total identified in acetonitrile extract	0.033 (25.1)	0.053 (83.4)	0.376 (98.8)	0.146 (17.4)	0.137 (43.4)

n.d not detected
In the case of milk and kidney, pyraclostrobin and the desmethoxy metabolite 500M07 (BF 500-3) were quantified as sum.

Characterised residues in milk: 0.086 mg/kg (67.8 % of TRR), sum of 15 minor HPLC peaks 0.083 mg/kg (65.4 % of TRR), each <= 0.021 mg/kg.

Characterisation of non-extractable radioactivity:
The non-extractable residues of liver and kidney from the high dose were further characterised by pronase treatment and acid hydrolysis (for results see Table B.7.2-12)
Liver:
In the case of liver, more than 30% of the TRR was released by pronase digestion. The digest

was further analysed by HPLC and consisted of 13 peaks between 0.45 % and 5.94 % of TRR (or 0.007 and 0.089 mg/kg) in the chlorophenyl labelled sample. Acid hydrolysis released 60.8 % of TRR, two metabolites were identified : 500M04 (26.47 % of TRR or 0.398 mg/kg) and 500M85 (13.87 % of TRR or 0.208 mg/kg).
In an additional analysis of purified pronase digest the metabolite 500M04 was identified (6.91 % of TRR or 0.104 mg/kg).
The results for the tolyl labelled sample was comparable. In the pronase extract nine unknown metabolites were found between 0.48 and 7.33 % of TRR (or 0.005 and 0.07 mg/kg).
Kidney:
Due to the lower amounts of non-extractables present (see Table B.7.2-12), the values for kidney were lower.
After acid hydrolysis of the non-extractable residues of the chlorophenyl labelled sample of kidney were found by HPLC analysis four unknown metabolites (0.12 % - 0.27 % of TRR or <0.001 - 0.001 mg/kg) and the metabolites 500M04 (2.14 % of TRR or 0.007 mg/kg) and 500M85 (2.27 % of TRR or 0.007 mg/kg).
In the pronase digest of the non-extractable residues of the chlorophenyl labelled kidney sample were found by HPLC analysis six unknown metabolites (0.14 % - 0.55 % of TRR or <0.001 - 0.02 mg/kg) and the metabolites 500M04 (0.68 % of TRR or 0.002 mg/kg) and 500M85 (2.18 % of TRR or 0.007 mg/kg).
In a purified pronase digest of the tolyl labelled kidney sample fourteen metabolites were detected by HPLC (0.12 % - 1.48 % of TRR or <0.001 - 0.005 mg/kg), no metabolite was identified.

Generally, more radioactivity was released by refluxing with hydrochloric acid. By applying these rigorous conditions, most of the non-extractable residue present in the chlorophenyl label was cleaved and converted to chloropyrazole derivatives.

Table B.7.2-12: Characterisation of the non-extractable radioactive residues (RRR) in goat liver and kidney after dosing with ^{14}C-pyraclostrobin (dose group: 50 mg/kg feed)

Sample	Released radioactivity [mg(kg] (% TRR)	
	Pronase treatment	Acid hydrolysis
Chlorophenyl label		
Liver	0.504 (33.5)	0.915 (60.8)
Kidney	0.041 (12.2)	0.057 (17.2)
Tolyl label		
Liver	0.446 (53.9)	0.506 (61.0)
Kidney	0.102 (32.4)	0.105 (33.2)

Storage stability:
In the case of milk, the sample was extracted 56 days after sampling and analysed by HPLC; after a storage interval of 159 days the extract was reanalysed.

In the case of kidney, the sample was first extracted 64 days after sampling and analysed. After a storage interval of 141 days the extract was reanalysed by HPLC. A frozen kidney sample was extracted after freezer storage of 259 days and analysed by HPLC.

A comparison of the extractability and the HPLC metabolite profiles indicated that there was no noticeable change in the nature of the radioactive residues during extract and sample storage over a period of about 5 month for milk and about 9 month for kidney

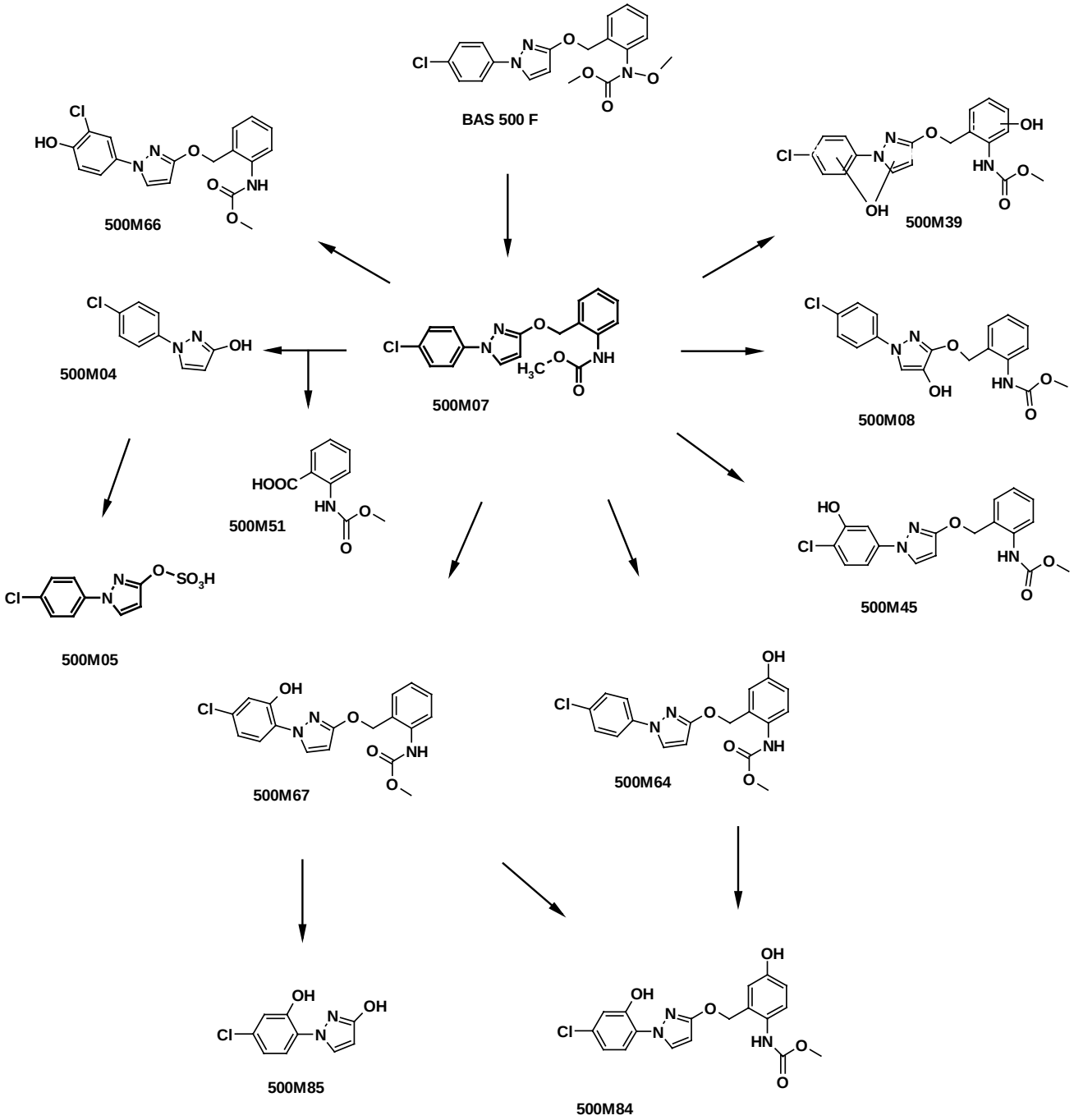
Conclusion:
The metabolism and distribution of ^{14}C-pyraclostrobin was investigated in lactating goats using material labelled either in the chlorophenyl or in the tolyl ring system. The metabolic pathway is shown in Figure B.7.2-1: Metabolic pathway of pyraclostrobin in the goat.

After administration of the test compound, most of the radioactivity was excreted. There was no indication of accumulation of ^{14}C-pyraclostrobin in goat milk and tissues. The total radioactive residues in the edible portions of the 12 mg/kg dose group which is close to the expected residue level in plant parts to be fed were low.
The parent compound, pyraclostrobin was main residue in nearly all samples under investigation. In addition, ^{14}C-pyraclostrobin is metabolised in goats by three key transformation steps:
(1) desmethoxylation at the oxime ether bond
(2) hydroxylation of the chlorophenyl, the pyrazole and/or the tolyl ring system and
(3) cleavage between both ring systems and subsequent oxidation of the two resulting molecule parts.
The same key transformation steps had been also observed in rat and in hen.

Three minor metabolites were detected in edible matrices (500M66, 500M67 and 500M85) which are not reported as metabolites in the rat. There are no toxicological studies available for these metabolites. Due to the low concentration of these metabolites , no further toxicological studies seem to be necessary.

The hydrolysis reaction with hydrochloric acid which was performed for further characterisation of the extractable and the non-extractable residue resulted for the chlorophenyl label in the formation of two chlorophenyl pyrazole products. Thus, the hydrolytic degradation reaction is regarded as suitable approach for the development of a common moiety method for goat matrices.

Figure B.7.2-1: Metabolic pathway of pyraclostrobin in the goat



B.7.2.2 Laying hens

B.7.2.2.1 Absorption, distribution and exretion of ^{14}C-pyraclostrobin

Report: RIP 2000-1021
Leibold, E.; et al. 1998(b)
^{14}C-BAS 500 F - Study of the absorption, distribution and excretion after repeated oral administration to laying hens

BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.
BASF RegDoc# 1998/10637

Test Material: [Chlorophenyl-UL-^{14}C]BAS 500 F (pyraclostrobin) and [Tolyl-UL-^{14}C]BAS 500 F (pyraclostrobin)

Guidelines: US, EPA Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue – Plants, Livestock (August 1996)

GLP: Yes (laboratory certified)

Acceptability: The study is considered to be acceptable.

The objectives of this study were:
- To determine rates and routes of excretion and distribution of ^{14}C pyraclostrobin in excreta, blood, plasma, eggs and tissues of laying hens;
- To generate samples of biological material following administration of ^{14}C pyraclostrobin (excreta, eggs, tissues) which were transferred to an investigation on the metabolism performed as a separate study.

Material and Methods:
The metabolism and distribution in laying hens was investigated following repeated oral administration of ^{14}C-pyraclostrobin at one dose level.

The test compound was administered to two groups of hens using two different radiolabels. The chlorophenyl label was applied at a dose of 12 mg/kg feed, and the tolyl label was fed at a dose of 13 mg/kg feed, both by gavage and on seven consecutive days. The dose levels based on dry matter and corresponded to 0.70 mg/kg body weight (chlorophenyl label) and 0.88 mg/kg body weight (tolyl label); they were orientated on the minimum dose level recommended by the relevant guidelines.
Details of the study design are summarised in Table B.7.2-13.

Table B.7.2-13: **Dosing of laying hens with ^{14}C-pyraclostrobin**

Dose group	Number of animals	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time
			mg/kg feed intake	mg/kg feed intake	mg/kg b.w.	Hours post last dose
A	11	7	12	12.14	0.70	23
B	11	7	12	12.72	0.88	23

Sampling and sample storage:
Excreta were collected in time intervals of 24 hours. Aliquots were taken for determination of radiocativity immediately after collection.
Eggs were collected in the morning before administration of the test substance and in the afternoon.

In order to get some information on the blood and plasma concentration of radioactivity, blood samples were taken on the last day of application at 1, 2, 4, 6, 8, and 23 hours post-dosing.
23 hours after the last dosing, animals were sacrificed and liver, kidney, fat, chest muscle, leg muscle, gastrointestinal tract (skin and contents) were checked for remaining radioactivity.
All samples were stored frozen.

Measurement of radioactivity:
Aliquots of liquid samples were mixed with scintillation cocktail and direct analysed for radioactivity. Other matrices were lyophilisized and/or homogenized and solubilisized before counting.

Findings:
During the study period, no behavioural or physical abnormalities were observed. Macroscopic examination at sacrifice revealed no abnormalities. Body weights, feed consumption and egg production remained virtually unchanged during the study period.

The material balance after dosing ^{14}C-pyraclostrobin with the two radiolabels accounted in total for 101.17 % of the dose for the chlorophenyl label and for 95.49% of the dose for the tolyl label [see Table B.7.2-14]. In case of both labels, 0.05% of the dose were excreted via eggs and 0.19% of the dose were distributed into organs and tissues. Excretion of radioactivity was very effective with 93% of the dose for the chlorophenyl label and with 87% of the dose for the tolyl label determined in excreta. Around 8% and 9% of the dose were recovered in the gastrointestinal tract and cage wash of the chlorophenyl label and of the tolyl label, respectively.

Table B.7.2-14: Material balance after administration of ^{14}C-pyraclostrobin to laying hens

	Material balance in % of total dose	
	Animal group A	Animal group B
Matrix	*Chlorophenyl label*	*Tolyl label*
Eggs	0.05	0.05
Liver	0.1	0.13
Fat	0.03	0.02
Other organs and Tissues	0.06	0.04
Excreta	93.30	86.59
GIT	7.47	8.49
Cage Wash	0.16	0.17
Total	101.17	95.49

After the seventh daily oral administration of the test substance (both label), the mean plasma level peaked at 1 hour post-dosing. Radioactivity in blood did not go in parallel with plasma concentrations.

Conclusion:
There was a rapid but probably not complete absorption of radioactivity after seven administrations from the gastrointestinal tract. The relatively high amounts of radioactivity in

the contents of the GI-tract and the relatively low concentration in plasma indicated limited bioavailibility.
Comparing the biokinetic properties of the chlorophenyl and the tolyl labelled test substance shows that they behave very similar.

B.7.2.2.2 Investigation of the Metabolism of ^{14}C-pyraclostrobin in laying hen

Report: RIP 2000-1018
Hafemann C.,Knoell H.-E. 1999
Metabolism of (^{14}C)BAS 500 F in laying hens
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep.
BASF RegDoc# 1999/11480

Test Material: [Chlorophenyl-UL-^{14}C]BAS 500 F (pyraclostrobin) and
[Tolyl-UL-^{14}C]BAS 500 F (pyraclostrobin)

Guidelines: US, EPA Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue – Plants, Livestock (August 1996)

GLP: Yes (laboratory certified)

Acceptability: The study is considered to be acceptable

The study had the objectives to identify and quantify the relevant metabolites of Pyraclostrobin (BAS 500 F) in eggs and tissues of laying hens.

Material and Methods:
The in-life part of the study and the sampling are reported under B.7.2.2.1.

Sample storage and preparation for analysis:
The tissue samples were homogenised and divided into subsamples. Excreta collected at 24 h intervals were separately pooled and homogenised. Egg-shells were discarded and egg white and egg yolk thoroughly homogenised, separately for ech 24 h pool.
All sample were stored frozen and extracted and analysed within one year.

Analysis:
For measurement of radioactivity see B.7.2.2.1.
Subsamples of excreta and eggs were subjected to an extraction procedure using acetonitrile, subsamples of liver were extracted with acetonitrile and water. Fat samples were subjected to extensive extraction with acetonitrile, n-hexane and water.
Acetonitrile extracts of eggs, fat and liver were subsequently partioned with n-hexane followed by HPLC analysis of the acetonitrile phase.
Muscle showed radioactive residues below 0.01 mg/kg and was no further processed.

For the characterization of the non-released radioactivity in liver, the radioactive residue that was not extractable with acetonitrile and water was further treated with pronase or subjected to acid hydrolysis.

Findings:
The total radioactive residues were determined for eggs and for muscle, fat and liver as edible tissues [see Table B.7.2-15]. Very low residue levels were detected for muscle with less than 0.01 mg/kg and for eggs with around 0.03 mg/kg in the case of both labels. Higher residue levels were measured for liver with 0.317 mg/kg for the chlorophenyl label and with 0.474 mg/kg for the tolyl label.

All results are calculated as parent equivalents.

Table B.7.2-15: Total radioactive residues in edible matrices after dosing of laying hens with ^{14}C-pyraclostrobin (nominal dose: 12 mg/kg based on feed intake)

Matrix	Animal group A *Chlorophenyl label*	Animal group B *Tolyl label*
	mg/kg	mg/kg
Eggs	0.026	0.031
Muscle	0.007	0.009
Fat	0.083	0.065
Liver	0.317	0.474

The extractability of all matrices by organic solvents is presented in Table B.7.2-16.

The aqueous extracts of fat were 3.3 % and 5.2 % of TRR (or 0.003 mg/kg) and of liver 8.7 % and 5.8 % of TRR (or 0.027 and 0.028 mg/kg) respectively for the two labels and were no further analysed.

The non-extractable liver residues of both labels were treated by pronase. 15.1% TRR (0.048 mg/kg) and 20.7% TRR (0.098 mg/kg) were solubilised for the chlorophenyl label and the tolyl label, respectively. Further purification of the released fraction by partition chromatography preceeded HPLC analysis.

Table B.7.2-16: Extractability of eggs and tissues of laying hens after dosing with ^{14}C pyraclostrobin at 12 mg/kg (nominal dose)

Matrix	Animal group A (*Chlorophenyl label*)			
	Organosoluble extract		Non-extractable residue	
	mg/kg	%TRR	mg/kg	%TRR
Eggs	(1) 0.017	66.2	0.010	38.5
Fat	(1) 0.057	69.4		
	(2) 0.022	26.7	0.002	1.8
Liver	(1) 0.136	42.8	0.136	42.7
Matrix	Animal Group B *(Tolyl label*)			
	Organosoluble extract		Non-extractable residue	
	mg/kg	%TRR	mg/kg	%TRR
Eggs	(1) 0.017	54.0	0.014	46.0
Fat	(1) 0.049	76.1		
	(2) 0.009	14.2	0.002	2.8
Liver	0.218	46.0	0.225	47.5

acetonitrile
n-hexane

Identification, characterisation and quantification of extractable radioactivity:

The acetonitrile phases obtained from eggs, fat and liver were analysed by HPLC for metabolite identities and quantities. A summary of all identified metabolites and their distribution in liver, fat and eggs is given in Table B.7.2-17.

Excreta have also been extracted and analysed by HPLC for subsequent isolation and identification of metabolites as reference substances. These reference substances were used for identification of metabolites by means of cochromatography with extracts of the edible tissues that showed much lower residue levels.
Metabolite isolates of excreta were all identified by mass spectrometry. The chemical structures of excreta metabolites 500M64, 500M66 and 500M77 were further specified by NMR.

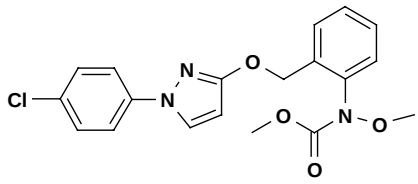
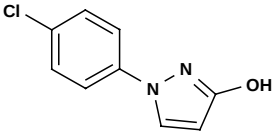
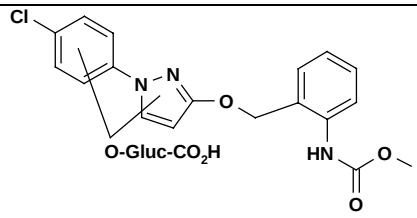
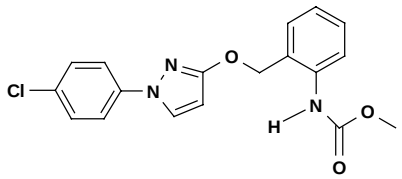
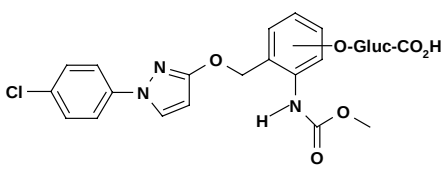
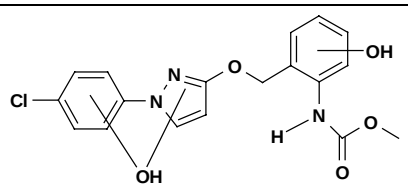
Liver:The major metabolite was the glucuronic acid conjugate 500M32 bound to the tolyl ring of the demethoxylated parent structure. It accounted for 10.9% TRR or 0.035 mg/kg in case of the chlorophenyl label and for 13.1% TRR or 0.062 mg/kg in case of the tolyl label. The parent compound could not be detected; the occurance of the metabolites 500M04 and 500M49 indicates the cleavage of the methylene ether brigde.

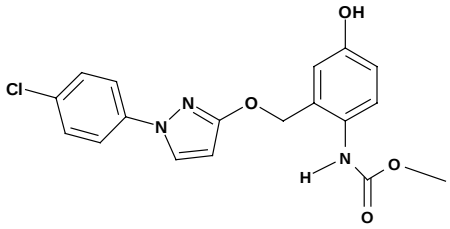
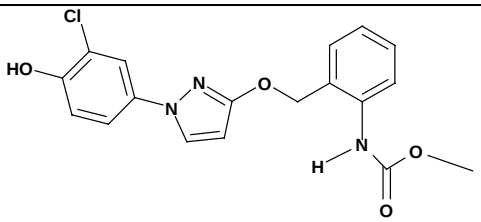
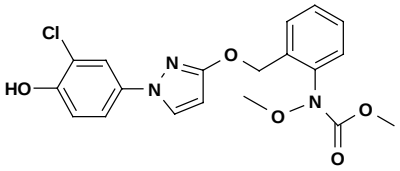
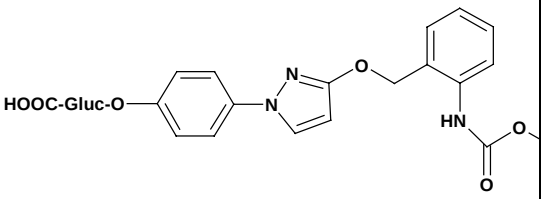
Fat: Fat contained as major residue the parent compound and its demethoxylated metabolite 500M07 (BF 500-3). Quantities of parent were 10.2% TRR or 0.008 mg/kg for the chlorophenyl label and 15.2% TRR or 0.010 mg/kg for the tolyl label. Metabolite 500M07 (BF 500-3) in fat accounted for 27.3% TRR or 0.022 mg/kg in case of the chlorophenyl label and for 38.9% TRR or 0.025 mg/kg in case of the tolyl label. Metabolite 500M64 accounted for 10.8 % and 7.8 % in the chlorophenyl and tolyl label respetively.

Eggs : The overall very low residues in eggs (0.03 mg/kg) mainly consisted of parent and its demethoxylated derivative 500M07 (BF 500-3). Parent and its demethylated compound accounted for 8.8% TRR or 0.002 mg/kg and for 11.2% TRR or 0.003 mg/kg, respectively, in case of the chlorophenyl label. In case of the tolyl label, parent and metabolite 500M07 (BF 500-3) accounted for 8.5% TRR or 0.003 mg/kg and for 8.3% TRR or 0.003 mg/kg, respectively.
One HPLC peak (15 % of TRR or 0.004 mg/kg)) was assigned to an unspecific glucuronic acid conjugate.

The other metabolites identified by mass spectrometry, were detected in edible tissues at levels below 0.0005 mg/kg up to 0.036 mg/kg related to TRR values of mainly less than 5% (Table B.7.2-17).

Table B.7.2-17: Summary of identified and quantified metabolites in edible matrices of laying hens after dosing with ^{14}C-pyraclostrobin *(chlorophenyl label)* (nominal dose level: 12 mg/kg based on feed intake)

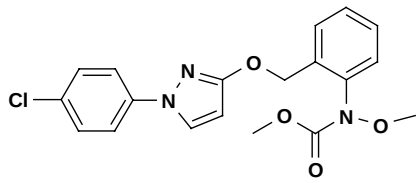
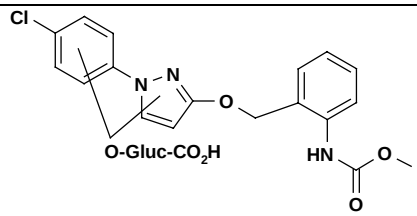
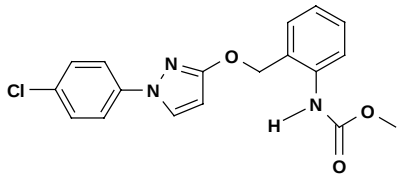
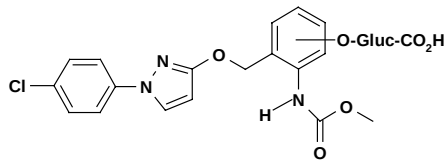
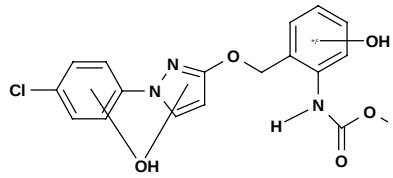
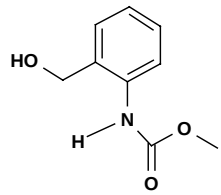
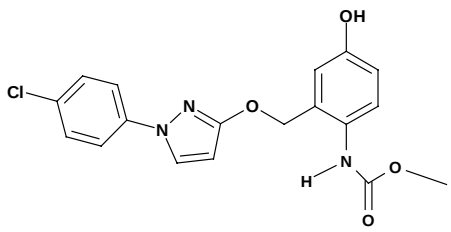
Metabolite code	Structure	Liver mg/kg (%TRR)	Fat mg/kg (%TRR)	Eggs mg/kg (%TRR)
Pyraclo-strobin		n.d.	0.008 (10.2)	0.002 (8.8)
500M04		0.004 (1.4)	0.002 (2.7)	0.001 (3.1)
500M06		0.013 (4.1)	n.d.	n.d.
500M07 (BF 500-3)		n.d.	0.022 (27.3)	0.003 (11.2)
500M32		0.035 (10.9)	n.d.	n.d.
500M39		0.003 (1.0)	n.d.	n.d.

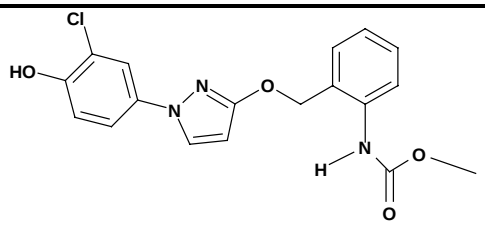
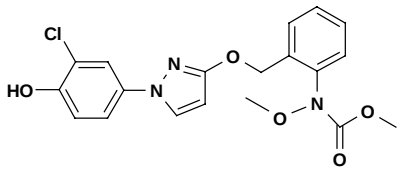
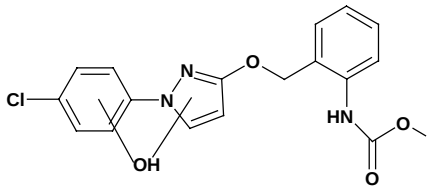
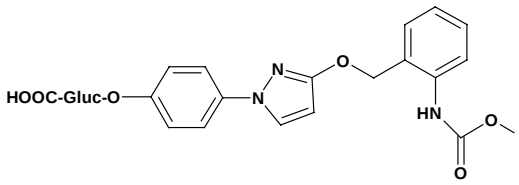
Metabolite code	Structure	Liver mg/kg (%TRR)	Fat mg/kg (%TRR)	Eggs mg/kg (%TRR)
500M64		0.009 (2.8)	0.009 (10.8)	0.001 (2.6)
500M66		0.012 (3.8)	n.d.	n.d.
500M77		0.006 (1.8)	0.001 (1.8)	n.d.
500M83		0.014 (4.5)	n.d.	n.d.
	Total identified in acetonitrile phase	0.096 (30.3)	0.042 (52.8)	0.007 (25.7)

n.d. not detected

Characterised residues in the acetonitrile phase:
Liver: 12.4 % of TRR or 0.038 mg/kg; 13 minor unknown metabolites in the range of 0.3 - 2.7 % of TRR or 0.001 - 0.008 mg/kg.
Fat: 16.6 % of TRR or 0.014 mg/kg; 8 minor unknown metabolites in the range of 0.8 - 3.9 % of TRR or 0.001 - 0.003 mg/kg.
Eggs: 36.5 % of TRR or 0.01 mg/kg; 7 minor unknown metabolites in the range of 0.9 - 15.2 % of TRR or < 0.001 - 0.004 mg/kg.

Table B.7.2-18: Summary of identified and quantified metabolites in edible matrices of laying hens after dosing with ^{14}C-pyraclostrobin (*tolyl label)* (nominal dose level: 12 mg/kg based on feed intake)

Metabolite code	Structure	Liver mg/kg (%TRR)	Fat mg/kg (%TRR)	Eggs mg/kg (%TRR)
Pyraclo-strobin		-	0.010 (15.2)	0.003 (8.5)
500M06		0.024 (5.0)	-	0.001 (2.6)
500M07 (BF 500-3)		-	0.025 (38.9)	0.003 (8.3)
500M32		0.062 (13.1)	-	-
500M39		0.002 (0.4)	n.d.	0.000 (1.3)
500M49		0.036 (7.5)	0.001 (1.7)	0.000 (0.7)
500M64		0.034 (7.3)	0.005 (7.8)	0.001 (1.9)

Metabolite code	Structure	Liver mg/kg (%TRR)	Fat mg/kg (%TRR)	Eggs mg/kg (%TRR)
500M66		0.009 (1.9)	n.d.	n.d.
500M77		0.009 (1.9)	0.001 (2.3)	0.000 (0.2)
500M80		0.003 (0.6)	n.d.	n.d.
500M83		0.021 (4.2)	n.d.	n.d.
	Total identified in the acetonitrile phase	0.2 (41.9)	0.042 (65.9)	0.008 (23.6)

n.d. not detected

Characterised residues in the acetonitrile phase:
Liver: 6 % of TRR or 0.029 mg/kg; 4 minor unknown metabolites in the range of 0.6 - 3.7 % of TRR or 0.004 - 0.018 mg/kg.
Fat: 10.2 % of TRR or 0.005 mg/kg; 4 minor unknown metabolites in the range of 1.8 - 3.8 % of TRR or 0.001 - 0.002 mg/kg.
Eggs: 27 % of TRR or 0.008 mg/kg ;11 minor unknown metabolites in the range of 0.5 - 8.4 % of TRR or < 0.001 - 0.003 mg/kg.

Characterisation of non-extrable radioactivity :
The non-extrable residues in liver were about 43 % of TRR and 48 % of TRR in the two labels respectively. Part of the non-extractable residues could be solubilized by pronase digestion: about 35 % (chlorophenyl label) and 41 % (tolyl label) was released suggesting that a considerable part was protein bound.
By chromatographic comparison, metabolites 500M04 and 500M64 were identified.

Acid hydrolysis of the non-extractable residue of liver under rigorous conditions released about 76 % of the bound radioactivity (chlorophenyl label). The hydrolysis products were

analysed by HPLC and one of the components identified by co-chromatography with BF 500-9 as 1-(4-hydroxy-3-chlorophenyl)-3-hydroxypyrazol.

No further attempt was made to characterise non-extractable radioactivity in other matrices because of the low concentration.

Storage stability:
Approximately 21 month (excreta, eggs) and about 7 month (liver) after the initial extractions, new subsamples of excreta, eggs and liver homogenate were extracted and analysed in the same manner as the original analyses. The extractability was very similar. Therefore, it was concluded that no binding of residues to the matrix took place upon storage in the freezer for about 0.5 - 1 month and 21 - 23 month.
The methanol extracts (excreta) and acetonitrile phases (eggs, liver) of the stored samples were analysed by HPLC. The chromatograms for excreta showed no significant differences. The chromatograms of eggs from the chlorophenyl label showed to be similar in the general pattern, but there were some differences in the percentage of relative area. Such differences were not observed for the tolyl label. Chromatographic pattern of liver were comparable.
It can be concluded, that the analysed residues were stable upon the storage period in this study.

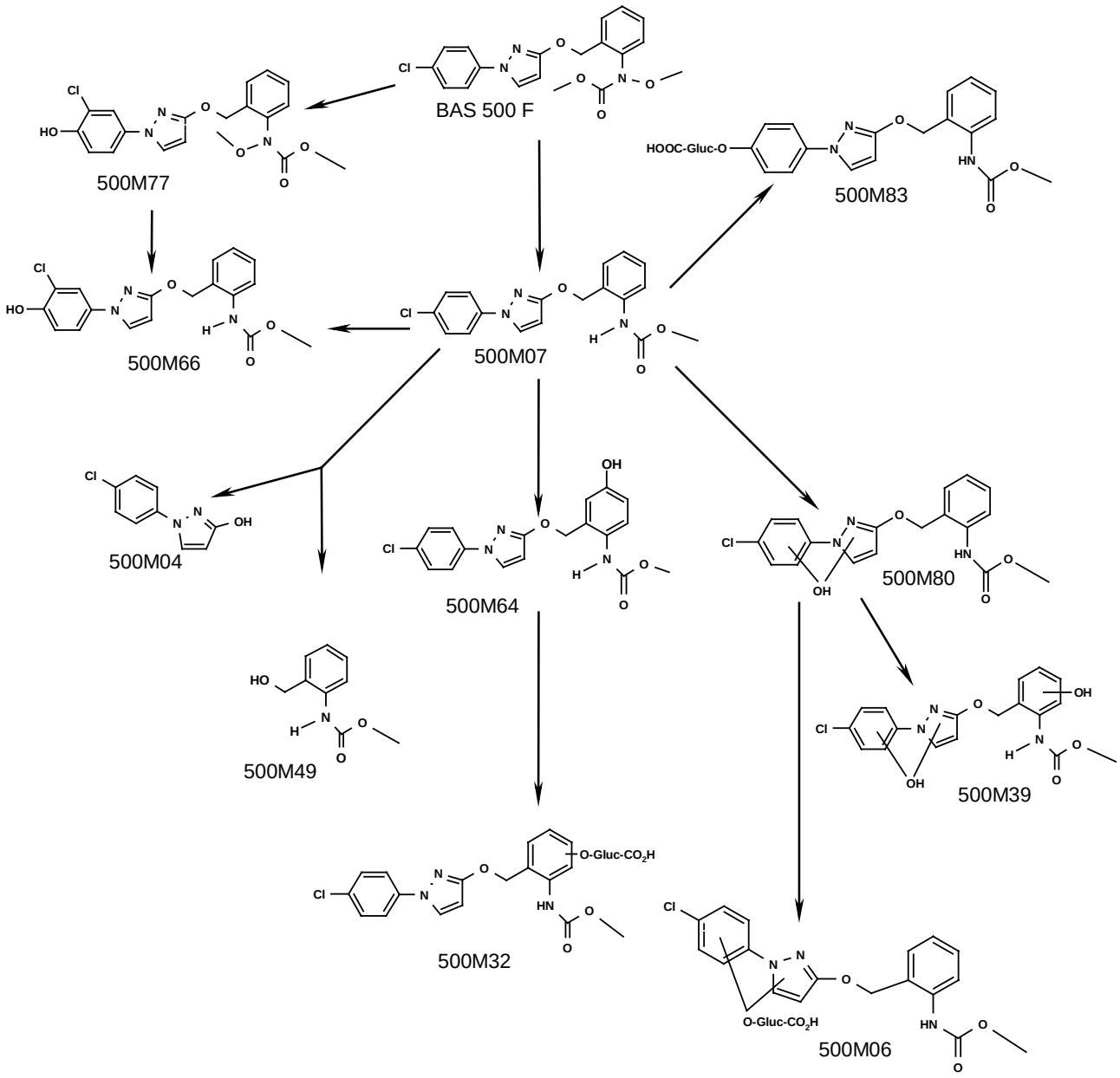
Conclusion:
Five routes of biotransformation were detected.
(1) the predominant transformation was the demethoxylation step.
(2) the demethoxylated metabolite was oxygenated at the tolyl ring followed by conjugation with glucuronic acid.
(3) the demethoxylated metabolite was hydroxylated at the chlorophenyl ring or at the pyrazol ring again followed by a conjugation reaction with glucuronic acid.
(4) the parent compound was hydroxylated at the chlorophenyl ring in para-position whereby the Cl-atom was shifted to the meta-position (NIH-shift).
(5) the parent compound was cleaved at the methylene ether bridge.

A specific variation was the substitution of the Cl-atom by glucuronic acid.

A proposed metabolic pathway for pyraclostrobin in laying hens is given in Figure B.7.2-2.

Tissues and eggs of hens that received an exaggerated dose of actually 12 mg/kg feed of [chlorophenyl-^{14}C]pyraclostrobin and of 13 mg/kg [tolyl-^{14}C]pyraclostrobin contained residues at low levels consisting of three major metabolites. The parent compound was found in fat and eggs but not in liver.
Four minor metabolites were detected in edible matrices of laying hens (500M66, 500M77, 500M80 and 500M83) which were not reported as metabolites in the rat. There are no toxicological studies availabe for these metabolites. Due to the very low concentration of these metabolites , no further toxicological studies seems to be necessary.

Figure B.7.2-2: Metabolic pathway of ^{14}C-pyraclostrobin in laying hens



B.7.2.3 Pigs

No metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly.

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

B.7.3.1 Plant matrices

Three metabolism studies were performed in three crop categories

fruits	grapes
root and tuber vegetables	potatoes
cereals	wheat

The metabolic pathways in these studies are comparable. The residue level in potato tubers and cereal grain is very low. In the other matrices there are only two compounds found in significant amounts (> 10 % TRR, > 0.05 mg/kg):

- Pyraclostrobin (BAS 500 F) - Grape fruits, potato green matter, wheat forage, wheat straw

Desmethoxy metabolite BF 500-3 (500M07) -Grape fruits, potato green matter, wheat forage, wheat straw

Both compounds formed the major part of the residue in most of the plant samples under investigation. Due to these results of the metabolism studies, the analytical methods developed for plant read both compounds, the desmethoxy metabolite 500M07 (synonym BF 500-3) and pyraclostrobin (BAS 500 F.)

In the course of the analysis of the residue samples from supervised trials, it turned out that the desmethoxy metabolite BF 500-3 appeared in small amounts compared to parent BAS 500 F. Furthermore BF 500-3 (500M07) is a main metabolite in the animal metabolism.
Therefore the desmethoxy metabolite BF 500-3 (500M07) should **not** be included into the definition of the relevant residue.

Residue definition for plants:

Parent Pyraclostobin (BAS 500 F)

B.7.3.2 Animal matrices

Metabolism studies were performed on lactating goats and laying hens. Pyraclostrobin (BAS 500 F) is intensively metabolised.
Goat:
The parent compound was the main residue in nearly all edible matrices of goat.

Laying hen:
In edible matrices of laying hens the parent compound was found in eggs and fat apart from the metabolite BF 500-3 (500M07), that accounted for about 30 - 40 % of the extractable residue in fat. The parent compound was not detected in hen liver.
Due to the low residue levels in edible matrices of hens in relation to the feeding level, no detectable residues have to be expected in practice.

Therefore, **the parent compound BAS 500 F (Pyraclostrobin)** is proposed as residue definition **for <u>monitoring</u> purposes**.

For <u>risk assesment</u> purposes, the following residue definition for **liver** (**except poultry liver**) and **milk fat** only is proposed :
BAS 500 F (Pyraclostrobin) and its metabolites analysed as the hydroxy pyrazoles BF 500-5 and BF 500-8, sum expressed as BAS 500 F (Pyraclostrobin)

B.7.4 Use pattern

The use pyraclostrobin containing products is intended in grapes and cereals in the northern and southern region of Europe. Other uses in vegetable and fruit crops are planned with the formulation BAS 516 00 F but for the moment they are not supported by the available residue data. Information to the different intended formulations is given in Table B.7.4-1
Details to the uses intended in Europe are summarised in Table B.7.4-2

Table B.7.4-1: Pyraclostrobin containing formulations

BAS-code	Content of BAS 500 F [kg/L]	Formulation type	Other active substance [kg/L]
BAS 500 00 F	0.250	EC	
BAS 500 01 F	0.250	EC	
BAS 512 00 F	0.133	SE	epoxiconazole, 0.050
BAS 513 00 F	0.133	SE	epoxiconazole, 0.050 kresoxim-methyl,0.067
BAS 516 00 F	0.067	WG	new active, 0.267
BAS 518 00 F	0.050	WG	metiram, 0.550

Table B.7.4-2: Uses intended in Europe

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grapes	France	BAS 500 00 F	F	p+d mildew	EC	250	row, SP	09-85	3	12	0.01	1000	0.100	35	
Grapes	Germany	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	11-81	3	12	0.01	400-1600	0.04-0.16	35	
Grapes	Italy	BAS 500 00 F	F	p+d mildew	EC	250	row, SP	60-80	3	12	0.01	1000	0.100	35	
Grapes	Portugal	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	16-71	3	12	0.01	1000	0.100	35	
Grapes	Spain	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	65-81	3	12	0.01	1000	0.100	35	
Turf	all EU MS	BAS 500 00 F	F	mold	EC	250	overall,SP		2	14	0.025-0.05	500-1000	0.250		
Cereals	France	BAS 500 01 F	F	leaf diseases	EC	250	overall,SP	31-65	2	appr. 20	0.100	250	0.250	30	
Cereals	Ireland	BAS 500 01 F	F	leaf diseases	EC	250	overall,SP	from GS12	2	appr. 20	max. 0.125	min. 200	0.250	35	
Cereals	United Kingdom	BAS 500 01 F	F	leaf diseases	EC	250	overall,SP	from GS12	2	appr. 20	max. 0.125	min. 200	0.250	35	
Cereals	Belgium	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	31-59	1-2	appr. 20	0.100	200	0.200	30	
Cereals	Germany	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	25-69	2	appr. 20	0.058	400	0.233	35	
Cereals	France	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	31-65	2	appr. 20	0.080	250	0.200	30	
Cereals	Ireland	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	from GS12	2	appr. 20	max. 0.1	min. 200	0.200	35	
Cereals	Nether-lands	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	30-59	1-2	appr. 20	0.080	250	0.200	35	
Cereals	United Kingdom	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	from GS12	2	appr. 20	max. 0.1	min. 200	0.200	35	
Cereals	Belgium	BAS 513 00 F	F	leaf diseases	SE	133	overall,SP	31-59	1-2	appr. 20	0.100	200	0.200	30	
Cereals	Germany	BAS 513 00 F	F	leaf diseases	SE	133	overall,SP	25-69	2	appr. 20	0.058	400	0.233	35	
Cereals	France	BAS 513 00 F	F	leaf diseases	SE	133	overall,SP	31-65	2	appr. 20	0.080	250	0.200	30	
Cereals	Ireland	BAS 513 00 F	F	leaf diseases	SE	133	overall,SP	from GS12	2	appr. 20	max. 0.1	min. 200	0.200	35	
Cereals	Nether-lands	BAS 513 00 F	F	leaf diseases	SE	133	overall,SP	30-59	1-2	appr. 20	0.080	250	0.200	35	
Cereals	United Kingdom	BAS 513 00 F	F	leaf diseases	SE	133	overall,SP	from GS12	2	appr. 20	max. 0.1	min. 200	0.200	35	
Grapes	Austria	BAS 518 00 F	F	p+d mildew	WG	50	row,SP	11-81	3	12	0.01	400-1600	0.04-0.16	35	
Grapes	Germany	BAS 518 00 F	F	p+d mildew	WG	50	row,SP	11-81	3	12	0.01	400-1600	0.04-0.16	35	
Grapes	Spain	BAS 518 00 F	F	p+d mildew	WG	50	row,SP	65-81	3	12	0.01	1000	0.100	35	
Grapes	Fance	BAS 518 00 F	F	p+d mildew	WG	50	row,SP		3	12	0.01	1000	0.100	35	
Grapes	Italy	BAS 518 00 F	F	p+d mildew	WG	50	row,SP	60-80	3	12	0.01	1000	0.100	35	

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.7.5 Identification of critical GAPs

From the intended uses the most critical ones are selected in Table B.7.5-1.

Table B.7.5-1: List of identified critical uses

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grapes - North Europe –															
Grapes	Germany	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	11-81	3	12	0.01	400-1600	0.04-0.16	35	
Grapes -South Europe –															
Grapes	Spain	BAS 500 00 F	F	p+d mildew	EC	250	row,SP	65-81	3	12	0.01	1000	0.100	35	
Cereals - North Europe –															
Cereals	Germany	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	25-69	2	appr. 20	0.058	400	0.233	35	
Cereals - South Europe –															
Cereals	France	BAS 512 00 F	F	leaf diseases	SE	133	overall,SP	31-65	2	appr. 20	0.080	250	0.200	30	

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

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

B.7.6.1 Analytical method used in the residue trials

The samples were analysed with the BASF Analytical Method Number 421/0 (Reinhard and Mackenroth, 1999, MET 2000-273; Perez and Perez, 2000, MET 2000-274) which is based on the determination of pyraclostrobin and its metabolite BF 500-3 using LC/MS/MS. For details see chapter B.5.
All recoveries (fortification level 0.02 ,1.0, 5.0 mg/kg) reported in the residue studies were acceptable (No results outside the range 70 – 110 %).

B.7.6.2 Storage stability

Report: Abdel-Baky, RIP 2000-2043

The deep freeze stability of pyraclostrobin (BAS 500 F) and its metabolite BF 500-3 in various plant matrices was investigated over a period of 19 month. Untreated samples were fortified with 1.0 mg/kg pyraclostrobin (BAS 500 F) and its metabolite BF 500-3. The samples were stored under the usual storage conditions for field samples (polyethylene bottle, -20°C). The samples were analysed with BASF Analytical Method Number 421/0 (D9808).

Findings:
Samples were taken and analysed after 1, 3, 6, 14 and 18 (19) months frozen storage (<-10°C). The results of these sampling dates are given in Table B.7.6-1. The results show that pyraclostrobin (BAS 500 F) and its metabolite BF 500-3 are stable in various plant matrices under storage conditions at least over a period of 18 (19) month.

Table B.7.6-1: Storage stability

Matrix	Average Relative Recovery[1] (%)					
	0-Month	1-Month	3-Month	6-Month	14-Month	18 Month [2]
Pyraclostrobin (BAS 500 F)						
Peanut Nutmeat	92	96	90	91	95	88
Peanut Oil	105	92	102	118	101	106
Wheat Grain	105	92	87	91	82	88
Wheat Straw	93	96	96	113	71	99
Sugarbeet Tops	103	100	97	100	91	98
Sugarbeet Roots	92	94	92	96	78	91
Tomatoes	104	98	96	90	91	96
Grape Juice	91	96	94	96	69	88

Matrix	Average Relative Recovery[1] (%)					
	0-Month	1-Month	3-Month	6-Month	14-Month	18 Month [2]
BF 500-3						
Peanut Nutmeat	94	96	112	92	92	84
Peanut Oil	104	92	102	122	103	120
Wheat Grain	101	90	86	86	79	89
Wheat Straw	90	91	99	104	63	97
Sugarbeet Tops	103	101	99	99	93	99
Sugarbeet Roots	87	97	97	94	78	91
Tomatoes	103	98	97	85	85	92
Grape Juice	92	95	92	94	80	93

[1] $\text{Relative Recovery} = \frac{\text{Average Stored Recovery}}{\text{Average Procedural Recovery}} \times 100$

[2] peanut nutmeat and oil: 19 month

B.7.6.3 Residues in grapes

Reports: Meumann, 1999, RIP 2000-1027; Meumann, 1999, RIP 2000-1028; Schulz, 1999, RIP 2000-1029

In grapes, a total of 16 trials was conducted in representative growing areas in Germany, Spain, France and Italy in the years 1997 and 1998 (8 each in Northern and Southern regions of EU). In all trials, the formulation BAS 500 00 F was applied eight times with increasing application rates from 0.24 to 0.64 l/ha which corresponds to 0.06 to 0.16 kg as/ha. By using this dosage the application rates were adopted to the growthstage of the plants.

In all trials, fruit samples were taken directly after the last application (0 DALA) as well as about 5, 6, 7 and 8 weeks thereafter.
The samples were analysed with BASF method no. 421/0 which quantifies the parent compound pyraclostrobin (BAS 500 F) and its metabolite BF 500-3. The limit of quantitation is 0.02 mg/kg each in all sample materials.

The overall average results of the procedural recovery experiments obtained with each analytical series were about 91 % for pyraclostrobin (BAS 500 F) and about 88 % for BF 500-3. Fortification levels were between 0.02 mg/kg and 5.0 mg/kg.

Findings
The results of the residue trials in grapes are summarised for North Europe in Table B.7.6-2 and for South Europe in Table B.7.6-3 .

One result obtained from the trials in grapes is that the residue found consists mainly of the parent compound pyraclostrobin (BAS 500 F). Adding up all the total residue values to a theoretic overall sum, the parent compound contributes to 92 % whereas the metabolite only amounts to 8 % of this sum. Therefore, for a more detailed discussion of the results, it is sufficient to look at the residues of the parent compound.

Within the examined range between 35 and 56 days there is no indication for a significant degradation of the residue. (Directly after the last application, the residues of pyraclostrobin

(BAS 500 F) ranged between 0.42 and 2.01 mg/kg. At the second sampling after about five weeks which is the intended pre-harvest interval, the residues of pyraclostrobin (BAS 500 F) were between 0.17 and 0.75 mg/kg. After about six weeks, pyraclostrobin (BAS 500 F) was found between < 0.02 and 0.84 mg/kg, after 7 weeks it ranged between 0.11 and 0.89 mg/kg.) Therefore, the highest value between 35 and 56 days is used for MRL calculation.

North EU

Critical GAP:

0.04-0.16 kg as/ha (0.01 kg as/hl), 3 applications, growth stage at last treatment: BBCH 81, PHI 35 days

Supporting residue data:

8 Trials with formulation BAS 500 00 F 1997 and 1998 to grapes.
fruit, PHI 35 – 56 days: 0.19, 0.25, 0.48, 0.57, 0.78, 0.82, 0.84, 0.89

MRL calculation

R_{max}:	1.48
R_{ber} (2 x R0.75):	1.67
STMR:	0.68

South EU

Critical GAP:

0.1 kg as/ha (0.01 kg as/hl), 3 applications, growth stage at last treatment: BBCH 81, PHI 35 days

Supporting residue data:

8 Trials with formulation BAS 500 00 F 1997 and 1998 to grapes.
fruit, PHI 35 – 56 days: 0.18, 0.20, 0.21, 0.34, 0.38, 0.48, 0.59, 0.72

MRL calculation

R_{max}:	1.02
R_{ber} (2 x R0.75):	1.13
STMR:	0.36

<u>MRL proposal</u>

The data presented for the intended uses in grapes is sufficient to set an MRL. Due to the higher residues in the trials from nothern Europe the MRL proposal is calculated on the basis of these results.

MRL proposal grapes: 2 mg/kg

Table B.7.6-2: Trials from northern Europe

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	: 250 g/l	Active ingredient	:	Pyraclostrobin
Formulation	(e.g. WP)	: EC	Crop / crop group	:	Grapes
Commercial product	(name)	: Pyraclostrobin (BASF 500 00 F)	Indoors / outdoors	:	Outdoor
Applicant		: BASF AG (BAS)	Other a. i. in formulation (common name and content)	:	--
			Residues calculated as	:	Pyraclostrobin Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 00 F	BF 500-3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/10980 DU2/04/97 Germany-69168 Wiesloch 00-01-31	Riesling	1) 1985 2) 20.06.-30.06.97 3) 15.10.97	0.057 0.059 0.058 0.059 0.102 0.121 0.159 0.158	286 298 294 297 513 613 800 797	0.019	10.06.97 24.06. 04.07. 16.07. 28.07. 08.08. 20.08. 02.09.	81	grapes	0.72 0.67 0.81 <u>0.82</u> 0.47	0.03 0.07 0.08 0.06 0.03	0.75 0.74 0.89 0.88 0.50	0 35 42 49 56	RIP 2000-1027
#BASF 99/10980 DU3/02/97 Germany-67269 Grünstadt 00-01-31	Riesling	1) 1937 2) 12.06.-27.06.97 3) 21.10.97	0.055 0.068 0.066 0066 0.103 0.114 0.162 0.161	276 345 330 333 517 575 819 813	0.019	12.06.97 24.06. 07.07. 18.07. 30.07. 11.08. 22.08. 04.09.	81	grapes	0.99 0.40 0.43 <u>0.48</u> 0.46	0.04 0.04 0.04 0.05 0.04	1.03 0.44 0.46 0.53 0.49	0 35 42 49 56	RIP 2000-1027

1	2	3	4			5	6	7	8			9	10
#BASF 99/10980 DU3/05/97 Germany-67269 Grünstadt 00-01-31	Portugieser	1) 1987 2) 09.06.- 27.06.97 3) 17.09.97	0.065 0.062 0.063 0.062 0.101 0.123 0.160 0.184	327 313 316 311 511 622 809 927	0.019	22.05.97 03.06. 16.06. 26.06. 07.07. 21.07. 01.08. 14.08.	79	grapes	0.88 <u>0.25</u> <0.02 0.24 0.23	0.04 0.02 <0.02 <0.02 0.02	0.92 0.27 <0.04 0.26 0.25	0 35 42 49 56	RIP 2000- 1027
#BASF 99/10980 FR4/04/97 France-21420 (North-France) Pernand- Vergelesse 00-01-31	Pinot Noir	1) 1978 2) 05.06.- 18.06.97 3) 22.09.97	0.055 0.056 0.060 0.063 0.096 0.119 0.154 0.161	278 281 307 319 481 602 581 607	0.019	15.05.97 27.05. 05.06. 18.06. 01.07. 24.07. 05.08.	79-81	grapes	0.84 0.41 <u>0.78</u> 0.28 0.32	0.04 0.03 0.07 0.03 0.02	0.88 0.44 0.85 0.31 0.34	0 35 42 49 56	RIP 2000- 1027
#BASF 99/10981 AGR/03/98 Germany-53474 Bad Neuenahr- Ahrweiler 00-01-31	Dornfelder	1) 1993 2) 18.06.- 06.07.98 3) 30.09.98	0.058 0.064 0.060 0.059 0.099 0.122 0.159 0.155	294 325 304 298 504 615 802 784	0.019	02.06.98 15.06. 24.06. 08.07. 21.07. 30.07. 13.08. 25.08.	81	grapes	2.01 0.44 0.54 <u>0.57</u> 0.55	0.12 0.08 0.11 0.09 0.09	2.13 0.52 0.65 0.66 0.64	0 35 42 49 56	RIP 2000- 1028
#BASF 99/10981 DU2/07/98 Germany-69168 Wiesloch 00-01-31	Spätburgunder	1) 19983 2) 04.06.- 25.06.98 3) 06.10.98	0.060 0.059 0.059 0.059 0.100 0.119 0.163 0.158	305 302 302 300 506 602 824 798	0.019	09.06.98 22.06. 03.07. 13.07. 27.07. 07.08. 20.08. 01.09	83	grapes	0.68 0.75 <u>0.84</u> 0.74 0.61	0.06 0.12 0.11 0.09 0.07	0.74 0.87 0.95 0.83 0.68	0 35 42 49 56	RIP 2000- 1028

1	2	3	4			5	6	7	8			9	10
#BASF 99/10981 DU3/05/98 Germany-67269 Grünstadt 00-01-31	Riesling	1) 1984 2) 15.06.- 25.06.98 3)17.10.98	0.060 0.065 0.066 0.062 0.100 0.121 0.163 0.156	301 329 332 313 506 614 823 785	0.019	16.06.98 29.06. 10.07. 22.07. 05.08. 14.08. 27.08. 08.09.	83	grapes	0.75 0.64 0.58 <u>0.89</u> 0.77	0.04 0.08 0.06 0.08 0.07	0.80 0.72 0.64 0.97 0.84	0 35 42 49 56	RIP 2000- 1028
#BASF 99/10981 FR4/01/98 France-21420 (North-France) Pernand Vergelesses 00-01-31	Pinot Noir	1) 1974 2) 08.06.- 19.06.98 3) 25.09.98	0.057 0.042 0.061 0.060 0.099 0.119 0.159 0.161	288 212 309 306 503 601 602 611	0.019	14.05.98 26.05. 08.06. 19.06. 01.07. 09.07. 23.07. 06.08.	81	grapes	0.60 0.17 0.18 0.18 <u>0.19</u>	0.04 0.03 0.03 0.03 0.03	0.64 0.20 0.21 0.21 0.22	0 35 42 48 55	RIP 2000- 1028

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-3: Trials from southern Europe

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 250 g/l
Formulation (e.g. WP) : EC
Commercial product (name) : Pyraclostrobin (BASF 500 00 F)
Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Grapes
Indoors / outdoors : Outdoor
Other a. i. in formulation (common name and content) : --
Residues calculated as : Pyraclostrobin
Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)			PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl				BAS 500 00 F	BF 500-3	TOTAL		
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/10980 FR3/03/97 France-30350 Aigremont (Southern-France) 00-01-31	Carignan	1) 1973 2) 19.05.- 31.05.97 3) 17.09.97	0.057 0.061 0.059 0.060 0.103 0.121 0.161 0.165	290 309 302 305 519 609 814 831	0.019	19.05.97 31.05. 12.06. 24.06. 05.07. 18.07. 30.07. 12.08.	81	grapes	0.42 0.20 0.15 0.19 <u>0.21</u>	0.02 <002 <0.02 <0.02 <0.02	0.44 0.22 0.17 0.21 0.21	0 35 42 49 56	RIP 2000-1027
#BASF 99/10980 FR4/05/97 France-31620 Froton (Southern-France) 00-01-31	Cabernet France/ 420 A	1) 1978 2) 16.05.- 28.06.97 3) 27.09.97	0.053 0.061 0.064 0.062 0.101 0.113 0.154 0.155	268 307 323 314 509 570 777 782	0.019	21.05.97 03.06. 13.06. 25.06. 08.07. 18.07. 31.07. 13.08.	83	grapes	1.35 <u>0.72</u> 0.55 0.58 0.50	0.07 0.07 0.05 0.05 0.05	1.42 0.79 0.60 0.63 0.55	0 35 42 49 56	RIP 2000-1027

1	2	3	4			5	6	7	8			9	10
#BASF 99/10981 AC/05/98 Spain-11500 Jerez de la Frontera 00-01-31	Palomino fino	1).1967 2) 10.05.- 05.06.98 3) 17.09.98	0.058 0.057 0.060 0.064 0.102 0.124 0.163 0.150	292 289 303 322 515 628 821 758	0.019	05.05.98 18.05. 29.05. 10.06. 22.06. 03.07. 16.07. 28.07.	79-81	grapes	0.46 <u>0.20</u> 0.14 0.11 0.11	0.06 0.04 0.03 0.02 0.03	0.52 0.24 0.17 0.13 0.14	0 35 42 49 56	RIP 2000- 1028
#BASF 99/10981 AC/06/98 Spain-11402 Jerez de la Frontera 00-01-31	Palomino fino	1) 02.1962 2) 10.05- 05.06.98 3) 15.09.98	0.055 0.059 0.060 0.063 0.100 0.115 0.164 0.161	279 300 303 319 506 580 825 814	0.019	05.05.98 18.05. 29.05. 10.06. 22.06. 03.07. 16.07. 28.07.	79-81	grapes	0.62 0.17 0.15 <u>0.18</u> 0.13	0.09 0.05 0.04 0.04 0.02	0.71 0.22 0.19 0.22 0.15	0 35 42 49 56	RIP 2000- 1028
#BASF 99/10981 FR3/01/98 France-30350 Aigremont (South-France) 00-01-31	Carignan	1) 1974 2) 02.06.- 09.06.98 3) 25.09.98	0.060 0.060 0.059 0.058 0.100 0.121 0.163 0.161	303 302 299 292 507 612 821 814	0.019	19.05.98 30.05. 12.06. 23.06. 06.07. 17.07. 30.07. 11.08.	81	grapes	0.78 0.42 <u>0.59</u> 0.41 0.42	0.03 0.03 0.05 0.03 0.05	0.81 0.45 0.64 0.44 0.47	0 35 42 49 56	RIP 2000- 1028
#BASF 99/10981 FR8/01/98 France-31620 Fronton (South-France) 00-01-31	Cabernet France/ 420A	1) 1978 2) 05.06.- 20.06.98 3) 05.10.98	0.056 0.063 0.061 0.063 0.100 0.123 0.171 0.160	284 316 307 318 502 618 861 802	0.019	28.05.98 08.06. 18.06. 03.07. 15.07. 28.07. 06.07. 19.08.	83	grapes	0.47 0.23 <u>0.34</u> 0.32 0.26	0.05 <0.02 0.04 0.03 <0.02	0.52 0.25 0.38 0.35 0.28	0 35 42 48 55	RIP 2000- 1028

1	2	3	4			5	6	7	8			9	10
#BASF 99/11638	Sangiovese	1)1980	0.059	490	0.012	12.05.98	80	grapes	1.32	0.03	1.25	0	RIP 2000-
9802R		2)01.06.-	0.059	488	0.012	25.05.			<u>0.38</u>	0.03	0.41	34	1029
		12.06.98	0.062	619	0.01	05.06.			0.23	<0.02	0.25	41	
Italy-27020		3)04.09.98	0.059	590	0.01	17.06.			0.19	<0.02	0.21	48	
S. Giorgo di			0.102	714	0.014	29.06.			0.15	<0.02	0.17	55	
Cesena			0.121	811	0.015	10.07.							
			0.162	1011	0.016	20.07.							
00-01-31			0.169	1057	0.016	01.08.							
#BASF 99/11638	Trebbiano	1)1963	0.064	539	0.012	12.05.98	80	grapes	1.74	0.07	1.81	0	RIP 2000-
9803R		2)03.06.-	0.061	508	0.012	26.05.			<u>0.48</u>	0.04	0.52	34	1029
		15.06.98	0.060	600	0.016	05.06.			0.45	0.04	0.49	41	
Italy-40055		3)04.09.98	0.060	603	0.01	17.06.			0.45	0.04	0.49	48	
Castenaso-fraz.			0.100	703	0.014	30.06.			0.40	0.03	0.43	55	
Bagnarola			0.118	785	0.015	10.07.							
			0.162	1009	0.016	21.07.							
00-01-31			0.162	1014	0.016	01.08.							

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.6.4 Residues in cereals

Reports: Beck, 1999, RIP 2000-1031
Beck, Benz, Mackenroth, 1999, RIP 2000-1072
Meumann, Benz, Mackenroth., 1999, RIP 2000-1073

In cereals, a total of 56 trials was conducted in representative growing areas in Belgium, Germany, Denmark, Spain, France, the Netherlands and Sweden in the years 1998 and 1999. Different varieties of cereals such as spring and winter barley, spring and winter wheat and durum wheat were used.
The results are discussed separately for barley (B.7.6.4.1) and wheat (B.7.6.4.2).
According to the extrapolation guidance document (Doc. 7525/VI/95-rev.5, 20/10/1999) these results can be used for the evaluation of the residue situation on other cereals (except maize and rice) too.

The fungicidal product BAS 500 01 F (250 g/l pyraclostrobin (BAS 500 F), EC) was tested in 1998 and 1999 conducting a total of 30 trials out of which 19 were situated in Northern regions of EU and 11 in the South. It was applied according to the critical GAP (2 x 0.250 kg as/ha, PHI 35 days)

Additionally, bridging studies testing the formulations BAS 512 00 F (133 g/l pyraclostrobin (BAS 500 F) and 50 g/l epoxiconazole, SE) and BAS 513 00 F (133 g/l pyraclostrobin (BAS 500 F), 50 g/l epoxiconazole and 67 g/l kresoxim-methyl, SE) were performed in 1998 with 13 trials each (8 in the North, 5 in the South). It was applied according to the critical GAP (2 x 0.233 kg Pyraclostobin/ha, 0.088 kg epoxiconazole/ha and 0.117 kg kresoxim-methyl/ha PHI 35 days)

In all trials, samples of whole plant without roots were taken directly after the last application (0 DALA). Samples of ears and haulms were taken about three weeks after the last application. At the third sampling date which was at the proposed PHI of 35 days, different sampling material was taken depending on the ripening situation: In 26 trials, ears and haulms were taken whereas in 30 trials grain and straw were sampled. Further samples of grain and straw were taken about 6 weeks after the last application and in 13 trials also after about 7 weeks.
The samples were analysed with BASF method no. 421/0 which quantifies the parent compound pyraclostrobin (BAS 500 F) and its metabolite BF 500-3. The limit of quantitation is 0.02 mg/kg each in all sample materials.
The overall average results of procedural recovery experiments obtained with each analytical series were at about 87 % for pyraclostrobin (BAS 500 F) (n=95) and at about 85 % for BF 500-3 (n=93). Fortification levels were between 0.02 mg/kg and 2.0 mg/kg.

Findings
Comparable to the situation in grapes, the parent compound pyraclostrobin (BAS 500 F) was also the main residue found in cereal matrices. Here, it amounted to about 87 % of a theoretic overall sum of all total residues. Specifically looking at grain as the most important matrix, the parent portion was lower but it still came to about 70 % of the overall sum of total residues in grain above LOQ. Therefore, the detailed discussion of the residue situation in cereals will also focus on the parent results.

Table B.7.6-4: Comparison of the three different formulations

Formulation	Initial residue (after second application)
BAS 500 01 F	2.67 and 14.6 mg/kg
BAS 512 00 F	3.10 and 10.3 mg/kg
BAS 513 00 F	2.8 to 10.9 mg/kg

Table B.7.6-4 shows that there are no significant differences between the formulations used in the residue trials. The results from the trials with slightly different application rates (0.250 kg as/ha in BAS 500 01 F and 0.233 kg as/ha in BAS 512 00 F and BAS 513 00 F) are comparable.
Therefore, the residue situation at the later sampling days can be discussed together regardless the formulation used.

Residues in straw
After about five weeks, the residues found in haulms (27 trials) ranged between 0.23 and 2.43 mg/kg regardless the cereal species. The results from those trials (n=29) where straw was taken at that sampling day were considerably higher ranging between 0.54 and 6.09 mg/kg. This higher residue level can be explained by the lower water content in straw compared to haulms.

Kresoxim-methyl, Epoxiconazol
The residue situation of kresoxim-methyl was according to former experiences: In none of the ears or grain samples taken at the third sampling or later residues above the limit of quantitation were found. These residues are in line with the EU-evaluation of kresoxim-methyl and are covered by the EU-MRL of 0.05 mg/kg cereals (Codex MRL proposals: 0.05 mg/kg wheat, rye; 0.1 mg/kg barley)

In case of epoxiconazole, the results of the third sampling were dependant on the material taken. In case of grain, only one out of 12 samples showed a residue of 0.06 mg/kg, all others were below the limit of quantitation. If whole ears were taken, four samples out of 14 were < 0.05 mg/kg, the residues of the others ranged between 0.07 and 0.29 mg/kg. These residues are covered by the established German MRL of 0.2 mg/kg for cereal grain. There are no EU or Codex proposals up to now.

B.7.6.4.1 Barley

North EU

Critical GAP
0.233 kg as/ha, 2 applications, growth stage at last treatment: BBCH 69, PHI: 35 d

Supporting residue data
11 Trials with formulation BAS 500 01 F 1998 and 1999 with winter (7) and spring (4) barley.
grain, PHI 35-42 days: < 0.02, < 0.02, 0.03, 0.04, 0.04, 0.04, 0.05, 0.07, 0.07, 0.09, 0.29
straw, PHI 35-42 days: 0.78, 0.78, 0.82, 1.72, 1.81, 2.15, 2.23, 2.77, 3.83, 4.38, 5.68

Additional trails were conducted simultanously with two different formulations. As all other parameter like time, location, variety are identical for all three formulations the results show clearly that the residue level in barley is indipendent of the tested formulation.

5 trials with formulation BAS 512 00 F with winter (2) and spring (3) barley.
grain, PHI 35-42 days: < 0.02, 0.03, 0.03, 0.07, 0.12
straw, PHI 35-42 days: 0.99, 1.48, 2.04, 3.2, 6.01

5 trials with formulation BAS 513 00 F with winter (2) and spring (3) barley.
grain, PHI 35-42 days: < 0.02, 0.03, 0.05, 0.07, 0.1
straw, PHI 35-42 days: 0.52, 1.57, 2.94, 3.26, 4.56

MRL calculation

R_{max}:	0.20
R_{ber} (2 x $R_{0.75}$):	0.14
STMR:	0.04

South EU

Critical GAP

The only use in sothern Europe is intended in France.
0.250 kg as/ha, 2 applications, growth stage at last treatment: BBCH 65, PHI: 30 d

Supporting residue data

3 Trials with formulation 500 01 F in 1999 with winter barley.
grain, PHI 35-43 days: 0.02, 0.03, 0.05
straw, PHI 35-43 days: 3.91, 4.87, 6.09

MRL calculation

There are not enough results with barley to calculate a MRL for southern Europe.

MRL proposal

The data presented for northern Europe is sufficient to set a MRL.
Due to missing results from southern Europe a MRL for barley is calculated on the basis of northern trials only.

MRL proposal barley and oats: 0.2 mg/kg

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Table B.7.6-5: Trials from northern Europe - spring barley – (solo formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 250g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 500 01 F
Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Spring barley
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content)
Residues calculated as : Pyraclostrobin (BAS 500 F)
Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 AGR/05/98 Belgium-3470 Kortenaken 00-01-31	Riviera	1)25.03.98 2)18.06.-25.06.98 3)03.08.98	0.244 0.255	296 310	0.082	29.05.98 19.06.	60	Whole plants without roots ears haulms grain straw grain straw	 5.68 0.26 0.88 0.04 1.85 0.05 1.81	 0.12 0.10 0.10 0.03 0.39 0.02 0.42	 5.80 0.36 0.98 0.07 2.24 0.07 2.23	 0 20 20 34 34 40 40	RIP 2000-1031
#BASF 99/11509 DU2/05/98 Germany-67149 Meckenheim 00-01-31	Scarlett	1)18.03.98 2)08.06.-15.06.98 3)15.07.98	0.244 0.225	296 273	0.082	18.05.98 22.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 5.35 0.27 0.93 0.32 1.42 <0.02 2.23	 0.15 0.08 0.12 0.08 0.16 <0.02 0.52	 5.50 0.35 1.05 0.40 1.58 <0.04 2.75	 0 21 21 36 36 42 42	RIP 2000-1031

1	2	3	4			5	6	7	8			9	10
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 HUS/03/98 Sweden-23791 Bjärred Borgeby Gard 00-01-31	Scarlett	1)30.03.98 2)02.07.- 10.07.98 3)10./11.08. 98	0.250 0.245	303 297	0.083	02.06.98 25.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 5.99 0.11 0.46 0.06 0.53 <0.02 0.78	 0.14 0.05 0.07 0.04 0.09 <0.04 0.16	 6.13 0.16 0.53 0.10 0.62 <0.04 0.94	 0 22 22 35 35 42 42	RIP 2000- 1031
#BASF 99/11824 FR2/05/99 France-62580 (Nothern France) Neuville Saint Vaast 00-01-31	Scarlett	1)13.03.1999 2) 10.06.- 15.06.99 3)28.07.1999	0.247 0.249	300 302	0.082	28.05.1999 14.06.	69	pl. w/o roots ears haulms grain straw grain straw	6.46 0.12 0.98 0.04 1.72 0.03 0.64	0.14 0.13 0.13 0.03 0.31 0.03 0.10	6.60 0.25 1.11 0.07 2.03 0.06 0.74	0 21 21 35 35 43 43	RIP 2000- 1072
#BASF 99/11824 HUS/02/99 Sweden-23791 Bjärred 00-01-31	Scarlett	1) 02.04.1999 2)18.06.- 02.07.99 3) 08.08.1999	0.270 0.239	327 289	0.082	10.06.1999 02.07.	69	pl. w/o roots ears haulms grain straw grain straw	6.43 1.91 4.09 0.29 5.68 0.22 3.72	0.15 0.52 0.58 0.08 1.58 0.08 1.36	6.58 2.43 4.67 0.37 7.26 0.30 5.08	0 21 21 35 35 43 43	RIP 2000- 1072
#BASF 99/11825 DU2/08/98 Germany-67149 Meckenheim 00-01-31	Scarlett	1)18.03.98 2)08.06.- 15.06.1998 3)15.07.98	0.232 0.249	281 301	0.083	20.05.1998 04.06.	59	pl. w/o roots ears haulms grain straw grain straw	4.88 0.21 2.54 0.04 4.38 0.03 2.58	0.19 0.05 0.20 <0.02 0.39 <0.02 0.50	5.07 0.26 2.74 0.06 4.77 0.05 3.08	0 22 22 35 35 41 41	RIP 2000- 1073

1	2	3	4			5	6	7	8			9	10
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11825 HUS/05/98 Sweden-23791 Bjärred 00-01-31	Scarlett	1)30.03.98 2)02.07.- 10.07.1998 3)11.08.1998	0.261 0.256	317 310	0.082	02.06.1998 25.06.	59	pl. w/o roots ears haulms ears haulms grain straw	4.46 0.10 0.35 0.08 0.54 <u>0.03</u> 0.78	0.14 0.05 0.07 0.04 0.12 <0.02 0.23	4.60 0.15 0.42 0.12 0.66 0.05 1.01	0 22 22 36 36 42 42	RIP 2000- 1073

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-6: Trials from northern Europe - spring barley – (bridging studies with other active ingredients in formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 133 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 512 00 F
Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Spring barley
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Epoxiconazole (50 g/l)
Residues calculated as : Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 AGR/05/98	Riviera	1)25.03.98	0.243	304	0.080	29.05.98	60	Whole plants					RIP 2000-1031
		2)18.06.-25.06.98	0.244	305		19.06.		without roots	4.80	0.13	4.93	0	
								ears	0.29	0.11	0.40	20	
Belgium-3470 Kortenaken		3)03.08.98						haulms	0.73	0.08	0.81	20	
								grain	<0.02	<0.02	<0.04	34	
								straw	2.04	0.42	2.46	34	
00-01-31								grain	<0.02	<0.02	<0.04	40	
								straw	1.44	0.27	1.71	40	
#BASF 99/11509 DU2/05/98	Scarlett	1)18.03.98	0.242	303	0.080	18.05.98	59	Whole plants					RIP 2000-1031
		2)08.06.-15.06.98	0.225	281		22.06.		without roots	6.01	0.26	6.27	0	
								ears	0.18	0.06	0.24	20	
Germany-67149 Meckenheim		3)15.07.98						haulms	1.39	0.14	1.53	20	
								ears	0.03	<0.02	0.05	35	
								haulms	2.55	0.36	2.91	35	
00-01-31								grain	0.03	<0.02	0.05	41	
								straw	1.48	0.25	1.73	41	

1	2	3	4			5	6	7	8			9	10
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 HUS/03/98 Sweden-23791 Bjärred Borgeby Gard 00-01-31	Scarlett	1)30.03.98 2)02.07.-10.07.98 3)10./11.08.98	0.249 0.240	312 300	0.080	02.06.98 25.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 6.22 0.12 0.36 0.06 0.58 <u>0.03</u> 0.99	 0.16 0.06 0.06 0.04 0.10 <0.02 0.23	 6.38 0.18 0.42 0.10 0.68 0.05 1.22	 0 22 22 35 35 42 42	RIP 2000-1031

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 133 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Spring barley
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Epoxiconazole (50 g/l)
Kresoxim-methyl (67 g/l)
Residues calculated as : Pyraclostrobin (BAS 500 F)
Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 AGR/05/98 Belgium-3470 Kortenaken 00-01-31	Riviera	1)25.03.98 2)18.06.-25.06.98 3)03.08.98	0.244 0.243	305 303	0.080	29.05.98 19.06.	60	Whole plants without roots ears haulms grain straw grain straw	 3.61 0.28 0.97 <u><0.02</u> 1.57 <0.02 2.69	 0.05 0.08 0.08 <0.02 0.25 <0.02 0.29	 3.66 0.36 1.05 <0.04 1.82 <0.04 2.98	 0 20 20 34 34 40 40	RIP 2000-1031

1	2	3	4			5	6	7	8			9	10
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 DU2/05/98 Germany-67149 Meckenheim 00-01-31	Scarlett	1)18.03.98 2)08.06.- 15.06.98 3)15.07.98	0.233 0.242	291 303	0.080	18.05.98 22.06.	59	Whole plants without roots ears haulms grain straw grain straw	 6.70 0.23 1.68 <u>0.05</u> 2.94 0.04 1.97	 0.10 0.05 0.13 <0.02 0.24 <0.02 0.23	 6.80 0.28 1.81 0.07 3.18 0.06 2.20	 0 20 20 35 35 41 41	RIP 2000- 1031
#BASF 99/11509 HUS/03/98 Sweden-23791 Bjärred Borgeby Gard 00-01-31	Scarlett	1)30.03.98 2)02.07.- 10.07.98 3)10./11.08. 98	0.266 0.238	332 297	0.080	02.06.98 25.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 3.01 0.09 0.39 0.06 0.34 <u>0.03</u> 0.52	 0.05 0.38 0.05 0.03 0.05 <0.02 0.14	 3.06 0.47 0.44 0.09 0.39 0.05 0.66	 0 22 22 35 35 42 42	RIP 2000- 1031

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-7 Trials from northern Europe - winter barley – (solo formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	:	250g/l	Active ingredient	: Pyraclostrobin
Formulation	(e.g. WP)	:	EC	Crop / crop group	: Winter barley
Commercial product	(name)	:	BAS 500 01 F	Indoors / outdoors	: outdoors
Applicant		:	BASF AG (BAS)	Other a. i. in formulation (common name and content)	: --
				Residues calculated as	: Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509	Fighter	1)15.09.97	0.253	307	0.082	27.04.98	59	Whole plants					RIP 2000-
OAT/02/98		2)24.05.-31.05.98	0.243	294	0.083	21.05.		without roots	6.89	0.20	7.09	0	1031
								ears	0.39	0.14	0.53	22	
Great Britain		3)16.07.98						haulms	1.81	0.52	2.33	22	
								grain	0.04	<0.02	0.06	35	
NN13 6DY								straw	2.15	0.79	2.94	35	
Brackley								grain	0.04	0.02	0.06	42	
								straw	2.82	1.07	3.89	42	
00-01-31													
#BASF 99/11509	Muscat	1)20.09.97	0.247	297	0.083	07.05.98	65	Whole plants					RIP
OAT/03/98		2)23.05.-02.06.98	0.255	310	0.082	27.05.		without roots	3.35	0.12	3.47	0	2000-
								ears	0.37	0.08	0.45	21	1031
Great Britain		3)15.07.98						haulms	1.18	0.13	1.31	21	
								grain	0.06	<0.02	0.08	35	
CV9 2JS								straw	4.42	0.52	4.94	35	
Atherstone								grain	0.07	<0.02	0.09	42	
00-01-31								straw	3.83	0.65	4.48	42	

1	2	3	4			5	6	7	8			9	10
#BASF 99/11824 D05/03/99 Germany-24625 Großharrie 00-01-31	Landi	1)01.10.1998 2)28.05.- 04.06.1999 3)09.07.1999	0.253 0.247	306 299	0.083	07.05.1999 28.05.	61	pl. w/o roots ears haulms ears haulms grain straw	6.03 0.37 1.73 0.15 1.36 <u>0.09</u> 2.77	0.16 0.14 0.34 0.07 0.24 0.04 0.63	6.19 0.51 2.07 0.22 1.60 0.13 3.40	0 20 20 34 34 42 42	RIP 2000- 1072
#BASF 99/11824 OAT/10/99 Great Britain-CV9 2JS Atherstone 00-01-31	Musket	1)28.09.1998 2)26.05.- 07.06.1999 3)14.07.1999	0.252 0.268	306 324	0.082 0.083	30.04.1999 01.06.	65	pl. w/o roots ears haulms grain straw grain straw	4.4 0.17 0.74 0.07 0.82 <u>0.07</u> 0.84	0.07 0.04 0.09 <0.02 0.12 <0.02 0.17	4.47 0.21 0.83 0.09 0.94 0.09 1.01	0 20 20 35 35 42 42	RIP 2000- 1072

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-8: Trials from northern Europe - winter barley – (bridging studies with other active ingredients in formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	:	133g/l	Active ingredient	: Pyraclostrobin
Formulation	(e.g. WP)	:	EC	Crop / crop group	: Winter barley
Commercial product	(name)	:	BAS 512 00 F	Indoors / outdoors	: outdoors
Applicant		:	BASF AG (BAS)	Other a. i. in formulation (common name and content)	: Epoxiconazole(50 g/l)
				Residues calculated as	: Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509	Fighter	1)15.09.97	0.232	289	0.080	27.04.98	59	Whole plants					RIP 2000-
OAT/02/98		2)24.05.-31.05.98	0.238	297		21.05.		without roots	6.83	0.14	6.97	0	1031
		3)16.07.98						ears	0.40	0.15	0.55	22	
Great Britain								haulms	1.73	0.44	2.17	22	
								grain	0.07	0.03	0.10	35	
NN13 6DY								straw	3.20	1.02	4.22	35	
Brackley								grain	0.05	0.02	0.07	42	
								straw	3.58	1.29	4.87	42	
00-01-31													
#BASF 99/11509	Muscat	1)20.09.97	0.235	294	0.080	07.05.98	65	Whole plants					RIP 2000-
OAT/03/98		2)23.05.-02.06.98	0.285	311	0.092	27.05.		without roots	5.68	0.11	5.79	0	1031
		3)15.07.98						ears	0.42	0.12	0.54	21	
Great Britain								haulms	1.21	0.15	1.36	21	
								grain	0.12	0.03	0.15	35	
CV9 2JS								straw	6.01	0.64	6.65	35	
Atherstone								grain	0.12	0.03	0.15	42	
								straw	5.18	0.63	5.81	42	
00-01-31													

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 133g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Winter barley
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Epoxiconazole(50 g/l) Kresoxim-methyl (67 g/l)
Residues calculated as : Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 OAT/02/98 Great Britain NN13 6DY Brackley 00-01-31	Fighter	1)15.09.97 2)24.05.-31.05.98 3)16.07.98	0.233 0.243	291 303	0.08	27.04.98 21.05.	59	Whole plants without roots ears haulms grain straw grain straw	 7.51 0.52 1.58 0.06 4.56 <u>0.07</u> 2.78	 0.13 0.15 0.28 0.03 1.65 0.03 0.69	 7.64 0.67 1.86 0.09 6.21 0.10 3.47	 0 22 22 35 35 42 42	RIP 2000-1031
#BASF 99/11509 OAT/03/98 Great Britain CV9 2JS Atherstone 00-01-31	Muscat	1)20.09.97 2)23.05.-02.06.98 3)15.07.98	0.244 0.236	304 295	0.080	07.05.98 27.05.	65	Whole plants without roots ears haulms grain straw grain straw	 5.10 0.38 0.86 0.09 3.14 <u>0.10</u> 3.26	 0.10 0.80 0.09 <0.02 0.67 <0.02 0.32	 5.20 1.18 0.95 0.11 3.81 0.12 3.58	 0 21 21 35 35 42 42	RIP 2000-1031

Table B.7.6-9: Trials from southern Europe - winter barley – (solo formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	:	250 g/l	Active ingredient	: Pyraclostrobin
Formulation	(e.g. WP)	:	EC	Crop / crop group	: Winter barley
Commercial product	(name)	:	BAS 500 01 F	Indoors / outdoors	: outdoors
Applicant		:	BASF AG (BAS)	Other a. i. in formulation (common name and content)	: --
				Residues calculated as	: Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)			PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl				BAS 500 F	BF 500 -3	TOTAL		
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11824 ALO/03/99 SPAIN-41799 Trajano 00-01-31	Apex	1)27.11.1998 2)12.04.-20.04.1999 3)05.06.1999	0.257 0.254	312 308	0.082	08.04.1999 16.04.	63-65	pl. w/o roots ears haulms ears haulms grain straw	9.65 0.16 1.65 0.29 1.34 <u>0.03</u> 3.91	0.51 0.08 0.27 0.15 0.27 <0.02 0.68	10.16 0.24 1.92 0.44 1.61 0.05 4.59	0 21 21 34 34 41 41	RIP 2000-1072
#BASF 99/11824 ALO/04/99 SPAIN-21880 Paterna 00-01-31	Irene	1)20.12.1998 2)18.04.-25.04.99 3)04.06.1999	0.223 0.253	270 307	0.083	09.04.1999 19.04.	61-63	pl. w/o roots ears haulms grain straw grain straw	11.5 0.15 4.32 <u>0.05</u> 6.09 0.03 6.92	0.75 0.07 1.51 0.03 2.06 0.02 1.81	12.25 0.22 5.83 0.08 8.15 0.05 8.73	0 21 21 35 35 43 43	RIP 2000-1072

1	2	3	4			5	6	7	8			9	10
#BASF 99/11824 ALO/05/99 SPAIN-21880 Paterna 00-01-31	Tina	1)20.12.1998 2)18.04.- 28.04.99 3)04.06.199	0.258 0.252	313 305	0.082	09.04.1999 19.04	59-61	pl. w/o roots ears haulms grain straw	14.6 0.59 3.35 <u>0.02</u> 4.87	1.06 0.33 1.29 <0.02 1.74	15.66 0.92 4.64 0.04 6.61	0 21 21 35 35	RIP 2000- 1072

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-10: Residues Epoxiconazol

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	:	50 g/l	Active ingredient	: Epoxiconazole
Formulation	(e.g. WP)	:	SE	Crop / crop group	: Spring barley
Commercial product	(name)	:	BAS 512 00 F	Indoors / outdoors	: outdoors
Applicant		:	BASF AG (BAS)	Other a. i. in formulation (common name and content)	: Pyraclostrobin(133 g/l)
				Residues calculated as	: Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 AGR/05/98 B-3470 Kortenaken 00-01-31	Riviera	1)25.03.98 2)18.06.-25.06.98 3)03.08.98	0.092 0.093	304 305	0.030	29.05.98 19.06.	60	Whole plants without roots ears haulms grain straw grain straw	 1.51 0.14 0.51 <0.05 1.79 <0.05 1.35	 0 20 20 34 34 40 40	RIP 2000-1031
#BASF 99/11509 DU2/05/98 D-67149 Meckenheim 00-01-31	Scarlett	1)18.03.98 2)08.06.-15.06.98 3)15.07.98	0.092 0.086	303 281	0.030	18.05.98 22.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 2.44 0.28 1.67 <0.05 3.12 <0.05 3.25	 0 20 20 35 35 41 41	RIP 2000-1031

1	2	3	4			5	6	7	8		9	10
#BASF 99/11509 HUS/03/98 S-23791 Bjärred Borgeby Gard 00-01-31	Scarlett	1)30.03.98 2)02.07.- 10.07.98 3)10./11.08. 98	0.094 0.091	312 300	0.030	02.06.98 25.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 1.8 0.10 0.51 0.07 0.39 <0.05 1.22	 0 22 22 35 35 42 42	RIP 2000-1031	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 50 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Epoxiconazole
Crop / crop group : Spring barley
Indoors / outdoors : outdoors
Other a. i. in formulation
(common name and content) : Pyraclostrobin (133 g/l), Kresoxim-methyl (67 g/l)
Residues calculated as : Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 AGR/05/98 B-3470 Kortenaken 00-01-31	Riviera	1)25.03.98 2)18.06.- 25.06.98 3)03.08.98	0.119 0.119	305 303	0.039	29.05.98 19.06.	60	Whole plants without roots ears haulms grain straw grain straw	 1.18 0.17 0.54 <0.05 1.41 <0.05 1.42	 0 20 20 34 34 40 40	RIP 2000-1031

1	2	3	4			5	6	7	8		9	10
#BASF 99/11509 DU2/05/98 D-67149 Meckenheim 00-01-31	Scarlett	1)18.03.98 2)08.06.-15.06.98 3)15.07.98	0.083 0.087	291 303	0.029	18.05.98 22.06.	59	Whole plants without roots ears haulms grain straw grain straw	 2.55 0.26 1.52 <0.05 2.71 <0.05 2.73	 0 20 20 35 35 41 41	RIP 2000-1031	
#BASF 99/11509 HUS/03/98 S-23791 Bjärred Borgeby Gard 00-01-31	Scarlett	1)30.03.98 2)02.07.-10.07.98 3)10./11.08.98	0.095 0.085	332 297	0.029	02.06.98 25.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 1.43 0.07 0.22 <0.05 0.31 <0.05 0.62	 0 22 22 35 35 42 42	RIP 2000-1031	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 50 g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 512 00 F
Applicant : BASF AG (BAS)

Active ingredient : Epoxiconazole
Crop / crop group : Winter barley
Indoors / outdoors : outdoors
Other a. i. in formulation
(common name and content) : Pyraclostrobin (133 g/l)
Residues calculated as : Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 OAT/02/98 NN13 6DY Hillfarm, Halse Nr. Brackley 00-01-31	Fighter	1)15.09.97 2)24.05.-31.05.98 3)16.07.98	0.088 0.090	289 297	0.030	27.04.98 21.05.	59	Whole plants without roots ears haulms grain straw grain straw	 4.08 <0.05 0.07 <0.05 0.15 <0.05 0.15	 0 22 22 35 35 42 42	RIP 2000-1031
#BASF 99/11509 OAT/03/98 CV9 2JS Chapel Farm Bentley, Atherstone 00-01-31	Muscat	1)20.09.97 2)23.05.-02.06.98 3)15.07.98	0.089 0.094	294 311	0.030	07.05.98 27.05.	65	Whole plants without roots ears haulms grain straw grain straw	 2.56 <0.05 0.14 <0.05 0.27 <0.05 0.33	 0 21 21 35 35 42 42	RIP 2000-1031

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 50 g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Epoxiconazole
Crop / crop group : Winter barley
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (133 g/l)
Kresoxim-methyl (67 g/l)
Residues calculated as : Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 OAT/02/98 NN13 6DY Hillfarm, Halse Nr. Brackley 00-01-31	Fighter	1)15.09.97 2)24.05.-31.05.98 3)16.07.98	0.083 0.087	291 303	0.029	27.04.98 21.05.	59	Whole plants without roots ears haulms grain straw grain straw	 2.75 0.17 0.54 <0.05 0.91 <0.05 0.59	 0 22 22 35 35 42 42	RIP 2000-1031
#BASF 99/11509 OAT/03/98 CV9 2JS Chapel Farm Bentley, Atherstone 00-01-31	Muscat	1)20.09.97 2)23.05.-02.06.98 3)15.07.98	0.087 0.084	304 295	0.029 0.028	07.05.98 27.05.	65	Whole plants without roots ears haulms grain straw grain straw	 1.95 0.15 0.39 <0.05 0.99 <0.05 0.92	 0 21 21 35 35 42 42	RIP 2000-1031

Table B.7.6-11: Residues Kresoxim-methyl

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 67g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F
Applicant : BASF AG (BAS)

Active ingredient : Kresoxim-methyl
Crop / crop group : Spring barley
Indoors / outdoors : Outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (133 g/l) Epoxiconazole (50 g/l)
Residues calculated as : Kresoxim-methyl

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 AGR/05/98 B-3470 Kortenaken 00-01-31	Riviera	1) 25.03.98 2) 18.06.-25.06.98 3) 03.08.98	0.119 0.119	305 303	0.039	29.05.98 19.06.	60	Whole plants without roots ears haulms grain straw grain straw	 1.78 0.06 0.23 <0.05 0.19 <0.05 0.18	 0 20 20 34 34 40 40	RIP 2000-1031
#BASF 99/11509 DU2/05/98 D-67149 Meckenheim 00-01-31	Scarlett	1) 18.03.98 2) 08.06.-15.06.98 3) 15.07.98	0.114 0.118	291 303	0.039	18.05.98 22.06.	59	Whole plants without roots ears haulms grain straw grain straw	 2.42 <0.05 0.25 <0.05 0.56 <0.05 0.34	 0 20 20 35 35 41 41	RIP 2000-1031

1	2	3	4			5	6	7	8		9	10
#BASF 99/11509 HUS/03/98 S-23791 Bjärred Borgeby Gard 00-01-31	Scarlett	1) 30.03.98 2) 02.07.-10.07.98 3) 10./11.08.98	0.130 0.116	332 297	0.039	02.06.98 25.06.	59	Whole plants without roots ears haulms ears haulms grain straw	 1.87 <0.05 0.07 <0.05 <0.05 <0.05 <0.05	 0 22 22 35 35 42 42	RIP 2000-1031	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 67 g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 513 00

Applicant : BASF AG (BAS)

Active ingredient : Kresoxim-methyl (67 g/l)
Crop / crop group : Winter barley
Indoors / outdoors : Outdoors
Other a. i. in formulation
(common name and content) : Pyraclostrobin (133 g/l)
Epoxiconazole(50 g/l
Residues calculated as : Kresoxim-methy

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 OAT/02/98 NN13 6DY Hillfarm, Halse Nr. Brackley 00-01-31	Fighter	1)15.09.97 2)24.05.-31.05.98 3)16.07.98	0.114 0.119	291 303	0.039	27.04.98 21.05.	59	Whole plants without roots ears haulms grain straw grain straw	 4.08 <0.05 0.07 <0.05 0.15 <0.05 0.15	 0 22 22 35 35 42 42	RIP 2000-1031

1	2	3	4			5	6	7	8		9	10
#BASF 99/11509 OAT/03/98 CV9 2JS Chapel Farm Bentley, Atherstone 00-01-31	Muscat	1)20.09.97 2)23.05.- 02.06.98 3)15.07.98	0.119 0.115	304 295	0.039	07.05.98 27.05.	65	Whole plants without roots ears haulms grain straw grain straw	 2.56 <0.05 0.14 <0.05 0.27 <0.05 0.33	 0 21 21 35 35 42 42	RIP 2000-1031	

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.6.4.2 Wheat

North EU

Critical GAP

0.233 kg as/ha, 2 applications, growth stage at last treatment: BBCH 69, PHI: 35 d

Supporting residue data

9 Trials with formulation BAS 500 01 F 1998 and 1999 with winter (8) and spring (1) wheat.
grain, PHI 33-42 days: < 0.02 (7), 0.03, 0.04, 0.04
straw, PHI 33-42 days: 0.87, 1.10, 1.19, 1.31, 1.96, 2.23, 2.34, 2.50, 3.14
One further trial (ACK/02/98, RIP 2000-1073) is not valid to support the critical GAP because of the long PHI (64 days).

Additional trails were conducted simultanously with two different formulations. As all other parameter like time, location, variety are identical for all three formulations the results show clearly that the residue level in wheat is independant of the tested formulation.

4 trials with formulation BAS 512 00 F with winter wheat.
grain, PHI 35-42 days: < 0.02 (3), 0.05
straw, PHI 35-42 days: 1.00, 1.40, 1.65, 1.90

4 trials with formulation BAS 513 00 F with winter wheat.
grain, PHI 35-42 days: < 0.02 (2), 0.02, 0.05
straw, PHI 35-42 days: 1.22, 1.31, 1.95, 2.13

MRL calculation

R_{max}:	0.05
R_{ber} (2 x $R_{0.75}$):	0.07
STMR:	< 0.02

South EU

Critical GAP

The only use in southern Europe is intended in France.
0.250 kg as/ha, 2 applications, growth stage at last treatment: BBCH 65, PHI: 30 d

Supporting residue data:

2 Trials with each formulation BAS 500 01 F, BAS 512 00 F and BAS 513 00 F in 1998 with winter wheat.
grain, PHI 42 days: < 0.02 (6)
straw, PHI 42 days: 1.67, 4.59 / 1.67, 4.95 / 1.53, 5.68

3 Trials with formulation BAS 500 01 F 1998 and 1999 (1) with durum wheat.
grain, PHI 35-43 days: < 0.02 (3)
straw, PHI 33-42 days: 0.75, 1.44, 2.07

In a further trial (FR3/02/98, RIP 2000-1072) sampling was done at an immature stage only so instead of grain only ears were analysed. Therefore the resulting high residue value (PHI 35 days, 0.14 mg/kg) is not appropriate for MRL calculation.

To prove the comparability of the formulations BAS 512 00 F and BAS 513 00 F in durum two trials each were conducted simultaniously 1998. As with BAS 500 01 F in one of these trials only ears were analysed. Nevertheless the residue level in these samples was low (PHI 35 days, 0.06 mg/kg (2))
grain, PHI 42 days: < 0.02 (2)
straw, PHI 42 days: 2.20 / 1.85

MRL calculation

all results: < 0.02 mg/kg grain

MRL proposal

The data presented is sufficient to set a MRL.
Due to the higher residues in the trials from nothern Europe the MRL proposal is calculated on the basis of these results.

MRL proposal wheat, rye, triticale: 0.1 mg/kg

Table B.7.6-12: Trials from northern Europe - spring wheat – (solo formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	: 250 g/l	Active ingredient	:	Pyraclostrobin
Formulation	(e.g. WP)	: EC	Crop / crop group	:	Spring wheat
Commercial product	(name)	: BAS 500 01 F	Indoors / outdoors	:	outdoors
Applicant		: BASF AG (BAS)	Other a. i. in formulation (common name and content)	:	--
			Residues calculated as	:	Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500-3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11824	Dragon	1)09.04.1999	0.254	309	0.082	07.06.1999	65-69	pl. w/o	4.98	0.13	5.11	0	RIP 2000-1072
ALB/01/99		2) 01.07.-12.07.99	0.254	308		07.07.		ears	0.36	0.12	0.48	21	
								haulms	0.99	0.20	1.19	21	
Denmark-5500		3)15.08.1999						grain	<0.02	<0.02	<0.04	35	
Middelfart								straw	0.59	0.13	0.72	35	
								grain	<0.02	<0.02	<0.04	42	
00-01-31								straw	0.87	0.28	1.15	42	

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-13: Trials from northern Europe - winter wheat – (solo formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 250g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 500 01 F
Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : winter wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : --
Residues calculated as : Pyraclostrobin (BAS 500 F)
Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 AGR/04/98 Netherland-5853 EJ Siebengewald 00-01-31	Brigadier	1)15.10.97 2)05.06.-17.06.98 3)07.08.98	0.239 0.251	290 304	0.082	15.05. 17.06.	69	whole plant without roots ears haulms grain straw grain straw grain straw	4.27 0.45 0.84 0.04 1.96 <0.02 2.24 <0.02 1.03	0.06 0.09 0.09 <0.02 0.24 <0.02 0.59 <0.02 0.24	4.33 0.54 0.93 0.06 2.20 <0.04 2.83 <0.04 1.27	0 20 20 35 35 44 44 51 51	RIP 2000-1031
#BASF 99/11509 D05/02/98 Germany-24625 Großharrie 00-01-31	Pepital	1)24.09.97 2)12.06.-22.06.98 3)10.08.98	0.265 0.231	321 280	0.083	18.05.98 22.06.	69-71	Whole plant without root ears haulms ears haulms grain straw	5.35 0.27 0.93 0.32 1.42 <0.02 2.23	0.15 0.08 0.12 0.08 0.16 <0.02 0.52	5.50 0.35 1.05 0.40 1.58 <0.04 2.75	0 21 21 36 36 42 42	RIP 2000-1031

1	2	3	4			5	6	7	8				9	10
#BASF 99/11509 FR2/03/98 France-62580 Nothern France Neuville Saint Vaast 00-01-31	Balthazar	1)08.11.97 2)04.06.- 12.06.98 3)08.08.98	0.248 0.254	300 308	0.083	14.05.98 08.06.	65	Whole plant without root ears haulms ears haulms grain straw grain straw	 4.92 0.36 0.52 0.19 0.25 <0.02 3.14 <0.02 2.47	 0.12 0.08 0.11 0.05 0.08 <0.02 0.24 <0.02 0.20	 5.04 0.44 0.63 0.24 0.33 <0.04 3.38 <0.04 2.67	 0 22 22 32 32 42 42 50 50	RIP 2000- 1031
#BASF 99/11509 OAT/01/98 Great Britain AB30IXJ Grampian Marykirk 00-01-31	Riband	1)02.10.97 2)03.07.- 25.07.98 3)16.09.98	0.226 0.194	273 235	0.083	05.06.98 25.07.	69-72	Whole plant without root ears haulms grain straw straw grain	 3.32 0.29 0.79 0.04 1.19 1.89 <0.02	 0.06 0.05 0.09 <0.02 0.26 0.76 <0.02	 3.38 0.34 0.88 0.06 1.45 2.65 <0.04	 0 20 20 33 33 47 47	RIP 2000- 1031
#BASF 99/11824 D07/01/99 Germany-88445 Oberding 00-01-31	Kanzler	1)13.10.98 2)10.06.- 20.06.99 3)27.07.99	0.261 0.252	317 305	0.082	25.05.1999 16.06.	69	pl.w/o roots ears haulms grain straw grain straw	4.71 0.24 1.55 <0.02 1.10 <0.02 1.16	0.10 0.07 0.33 <0.02 0.44 <0.02 0.45	4.81 0.31 1.88 <0.04 1.54 <0.04 1.61	0 20 20 34 34 41 41	RIP 2000- 1072
#BASF 99/11824 OAT/08/99 Great Britain- TQ7 2BU Kinesbridge 00-01-31	Hussar	1)01.10.98 2)10.06.- 30.06.99 3)12.08.99	0.253 0.241	307 292	0.082	20.05.1999 30.06.	69	pl. w/o roots ears haulms grain straw grain straw	3.54 0.18 1.3 <0.02 2.34 <0.02 2.33	0.06 0.05 0.21 <0.02 0.46 <0.02 0.61	3.6 0.23 1.51 <0.04 2.80 <0.04 2.94	0 20 20 36 36 43 43	RIP 2000- 1072

1	2	3	4			5	6	7	8			9	10
#BASF 99/11824 OAT/09/99 Great Britain- NN13 6DY Brackley 00-01-31	Consort	1)02.10.98 2)16.06.- 25.06.1999 3)08.10.99	0.240 0.242	290 293	0.082	06.05.1999 14.06.	65	pl.w/o roots ears haulms grain straw grain straw	2.67 0.23 1.00 0.03 1.31 <0.02 1.59	0.03 0.07 0.16 <0.02 0.26 <0.02 0.30	2.70 0.30 1.16 0.05 1.57 <0.04 1.89	0 20 20 35 35 42 42	RIP 2000- 1072
#BASF 99/11825 ACK/02/98 Germany-16356 Blumberg 00-01-31	Flair	1)21.10.1997 2)02.06.- 08.06.1998 3)05.08.1998	0.245 0.253	297 307	0.082	14.05.1998 02.06.	61	pl. w/o roots ears haulms ears haulms ears haulms grain straw	8.93 0.26 0.77 0.15 0.94 0.10 0.66 <0.02 0.67	0.18 0.06 0.21 0.03 0.31 0.03 0.27 <0.02 0.21	9.11 0.32 0.98 0.18 1.25 0.13 0.93 <0.04 0.88	0 21 21 35 35 42 42 64 64	RIP 2000- 1073
#BASF 99/11824 HUS/04/98 Sweden-23791 Bjärred 00-01-31	Meridien	1)18.09.1997 2)09.06.- 22.06.1998 3)19.08.98	0.254 0.244	308 297	0.082	01.06.1998 21.06.	69	pl. w/o roots ears haulms ears haulms grain straw	5.21 0.15 0.50 0.09 1.81 <0.02 2.50	0.13 0.06 0.18 0.04 0.52 <0.02 1.2	5.34 0.21 0.68 0.13 2.33 <0.04 3.70	0 22 22 36 36 42 42	RIP 2000- 1073

Table B.7.6-14: Trials from southern Europe - winter wheat – (solo formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	: 250g/l	Active ingredient	:	Pyraclostrobin
Formulation	(e.g. WP)	: EC	Crop / crop group	:	winter wheat
Commercial product	(name)	: BAS 500 01 F	Indoors / outdoors	:	outdoors
Applicant		: BASF AG (BAS)	Other a. i. in formulation (common name and content)	:	--
			Residues calculated as	:	Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 ALO/07/98	Cartaga	1)03.12.97	0.243	295	0.082	16.03.	69	Whole plant					RIP 2000-1031
		2)20.03.-27.03.98	0.230	279		27.03.		without roots	10.30	0.62	10.92	0	
								ears	0.39	0.13	0.52	21	
Spain-41727 Maribanez		3)End of May						haulms	2.66	0.80	3.46	21	
								ears	0.20	0.07	0.27	33	
								haulms	1.99	0.64	2.63	33	
00-01-31								grain	<0.02	<0.02	<0.04	42	
								straw	4.95	1.77	6.72	42	
#BASF 99/11509 ALO/08/98	Cajense	1)10.12.97	0.248	301	0.082	24.03.98	69	Whole plant					RIP 2000-1031
		2)30.03-08.04.98	0.249	302		08.04.		without root	4.18	0.12	4.30	0	
								ears	0.48	0.26	0.74	22	
Spain-41500 Alcala de Guadaira		3) End of May						haulms	0.74	0.17	0.91	22	
								ears	0.15	0.08	0.23	37	
								haulms	0.44	0.12	0.56	37	
								grain	<0.02	<0.02	<0.04	42	
00-01-31								straw	1.67	0.61	2.28	42	

Table B.7.6-15: Trials from northern Europe - winter wheat – (bridging studies with other active ingredients in formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	:	133 g/l	Active ingredient	: Pyraclostrobin
Formulation	(e.g. WP)	:	SE	Crop / crop group	: winter wheat
Commercial product	(name)	:	BAS 512 00 F	Indoors / outdoors	: outdoors
Applicant		:	BASF AG (BAS)	Other a. i. in formulation (common name and content)	: Epoxiconazole (50 g/l)
				Residues calculated as	: Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 AGR/04/98	Brigadier	1)15.10.97	0.247	309	0.080	15.05.	69	whole plant					RIP 2000-1031
		2)05.06.-17.06.98	0.236	294		17.06.		without roots	4.04	0.07	4.11	0	
								ears	0.34	0.08	0.42	20	
Netherland-5853 EJ Siebengewald		3)07.08.98						haulms	0.98	0.09	1.07	20	
								grain	<0.02	<0.02	<0.04	35	
								straw	1.65	0.21	1.86	35	
00-01-31								grain	<0.02	<0.02	<0.04	44	
								straw	2.18	0.25	2.43	44	
								grain	<0.02	<0.02	<0.04	51	
								straw	0.81	0.20	1.01	51	
#BASF 99/11509 D05/02/98	Pepital	1)24.09.97	0.239	298	0.080	18.05.98	69-71	Whole plant					RIP 2000-1031
		2)12.06.-22.06.98	0.244	304		22.06.		without root	5.75	0.12	5.87	0	
								ears	0.21	0.06	0.27	21	
Germany-24625 Großharrie		3)10.08.98						haulms	0.87	0.10	0.97	21	
								ears	0.46	0.10	0.56	36	
								haulms	1.17	0.12	1.29	36	
00-01-31								grain	<0.02	<0.02	<0.04	42	
								straw	1.40	0.34	1.74	42	
								grain	<0.02	<0.02	<0.04	49	
								straw	1.73	0.44	2.17	49	

1	2	3	4			5	6	7	8			9	10
#BASF 99/11509 FR2/03/98 France-62580 (North France) Neuville Saint Vaast 00-01-31	Balthazar	1)08.11.97 2)04.06.98 12.06. 3)08.08.98	0.243 0.244	303 305	0.080	14.05.98 08.06.	65	Whole plant without root ears haulms ears haulms grain straw grain straw	 6.49 0.26 0.62 0.14 0.23 <0.02 1.00 <0.02 1.91	 0.12 0.07 0.11 0.04 0.07 <0.02 0.22 <0.02 0.88	 6.61 0.33 0.73 0.18 0.30 <0.04 1.22 <0.04 2.79	 0 22 22 32 32 42 42 50 50	RIP 2000- 1031
#BASF 99/11509 OAT/01/98 Great Britain AB30IXJ Grampian Marykirk 00-01-31	Riband	1)02.10.97 2)03.07.- 25.07.98 3)16.09.98	0.226 0.232	282 289	0.080	05.06.98 25.07.	69-72	Whole plant without root ears haulms grain straw straw grain	 3.10 0.29 1.12 0.05 1.90 2.23 0.03	 0.04 0.05 0.15 <0.02 0.87 0.85 <0.02	 3.14 0.34 1.27 0.07 2.77 3.08 0.05	 0 20 20 33 33 47 47	RIP 2000- 1031

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 133 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : winter wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Epoxiconazole (50 g/l), Kresoxim-methyl (67 g/l)
Residues calculated as : Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 AGR/04/98	Brigadier	1)15.10.97	0.231	289	0.080	15.05.	69	whole plant					RIP 2000-1031
		2)05.06.-17.06.98	0.238	297		17.06.		without roots	2.80	0.02	2.82	0	
								ears	0.35	0.06	0.41	20	
Netherland-5853 EJ Siebengewald		3)07.08.98						haulms	0.92	0.07	0.99	20	
								grain	0.02	<0.02	0.04	35	
								straw	1.31	0.15	1.46	35	
00-01-31								grain	<0.02	<0.02	<0.04	44	
								straw	1.48	0.17	1.65	44	
								grain	<0.02	<0.02	<0.04	51	
								straw	0.93	0.19	1.12	51	
#BASF 99/11509 D05/02/98	Pepital	1)24.09.97	0.239	299	0.080	18.05.98	69-71	Whole plant					RIP 2000-1031
		2)12.06.-22.06.98	0.242	303		22.06.		without root	8.23	0.07	8.3	0	
								ears	0.22	0.06	0.28	21	
Germany-24625 Großharrie		3)10.08.98						haulms	0.68	0.08	0.76	21	
								ears	0.43	0.08	0.51	36	
								haulms	1.00	0.10	1.10	36	
00-01-31								grain	<0.02	<0.02	<0.04	42	
								straw	1.95	0.38	2.33	42	
								grain	<0.02	<0.02	<0.04	49	
								straw	1.57	1.57	1.93	49	

1	2	3	4			5	6	7	8				9	10
#BASF 99/11509 FR2/03/98 France-62580 Nothern France Neuville Saint Vaast 00-01-31	Balthazar	1)08.11.97 2)04.06.98 12.06. 3)08.08.98	0.238 0.252	297 315	0.080	q	65	Whole plant without root ears haulms ears haulms grain straw grain straw	 4.06 0.19 0.56 0.14 0.23 <0.02 1.22 <0.02 2.05	 0.07 0.05 0.10 0.04 0.06 <0.02 0.21 <0.02 0.25	 4.13 0.24 0.66 0.18 0.29 <0.04 1.43 <0.04 0.25	 0 22 22 32 32 42 42 50 50	RIP 2000- 1031
#BASF 99/11509 OAT/01/98 Great Britain AB30IXJ Grampian Marykirk 00-01-31	Riband	1)02.10.97 2)03.07.- 25.07.98 3)16.09.98	0.246 0.236	307 294	0.080	05.06.98 25.07.	69-72	Whole plant without root ears haulms grain straw straw grain	 3.93 0.37 0.89 0.05 2.13 0.02 2.73	 0.02 0.07 0.07 <0.02 0.88 <0.02 0.090	 3.95 0.44 0.96 0.07 3.01 0.04 3.63	 0 20 20 33 33 47 47	RIP 2000- 1031

Table B.7.6-16: Trials from southern Europe - winter wheat – (bridging studies with other active ingredients in formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	: 133 g/l	Active ingredient	:	Pyraclostrobin
Formulation	(e.g. WP)	: SE	Crop / crop group	:	winter wheat
Commercial product	(name)	: BAS 512 00 F	Indoors / outdoors	:	outdoors
Applicant		: BASF AG (BAS)	Other a. i. in formulation (common name and content)	:	Epoxiconazole (50 g/l)
			Residues calculated as	:	Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509	Cartaga	1)03.12.97	0.239	299	0.080	16.03.	69	Whole plant					RIP 2000-1031
ALO/07/98		2)20.03.-27.03.98	0.252	314		27.03.		without roots	10.30	0.62	10.92	0	
								ears	0.39	0.13	0.52	21	
Spain-41727		3)End of May						haulms	2.66	0.80	3.46	21	
Maribanez								ears	0.20	0.07	0.27	33	
								haulms	1.99	0.64	2.63	33	
00-01-31								grain	<0.02	<0.02	<0.04	42	
								straw	4.95	1.77	6.72	42	
#BASF 99/11509	Cajense	1)10.12.97	0.241	301	0.080	24.03.98	69	Whole plant					RIP 2000-1031
ALO/08/98		2)30.03-08.04.98	0.244	304		08.04.		without root	6.22	0.19	6.41	0	
								ears	0.38	0.21	0.59	22	
Spain-41500		3) End of May						haulms	0.85	0.21	1.06	22	
Alcala de Guadaira								ears	0.13	0.09	0.22	37	
								haulms	0.64	0.19	0.83	37	
								grain	<0.02	<0.02	<0.04	42	
00-01-31								straw	1.67	0.61	2.28	42	

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 133 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : winter wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Epoxiconazole (50 g/l), Kresoxim-methyl (67 g/l)
Residues calculated as : Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 ALO/07/98 Spain-41727 Maribanez 00-01-31	Cartaga	1)03.12.97 2)20.03.-27.03.98 3)End of May	0.239 0.243	299 304	0.080	16.03. 27.03.	69	Whole plant without roots ears haulms ears haulms grain straw	10.90 0.40 2.71 0.26 2.01 <0.02 5.68	0.59 0.10 0.55 0.07 0.53 <0.02 1.54	11.49 0.50 3.26 0.33 2.54 <0.04 7.22	0 21 21 33 33 42 42	RIP 2000-1031
#BASF 99/11509 ALO/08/98 Spain-41500 Alcala de Guadaira 00-01-31	Cajense	1)10.12.97 2)30.03-08.04.98 3) End of May	0.240 0.246	300 307	0.080	24.03.98 08.04.	69	Whole plant without root ears haulms ears haulms grain straw	4.18 0.48 0.74 0.15 0.44 <0.02 1.53	0.12 0.26 0.17 0.08 0.12 <0.02 0.61	4.30 0.74 0.91 0.23 0.56 <0.04 2.14	0 22 22 37 37 42 42	RIP 2000-1031

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-17: Trials from southern Europe – durum wheat – (solo formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	: 250g/l	Active ingredient	:	Pyraclostrobin
Formulation	(e.g. WP)	: EC	Crop / crop group	:	Durum wheat
Commercial product	(name)	: BAS 500 01 F	Indoors / outdoors	:	outdoors
Applicant		: BASF AG (BAS)	Other a. i. in formulation (common name and content)	:	
			Residues calculated as	:	Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 ALO/09/98 E-41500 Alcala de Guadaira 00-01-31	Vitrou	1)08.12.98 2)30.03.-08.04.98 3)End of May	0.236 0.235	286 284	0.083	24.03.98 08.04.	69	whole plant without roots ears haulms ears haulms grain straw	 7.79 0.60 0.92 0.17 0.67 <0.02 1.44	 0.30 0.28 0.27 0.10 0.17 <0.02 0.52	 8.09 0.88 1.19 0.27 0.84 <0.04 1.96	 0 22 22 36 36 42 42	RIP 2000-1031
#BASF 99/11509 FR3/02/98 F-30730 Southern France Gajan 00-01-31	Ardenti	1)25.10.98 2)12.05.-23.05.98 3)01.07.98	0.246 0.234	299 284	0.082	17.04.98 05.05.	61	whole plant without roots ears haulms ears haulms	 5.75 0.29 1.21 0.14 1.93	 0.21 0.12 0.54 0.05 0.64	 5.96 0.41 1.75 0.19 2.57	 0 21 21 35 35	RIP 2000-1031

1	2	3	4			5	6	7	8			9	10
#BASF 99/11824 FR3/05/99 F-30700 St. Maximin Southern France 00-01-31	Arstar	1)12.11.1998 2) 10.05.- 20.05.199 3)10.07.99	0.242 0.229	294 278	0.082	16.04.1999 13.05.	61	pl. w/o roots ears haulms grain straw grain straw	5.41 0.33 1.33 <u><0.02</u> 2.07 <0.02 1.9	0.08 0.11 0.36 <0.02 0.61 <0.02 0.58	5.49 0.44 1.69 <0.04 2.68 <0.04 2.48	0 21 21 35 35 42 42	RIP 2000- 1072
#BASF 99/11825 FR3/03/98 F-30700 St. Maximin Southern France 00-01-31	Ardente	1)01.11.1997 2)10.05.- 20.05.1998 3)01.07..1998	0.238 0.256	288 310	0.083	17.04.1998 29.04.	59	pl. w/o roots ears haulms ears haulms grain straw	7.77 0.10 0.81 0.03 0.40 <u><0.02</u> 0.75	0.53 0.02 0.19 <0.02 0.10 <0.02 0.26	8.30 0.12 1.00 0.05 0.5 <0.04 1.01	0 21 21 35 35 43 43	RIP 2000- 1073

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

Table B.7.6-18: Trials from southern Europe – durum wheat – (bridging studies with other active ingredients in formulation)

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i.	(g/kg or g/l)	:	133 g/l	Active ingredient	: Pyraclostrobin
Formulation	(e.g. WP)	:	SE	Crop / crop group	: Durum wheat
Commercial product	(name)	:	BAS 512 00 F	Indoors / outdoors	: outdoors
Applicant		:	BASF AG (BAS)	Other a. i. in formulation (common name and content)	: Epoxiconazole (50 g/l)
				Residues calculated as	: Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509	Vitrou	1)08.12.98	0.244	304	0.080	24.03.98	69	whole plant					RIP 2000-
ALO/09/98		2)30.03.-08.04.98	0.248	310		08.04.		without roots	6.70	0.34	7.04	0	1031
		3)End of May						ears	0.53	0.29	0.82	22	
E-41500								haulms	1.38	0.56	1.94	22	
Alcala de								ears	0.35	0.18	0.53	36	
Guadaira								haulms	1.30	0.54	1.84	36	
								grain	<u><0.02</u>	<0.02	<0.04	42	
00-01-31								straw	2.20	0.99	3.19	42	
#BASF 99/11509	Ardenti	1)25.10.98	0.211	264	0.080	17.04.98	61	whole plant					RIP 2000-
FR3/02/98		2)12.05.-23.05.98	0.230	287		05.05.		without roots	4.69	0.14	4.83	0	1031
		3)01.07.98						ears	0.21	0.07	0.28	21	
F-30730								haulms	0.96	0.21	1.17	21	
Southern France								ears	0.06	0.02	0.08	35	
Gajan								haulms	0.76	0.21	0.97	35	
00-01-31													

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 133 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Durum wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Epoxiconazole (50 g/l) Kresoxim-methyl (67 g/l)
Residues calculated as : Pyraclostrobin (BAS 500 F) Metabolite BF 500-3 calculated as Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11509 ALO/09/98 E-41500 Alcala de Guadaira 00-01-31	Vitrou	1)08.12.98 2)30.03.-08.04.98 3)End of May	0.239 0.241	299 301	0.08	24.03.98 08.04.	69	whole plant without roots ears haulms ears haulms grain straw	 6.62 0.48 1.54 0.29 1.12 <u>≤0.02</u> 1.85	 0.19 0.22 0.63 0.14 0.46 <0.02 0.87	 6.81 0.70 2.17 0.43 1.58 <0.04 2.72	 0 22 22 36 36 42 42	RIP 2000-1031
#BASF 99/11509 FR3/02/98 F-30730 Southern France Gajan 00-01-31	Ardenti	1)25.10.98 2)12.05.-23.05.98 3)01.07.98	0.234 0.242	292 302	0.08	17.04.98 05.05.	61	whole plant without roots ears haulms ears haulms	 3.85 0.18 0.75 0.06 0.59	 0.09 0.06 0.14 0.02 0.10	 3.94 0.24 0.89 0.08 0.69	 0 21 21 35 35	RIP 2000-1031

Table B.7.6-19: Residues Epoxiconazol

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 50 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 512 00 F
Applicant : BASF AG (BAS)

Active ingredient : Epoxiconazole
Crop / crop group : winter wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (BAS 500 F)
Residues calculated as : Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 AGR/04/98 NL-5853 EJ Siebengewald 00-01-31	Brigadier	1)15.10.97 2)05.06.-17.06.98 3)07.08.98	0.094 0.090	309 294	0.030 0.030	15.05. 17.06.	69	whole plant without roots ears haulms grain straw grain straw grain straw	 0.79 0.11 0.47 <0.05 0.94 <0.05 1.00 <0.05 0.74	 0 20 20 35 35 44 44 51 51	RIP 2000-1031
#BASF 99/11509 ALO/07/98 E-41727 Maribanez 00-01-31	Cartaga	1)03.12.97 2)20.03.-27.03.98 3)End of May	0.091 0.096	299 314	0.030 0.030	16.03. 27.03.	69	Whole plant without roots ears haulms ears haulms grain straw	 5.15 0.40 1.88 0.22 1.72 <0.05 3.23	 0 21 21 33 33 42 42	RIP 2000-1031

1	2	3	4			5	6	7	8	9	10
#BASF 99/11509 ALO/08/98 E-41500 Alcala de Guadaira 00-01-31	Cajense	1)10.12.97 2)30.03- 08.04.98 3) End of May	0.092 0.093	301 304	0.031 0.031	24.03.98 08.04.	69	Whole plant without root ears haulms ears haulms grain straw	 2.85 0.28 0.98 0.14 0.90 <0.05 1.79	 0 22 22 37 37 42 42	RIP 2000-1031
#BASF 99/11509 D05/02/98 D-24625 Großharrie 00-01-31	Pepital	1)24.09.97 2)12.06.- 22.06.98 3)10.08.98	0.091 0.093	298 304	0.031 0.031	18.05.98 22.06.	69-71	Whole plant without root ears haulms ears haulms grain straw grain straw	 1.91 0.18 0.35 <0.05 0.19 <0.05 0.58 <0.05 0.71	 0 21 21 36 36 42 42 49 49	RIP 2000-1031
#BASF 99/11509 FR2/03/98 F-62580 (N-FR) Neuville Saint Vaast 00-01-31	Balthazar	1)08.11.97 2)04.06. - 12.06.98 3)08.08.98	0.092 0.093	303 305	0.030 0.030	14.05.98 08.06.	65	Whole plant without root ears haulms ears haulms grain straw grain straw	 2.35 0.15 0.49 0.14 0.39 <0,.05 1.44 <0.05 1.42	 0 22 22 32 32 42 42 50 50	RIP 2000-1031
#BASF 99/11509 OAT/01/98 AB30IXJ Grampian Prrbeadlie Farm,Maykirk 00-01-31	Riband	1)02.10.97 2)03.07.- 25.07.98 3)16.09.98	0.086 0.088	282 289	0.030 0.030	05.06.98 25.07.	69-72	Whole plant without root ears haulms grain straw straw grain	 1.14 0.07 0.29 <0.05 0.63 <0.05 0.74	 0 20 20 33 33 47 47	RIP 2000-1031

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 50 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Epoxiconazole
Crop / crop group : winter wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (133 g/l)
Kresoxim-methyl (67 g/l)
Residues calculated as : Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 AGR/04/98 NL-5853 EJ Siebengewald 00-01-31	Brigadier	1)15.10.97 2)05.06.-17.06.98 3)07.08.98	0.083 0.085	289 297	0.029	15.05. 17.06.	69	whole plant without roots ears haulms grain straw grain straw grain straw	 0.89 0.14 0.37 <0.05 0.83 <0.05 0.73 <0.05 0.69	 0 20 20 35 35 44 44 51 51	RIP 2000-1031
#BASF 99/11509 ALO/07/98 E-41727 Maribanez 00-01-31	Cartaga	1)03.12.97 2)20.03.-27.03.98 3)End of May	0.085 0.087	299 304	0.029	16.03. 27.03.	69	Whole plant without roots ears haulms ears haulms grain straw	 4.7 0.31 1.50 0.26 1.50 <0.05 2.98	 0 21 21 33 33 42 42	RIP 2000-1031

1	2	3	4			5	6	7	8	9	10
#BASF 99/11509 ALO/08/98 E-41500 Alcala de Guadaira 00-01-31	Cajense	1)10.12.97 2)30.03- 08.04.98 3) End of May	0.086 0.088	300 307	0.029	24.03.98 08.04.	69	Whole plant without root ears haulms ears haulms grain straw	 2.46 0.26 0.89 0.11 0.77 <0.05 1.74	 0 22 22 37 37 42 42	RIP 2000-1031
#BASF 99/11509 D05/02/98 D-24625 Großharrie 00-01-31	Pepital	1)24.09.97 2)12.06.- 22.06.98 3)10.08.98	0.085 0.087	299 303	0.029	18.05.98 22.06.	69-71	Whole plant without root ears haulms ears haulms grain straw grain straw	 1.66 0.14 0.22 <0.05 0.17 <0.05 0.49 <0.05 0.65	 0 21 21 36 36 42 42 49 49	RIP 2000-1031
#BASF 99/11509 FR2/03/98 F-62580 (N-FR) Neuville Saint Vaast 00-01-31	Balthazar	1)08.11.97 2)04.06. - 12.06.98 3)08.08.98	0.085 0.090	297 315	0.029	14.05.98 08.06.	65	Whole plant without root ears haulms ears haulms grain straw grain straw	 1.76 0.18 0.66 0.13 0.26 <0.05 1.35 <0.05 1.48	 0 22 22 32 32 42 42 50 50	RIP 2000-1031
#BASF 99/11509 OAT/01/98 AB30IXJ Grampian Prrbeadlie Farm,Maykirk 00-01-31	Riband	1)02.10.97 2)03.07.- 25.07.98 3)16.09.98	0.088 0.084	307 294	0.029	05.06.98 25.07.	69-72	Whole plant without root ears haulms grain straw straw grain	 1.1 0.07 0.28 <0.05 0.78 <0.05 0.67	 0 20 20 33 33 47 47	RIP 2000-1031

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 50 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 512 00 F
Applicant : BASF AG (BAS)

Active ingredient : Epoxiconazole
Crop / crop group : Durum wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (BAS 500 F)
Residues calculated as : Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 ALO/09/98 E-41500 Alcala de Guadaira 00-01-31	Vitrou	1)08.12.98 2)30.03.-08.04.98 3)End of May	0.093 0.094	304 310	0.030	24.03.98 08.04.	69	whole plant without roots ears haulms ears haulms grain straw	 2.72 0.26 0.87 0.15 0.80 <0.05 0.97	 0 22 22 36 36 42 42	RIP 2000-1031
#BASF 99/11509 FR3/02/98 F-30730 (S-FR) Gajan 00-01-31	Ardenti	1)25.10.98 2)12.05.-23.05.98 3)01.07.98	0.080 0.087	264 287	0.030	17.04.98 05.05.	61	whole plant without roots ears haulms ears haulms	 1.08 0.17 0.42 <0.05 0.28	 0 21 21 35 35	RIP 2000-1031

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 50 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Epoxiconazole
Crop / crop group : Durum wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (BAS 500 F) (133 g/l)
Kresoxim-methyl (67 g/l)
Residues calculated as : Epoxiconazole

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 ALO/09/98 E-41500 Alcala de Guadaira 00-01-31	Vitrou	1)08.12.98 2)30.03.-08.04.98 3)End of May	0.085 0.086	299 301	0.028	24.03.98 08.04.	69	whole plant without roots ears haulms ears haulms grain straw	 2.72 0.26 0.87 0.15 0.80 <0.05 0.97	 0 22 22 36 36 42 42	RIP 2000-1031
#BASF 99/11509 FR3/02/98 F-30730 (S-FR) Gajan 00-01-31	Ardenti	1)25.10.98 2)12.05.-23.05.98 3)01.07.98	0.083 0.086	292 302	0.028	17.04.98 05.05.	61	whole plant without roots ears haulms ears haulms	 1.08 0.17 0.42 <0.05 0.28	 0 21 21 35 35	RIP 2000-1031

Table B.7.6-20: Residues Kresoxim-methyl

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 67 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Kresoxim-methyl
Crop / crop group : winter wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (133 g/l), Epoxiconazol (50 g/l)
Residues calculated as : Kresoxim-methyl

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)		(d)	(e)
#BASF 99/11509 AGR/04/98 NL-5853 EJ Siebengewald 00-01-31	Brigadier	1)15.10.97 2)05.06.-17.06.98 3)07.08.98	0.113 0.116	289 297	0.039	15.05. 17.06.	69	whole plant without roots ears haulms grain straw grain straw grain straw	 1.27 0.09 0.10 <0.05 0.20 <0.05 0.13 <0.05 0.11	 0 20 20 35 35 44 44 51 51	RIP 2000-1031
#BASF 99/11509 ALO/07/98 E-41727 Maribanez 00-01-31	Cartaga	1)03.12.97 2)20.03.-27.03.98 3)End of May	0.117 0.119	299 304	0.039	16.03. 27.03.	69	Whole plant without roots ears haulms ears haulms grain straw	 4.48 0.07 0.27 <0.05 0.22 <0.05 0.15	 0 21 21 33 33 42 42	RIP 2000-1031

1	2	3	4			5	6	7	8	9	10
#BASF 99/11509 ALO/08/98 E-41500 Alcala de Guadaira 00-01-31	Cajense	1)10.12.97 2)30.03- 08.04.98 3) End of May	0.117 0.120	300 307	0.039	24.03.98 08.04.	69	Whole plant without root ears haulms ears haulms grain straw	 2.8 0.05 0.08 <0.05 <0.05 <0.05 0.07	 0 22 22 37 37 42 42	RIP 2000-1031
#BASF 99/11509 D05/02/98 D-24625 Großharrie 00-01-31	Pepital	1)24.09.97 2)12.06.- 22.06.98 3)10.08.98	0.117 0.118	299 303	0.039	18.05.98 22.06.	69-71	Whole plant without root ears haulms ears haulms grain straw grain straw	 2.76 <0.05 <0.05 <0.05 0.38 <0.05 <0.05 <0.05 <0.05	 0 21 21 36 36 42 42 49 49	RIP 2000-1031
#BASF 99/11509 FR2/03/98 F-62580 (N-FR) Neuville Saint Vaast 00-01-31	Balthazar	1)08.11.97 2)04.06. - 12.06.98 3)08.08.98	0.116 0.123	297 315	0.039	14.05.98 08.06.	65	Whole plant without root ears haulms ears haulms grain straw grain straw	 1.67 <0.05 0.09 <0.05 <0.05 <0.05 0.16 <0.05 0.28	 0 22 22 32 32 42 42 50 50	RIP 2000-1031
#BASF 99/11509 OAT/01/98 AB30IXJ Grampian Prrbeadlie Farm,Maykirk 00-01-31	Riband	1)02.10.97 2)03.07.- 25.07.98 3)16.09.98	0.120 0.115	307 294	0.039	05.06.98 25.07.	69-72	Whole plant without root ears haulms grain straw straw grain	 1.48 0.06 <0.05 <0.05 0.08 <0.05 0.05	 0 20 20 33 33 47 47	RIP 2000-1031

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 67 g/l
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 513 00 F

Applicant : BASF AG (BAS)

Active ingredient : Kresoxim-methyl
Crop / crop group : Durum wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : Pyraclostrobin (133 g/l) Epoxiconazole (50 g/l)
Residues calculated as : Kresoxim-methyl

1	2	3	4			5	6	7	8	9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Remarks
	(a)	(b)				(c)		(a)			(d)
#BASF 99/11509 ALO/09/98 E-41500 Alcala de Guadaira 00-01-31	Vitrou	1)08.12.98 2)30.03.-08.04.98 3)End of May	0.117 0.118	299 301	0.039	24.03.98 08.04.	69	whole plant without roots ears haulms ears haulms grain straw	 1.80 <0.05 0.09 <0.05 <0.05 <0.05 <0.05	 0 22 22 36 36 42 42	RIP 2000-1031
#BASF 99/11509 FR3/02/98 F-30730 (S-FR) Gajan 00-01-31	Ardenti	1)25.10.98 2)12.05.-23.05.98 3)01.07.98	0.114 0.118	292 302	0.039	17.04.98 05.05.	61	whole plant without roots ears haulms ears haulms	 0.93 0.06 0.11 <0.05 <0.05	 0 21 21 35 35	RIP 2000-1031

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

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

B.7.7.1 Effects on the nature of residue

Report: Scharf, 1998, RIP 2000-1078

Test system:
To estimate the degradation behaviour of pyraclostrobin during industrial processing or household preparation, different processes (pasteurisation, baking, brewing, boiling and sterilisation) have to be simulated. The amount and nature of products formed during the different processes have to be determined.
The test was performed with both labels (tolyl label and chlorophenyl label). The test substances were dissolved in aqueous buffer solutions of different pH-values. To avoid an influence of light, the glassware was wrapped. In order to simulate the process of pasteurisation, the test solutions were heated for 20 minutes at 90°C. For simulation of baking, brewing and boiling, the test substances were treated in a four-neck, round-bottom flask under reflux at 100°C for 60 minutes. For simulation of sterilisation the box with 5 sample vessels was treated at about 120°C in an autoclave for 20 minutes.

For analysis, aliquots were taken right before starting a test and at the end of the test for LSC-measurements after cooling of the solution. Additionally, aliquots for HPLC-analysis were taken before and at the end of a test.

Findings:
The results are summarised in Table B.7.7-1

Table B.7.7-1: Recovery data after the simulation of processing

Process	**Test conditions**	**Chlorophenyl label** % TAR* after test	**Tolyl label** % TAR* after test
Pasteurisation	pH 4, 90 °C	98.1	103.9
Baking, brewing and boiling	pH 5, 100 °C	110.9	98.1
Sterilisation	pH 6, 120 °C	97.4	96.1

* Total applied radioactivity

By means of HPLC-analysis it could be shown that pyraclostrobin (BAS 500 F) remained unchanged during all the different tests.

Conclusion:
Pyraclostrobin (BAS 500 F) was not degraded neither during the simulation of pasteurisation (pH 4, 90°C) nor during the simulation of baking, boiling, brewing (pH 5, 100°C) or during sterilisation (pH 6, 120°C). Because no degradation occurred, no degradation products were observed.

B.7.7.2 Effects on the residue level

B.7.7.2.1 Residues in grapes

Report: Meumann, 1999, RIP2000-1079

Test system:
During the 1998 growing season 4 field trials, 2 each with varieties of red and white grapes were conducted in different representative wine growing areas in Germany to determine the residue levels of Pyraclostobin (BAS 500 F) and its metabolite BF 500-3 in grapes and grape process fractions (juice, wine, pomace).
The test substance, BAS 500 00 F fungicide, which contains 250 g/l of Pyraclostobin, was applied eight times in spray intervals of 12 days with increasing application rates (0.1 kg as/ha – 0.16 kg as/ha, for details see: Table B.7.7-4).

Fruit samples were collected immediately after the last application as well with a PHI of 35 days. The grapes harvested at normal maturity, were processed into juice, wet pomace, and wine according to standard processing procedures.

The fruits and processing products were analysed using BASF Analytical Method Number D9808 (which is identical with 421/0) at BASF Corporation (USA). The method is based on the determination of Pyraclostobin (BAS 500 F) and its metabolite BF 500-3 using LC/MS/MS. The method quantifies the residue of Pyraclostobin (BAS 500 F) and BF 500-3, with a limit of quantification of 0.02 mg/kg for each analyte.

Findings:
Immediately after the last application, residues of the parent compound were found in the range of 0.43 to 0.96 mg/kg. In the following weeks (35 DALA), these residues decreased to 0.36 to 0.78 mg/kg, with the normal variation for biological material [see Table B.7.7-2].

With the exception of one sample, there were no Pyraclostobin (BAS 500 F) and BF 500-3 residues detected above the limit of quantification (0.02 mg/kg) in all must and wine samples. Residues found in grapes will be concentrated in pomace during processing.

The results of procedural recovery experiments obtained with each analytical set ranged from 71 % to 107 % (averaged 93 %) for Pyraclostobin (BAS 500 F) and 63 % to 104 % (averaged 90 %) for BF 500-3, and the fortification levels were 0.02 and 1.0 mg/kg.

Table B.7.7-2: Residues of Pyraclostobin (BAS 500 F) and BF 500-3 in grape process fractions (wine)

Matrix	Days after last application (DALA)	Pyraclostrobin	Concentration factor
Whole Grapes	0 35	0.43 – 0.96 0.74, 0.76, 0.78, 0.36	
Cold Must	35	< 0.02	0.03
Heated Must	35	< 0.02 (3) 0.04	0.03
Wet Pomace	35	2.86, 2.81, 1.94, 2.00	3.8, 3.7, 2.5, 5.5 (3.9)
Wine from Cold Must	35	< 0.02	0.03
Wine from Heated Must	35	< 0.02	0.03

Conclusion:
The concentration factors for total residues from grape to must and wine were < 1 indicating that residues did not concentrate. Concentration factors in the range of 2.5 to 5.5 (mean value 3.9) were calculated for the transition of residues from the whole grape to pomace.

Report: Wofford, Abdel-Baky, Riley,1999, RIP2000-1080

Test system:
A grape processing study was conducted to determine the distribution of Pyraclostobin (BAS 500 F) residues in grape process fractions. Grape vines at a site in California, one of the principal grape growing regions of the United States, were treated with the test substance BAS 500 00 F. The trial consisted of one untreated control plot (Treatment 1) and three treated plots that received six foliar spray applications of the test substance, BAS 500 00 F fungicide, at 7 day intervals, beginning 49 days prior to harvest.

One treated plot targeted the US proposed label rate per application of 168 g as/ha (corresponding to 0.15 lb as/A, treatment 2, 1X), and the remaining treated plots targeted exaggerated rates of 336 g as/ha (corresponding to 0.30 lb as/A, treatment 3, 2X), and 841 g as/ha (corresponding to 0.75 lb as/A, treatment 4, 5X) to increase the probability that residue would be detected in the samples

Whole grape samples were collected as the Raw Agricultural Commodity (RAC) at normal crop maturity, 14 days after the last application (DALA), and delivered the same day to the local processing facility. Only grapes from the control and 5X plots were harvested and processed according to typical commercial practices. The grape juice processing steps included crushing, destemming, depectinisation, extraction, pasteurising and Argol settling, filtering and canning. The processing steps for raisins included sun drying, destemming, cap stem removal, and washing. After processing, samples were shipped frozen to the BASF Corporation Agricultural Products Center.

Whole grape, juice and raisin commodities were analysed by BASF Analytical Method Number D9808 which is identical with method 421/0. Representative samples from the control plot and 5X treatment were analysed.

Findings:
The total (pyraclostrobin (BAS 500 F) and BF 500-3) average residues in whole grapes, juice and raisins, were 3.11, 0.042 and 9.53 mg/kg, respectively. The residues determined in the grape RAC samples were compared to those of the process fractions in order to determine if residues concentrated as a result of the processing. The results are summarised in Table B.7.7-3.
A summary of the grape residues for the 5X treated plot (application rate 6 x 841 g as/ha) are given in Table B.7.7-3.
The efficiency of the method was determined by fortifying control grapes, juice and raisin samples with Pyraclostobin (BAS 500 F) and BF 500-3. The overall average recovery was 96 ± 11 % (n = 28).

Table B.7.7-3: Residues of Pyraclostobin (BAS 500 F) and BF 500-3 in grape process fractions (juice and raisins)

Matrix	Residue		Concentration factor [1]
	Total	Pyraclostrobin	
Whole Grape	3.11	2.99	
Grape Juice	0.04	0.02	0.013 / 0.007
Raisins	9.53	8.11	3.1 / 2.7

[1)] Concentration Factor = Avarage Residue in Process Fractions / Average Residue in RAC

Conclusion
The concentration factors for total residues from grape to juice was < 1 indicating that residues did not concentrate. The concentration factor for total residues from whole grapes to raisins was 3.1 (2.7 for pyraclostrobin). This concentration could be explained by the loss of water during processing.

Table B.7.7-4: Processing of grapes – summary of residue data –

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 250 g/l
Formulation (e.g. WP) : EC
Commercial product (name) : Pyraclostrobin BASF 500 00 F (004928-00)
Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Grapes
Indoors / outdoors : Outdoor
Other a. i. in formulation (common name and content) : --
Residues calculated as : Pyraclostrobin (metabolite BF 500-3 calculated as Pyraclostrobin)

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF	Spätburgunder	1)1983	0.057	286	0.02	09.06.1998	83	fruit	0.96	0.09	1.05	0	RIP2000-1079
99/10982		2)04.06.-	0.061	308		22.06.		fruit	0.74	0.07	0.81	35	
DU2/02/98		25.06.1998	0062	313		03.07.		cold must	<0.02	<0.02	<0.04	35	
		3)06.10.1998	0.058	293		13.07.		heated must	0.04	<0.02	0.06	35	
Germany-69168			0.100	509		27.07.		wet pomace	2.86	0.38	3.24	35	
Wiesloch			0.117	588		07.08.		wine f. cold must	<0.02	<0.02	<0.04	35	
			0.163	822		20.08.							
00-01-31			0.163	823		01.09.		wine f. heated must	<0.02	<0.02	<0.04	35	
#BASF	Ruländer	1)1986	0.059	298	0.02	16.06.1998	81-83	fruit	0.62	0.04	0.65	0	RIP 2000-1079
99/10982		2)04.06.-	0.061	306		29.06.		fruit	0.76	0.04	0.80	35	
DU2/03/98		20.06.1998	0.060	300		10.07.		cold must	<0.02	<0.02	<0.04	35	
		3)13.10.1998	0.060	300		22.07.		heated must	<0.02	<0.02	<0.04	35	
Germany-69168			0.102	515		05.08.		wet pomace	2.81	0.31	3.12	35	
Wiesloch			0.119	601		14.08.		wine from cold must	<0.02	<0.02	<0.04	35	
			0.161	812		27.08.							
00-01-31			0.159	800		08.09.		wine from heated must	<0.02	<0.02	<0.04	35	

1	2	3	4			5	6	7	8			9	10
#BASF 99/10982 DU3/02/98 Germany-67269 Grünstadt 00-01-31	Portugieser	1)1987 2)10.06.- 22.06.1998 3)04.10.1998	0.066 0.063 0.062 0.062 0.095 0.124 0.162 0.164	333 317 311 311 479 627 815 829	0.02	26.05. 08.06. 19.06. 30.06. 13.07. 24.07. 06.08. 18.08.	81	fruit fruit cold must heated must wet pomace wine from cold must wine from heated must	0.73 0.78 <0.02 <0.02 1.94 <0.02 <0.02	0.11 0.14 <0.02 <0.02 0.27 <0.02 <0.02	0.84 0.93 <0.04 <0.04 2.21 <0.04 <0.04	0 35 35 35 35 35 35	RIP 2000-1079
#BASF 99/10982 DU3/03/98 Germany-67269 Grünstadt 00-01-31	Müller-Thurgau	1)1987 2)10.06.- 22.06.1998 3)01.10.1998	0.060 0.060 0.062 0.059 0.097 0.117 0.161 0.160	304 303 310 298 491 589 810 809	0.02	26.05.1998 08.06. 19.06. 30.06. 13.07. 24.07. 06.08. 18.08.	81	fruit fruit cold must heated must wet pomace wine from cold must wine from heated must	0.43 0.36 <0.02 <0.02 2.00 <0.02 <0.02	0.06 0.04 <0.02 <0.02 0.19 <0.02 <0.02	0.49 0.39 <0.04 <0.04 2.19 <0.04 <0.04	0 35 35 35 35 35 35	RIP 2000-1079
1999/5011 RCN 97137 USA-Tulare County, California 00-01-31	Thompson Seedless	1)1967 2)-- 3)29.08.97	0.839 0.832 0.841 0.844 0.841 0.840	2366 2319 2394 2356 2366 2375	0.035	11.07.1997 18.07. 25.07. 01.08. 08.08. 15.08.	14 days before harvest	Whole grape Grape Juice Raisin	3.60 2.37 0.024 <0.02 7.63 8.58	0.151 0.084 <0.02 <0.02 1.43 1.43	3.75 2.45 0.044 0.040 9.05 10.01	14	RIP 2000-1080

B.7.7.2.2 Residues in barley

Report: Schulz, Scharm, 2000, RIP2000-1082
Schulz, Scharm, 2000, RIP2000-1083

Test system:
The barley processing basic study was conducted to investigate the pyraclostrobin (BAS 500 F) residues in barley grain and in the process fractions involved in beer and pot barley production. Therefore, barley plants at a German test site were treated twice with 2 l/ha (0.5 kg as/ha) BAS 500 01 F each which represents the twofold of the recommended rate in order to increase the probability of finding residues. The applications were carried out in a 14 days interval, starting at 49 days before expected harvest at BBCH growth stage 39. The last application was performed 35 days before expected harvest which is the pre-harvest-interval, at BBCH growth stage 59.
Barley plants were sampled immediately after the last application, ears and haulms were taken 42 days thereafter. Grain was harvested 45 days after the last application at BBCH growth stage 99 which was 10 days after the scheduled time of 35 DALA due to bad weather conditions.

The processing was carried out in pilot plants simulating commercial practice.

Pot barley production was carried out at Bundesforschungsanstalt für Getreide-, Kartoffel- und Fettforschung, Institut für Müllerei- und Bäckereitechnologie (BAGKF), Detmold, Germany. Pot barley is manufactured by gradually removing the hull and outer portion of the barley kernel by abrasive action whereby 7 % - 14 % of the weight of the grain are removed. The material removed is called pearling dust and is needed for the mass balance of the residues.
The malting of barley grain was carried out at Weissheimer Malz, Andernach, Germany. It comprises the steps steeping, germination and kilning after which the product is separated into malt (for brewing) and malt germs (for mass balance only).
The production of beer form the malt obtained was carried out at Binding Brauerei AG, Frankfurt am Main, Germany. After grinding and mashing of the malt and lautering (separation of the spent barley), the wort (extract) was boiled and hop was added. This was followed by separation of the trub, fermentation and maturing. Finally, the beer was filtered and bottled. From this process, the matrices spent grain, trub (flocs), beer yeast and beer were analysed for residues.

In the barley processing follow-up study, barley plants were treated at the same application rate (2 l/ha or 0.5 kg as/ha BAS 500 01 F) and at comparable timing in three trials at different German locations. After the same sampling scheme, the raw agricultural commodities plant without roots, ears, haulms and grain were analysed and the process fractions malt and beer were produced according to the processes mentioned above.
All samples were analysed for residues of pyraclostobin (BAS 500 F) and its metabolite BF 500-3. For the barley raw agricultural commodities such as plant without roots, ears, haulms and grain, BASF method no. 421/0 was used. The process fractions pot barley, pearling dust, malt, malt germs, spent grain, trub (flocs), beer yeast and beer were analysed according to BASF method no. 453/0. Both methods determine the parent compound pyraclostobin (BAS 500 F) and its metabolite BF 500-3 by means of HPLC-MS/MS with a limit of quantitation of 0.02 mg/kg for each analyte.

Findings:

The samples of plant without roots taken directly after the last application showed total residues of pyraclostobin (BAS 500 F) between 11.51 and 16.13 mg/kg. The grain sampled at 45 DALA contained residues of the parent compound between 0.03 and 0.04 mg/kg and of the metabolite BF 500-3 between 0.02 and 0.04 mg/kg. There was no significant difference between the grain analysed at harvest time and the grain stored for processing.

In pot barley, no residues of the parent compound above the limit of quantitation could be seen. The metabolite was present in 0.04 mg/kg. Therefore, no concentration was observed in the edible portion of the pot barley production. In pearling dust, however, consisting of the outer layer of the barley kernel removed during the process and so being a waste product, 0.33 mg/kg pyraclostobin (BAS 500 F) and 0.20 mg/kg BF 500-3 resulting in a total residue of 0.53 mg/kg were found. This leads to a concentration factor 7.57.

In the malt and beer production, the following residue situation was found: The malt contained 0.03 to 0.04 mg/kg pyraclostobin (BAS 500 F) and 0.03 to 0.05 mg/kg BF 500-3. This means a slight concentration with factors ranging between 1.17 to 1.33. In malt germs, pyraclostobin (BAS 500 F) was found in 0.07 mg/kg and BF 500-3 in 0.03 mg/kg. Therefore, the concentration factor was 1.43. The highest residues were present in the spent grain. Here, 0.30 mg/kg pyraclostobin (BAS 500 F) and 0.25 mg/kg BF 500-3 lead to a concentration factor of 7.86. In the trub (flocs) no residues of pyraclostobin (BAS 500 F) but 0.15 mg/kg of BF 500-3 were found. The total residue of 0.17 mg/kg resulted in a concentration factor of 2.43. Also, in beer yeast no residues of pyraclostobin (BAS 500 F) and 0.08 mg/kg of BF 500-3 lead to a total residue of 0.10 mg/kg and a concentration factor of 1.43. The final product beer being the only consumer product of the whole process and therefore the most important matrix did not show any residues of either the parent compound or the metabolite above the limit of quantitation. This also means that no concentration of pyraclostobin (BAS 500 F) residues took place in the final product to be consumed.

The efficiency of the methods was determined by fortification experiments. The average results obtained with method No. 421/0 analysing the RAC were 90% for pyraclostobin (BAS 500 F) and 86% for BF 500-3 (n=16 each). The recovery rates of method No. 453/0 used for the processing fractions averaged at 100% for pyraclostobin (BAS 500 F) (n=20) and 96% for BF 500-3 (n=19).

A summary of the residue results obtained in both the balance and the follow-up study is given in Table B.7.7-5. The concentration factors are summarised in Table B.7.7-6.

Table B.7.7-5: Residues of pyraclostobin (BAS 500 F) and BF 500-3 in barley RAC and process fractions

Study	Portion analysed	DALA [1]	Residues [mg/kg] Pyraclostobin (BAS 500 F)			BF 500-3 [2]			Total Residues3) [mg/kg]		
Raw agricultural commodities											
Basic study	pl. w/o roots	0	13.0			0.45			13.45		
	haulms	42	2.38			1.51			3.89		
	ears	42	0.07			0.07			0.14		
	grain	45	0.04			0.04			0.08		
Follow-up study	Pl. w/o roots	0	11.10	15.60	15.80	0.41	0.53	0.31	11.51	16.13	16.11
	haulms	42	2.34		5.23	1.62		1.81	3.96		7.04
	ears	42	0.13		0.24	0.15		0.23	0.28		0.47
	grain	45	0.03	0.03	0.03	0.03	0.03	0.02	0.06	0.06	0.05
Pot barley fractions											
Basic study	grain [4]	-	0.03			0.04			0.07		
	Pot barley	-	< 0.02			0.04			0.06		
	pearling dust	-	0.33			0.20			0.53		
Beer fractions											
Basic study	grain[5]	-	0.03			0.04			0.07		
	malt	-	0.04			0.05			0.09		
	malt germs	-	0.07			0.03			0.10		
	spent grain [6]	-	0.30			0.25			0.55		
	trub (flocs) [7]	-	< 0.02			0.15			0.17		
	beer yeast [7]	-	< 0.02			0.08			0.10		
	beer	-	< 0.02			< 0.02			< 0.04		
Follow-up study	grain [5]	-	0.03	0.03	0.03	0.03	0.03	0.02	0.06	0.06	0.05
	Malt	-	0.03	0.04	0.03	0.04	0.04	0.03	0.07	0.08	0.06
	beer	-	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.04	<0.04	<0.04

1) days after last application 2) calculated as pyraclostobin (BAS 500 F) equivalents 3) for calculation purposes, < 0.02 mg/kg were set at 0.02 mg/kg 4) before processing 5) stored for beer processing 6) based on dry weight 7) centrifuged sediment

Table B.7.7-6: Concentration factors found after processing

Study	Portion analysed	Pyraclostobin (BAS 500 F) [mg/kg]			Concentration factors [1]		
Pot barley fractions							
Basic study	grain	0.03			1		
	pot barley	<0.02			0.7		
	pearling dust	0.33			11		
Beer fractions							
Basic study	grain	0.03			1		
	malt	0.04			1.3		
	malt germs	0.07			2.3		
	spent grain	0.30			10		
	trub (flocs)	<0.02			0.7		
	beer yeast	<0.02			0.7		
	beer	<0.02			0.7		
Follow-up study	grain	0.03	0.03	0.03	1	1	1
	malt	0.03	0.04	0.03	1	1.3	1
	beer	<0.02	<0.02	<0.02	0.7	0.7	0.7

[1] Concentration Factor = Avarage Residue in Process Fractions / Average Residue in RAC

Conclusion:
In the process fractions obtained from pot barley and beer production such as pearling dust, malt, malt germs, spent grain, trub (flocs) and beer yeast which are all not meant for final consumption, the residues of pyraclostobin (BAS 500 F) show a certain concentration with factors ranging between 1.29 and 7.86. However, in the final products to be consumed such as pot barley and beer, no concentration of pyraclostobin (BAS 500 F) residues is observed expressed by concentration factors <1.

B.7.7.2.3 Residues in wheat

Report: Versoi, Abdel-Baky, Riley,.1999, RIP2000-1084

Test system:
A wheat processing trial was conducted to determine the distribution of pyraclostobin (BAS 500 F) residues in wheat processed fractions. Wheat plants at a site in Washington, one of the principal wheat growing regions of the United States, were treated with the test substance BAS 500 00 F. The trial consisted of one untreated control plot (treatment 1) and three treated plots that received two foliar spray applications of the test substance, BAS 500 00 F fungicide. One treated plot targeted the US proposed label rate per application of 112 g as/ha (corresponding to 0.10 lb as/A, treatment 2, 1X), and the remaining treated plots targeted exaggerated rates of 673 g as/ha (corresponding to 0.60 lb as/A, treatment 3, 3X) and 1120 g as/ha (corresponding to 1.0 lb ai/A, treatment 4, 5X) to increase the probability that residues would be detected in the samples. The first application to all treated plots was made when the flag leaf was just visible on the plants and the second application was made at 50 % head emergence but no less than 40 days before the expected grain harvest.

Wheat grain samples were collected as the Raw Agricultural Commodity (RAC) at normal crop maturity and transported the same day at ambient temperature to the processing facility at Englar Food Laboratories, Inc. in Moses Lake, Washington. Only wheat from the control, 1X, and 5X treated plots were harvested and processed according to typical commercial practices. The wheat processing included screening/cleaning, aspirating, tempering with water, and milling to produce flour, bran, middlings, and shorts. The dry cleaned wheat grain was lightly ground and placed through several screenings to separate wheat germ. After processing, the samples (wheat grain, flour, bran, middlings, shorts, and germ commodities) were analysed according to the analytical method number D9808 (which is identical with the analytical method 421) at BASF Corporation. The method is based on the determination of pyraclostobin (BAS 500 F) and its metabolite BF 500-3 using LC/MS/MS. Representative grain and processed fraction samples from the control plot and two independent replicates from the 5X treatment were analysed.

Findings:
The total (pyraclostobin (BAS 500 F) + BF 500-3) average residues in wheat grain and germ were 0.06 mg/kg and 0.05 mg/kg, respectively. Residues in flour, bran, middlings, and shorts were less than the limit of quantitation (< 0.02 mg/kg). The residues determined in the wheat grain samples were compared to those of the process fractions in order to determine if residues concentrated as a result of the processing. The efficiency of the method was determined by fortifying control grain, flour, bran, middlings, shorts, germ, and aspirated grain fractions with pyraclostobin (BAS 500 F) and BF 500-3. The overall average recovery was 89 ± 17 % (n=30).
A summary of wheat residues for the 5X treated plot are given below (Table B.7.7-7)

Table B.7.7-7: Residues of pyraclostobin (BAS 500 F) and BF 500-3 in wheat process fractions

Matrix	Average residue [mg/kg][1]		Total residue [mg/kg]	Concentration factor[1)]
	Analyte			
	Pyraclostobin (BAS 500 F)	BF 500-3		
Grain	0.035	< 0.02	0.06	1.0
Flour	< 0.02	< 0.02	< 0.04	0.06
Bran	< 0.02	< 0.02	< 0.04	0.06
Middlings	< 0.02	< 0.02	< 0.04	0.06
Shorts	< 0.02	< 0.02	< 0.04	0.06
Germ	0.027	< 0.02	0.05	0.8

[1)] Pyraclostrobin only
[2)] Concentration Factor = Avarage Residue in Process Fractions / Average Residue in RAC

Conclusion:
There are only very low residues of pyraclostrobin to be expected in wheat. In the trials conducted to get grain for processing only values near the limit of quantification were analysed in the RAC. In all commodities except germ no concentration of residues was observed.
In germs, the most lipophilic matrix, residues were detected in the same magnitude as in the raw agriculture commodity.
This result indicate a possible accumulation of pyraclostrobin in oil made from wheat germs. To get more information about this residue behaviour two extra processing studies with wheat should be conducted (follow up studies for wheat germs only).

Table B.7.7-8: Processing of grain – summary of residue data barley and wheat–

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 250 g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 500 01 F (ZA 004928-00)
Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Winter barley
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : --
Residues calculated as : Pyraclostrobin (BAS 500 F) metabolite BF 500-3

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.i. / ha	Water l / ha	kg a.i. / hl	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) BAS 500 F	BF 500 -3	TOTAL	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 99/11826 AT-99/023-0 Germany-65510 Hünstetten-Ketternschwal-bach 00-01-31	Scarlett	1) 26.03.1999 2) 20.06.-05.07.99 3) 30.07.99	0.507 0.519	304 312	0.17	02.06.1999 16.06.	59	pl. w/o roots haulms ears grain <u>Beer Process Fractions:</u> grain brewing malt malt germ spent grain trup beere yeast beer <u>Pearl Barley Process Fractions:</u> grain pearl barley pearling dust	13.0 2.38 0.07 0.04 0.03 0.04 0.07 0.3 <0.02 <0.02 <0.02 0.03 <0.02 0.33	0.45 1.51 0.07 0.04 0.04 0.05 0.03 0.25 0.15 0.08 <0.02 0.04 0.04 0.2	13.45 3.89 0.14 0.08 0.07 0.09 0.1 0.55 0.17 0.1 <0.04 0.07 0.06 0.53	0 42 42 45 -- -- 	RIP 2000-1082

1	2	3	4			5	6	7	8			9	10
#BASF 99/11827 AT-99/022-1 Germany-65510 Hünstetten-Ketternschwa bach 00-01-31	Scarlett	1) 26.03.1999 2) 20.06.-05.07.99 3) 30.07.1999	0.503 0.514	302 308	0.17	02.06.1999 16.06.	59	pl. w/o roots haulms ears grain brewing malt beer	11.1 2.34 0.13 0.03 0.03 <0.02	0.41 1.62 0.15 0.03 0.04 <0.02	11.51 3.96 0.28 0.06 0.07 <0.04	0 42 42 45 -- --	RIP 2000-1083
#BASF 99/11827 AT-99/022-2 Germany-56368 Niedertiefen-bach 00-01-31	Scarlett	1) 15.03.1999 2)14.06.-02.07.99 3) 25.07.1999	0.517 0.497	310 298	0.17	02.06.1999 12.06.	59	pl. w/o roots grain brewing malt beer	15.6 0.03 0.04 <0.02	0.53 0.03 0.04 <0.02	16.13 0.06 0.08 <0.04	0 44	RIP 2000-1083
#BASF 99/11827 AT-99/022-3 Germany-23845 Grabau 00-01-31	Alexis	1) 03.04.1999 2) 16.06.-24.06.99 3) 08.08.1999	0.510 0.490	306 294	0.17	02.06.1999 14.06.	59-61	pl. w/o roots haulms ears grain brewing malt beer	15.8 5.23 0.24 0.03 0.03 <0.02	0.31 1.81 0.23 0.02 0.03 <0.02	16.11 7.04 0.47 0.05 0.06 <0.04	0 44 44 45	RIP 2000-1083

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Content of a.i. (g/kg or g/l) : 251.2 g/l
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 500 00 F (ZA 004928-00)
Applicant : BASF AG (BAS)

Active ingredient : Pyraclostrobin
Crop / crop group : Spring wheat
Indoors / outdoors : outdoors
Other a. i. in formulation (common name and content) : --
Residues calculated as : Pyraclostrobin (BAS 500 F) metabolite BF 500-3

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)			PHI (days)	Remarks
			kg a.i. / ha	Water l / ha	kg a.i. / hl				BAS 500 F	BF 500 -3	TOTAL		
	(a)	(b)				(c)		(a)				(d)	(e)
#BASF 1999/5122 RNC 98208 USA-Ephrata, Grant Co., Washington 00-01-31	Penewawa	1) 10.04.1998 2) -- 3) 30.07.1998	0.22 0.22 0.67 0.67 1.12 1.12	200 202 199 200 201 201	0.11 0.34 0.56	29.05.1998 08.06.	--	grain flour bran middlings shorts germ	0.035 <0.02 <0.02 <0.02 <0.02 0.027	0.02 <0.02 <0.02 <0.02 <0.02 <0.02	0.06 <0.04 <0.04 <0.04 <0.04 0.05	52	RIP 2000-1084

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

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

B.7.8.1 Ruminants

Reports: Schat, Beelen, 1999, RIP2000-1075
Tilting, 2000, RIP2000-1076

Test system:
Fourteen lactating Friesian cows (*Bos taurus*) aged between 4 and 9 years and in the weight range 500 to 650 kg before treatment were used in the study. The feeding was performed at TNO Voeding in Zeist (NL). Animals were selected for use in the study on the basis of health, condition and temperament (suitability for individual bucket unit milking).

Feeding and husbandry:
The animals were housed in a tie-stall with one empty stand between the treatment groups under usual husbandry conditions. The animals were individually fed a commercial concentrate twice daily at milking times. Before being offered the concentrate was soaked with the test substance for 15 to 30 min. All cows received grass silage and water ad libitum per treatment group.

Derivation of delected dose levels:
The feeding levels were estimated by taking into account the preliminary residue data available at the beginning of this study. The following commodities were considered as relevant feeding stuff for Europe: grain and straw from cereals and hay which may origin from the intended use in turf or from a possible early emergency harvest. Due to the low residue in grain it turned out that main contributions to residue intake were cereal hay and straw.
The calculation of feed-intake is shown in Table B.7.8-1. It is based on standard values for feed intake (20 kg feed consumption dry matter basis) and an average body weight of 550 kg as given in the EU residue guideline (EU-document 77031/VI/95 rev.4, 22/7/96). The anticipated residue intake for beef cattle in mg/kg feed (dry matter) was approximately 7 mg/kg. For the USA, a similar calculation was carried out yielding to a residue intake of approximately 7.7 mg/kg feed for dairy cattle.

Therefore, it was concluded that a feeding level of 7.0 mg/kg feed (dry matter) would reflect a realistic 1 x residue situation in feed, leading to feeding levels of 21 mg/kg feed (dry matter) for the 3 x level and 70 mg/kg feed (dry matter) for the 10 x level. These nominal doses corresponded to 140, 420 and 1400 mg pyraclostrobin (BAS 500 F) per cow and day.

Table B.7.8-1: Calcualation of the feed burden for cattle (according to the EC table)

Feed item	% Dry matter	% in diet	Residue level (mg/kg)	Feed burden (mg/kg)
Dairy Cow				
Cereal Straw	86	20	6	1.4
Cereal grain	86	40	0.3	0.14
(Cereal) hay	85	40*	6	2.8
Total diet (mg/kg)				4.3
Beef Cattle				
Cereal straws	86	50	6	3.5
(Cereal) hay	85	50**	6	3.5
Total diet (mg/kg)				7.0

* Cereal hay was considered as part of a cows diet, but an intake of 100 % as given in the EC feed tables for (grass) hay is highly unlikely with respect to the intended uses of pyraclostrobin as fungicide. Therefore, the remaining 40 or 50 % of the diet were used to take a contribution via this pathway into account.

Dose preparation
The test substance was dissolved in maize oil every week. The application formulation contained the test substance at a nominal concentration of 14 mg as/g. The chemical stability and the homogeneity were weekly assessed using one sample of the solution from the first week.

Dose administration
Animals were dosed twice daily. The maize oil suspension (5.5 to 55 ml) was added to 2.0 or 3.0 kg of concentrate ration and fed to the animals during milking. A 55 ml aliquot of maize oil only was similarly added to the ration for control cows. The achieved daily intake in terms of mg/kg body weight is listed in Table B.7.8-2. Taking into account the individual body weights, actual average feed intake (determined groupwise for silage plus 4 kg concentrate), and actual amount of test substance given to the animals, the actual doses can be calculated on a mg/kg/bw, a mg/kg dry feed and a mg/animal and day basis [see Table B.7.8-2].

The dosing period was of four weeks duration (28 days). Three cows in each test group were sacrificed one day after the final dose administration (zero withdrawal). Two cows of dose group T-4 were maintained on basal diet and were sacrificed on days 31 and 37 respectively, *ie* after two and seven days withdrawal.

Table B.7.8-2: Calculation of the actual dose levels

Cow (BASF Number)	Nominal dose: mg/kg feed	Actual dose: mg/animal/day	Actual concentration: mg/kg-bw	Actual concentration*: mg/kg-feed
3590 (1)	0	0	0	0
2070 (2)	0	0	0	0
4540 (3)	0	0	0	0
Average group 1	**0**	**0**	**0**	**0**
8604 (4)	7	134.3	0.22	8.8
3595 (5)	7	134.4	0.22	8.8
3454 (6)	7	134.7	0.21	8.8
Average group 2	**7**	**134.5**	**0.22**	**8.8**
3603 (7)	21	104.4	0.71	27.1
6559 (8)	21	402.1	0.63	27.2
1486 (9)	21	406.0	0.67	27.4
Average group 3	**21**	**403.2**	**0.67**	**27.2**
7022 (10)	70	1343.4	2.19	89.6
3602 (11)	70	1343.2	2.40	89.5
1465 (12)	70	1342.8	2.37	89.5
1804 (13)*	70	1343.2	2.51	89.5
3619 (14)*	70	1347.5	2.53	89.8
Average group 4	**70**	**1344.0**	**2.40**	**89.6**

* Animals from withdrawal group, calculation for dosing period only.

Milking and milk sampling:
All cows were machine milked twice daily into individual bucket units and the milk yield (kg) was recorded. Individual milk samples (4 x 50 g) were taken from each 24-hour milk production form day –3 to termination of the study. Changes in milk yield were considered to be within the normal limits; mean milk production showed a slight overall decline in all groups over the experimental period and no treatment-related group differences were observed.

On day 26 additional five litre samples were taken from the daily bulk milk production of each individual cos. Samples were separated by centrifugation into cream and skimmed milk.

Terminal procedures:
All cows were sacrificed using a “shoot mask” followed by bleading to death. A macroscopic *post mortem* examination was carried out and samples of the following tissues were retained.

Liver: 2 samples
Kidneys: 2 samples
Muscle left thigh

All tissue samples were rinsed with water, placed in polyethylene bags and stored at approximately -20°C. The samples were transferred to TNO Wageningen where they were chopped. Milk and tissues from the treated and the control group were were submitted to BASF for assay.

Findings

Bodyweight
The majority of cows showed a slight weight loss during the course of the experimental period. Bodyweight changes were considered to be within normal limits and no treatment-related group differences were observed.

Residue analysis
Analysis of the samples was carried out with two methods. BASF method 439 was used to determine the amount of pyraclostrobin (BAS 500 F) which can be used as marker compound and the common moiety BASF method 446 was applied to determine residues including metabolites for risk assessment (worst case estimate). The principle of the total method 446 is the cleavage of pyraclostrobin (BAS 500 F) and its metabolites to form hydroxy pyrazoles BF 500-5 and BF 500-8 which can be determined by GC-MS (milk and milk products) or by LC-MS/MS (tissues). The total residues determined according to method 446 are expressed as parent equivalents. The limit of quantification for pyraclostrobin (BAS 500 F) in method 439 is 0.01 mg/kg in milk and milk products and 0.05 mg/kg in tissues. For method 446, the limit of quantification is 0.01 mg/kg for each of both analytes in milk and milk products and 0.05 mg/kg for each of both analytes in tissues.

Residues in whole milk, skimmed milk and cream
No residues were detected in samples from the control group.

As shown in Table B.7.8-3, no pyraclostrobin (BAS 500 F) residues could be detected in the samples, even from the 10 x dose level, except for cream samples where residues in the range of 0.02 mg/kg to 0.04 mg/kg occurred.

Table B.7.8-3: Summary of group mean milk results (parent method)

	Group mean pyraclostrobin (BAS 500 F) residues in milk (mg/kg)			
Day of study	**Group 1 (control)**	**Group 2 1 x**	**Group 3 3 x**	**Group 4 10 x**
-1	< 0.01	< 0.01	< 0.01	< 0.01
1	< 0.01	< 0.01	< 0.01	< 0.01
4	n.a.	n.a.	n.a.	< 0.01
7	n.a.	n.a.	n.a.	< 0.01
10	n.a.	n.a.	n.a.	< 0.01
12	< 0.01	< 0.01	< 0.01	< 0.01
15	n.a.	n.a.	n.a.	< 0.01
18	n.a.	n.a.	n.a.	< 0.01
21	n.a.	n.a.	n.a.	< 0.01
24	n.a.	n.a.	n.a.	< 0.01
27	< 0.01	< 0.01	< 0.01	< 0.01
30	n.a.	n.a.	n.a.	< 0.01
33	n.a.	n.a.	n.a.	< 0.01
35	n.a.	n.a.	n.a.	< 0.01
Skim milk	< 0.01	< 0.01	< 0.01	< 0.01
Cream	< 0.01	< 0.01	< 0.01	0.033

The results obtained with the common moiety method 446 are summarised in Table B.7.8-4. In the 1 x dose group (daily dose of 140 mg/animal), only the cream samples showed residues above the limit of quantification (average: 0.025 mg/kg in cream). In the 3 x dose group (daily dose of 420 mg/animal), the situation is similar, but residues were higher (average in cream 0.037 mg/kg). Residues in milk were close to the limit of quantitation of 0.02 mg/kg with a few samples above that limit.

In the exaggerated 10 x dose group, residues up to 0.18 mg/kg were detected in milk, which mainly consisted of hydroxylated metabolites. As expected, the residue concentrations were higher in cream than in milk or skim milk, but the concentration effect was only moderate. The average total residues in milk (day 27), skim milk and cream were 0.086 mg/kg, 0.068 mg/kg, and 0.195 mg/kg respectively.

It can be seen that in the 10 x group a plateau is reached very soon (after 7 days after beginning of dosing) and the average residue level remains constant at the 0.1 mg/kg level. It drops off rapidly after dosing is stopped.

Table B.7.8-4: Summary of group mean milk (total method)

	Group mean pyraclostrobin (BAS 500 F) residue in milk (mg/kg)			
Day of dosing	**Group 1 (control)**	**Group 2 1 x**	**Group 3 3 x**	**Group 4 10 x**
-1	< 0.02	< 0.02	< 0.02	< 0.02
1	< 0.02	< 0.02	< 0.02	< 0.02
4	< 0.02	< 0.02	< 0.02	0.0928
7	< 0.02	< 0.02	< 0.02	0.100
10	< 0.02	< 0.02	< 0.02	0.0823
12	< 0.02	< 0.02	< 0.02	0.0952
15	< 0.02	< 0.02	< 0.02	0.0964
18	< 0.02	< 0.02	< 0.02	0.1001
21	< 0.02	< 0.02	< 0.02	0.0919
24	< 0.02	< 0.02	< 0.02	0.0834
27	< 0.02	< 0.02	< 0.02	0.0862
30	n.a.	n.a.	n.a.	0.0541*
33	n.a.	n.a.	n.a.	< 0.02**
35	n.a.	n.a.	n.a	< 0.02**
Skim Milk	< 0.02	< 0.02	< 0.02	0.068
Cream	< 0.02	0.025	0.037	0.195

* Individual result cow 13; cow 14 < 0.02 mg/kg

** Individual results cow 14

Residues in tissues (Muscle, liver, kidney and fat)

Residues in tissues were determined according to method 439 for determination of the parent molecule and to LC-MS/MS method 446/1 for the determination of the total residue.

As shown in Table B.7.8-5 , no parent pyraclostrobin (BAS 500 F) was detected in any tissue sample.

Table B.7.8-5: Summary of residue levels of pyraclostrobin (BAS 500 F) in tissues (parent method 439)

Treatment group	Group mean pyraclostrobin (BAS 500 F) residue (mg/kg)			
	Muscle	Liver	Kidney	Fat
1 (Control)	< 0.05	< 0.05	< 0.05	< 0.05
2 (1 x)	< 0.05	< 0.05	< 0.05	< 0.05
3 (3 x)	< 0.05	< 0.05	< 0.05	< 0.05
4 (10 x)	< 0.05	< 0.05	< 0.05	< 0.05
4 (10 x 2 days withdrawal)	< 0.05	< 0.05	< 0.05	< 0.05
4 (10 x 7 days withdrawal)	< 0.05	< 0.05	< 0.05	< 0.05

With the common moiety method, residues could be detected in kidney and liver with highest residues found in liver. In fat and muscle, no residues have been detected at any dose level.
In kidneys, residues were only found in the 10 x group. Average residues in kidney samples were 0.32 mg/kg. In liver, the highest residues were found mainly consisting of hydroxylated metabolites. The following average values were detected: 1 x group = 0.20 mg/kg, 3 x group = 0.52 mg/kg and 10 x group = 2.5 mg/kg. The withdrawal cows (daily dose of 1400 mg/day and animal) showed a rapid decline of residues. After seven days of withdrawal, residues could only be detected in liver (0.5 mg/kg). Therefore, it can be concluded that pyraclostrobin (BAS 500 F) and its metabolites were eliminated rapidly from the animal.

Table B.7.8-6: Summary of residue levels of pyraclostrobin (BAS 500 F) and its metabolites in tissues (total method 446/1)

Treatment group	Group mean pyraclostrobin (BAS 500 F) residue (mg/kg)			
	Muscle	Liver	Kidney	Fat
1 (Control)	< 0.1	< 0.1	< 0.1	< 0.1
2 (1 x)	< 0.1	0.20	< 0.1	< 0.1
3 (3 x)	< 0.1	0.524	< 0.1	< 0.1
4 (10 x)	< 0.1	2.484	0.381	< 0.1
4 (2 days withdrawal)	< 0.1	1.476	0.107	< 0.1
4 (7 days withdrawal)	< 0.1	0.495	< 0.1	< 0.1

Conclusion
A residue transfer study with pyraclostrobin (BAS 500 F) was conducted in cows. The animals were dosed with 7, 21 and 70 mg/kg feed (dry matter) equal to 140, 420 and 1400 mg/animal and day for a period of 28 days. In the dose group relevant under normal agricultural conditions (7 mg/kg feed), no residues could be detected in milk, meat, fat and kidney. and tissues. Low residues of pyraclostrobin (BAS 500 F) metabolites may occur in liver.

In the 10 x group (70 mg/kg feed) residues could be detected, the highest levels occurred in liver.

B.7.8.2 Poultry

A feeding study in poultry is only required,

(1) when significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to poultry,

and

(2) when metabolism studies indicate that significant residues (above the limit of determination) may occur in any edible animal tissue taking into account the residue levels in potential feedingstuffs obtained at the 1 x dose rate.

Only the first prerequisite is fulfilled:

(1) The highest residues in cereal grain, which is the only part of cereals that is fed to poultry, were found in barley (0.3 mg/kg), when barley had been treated according to current GAP (*cf.* point 6.3).

(2) The worst case scenario is the assumption that chicken are fed with 70% barley grains in the total diet. The calculated dose in terms of mg per animal and day is very low (see Table B.7.8-7).

Table B.7.8-7: Calculation of daily intake of pyraclostrobin (BAS 500 F) in poultry

	% dry matter in feed	**Intake of fresh feed**	**Residues in fresh feed**	**Residue intake mg/kg bw/day**
Poultry: bw 1.9 kg, feed intake 0.12 kg				
Grain	86	70 % (0.10 kg)	0.3 mg/kg	0.016
Total:				**0.016**

The metabolism study in laying hens was performed at dose levels of approximately 12 mg per kg feed. This corresponds to ca. 40 x dose rate (see Table B.7.8-8). Based on this overdosing factor, the expected total residues in eggs and edible tissues from chicken can be extrapolated from the total radioactive residues found in the hen metabolism study. This again is a worst case scenario, since total radioactive residues also include non-extractable residues and metabolites not accounted for by the residue analytical method. Nevertheless the extrapolation shows that no residues above the LOQ of the residue analytical method (0.05 mg/kg) are expected (see Table B.7.8-9).

Table B.7.8-8: Calculation of overdosing factor (nominal values)

	Hen metabolism	**Potential intake poultry**
mg/kg body weight	0.76	0.016
mg/kg feed	12	0.3
Overdosing factor: dose in metabolism study / potential intake		
mg/kg body weight		47
mg/kg feed		40

Table B.7.8-9: Potential transfer of residues to poultry tissues and eggs

	Total radioactive residue (mg/kg) from metabolism studies		Extrapolated total residue from actual intake (mg/kg)	
Matrix	Chlorophenyl label	Tolyl label	Based on chlorophenyl label	Based on tolyl label
Eggs	0.026	0.031	0.00065	0.00078
Muscle	0.007	0.009	0.00018	0.00023
Liver	0.317	0.474	0.0079	0.0118
Fat	0.083	0.065	0.0021	0.0021

B.7.8.2.1 Pigs

A feeding study in pigs is only required, if the metabolic pathways differ significantly in pigs as compared to ruminants. This is not expected to be the case, since there was no significant difference found between the metabolic pathways in rats and goats.

B.7.8.3 Storage stability in matrices of animal origin

Report: Tilting, 2000, RIP2000-2042

Test systems
The deep freeze stability of pyraclostrobin (BAS 500 F), and BF 500-10 (a model compound for metabolites showing hydroxylation in the 2 position of the chlorophenyl ring) in animal matrices is currently under investigation over a period of 240 days. Untreated samples of muscle, liver and milk from the cow were fortified with 0.5 mg/kg (0.1 mg/kg in case of milk) pyraclostrobin (BAS 500 F), or a mixture of 0.5 mg/kg (0.1 mg/kg in case of milk) pyraclostrobin (BAS 500 F) and the same amount of BF 500-10. All samples were stored under the usual storage conditions for samples (polyethylene bottles, -20°C). At different intervals, samples were analysed with BASF methods no. 439 and 446.
The average procedural recoveries and their relative standard deviations obtained with BASF method 439 at 0.5 mg/kg or 0.1 mg/kg respectively, were about 89.7 % (+/- 10.3; n= 12) in liver, 90.5 % (+/- 7.1; n = 12) in muscle, and 92.5 % (+/- 5.6; n = 12) in milk.
The recoveries obtained with BASF method 446 for pyraclostrobin (BAS 500 F) were: 81.2 % (+/- 14.1; n = 12) in liver, 80.3 % (+/- 14.9; n = 12) in muscle, and 86.2 % (+/- 18.2; n = 11) in milk.
For BF 500-10 these recoveries were 81.2 % (+/- 10.3; n = 12) in liver, 71.7 % (+/- 12.1; n = 12) in muscle, and 81.1 % (+/- 15.5; n= 11) in milk.

The analytical results used for the stability calculation were corrected for individual procedural recoveries.

Findings
The results after 8 months show no significant decrease in concentration for pyraclostrobin (BAS 500 F) in the analytes for liver, muscle and milk.
The concentrations of BF 500-10 show some degradation during the course of the study.

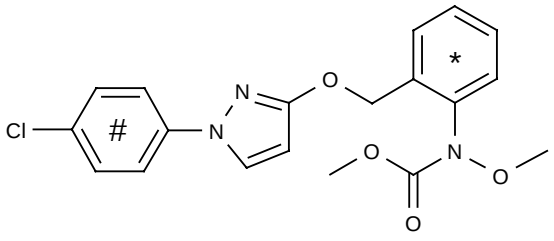
Table B.7.8-10: Summary of results from the storage stability study

Matrix	Days of storage					
	0	**30**	**60**	**90**	**120**	**240**
Average results for pyraclostrobin (BAS 500 F) analysed with method 439 (mg/kg)						
Liver	0.530	0.505	0.486	0.562	0.465	0.422
Muscle	0.505	0.452	0.482	0.460	0.525	0.500
Milk	0.101	0.099	0.097	0.112	0.106	0.104
Average results for pyraclostrobin (BAS 500 F) analysed with method 446 (mg/kg)						
Liver	0.512	0.474	0.525	0.492	0.462	0.423
Muscle	0.531	0.414	0.477	0.513	0.454	0.455
Milk	0.109	0.085	0.125	0.097	0.090	0.074
Average results for BF 500-10 analysed with method 446 (mg/kg)						
Liver	0.509	0.522	0.479	0.482	0.448	0.354
Muscle	0.524	0.433	0.482	0.441	0.439	0.433
Milk	0.119	0.098	0.126	0.103	0.095	0.063

Conclusion
The storage stability of pyraclostrobin (BAS 500 F) in samples of animal origin under deep freeze conditions is proved over a period of 240 days.
BF 500-10 show some degradation during the course of the study but as all samples from the cow feeding study were analysed within 6 month, residues werde sufficiently stable during that period of storage.

B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6; Annex IIIA 8.5)

The residues of pyraclostrobin (BAS 500 F) in succeeding crops were investigated using two different ^{14}C-labels (tolyl- and chlorophenyl-label) of the test substance. The molecular structure and the positions of the labels are shown below:



* tolyl label
\# chlorophenyl label

Since the actual study gives more detailed information, no theoretical consideration of the nature and the level of residues in succeeding crops has been performed.

Report: Veit, 2000, RIP2000-1085

Test system
The residue levels and the nature of the residues in three different succeeding crops were investigated following application of ^{14}C-pyraclostrobin (BAS 500 F) (tolyl and chlorophenyl label). The test compound was applied, as an acetonic solution, to the surface of a bare, loamy sand soil at an application rate equivalent to 900 g as/ha. In addition, the study was also performed with an application rate of 1500 g as/ha to cover to the maximum recommended use rate in the US. In the following only the distribution of the radioactive residues from the use rate relevant for Europe (900 g as/ha) are discussed in detail.

After application, the soil was aged for 30 days (simulating an emergency plant back; 30 DAT), 120 days (simulating a fall plant back; 120 DAT) and 365 days (365 DAT) under natural climatic conditions. After those time intervals, ploughing was simulated by mixing the top layer of 20 cm soil. Afterwards, the following crops were sowed or planted:
radish
lettuce
wheat
Food and feed items of mature crops were harvested, processed and analyzed by combustion and subsequent radioactivity measurement for the determination of the total radioactive residues (TRR) in the raw agricultural commodities (RAC's). In addition, soil samples were taken after application, ploughing and after harvest of mature crops.

The soil characteristic is summarised in Table B.7.9-1.

Table B.7.9-1: Soil used to investigate radioactive residues in succeeding crops

Origin of soil	Landwirtschaftliche Versuchsstation, Li 35 b
Soil type	Loamy sand (German scheme)
% Organic matter	0.8 %
Textural analysis: Sand Silt Clay	 89 % 6 % 5 %
Cation exchange capacity mVal/100 g	5.3
Soil pH	6.4

The total radioactive residues (TRR) of each sample were determined by combustion analysis. All samples were extracted with methanol and, in some cases, an additional water extraction and/ or a subsequent aqueous ammonia extraction were added. The remaining residual radioactive residues were treated with DMSO, sodium hyroxide and/ or different enzymes to release part of the remaining radioactivity. All methanol extracts yielding residues $\geq$0.009 mg/kg and, in addition, the methanol extract of wheat grain with a lower concentration were analysed by high pressure liquid chromatography.

Findings:
The distribution of the total radioactive residues (TRR), the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) in the individual samples are summarised in Table B.7.9-2 and Table B.7.9-3. A comparison to the results of the higher application rate relevant for the US (1500 g as/ha) showed that for most of the plant matrices, no major differences in the residue levels could be detected.

The low residue levels in the crops indicated that only a small portion of the available radioactive residues in the soil were translocated from the soil, through the roots and into the plant.

Table B.7.9-2: Quantitative distribution of radioactive residues in rotational crops after treatment with ^{14}C-pyraclostrobin (BAS 500 F) (tolyl and chlorophenyl label)

Crop parts	TRR	MeOH		ERR ($MeOH + H_2O + NH_3$)		RRR	
Days after sowing / planting DAP	mg/kg	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Plant back interval: 30 DAT							
Tolyl label							
Radish Plant 48	0.025	0.010	39.5	0.010	39.5	0.013	52.5
Radish Roots 48	0.025	0.012	45.9	0.012	45.9	0.011	44.7
Lettuce Head 61	0.013	0.005	42.1	0.005	42.1	0.007	55.3
Wheat Straw 167	0.114	0.019	16.6	0.030	26.0	0.072	63.2
Wheat Grain 167	0.082	0.005	6.5	0.020	24.0	0.060	73.7
Chlorophenyl label							
Radish Plant 47	0.028	0.011	38.8	0.014	49.2	0.007	26.8
Radish Roots 47	0.040	0.018	44.3	0.020	48.8	0.017	43.5
Lettuce Head 60	0.011	0.005	42.3	0.005	45.8	0.004	40.6
Wheat Straw 166	0.112	0.024	21.4	0.030	26.8	0.071	63.2
Wheat Grain 166	0.078	0.003	4.5	0.006	9.0	0.065	84.2
Plant back interval: 120 DAT							
Tolyl label							
Radish Plant 65	0.009	0.003	35.2	0.003	35.2	0.006	67.9
Radish Roots 65	0.008	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Lettuce Head 76	0.011	0.004	37.1	0.004	37.1	0.007	62.1
Wheat Straw 157	0.081	0.009	11.6	0.014	17.4	0.055	68.2
Wheat Grain 157	0.089	0.006	6.3	0.020	22.1	0.064	71.6
Chlorophenyl label							
Radish Plant 64	0.011	0.004	32.1	0.005	37.5	0.004	38.8
Radish Roots 64	0.006	0.003	43.1	0.003	47.4	0.002	36.2
Lettuce Head 75	0.009	0.003	34.8	0.004	43.6	0.004	45.0
Wheat Straw 156	0.079	0.012	15.0	0.015	18.9	0.063	79.9
Wheat Grain 156	0.079	0.005	6.6	0.009	11.7	0.058	72.9

Crop parts Days after sowing / planting DAP	TRR mg/kg	MeOH mg/kg	 % TRR	ERR (MeOH + H_2O + NH_3) mg/kg	 % TRR	RRR mg/kg	 % TRR
Plant back interval: 365 DAT							
Tolyl label							
Radish Plant 48	0.010	0.002	23.6	0.002	23.6	0.004	43.4
Radish Roots 48	0.014	0.006	40.4	0.006	40.4	0.007	48.3
Lettuce Head 62	0.017	0.006	32.3	0.006	32.3	0.007	40.2
Wheat Straw 153	0.067	0.009	12.9	0.014	21.2	0.046	68.8
Wheat Grain 153	0.013	0.001	4.6	0.004	25.6	0.007	53.7
Chlorophenyl label							
Radish Plant 47	0.006	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Radish Roots 47	0.004	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Lettuce Head 61	0.007	0.003	39.6	0.003	39.6	0.003	47.9
Wheat Straw 152	0.069	0.021	30.8	0.021	30.8	0.046	67.0
Wheat Grain 152	0.010	0.001	5.5	0.001	5.5	0.009	94.7

TRR = total radioactive residues
ERR = extractable radioactive residues
RRR = residual radioactive residues
n. d. = not determined

After a plant back interval of 30 days, the highest total radioactive residues were found in wheat straw (0.114/ 0.112 mg/kg tolyl-/ chlorophenyl-label). In wheat grain, the residue levels were lower with a concentration of 0.082/ 0.078 mg/kg tolyl-/ chlorophenyl-label. The lowest residue levels were detected in lettuce head (0.013/ 0.011 mg/kg tolyl-/ chlorophenyl-label). The residue level in radish roots reached a concentration of 0.025/ 0.040 mg/kg tolyl-/ chlorophenyl-label.

After longer plant back intervals, the residue levels in radish roots decreased to 0.008/ 0.006 mg/kg tolyl-/ chlorophenyl-label for a plant back interval of 120 days and to 0.014/ 0.004 mg/kg tolyl-/ chlorophenyl-label for a plant back interval of 365 days. In lettuce head, the residue levels did not change significantly after longer plant back intervals. The residue levels in wheat grain after a plant back interval of 120 days were similar to those after a plant back interval of 30 days. In this matrix, the residue levels decreased significantly after a plant back interval of 365 days to 0.013/ 0.010 mg/kg tolyl-/ chlorophenyl-label. The residue levels in wheat straw declined continously with subsequent plant back periods (120 DAT: 0.081/ 0.079 mg/kg tolyl-/ chlorophenyl-label; 365 DAT: 0.067/ 0.069 mg/kg tolyl-/ chlorophenyl-label).

A MeOH extraction could only release $\leq$45.9 % of the total radioactive residues (TRR), for all matrices. In the dry plant matrices, such as wheat straw and grain, the extractability with MeOH was even lower ($\leq$30.8 % TRR for wheat straw and $\leq$6.6 % TRR for wheat grain).

Extra extraction steps with water and/ or an aqueous ammonia solution released only a very low amount of additional radioactive residues.

The low extractability levels indicated that a portion of the radioactivity remained in the residual radioactive residues (RRR) of the samples under investigation. Because of the overall

low residue levels in radish and lettuce samples, the concentration of the radioactivity in the residual residues were ≤0.017 mg/kg. The residue levels in the non-extractable residues of wheat straw and wheat grain were higher. For wheat straw, the concentration levels in those residues ranged from 0.046 mg/kg - 0.072 mg/kg (≥63.2 % TRR). For wheat grain, the concentration levels ranged from 0.007 mg/kg - 0.065 mg/kg (≥53.7 % TRR); with the lowest concentration after a plant back interval of 365 days.

Residual radioactive residues from radish plant, radish roots and lettuce head were treated with a macerozyme (enzyme mix: cellulase, pectinase and hemicellulase) incubation to release additional radioactive residues. An additional 8.4 - 13.6 % of the remaining radioactivity in the residues of radish roots could be released with this method. These results indicate that a portion of the radioactive residues were connected with natural products, such as cellulose or hemicellulose.

The major part of the radioactivity in the residual residues of wheat straw could be released with extraction and precipitation methods for cellulose and lignin. After plant back intervals of 30 and 120 days, the concentration of radioactive residues in the cellulose fraction ranged from 0.018 mg/kg to 0.020 mg/kg (>17.0 % TRR) and after 365 days this concentration decreased to 0.014/ 0.008 mg/kg (21.2/ 11.6 % TRR) tolyl-/chlorophenyl-label .

Another portion of the radioactivity in the residual residues was found in the lignin fractions (lignin solid: <0.001 - 0.021 mg/kg; lignin liquid: 0.004 - 0.033 mg/kg).
For the residual radioactive residues of wheat grain, additional extractions, precipitation and enzyme incubation steps were added to determine the concentration of radioactive residues in the starch fraction. The radioactive residues in the starch fractions accounted for 0.001 to 0.036 mg/kg for all wheat grain samples. The lowest concentrations were observed after a plant back interval of 365 days. In addition, low concentrations of radioactive residues were detected in the cellulose fraction, in the lignin solid and lignin liquid fractions.

Table B.7.9-3: Total radioactive residues in soil samples after treatment with ^{14}C-pyraclostrobin (BAS 500 F) (tolyl and chlorophenyl label)

Soil samples	Tolyl label TRR [mg/kg]	Chlorophenyl label TRR [mg/kg]
After application		
0 DAT	8.621	9.681
Plant back intervals (after soil aging and ploughing)		
30 DAT	0.315	0.373
120 DAT	0.339	0.351
365 DAT	0.304	0.309
After harvest of mature crops		
Plant back interval: 30 DAT		
Radish	0.273	0.356
Lettuce	0.338	0.371
Wheat	0.347	0.367
Plant back interval: 120 DAT		
Radish	0.300	0.320
Lettuce	0.289	0.310
Wheat	0.260	0.305

Soil samples	Tolyl label TRR [mg/kg]	Chlorophenyl label TRR [mg/kg]
Plant back interval: 365 DAT		
Radish	0.287	0.273
Lettuce	0.191	0.284
Wheat	0.242	0.242

Soil samples analysed after three time periods of soil aging did not show a major decrease of the residue levels after longer time intervals. After harvest of the mature crops, only a slightly decrease in the residue level of the remaining soil could be detected.

Table B.7.9-4: Investigation of the nature of the residues in rotational crops after treatment with ^{14}C-pyraclostrobin (BAS 500 F) (tolyl and chlorophenyl label)

Crop parts DAP	TRR [mg/kg]	RRR [mg/kg] [% TRR]	MeOH [mg/kg] [% TRR]	Parent + Desmethoxy [mg/kg] (% TRR)	Metabolites [combined in regions defined by retention times) [mg/kg] / [% TRR]
Tolyl label					
Plant back interval: 30 DAT					
Radish Plant 48	0.025	0.013 (52.5 %)	0.010 (39.5 %)	0.0011 (4.4 %)	polar region: 0.0021 / 8.1 % medium polar region a: 0.0051 / 20.4 % (3 peaks)
Radish Roots 48	0.025	0.011 (44.7 %)	0.012 (45.9 %)	0.0024 (9.0 %) 0.0002 (0.8 %)	polar region: 0.0078 / 29.9 % medium polar region a: 0.0004 / 1.7 % medium polar region b: 0.0012 / 4.6 % (2 peaks)
Wheat Straw 167	0.114	0.072 (63.2 %)	0.019 (16.6 %)	0.0120 (10.5 %)	polar region: 0.0070 / 6.1 % (2 peaks)
Plant back interval: 365 DAT					
Wheat Straw 153	0.067	0.046 (68.8 %)	0.009 (12.9 %)	0.0010 (1.4 %)	polar region: 0.0022 / 3.2 % medium polar region c: 0.0058 / 8.3 % (2 peaks)

polar region: retention time from $\geq$ 7.40 - $\leq$ 10.11 min
medium polar region a: retention time from $\geq$ 22.25 - $\leq$ 27.30 min
medium polar region b: retention time from $\geq$ 35.60 - $\leq$ 37.53 min
medium polar region c: retention time from $\geq$ 45.36 - $\leq$ 46.88 min

Crop parts DAP	TRR [mg/kg]	RRR [mg/kg] [% TRR]	MeOH [mg/kg] [% TRR]	Parent + Desmethoxy [mg/kg] (% TRR)	Metabolites (combined in regions defined by retention times) [mg/kg] / [% TRR]
Chlorophenyl label					
Plant back interval: 30 DAT					
Radish Plant 47	0.028	0.007 (26.8 %)	0.011 (38.8 %)	0.0103 (36.4 %)	polar region: 0.0001 / 0.3 % medium polar region b: 0.0006 / 2.2 % (2 peaks)
Radish Roots 47	0.040	0.017 (43.5 %)	0.018 (44.3 %)	0.0106 (26.0 %)	polar region: 0.0046 / 11.3 % medium polar region b: 0.0026 / 6.3 % (2 peaks) nonpolar region: 0.0003 / 0.6 % (2 peaks)
Wheat Straw 166	0.112	0.071 (63.2 %)	0.024 (21.4 %)	0.0147 (13.1 %)	polar region: 0.0032 / 2.9 % (2 peaks) medium polar region a: 0.0037 / 3.3 % medium polar region b: 0.0012 / 1.1 % (2 peaks) medium polar region c: 0.0012 / 1.1 %) (3 peaks)
Plant back interval: 120 DAT					
Wheat Straw 156	0.079	0.063 (79.9 %)	0.012 (15.0 %)	0.0011 (1.4 %)	polar region: 0.0027 / 3.4 % medium polar region a: 0.0042 / 5.1 % (4 peaks) medium polar region b: 0.0016 / 2.0 % medium polar region c: 0.0025 / 3.1 %
Wheat Grain 156	0.079	0.058 (72.9 %)	0.005 (6.6 %)	n. d.	polar region: 0.0050 / 6.6 %
Plant back interval: 365 DAT					
Wheat Straw 152	0.069	0.046 (67.0 %)	0.021 (30.8 %)	0.0023 (3.3 %)	polar region: 0.0019 / 2.7 % (3 peaks) medium polar region a: 0.0020 / 2.9 % medium polar region c: 0.0149 / 21.8 % (3 peaks)

polar region:	retention time from ≥ 8.13 - ≤ 12.78 min
medium polar region a:	retention time from ≥ 26.32 - ≤ 29.55 min
medium polar region b:	retention time from ≥ 33.27 - ≤ 37.41 min
medium polar region c:	retention time from ≥ 43.64 - ≤ 47.20 min
nonpolar region:	retention time from ≥ 51.70 - ≤ 64.40 min

The MeOH extractable radioactive residues were characterised by different HPLC methods and in some cases by TLC, provided that the concentration levels were high enough for detection.

Because of the overall low radioactive residues, only the parent compound (^{14}C-pyraclostrobin (BAS 500 F)) and the desmethoxy metabolite (500M07) were identified by co-

chromatography. All the other degradation products were combined in regions defined by retention times and polarity, where each region consisted of one or more peaks (the concentration of individual peaks was very low). For all samples under investigation, a total of 5 different regions could be defined (polar region, medium polar regions a-c and a nonpolar region). Nonpolar metabolites known from soil degradation studies were not observed in the different plant matrices from any of the 3 different plant back intervals. Those metabolites were apparently not translocated from the soil, through the roots to the other parts of the plant.

After a plant back interval of 30 days, a major part of the radioactivity in the extracts of radish roots was detected in the polar region. Parent was found in a concentration range of 0.0024/ 0.0106 mg/kg (tolyl-/chlorophenyl-label). Minor portions of the radioactive residues were found in the polar and in the medium polar regions a and b.

In the extracts of wheat straw after a plant back interval of 30 days, parent was detected at a concentration of 0.0120/ 0.0147 mg/kg (10.5/ 13.1 % TRR) for the tolyl- and chlorophenyl-label. In addition, the polar region and, in case of the chlorophenyl-label, the medium polar regions a and b could be observed.

After a plant back interval of 120 days, the concentration of parent was lower in the extract of wheat straw (chlorophenyl-label: 0.0011 mg/kg or 1.4 % TRR). In addition, the polar region and the medium polar regions a and b could be observed. After a plant back interval of 365 days, the concentration of parent in the extract of wheat straw ranged from 0.0010 to 0.0023 mg/kg (1.4/ 3.3 % TRR; tolyl-/ chlorophenyl-label). In addition, the polar region and the medium polar regions a and c could be observed. The medium polar region c was only found in the wheat samples.
In the case where the MeOH extracts of wheat grain was analyzed, the parent compound could not be detected and the radioactivity was observed in the very polar region. This region included more than one peak.

Metabolic pathway:
For succeeding crops, the proposed metabolic pathway involves demethoxylation, a further degradation to various medium polar and polar metabolites and afterwards, most likely, conjugation reactions and final incorporation and/ or association into natural products, such as starch, cellulose and/ or lignins.
There are no new metabolites in significant amounts identified which are not already known from the metabolism in plants.

Conclusion:
The total radioactive residues in the edible parts of succeeding crops destined for human consumption are very low (radish roots, lettuce: $\leq$ 0.040 mg/kg; wheat grain: $\leq$ 0.089 mg/kg) after all 3 plant back intervals.

There is no accumulation of pyraclostrobin (BAS 500 F) or its degradation products in the parts of plants used for human food or animal feed consumption.
In the case of root vegetables (radish), leafy vegetables (lettuce), the concentration of parent was $\leq$ 0.0106 mg/kg. For wheat straw, the concentration of parent was $\leq$ 0.0147 mg/kg and in wheat grain parent was not detectable.

The levels of individual metabolites present were below 0.01 mg/kg.

Due to the low concentration of pyraclostrobin (BAS 500 F) and its degradation products in succeeding crops, no field trials are required.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post-harvest uses (Annex IIA 6.8; Annex IIIA 8.7)

PHI Grapes: 35 days
PHI Cereals: 35 day (30 days in France and Belgium)
For details see B.7.4 (intended uses).

B.7.11 Community MRLs and MRLs in EU Member States (Annex IIIA 12.2)

There are currently (2001) no harmonised EU MRL's established for pyraclostrobin and no MRL's established in any EU member state.

There are no CODEX MRL's for pyraclostrobin.

B.7.12 Proposed EU MRLs and justification for the acceptability of those residues (Annex IIA 6.7; Annex IIIA 8.6)

Proposed MRL's based on an assessment of the GAP and residue data submitted		
Commodity	**proposed MRL [mg/kg]**	**Data requirements**
grapes	2	none
wheat, rye, triticale	0.1	none
barley, oats	0.2	further trials with barley from southern Europe

B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues

B.7.13.1 Banana

Report: Wofford, Abdel-Baky, Riley, 1999, RIP2000-1030

Material and methods:
In banana, a total of 12 trials were conducted in the major banana-growing regions of Central and South America, such as Costa Rica, Ecuador, Colombia, Martinique, Guatemala and Mexico in 1999. In all trials, the formulation BAS 500 00 F was applied eight times with an application rate of 0.100 kg as/ha each. According to the regional agricultural practice, the

bananas were treated both bagged and unbagged and collected separately. In all trials, samples of whole bananas with peel were taken directly after the last application (0 DALA).
The samples were analysed with BASF method no. 421/0 which quantifies the parent compound pyraclostrobin (BAS 500 F) and its metabolite BF 500-3. The limit of quantitation is 0.02 mg/kg in all sample materials.
The average results of the procedural recovery experiments obtained with each analytical series were about 92% for pyraclostrobin (BAS 500 F) and about 88% for BF 500-3. Fortification levels were between 0.02 mg/kg and 1.0 mg/kg.

Findings:
None of the banana samples taken directly after the last application and treated either bagged or unbagged did show any residue of both the parent substance or its metabolite BF 500-3 above the limit of quantitation.

Conclusion:
On basis of the data submitted an import tolerance for residues of pyraclostrobin on bananas should be set on the limit of determination (0.02 mg/kg*).
In all samples , analysed with and without peel, no residues were detected. This is a surprising result due to the fact that the sampling was done direct after the application (day 0). The notifier has checked the studies again but there was no mistake (field or analysis) to be identified.
(RIP2000-1185)

Table B.7.13-1: Residues of pyraclostrobin (BAS 500 F) and BF 500-3 after application of BAS 500 00 F in banana

CROP	Application				Residues [1) 2)] (mg/kg)					Ref.
Country, year[2)] (trial no.)	Formulation	No	kg as/ha	kg as/hl	Matrix	Day	Pyraclostrobin (BAS 500 F)	BF 500-3	Total residue	Report no.
BANANA										
Costa Rica 1999, (RCN 98292)	BAS 500 00 F	8	0.100	0.46	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Costa Rica 1999, (RCN 98293)	BAS 500 00 F	8	0.100	0.44	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Costa Rica 1999, (RCN 98294)	BAS 500 00 F	8	0.100	0.44	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Ecuador 1999, (RCN 98295)	BAS 500 00 F	8	0.140	0.50	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Ecuador 1999, (RCN 98296)	BAS 500 00 F	8	0.120	0.50	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Ecuador 1999, (RCN 98297)	BAS 500 00 F	8	0.12	0.50	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Colombia 1998/99, (RCN 98298)	BAS 500 00 F	8	0.09	0.43	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Colombia 1998/99, (RCN 98299)	BAS 500 00 F	8	0.09	0.43	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Martinique 1999, (RCN 98300)	BAS 500 00 F	8	0.100	0.36	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Martinique 1999, (RCN 98301)	BAS 500 00 F	8	0.100	0.37	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)

CROP	Application				Residues [1) 2)] (mg/kg)					Ref.
Country, year[2)] (trial no.)	Formulation	No	kg as/ha	kg as/hl	Matrix	Day	Pyraclostrobin (BAS 500 F)	BF 500-3	Total residue	Report no.
Guatemala 1999, (RCN 98302)	BAS 500 00 F	8	0.100	0.45	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)
Mexico 1999, (RCN 98303)	BAS 500 00 F	8	0.108	0.38	banana [3)]	0	< 0.02	< 0.02	< 0.04	RIP2000-1030 (BASF #1999/5095)

1. All residues are expressed as pyraclostrobin (BAS 500 F) equivalents. Residues are not corrected for recoveries
2. Average of duplicate analyses
3. Whole, with peel

B.7.14 Basis for differences, if any, in conclusion reached having regard to established or proposed Codex MRLs

There are currently (2001) no CODEX MRL's for pyraclostrobin.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

B.7.15.1 Chronic Dietary Risk Assessment

The dietary risk assessment is based on an ADI value of 0.04 mg/kg bw/d as proposed in the dossier. Where no MRL is proposed a value of 0.02 mg/kg for food of plant origin is used in the risk calculation

Table B.7.15-1: Assessment of the TMDI – German diet -

TMDI-CALCULATION

Active substance: **Pyraclostrobin**
ADI (mg/kg bw): **0.04**

Mean food consumption (g/d) of a 4 to 6 years old girl

Food	raw[1]	processed[2]	whole	MRL (mg/kg)	Intake (mg/kg bw)
Food of plant origin	105.8	386.2	492.0		
1. FRUITS AND TREE NUTS	72.0	89.3	161.3		
(i) Citrus fruit and citrus juice	18.0	26.5	44.5	0.02	0.00006593
(ii) Tree nuts	1.4	3.8	5.2	0.02	0.00000770
(iii) Pome fruit	13.0	35.6	48.6	0.02	0.00007200
(iv) Stone fruit	8.7	10.7	19.4	0.02	0.00002874
(v) Berries and small fruit3	9.0	8.6	17.6		
a) Table and wine grapes					
Table grapes	6.1	2.6	8.7	2.00	0.00128889
Wine grapes*					
b) Strawberries	1.6	3.2	4.8	0.02	0.00000711
c) Cane fruit	0.4	0.4	0.8	0.02	0.00000119
d) Other small fruit and berries	0.8	2.4	3.2	0.02	0.00000474
e) Wild berries and wild fruit	0.1		0.1	0.02	0.00000015
(vi) Miscellaneous fruit	21.3	3.5	24.8	0.02	0.00003674

Food	raw[1]	processed[2]	whole	MRL (mg/kg)	Intake (mg/kg bw)
2. VEGETABLES	33.0	75.4	108.4	0.02	0.00016059
3. PULSES		1.5	1.5	0.02	0.00000222
4. OIL SEEDS	0.3	11.0	11.3	0.02	0.00001674
5. POTATOES		71.1	71.1	0.02	0.00010533
6. TEA*					
7. HOPS*					
8. CEREALS	0.2	107.8	108.0		
Maize		2.0	2.0	0.02	0.00000296
Other cereals = other cereal products	0.2	105.8	106.0	0.20	0.00157037
9. SPICES (without ginger)	0.1	0.1	0.2	0.02	0.00000030
10. GINGER		0.2	0.2	0.02	0.00000030
11. TEA LIKE PRODUCTS		0.3	0.3	0.02	0.00000044
12. COCOA BEANS		29.4	29.4	0.02	0.00004356
13. SUGAR BEET		0.3	0.3	0.02	0.00000044
Intake whole (mg/kg bw):	0.0034				
Percent of ADI (%):	**8.54**				

Explanations:

1. raw = without any preparation/processing
2. processed = e.g. washed, peeled, cooked, baked, preserves
3. strawberries, cane fruit and other small fruit and berries without wild fruit and wild berries

* Food which is normally not consumed by a 4 to 6 years old girl

Mean food consumption (g/d) of a 36 to 50 years old woman

Wine grapes (wine)		97.6	97.6	2.00	0.00325333
Tea		1.1	1.1	0.02	0.00000037
Hops		4.9	4.9	0.02	0.00000163
Coffee beans (raw)		26.5	26.5	0.02	0.00000883
Intake whole (mg/kg bw):	0.0033				
Percent of ADI (%):	**8.16**				

Table B.7.15-2: Assessment of the TMDI – WHO diet –

TMDI-CALCULATION

Active substance: **Pyraclostrobin**
ADI (mg/kg bw): **0.04**

Mean food consumption in g/d (WHO European diet (1998))

Food	Consumption (g/day)	MRL (mg/kg)	Intake (mg/kg bw)
FOOD OF PLANT ORIGIN	1253.4		
1. FRUITS AND TREE NUTS	287.8		
(i) Citrus fruit	49.1	0.02	0.00001637
(ii) Tree nuts	4.1	0.02	0.00000137
(iii) Pome fruit	51.4	0.02	0.00001713
(iv) Stone fruit	23.3	0.02	0.00000777
(v) Berries and small fruit[1]	121.2		
a) Table and wine grapes	113.8	2.00	0.00379333
Table grapes[3]	16.0		
Wine grapes (wine)[3]	97.8		
b) Strawberries	5.3	0.02	0.00000177
c) Cane fruit	0.5	0.02	0.00000017
d) Other small fruit and berries	1.6	0.02	0.00000053
e) Wild berries and wild fruit		0.02	0.00000000
(vi) Miscellaneous fruit	38.7	0.02	0.00001290
2. VEGETABLES	339.0	0.02	0.00011300
3. PULSES	9.4	0.02	0.00000313
4. OIL SEEDS	28.3	0.02	0.00000943
5. POTATOES	240.8	0.02	0.00008027
6. TEA	2.3	0.02	0.00000077
7. HOPS[2]	4.9	0.02	0.00000163

Food	Consumption (g/day)	MRL (mg/kg)	Intake (mg/kg bw)
8. CEREALS	223.3		
Maize (corn and oil)	10.1	0.02	0.00000337
Rice	11.8	0.02	0.00000393
Oats	2.0	0.20	0.00000667
Barley	19.8	0.20	0.00006600
Rye	1.5	0.10	0.00000250
Wheat	178.1	0.10	0.00029683
Triticale			
Buckwheat			
Millet			
Sorghum			
9. SPICES (without ginger)	0.4	0.02	0.00000013
10. GINGER	0.1	0.02	0.00000003
11. TEA LIKE PRODUCTS		0.02	0.00000000
12. COCOA BEANS	3.1	0.02	0.00000103
13. SUGAR BEET	106.1	0.02	0.00003537
14. COFFEE BEANS	7.9	0.02	0.00000263
FOOD OF ANIMAL ORIGIN	608.7		
(i) Eggs	37.5	0.05	0.00003125
(ii) Milk	342.6	0.01	0.00005710
(iii) Meat	205.3	0.05	0.00017108
(iv) Edible offals	12.6	0.05	0.00001050
(v) Fat	10.7	0.05	0.00000892
Intake whole (mg/kg bw):	0.004757		
Percent of ADI (%):	**11.89**		

Explanations:
1. strawberries, cane fruit and other small fruit and berries without wild fruit and wild berries
2. value from German food consumption
3. 31st session of the CCPR

The values for food of animal origin: LOD for parent compound.

Conclusion
The calculations of the TMDI leads to a low utilisation of the ADI as well with the German diet (child 8.5 % + adult woman 8.2 %, Table B.7.15-1) as with the WHO diet (11.9 %, Table B.7.15-2). It is therefore not necessary to refine the risk assessment (IEDI, NEDI).
A chronic dietary consumer risk is highly unlikely.

B.7.15.2 Acute Dietary Risk Assessment

The acute dietary risk assessment is only necessary in case of grapes because no residues could be detected in bananas and for cereals the residues are low and the variability equals 1.
The acute dietary risk assessment is based on an ARfD value of 0.04 mg/kg bw and the UK-consumption data.
For acute risk assessment only table grapes are relevant. As table grapes are normally grown in Southern Europe only, the highest residue was taken from the results of residue trials conducted in South Europe.

Table B.7.15-3: NESTI calculation – UK, adult -

National estimate of short term intake (NESTI)

Active substance: **Pyraclostrobin**
ARfD (mg/kg bw): **0.04**

Food portion sizes of UK adults aged 16 to 64 years (97.5th percentile)

Food	portion size	unit weight	proc. factor	variab. factor	HR (mg/kg)	Intake (mg/kg) bw	Percent of ARfD (%)
1. FRUITS AND TREE NUTS							
(v) Berries and small fruit							
a) Table and wine grapes							
Table grapes	190.0		1.0	5.0	0.72	0.009758	**24.39**

Table B.7.15-4: NESTI calculation – UK, toddler -

National estimate of short term intake (NESTI)

Active substance: **Pyraclostrobin**
ARfD (mg/kg bw): **0.04**

Food portion sizes of UK toddlers aged 1.5 to 4.5 years (97.5th percentile)

Food	portion size	unit weight	proc. factor	variab. factor	HR (mg/kg)	Intake (mg/kg bw)	Percent of ARfD (%)
1. FRUITS AND TREE NUTS							
(v) Berries and small fruits							
a) Table and wine grapes							
Table grapes	158.0		1.0	5.0	0.72	0.039228	**98.07**

Conclusion
The acute dietary consumer risk assessment shows that the IESTI for children (toddlers) is 98 % of the ARfD for grapes. The figures for adults are smaller: 24 %. This leads to the conclusion that an acute dietary consumer risk is unlikely.

B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)

B.7.16.1 Metabolism in plants

The metabolism in plants has been studied in grapes, wheat and potatoes. The metabolic pattern is similar in all three crop groups. Therefore the metabolism in plants is considered to be proved.
Beside the parent compound which is the main residue in all commodities there is only one metabolite BF 500-3 (500M07) of quantitative significance. Since the residue level of this compound is clearly below the level of the parent compound and BF 500-3 (500M07) is a main metabolite in the animal metabolism too there is no need to include this compound in the residue definition in plants.

Residue definition plant: **Pyraclostrobin**

B.7.16.2 Metabolism in livestock

Metabolism studies were performed on lactating goats and laying hens. BAS 500 F (pyraclostrobin) is intensively metabolised; the parent compound was the main residue in nearly all edible matrices of goat.
In edible matrices of laying hens the parent compound was found in eggs and fat apart from the metabolite BF 500-3 (500M07), that accounted for about 30 - 40 % of the extractable residue in fat. The parent compound was not detected in hen liver.
Due to the low residue levels in edible matrices of hens in relation to the feeding level, no detectable residues have to be expected in poultry in practice.

Residue definition livestock for monitoring purposes: **Pyraclostrobin**

For risk assesment purposes, the following residue definition for **liver** (**except poultry liver**) and **milk fat** only is proposed :
BAS 500 F (pyraclostrobin) and its metabolites analysed as the hydroxy pyrazoles BF 500-5 and BF 500-8, sum expressed as BAS 500 F (pyraclostrobin)

B.7.16.3 Residues in treated crops, proposed MRL's

There is a sufficient number of trials presented to support the GAP's of pyraclostrobin in grapes and wheat (rye, triticale).
For barley (oats) the data set is sufficient for north Europe only. For the use of pyraclostrobin in south Europe there are only three trials supporting the GAP in France.

Proposed MRL's:

grapes	2 mg/kg
wheat, rye, triticale	0.1 mg/kg
barley, oats	0.2 mg/kg
other products of plant origin	0.02 * mg/kg

* indicates lower limit of quantification

B.7.16.4 Residues in food of animal origin, proposed MRL's

On the basis of the metabolism studies on lactating goats and laying hens and the cow feeding study, where no detectable residues of the parent compound in the 1x and 3x dose group were found, MRLs can be proposed at the limit of quantification of the anaytical method.

Proposed MRL's (pyraclostrobin):

Milk	0.01 * mg/kg
other products of animal origin	0.05 * mg/kg

* indicates lower limit of quantification

B.7.16.5 Stability of residues prior to analysis

Plant matrices
The information presented indicates that the residues of Pyraclostrobin are stable over a period of at least 18 month..

Animal matrices
The storage stability of pyraclostrobin (BAS 500 F) in samples of animal origin under deep freeze conditions is proven for a period of 8 month.
Results with compound BF 500-10 (a model compound for metabolites showing hydroxylation in the 2 position of the chlorophenyl ring) indicate a slow degradation, but nevertheless it is stable enough for the submitted feeding study (all samples were analysed within 6 month).

B.7.16.6 Residues in succeeding crops

On basis of a rotational crop study with radiolabelled pyraclostrobin it is not anticipated that residues of pyraclostrobin that exceed the LOQ of 0.02 mg/kg will occur in rotational crops.

B.7.16.7 Estimates of dietary exposure to pyraclostrobin

The chronic dietary consumer risk was assessed using the regional diets of Germany and of WHO.
On basis of the currently intended uses the utilisation of the proposed ADI (0.04 mg/kg bw/d) is low.
(German diet: child 8.5 % + adult woman 8.2 %, WHO diet: 11.9 %).

The acute dietary risk assessment is necessary for grapes only. It leads to an utilisation of the ARfD (0.04 mg/kg bw) of 98% for toddlers and 24% for adults for table grapes.

Neither an acute nor a chronic dietary consumer risk can be identified.

B.7.17 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
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AIIA-6.0	Abdel-Baky, S.; Riley M. E.	1999	Freezer Storage stability of BAS 500 F and BF 500-3 in Various plant matrices including processed commodities. BASF 99/5064 GLP, unpublished RIP2000-1074	Y	BAS
AIIA-6.0	Tilting, N.	2000	Investigation of the Stability of Residues of BAS 500 F (Reg. No. 304428) in Sample Materials of Animal Origin Under usual Storage Conditions. Reg. Doc.# 2000/1017116 GLP, unpublished RIP2000-2042	Y	BAS
AIIA-6.0	Tilting, N.; Knoell, H.-E.	2000	Investigation of the Stability of Residues of BAS 500 F (Reg. No. 304428) in Sample Materials of Animal Origin Under usual Storage Conditions. 2000/1000002 GLP, unpublished RIP2000-1077	Y	BAS
AIIA-6.1	Bross, M.; Mackenroth, C.	1999	The Metabolism of 14C-BAS 500 F (14C-Reg.No.304428) in Potato. 1999/11419 GLP, unpublished RIP2000-1051	Y	BAS

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-6.1	Bross, M.; Mackenroth, C.	2000	Report Amendment No. 1 to Final Report: The Metabolism of 14C-BAS 500 F (14C-Reg.No. 304428) in Potato. 2000/1000048 GLP, unpublished RIP2000-1041	Y	BAS
AIIA-6.1	Hamm, R. T.	2000	Report Amendment No. 1 to Final Report Metabolism of BAS 500 F in Grapes. BASF 2000/1000201 GLP, unpublished RIP2000-1275	Y	BAS
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AIIA-6.1	Reinhard, K.	1999	Metabolism of 14C-BAS 500 F in Wheat. BASF 1999/11137 GLP, unpublished RIP2000-1009	Y	BAS
AIIA-6.1	Reinhard, K.; Mackenroth, C.	2001	Extractabilitiy of 14C-BAS 500 F residues from Wheat and Grape Matrices with Aqueous Methanol (according to Method No. 421/0). 35512; BASF 1999/11700 GLP, unpublished RIP2001-73	N	BAS
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AIIA-6.2	Bross, M.; Tilting, T.	2000	Investigation of the Metabolism of 14C-BAS 500 F in the Goat. 2000/1000004 GLP, unpublished RIP2000-1020	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
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AIIA-6.2	Leibold, E.; Hoffmann, H. D.; Hildebrand, B.	1998	14C-BAS 500 F - Study of the Absorption, Distribution and Excretion after repeated Oral Administration to Layin Hens. BASF 98/10637 GLP, unpublished RIP2000-1021	Y	BAS
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AIIA-6.2	Tilting, N.; Knoell, H.-E.	2000	14C-Validation of Method 446 for the Determination of BAS 500 F (Reg. No. 304428) and its Metabolites in Matrices of Animal Origin. 35907;2000/1000001 GLP, unpublished RIP2001-75	N	BAS
AIIA-6.3	Beck, J.	1999	Study on the residue behavior of BAS 500 00 F, epoxiconazole and kresoxim-methyl in cereals after treatment with BAS 500 01 F, BAS 512 00 F and BAS 513 00 F under field conditions in Belgium, France, Germany, Great Britain, Spain, Sweden and the Netherlands. BASF 99/11509 GLP, unpublished RIP2000-1031	Y	BAS
AIIA-6.3	Beck, J.; Benz, A.; Mackenroth, C.	1999	Study on the residue behavior of Bas 500 F in cereals after treatment with Bas 500 01 F under field onditions in Denmark, France, Germany, Great Britain, Spain and Sweden. BASF 99/11824 GLP, unpublished RIP2000-1072	Y	BAS

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AIIA-6.3	Haughey, D.; Abdel-Baky, S.; Riley , M. E.	2000	The magnitude of BAS 500 F residues in pistachios. Study No. 61661; Reg. No. 1999/5150 GLP, unpublished RIP2000-1526	Y	BAS
AIIA-6.3	Haughey, D.; Abdel-Baky, S.; Riley , M. E.	1999	Magnitude of BAS 500 F residues in strawberries. Study No. 55223; Reg. No. 1999/5140 GLP, unpublished RIP2000-1528	Y	BAS
AIIA-6.3	Haughey, D; Abdel-Baky, S.; Riley , M. E.	1999	The magnitude of BAS 500 F residues in sugarbeets. Study No.55185; Reg. No. 1999/5157 GLP, unpublished RIP2000-1541	N	BAS
AIIA-6.3	Haughey, D.; Abdel-Baky, S.; Riley , M. E.	2000	The magnitude of BAS 500 F residues in pecans. Study No. 61660; Reg. No. 1999/5152 GLP, unpublished RIP2000-1525	Y	BAS
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AIIA-6.3	Schulz, H.	1999	Determination of the residues of BAS 500 F in Grapes following Treatment with BAS 500 00 F under field conditions in Italy 1998. BASF 99/11638 GLP, unpublished RIP2000-1029	Y	BAS
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AIIA-6.3	Versoi, P. L.; Abdel-Baky, S.; Riley , M. E.	2000	Magnitude of BAS 500 F residues in potatoes. Study No.46325; Reg. No. 1999/5148 GLP, unpublished RIP2000-1540	Y	BAS
AIIA-6.3	Versoi, P. L.; Abdel-Baky, S.; Riley , M. E.	2000	Magnitude of BAS 500 F residues in dry field peas. Study No.46591; Reg. No. 1999/5154 GLP, unpublished RIP2000-1537	Y	BAS
AIIA-6.3	Versoi, P. L.; Abdel-Baky, S.; Riley , M. E.	2000	Magnitude of BAS 500 F residues in lentils. Study No.46590; Reg. No. 1999/5159 GLP, unpublished RIP2000-1536	Y	BAS
AIIA-6.3	Versoi, P.L.; Abdel-Baky, S.; Riley , M. E.	2000	The magnitude of BAS 500 F residues in dry bulb and green onions. Study No.46694; Reg. No. 1999/5158 GLP, unpublished RIP2000-1532	Y	BAS
AIIA-6.3	Versoi, P.L.; Abdel-Baky, S.; Riley , M. E.	2000	Magnitude of BAS 500 F residues in radishes. Study No.61658; Reg. No. 1999/5149 GLP, unpublished RIP2000-1531	Y	BAS

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AIIA-6.3	Versoi, P.L.; Abdel-Baky, S.; Riley , M. E.	1999	The magnitude of BAS 500 F residues in red raspberries and highbush blueberries. Study No.46911; Reg. No. 1999/5143 GLP, unpublished RIP2000-1529	Y	BAS
AIIA-6.3	Wofford, J. T.; Abdel-Baky, S.; Riley , M. E.	1999	Magnitude of BAS 500 F residues in peanuts. Study No.98026; Reg. No. 1999/5078 GLP, unpublished RIP2000-1539	Y	BAS
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AIIA-6.3	Wofford, J.T.; Abdel-Baky, S.; Riley , M. E.	1999	Magnitude of BAS 500 F residues in tomatoes. Study No.46694; Reg. No. 1999/5158 GLP, unpublished RIP2000-1533	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
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AIIA-6.4	Schat, B.; Beelen, G. M.	1999	Feeding Study with BAS 500 F (Reg. no. 304428) in Lactating Dairy Cows. 1999/11895 GLP, unpublished RIP2000-1075	Y	BAS
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AIIA-6.5.2	Meumann, H.	1999	Study on the residue behavior of BAS 500 F in grapes and grape process fractions after treatments with BAS 500 00 F under field conditions in Germany. BASF 99/10982 GLP, unpublished RIP2000-1079	Y	BAS
AIIA-6.5.2	Schulz, H.; Scharm, M.	2000	Determination of the residues of BAS 500 F in Barley and processed products following treatment with BAS 500 01under field conditions in Germany. BASF 99/11826 GLP, unpublished RIP2000-1082	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
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AIIA-6.5.2	Wofford,T.; Abdel-Baky, S.; Riley, M. E.	1999	The magnitude of BAS 500 F Residues in Grape Process Fractions. 1999/5011 GLP, unpublished RIP2000-1080	Y	BAS
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Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Pyraclostrobin

B-8: Environmental fate and behaviour

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.8 Environmental fate and behaviour 343

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1) 343
B.8.1.1 Route of degradation 343
B.8.1.1.1 Aerobic conditions 343
B.8.1.1.2 Bound residues 347
B.8.1.1.3 Supplementary studies 348
B.8.1.2 Rate of degradation 354
B.8.1.2.1 Laboratory conditions 354
B.8.1.2.2 Field conditions 357
B.8.1.2.3 Storage stability of soil residues 361
B.8.2 Adsorption, desorption and mobility in soil (Annex IIA 7.1.2, 7.1.3; Annex IIIA 9.1.2) 362
B.8.2.1 Adsorption and desorption 362
B.8.2.2 Mobility in soil 366
B.8.2.2.1 Column leaching studies 366
B.8.2.2.2 Aged residue column leaching 368
B.8.2.2.3 Lysimeter studies or field leaching studies 369
B.8.3 Predicted environmental concentrations in soil (Annex IIIA 9.1.3) 370
B.8.3.1 Notifier's calculation 370
B.8.3.2 Rapporteur's calculation 370
B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2.1, 9.2.3) 373
B.8.4.1 Rate and route of degradation in aquatic systems 373
B.8.4.1.1 Hydrolytic degradation 373
B.8.4.1.2 Photochemical degradation 374
B.8.4.1.3 Biological degradation 378
B.8.4.1.4 Degradation in the saturated zone 391
B.8.5 Impact on water treatment procedures (Annex IIIA 9.2.2) 391
B.8.6 Predicted environmental concentrations in surface water and in ground water (Annex IIIA 9.2.1, 9.2.3) 391
B.8.6.1 Predicted environmental concentration in groundwater (PEGgw) 391
B.8.6.2 Predicted environmental concentration in surface water (PECsw) 392
B.8.6.2.1 Notifiers calculation 392
B.8.6.2.2 Rapporteur's PECsw calculations 393
B.8.6.3 Predicted environmental concentrations in sediment PECsed 396
B.8.7 Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3) 398
B.8.8 Predicted environmental concentrations in air (Annex IIIA 9.3) 399
B.8.9 Definition of the residue (Annex IIA 7.3) 399
B.8.9.1 Soil 399
B.8.9.2 Water 400
B.8.10 References relied on 403

B.8 Environmental fate and behaviour

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1)

B.8.1.1 Route of degradation

B.8.1.1.1 Aerobic conditions

Ebert D, 1998, BOD2000-636 and Ebert D, 1999, BOD2000-637)

GLP: yes
Guidelines: SETAC Europe, US-EPA

The aerobic soil metabolism of [^{14}C]-pyraclostrobin was investigated in a loamy sand soil (Table B.8.1-1). The specific radioactivity was 4.5 MBq/mg with a radiochemical purity > 93% for the tolyl-label and 4.14 MBq/mg with a radioachemical purity of >98% for the chlorophenyl-label. At the beginning of the study with the tolyl-label, the radiochemical purity was determined to be 100% by HPLC. However, with an improved HPLC-system it became obvious that the treatment solution contained some impurity of about 6%, which was identified as "des-methoxy"-pyraclostrobin (BF 500-3). The remaining purity of about 94% was considered to be sufficient for evaluating the behaviour of the substance in soil and the study was continued.

A concentration of 0.33 mg [^{14}C]-pyraclostrobin/kg dry soil was used in both metabolism studies. This is equivalent to the maximum single application rate of 250 g active substance/ha, assuming an equal distribution in the top 5 cm soil layer and a soil density of 1.5 g/cm^3.

The incubation conditions were: aerobic, in the dark, 20°C, 40% maximum water holding capacity. A system with continuous aeration and trapping of volatiles was used.

Table B.8.1-1: Soils used to investigate the degradation and metabolism of pyraclostrobin

	tolyl-label	chlorophenyl-label
Soil designation	Bruch West	Bruch West
Textural class (German scheme)	loamy sand	loamy sand
Textural class (USDA scheme)	sandy loam	sandy loam
Origin	Limburgerhof, Germany	Limburgerhof, Germany
Particle size distribution [%] (German scheme): 0.063 – 2 mm 0.002 – 0.063 mm < 0.002 mm	 62 28 10	 66 21 13
Organic C [%]	1.8	2.0
Microbial biomass [mg C/100g dry soil]	49.9	45.7
CEC [mVal/100g]	13.9	15.6
pH[$CaCl_2$]	7.3	7.5
MWC [g H_2O/100g dry soil]	43	44
FC [g H_2O/100g dry soil], 0.33 bar	19	19
Reference	BOD2000-636	BOD2000-637

Results:

The distribution of radioactivity at different sampling dates is shown in Table B.8.1-2. The average recoveries were 93.6% of the total applied radioactivity (TAR) for the tolyl label and 100.4% TAR for the chlorophenyl label. The mineralization rates of pyraclostrobin to $^{14}CO_2$ were similar for both labels and reached 8 and 11% TAR after 360 days.

Pyraclostrobin was quickly degraded in soil, although it is still detectable in low amounts after 360 days. Already within the first three days, two metabolites appeared which were identified as dimeric structures of the active substance. Two pyraclostrobin molecules react with each other forming an azoxy-like (BF 500-6) and an azo-like (BF 500-7) structure. Both metabolites appear in isomeric forms (cis-trans-isomers). The BF 500-6 reaches a maximum of 16% TAR (chlorophenyl-label, sum of both isomers) after 180 days, whereas the BF 500-7 never exceeded 7% TAR (sum of both isomers). The cis-isomers appeared only in very low amounts and never exceeded 2% TAR. The de-methoxylated pyraclostrobin (BF 500-3) was not considered as a real soil metabolite in this studies, since it was already present in the application solutions. It degraded completely so that it could not be detected at the last two sampling times. With the chlorophenyl-label, very low amounts of BF 500-5 could be detected, indicating that a cleavage of the ether bond took place. The corresponding tolyl moiety could never be found, obviously because the methoxy-amino-carbamate group at the tolyl ring preferably reacts with the humic substances. The structures of the metabolites are given in the proposed metabolic pathway for pyraclostrobin in soil (Figure B.8.1-1).

The amounts of bound residues were rather high and increased steadily to 59% TAR with the tolyl label and to 65% TAR with the chlorophenyl label. The highest amounts of radioactivity were located in the NaOH-insoluble humins and the high-molecular humic acids. When analyzing the fulvic acids by HPLC-chromatography, neither pyraclostrobin nor metabolites could be detected. In the chlorophenyl-label study, the NaOH-extraction resulted in a release of BF 500-5 (maximum 8% TAR), since the treatment of the humic substances with 0.5N NaOH also hydrolyzes the already bound pyraclostrobin derivatives. The binding of pyraclostrobin is supposed to happen via reactions at the methoxy-carbamate group of the tolyl-ring, which is then tightly bound to the humic substances (see also "Anaerobic soil metabolism"). Only the chlorophenyl moiety can be split apart by harsh NaOH-treatment. A "control" extraction of the bound residues with water did not result in any release of BF 500-5.

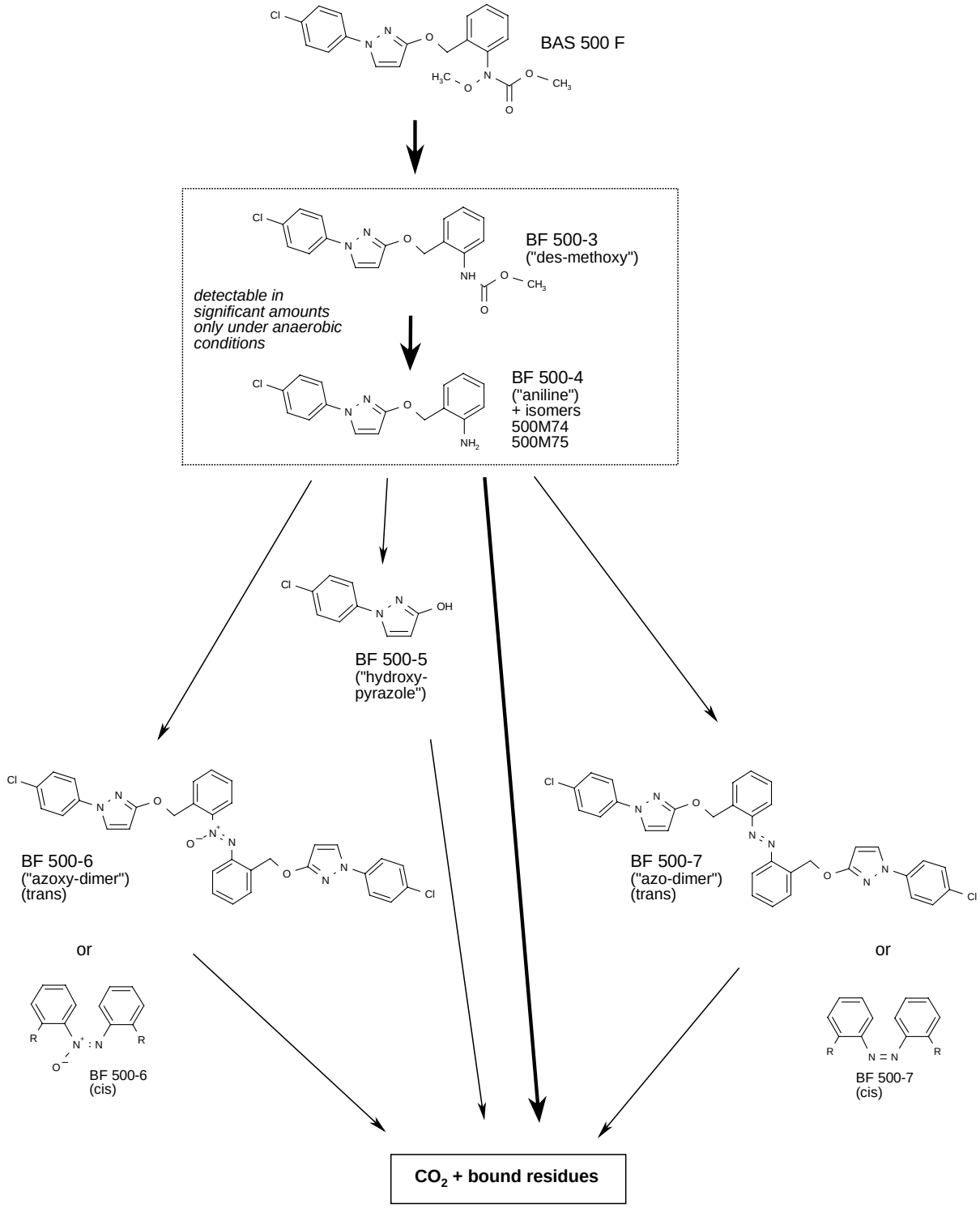
Table B.8.1-2: Recovery of radioactivity in % TAR (total applied radioactivity) and distribution of metabolites after application of [^{14}C]-pyraclostrobin to soil and incubation under aerobic conditions

DAT	$^{14}CO_2$	pyraclostrobin	BF 500-6 * (500M01)	BF 500-7 * (500M02)	BF 500-5 (500M04)	BF 500-3 (500M07)	others	bound residues	total
tolyl-label									
0	ns	95.5	0.0	0.0		6.4	0.0	0.3	102.2
1	0.0	87.8	0.0	0.0		5.1	0.0	3.5	96.4
3	0.0	76.5	1.9	1.0		4.8	0.0	9.6	93.8
7	0.3	61.1	5.1	2.9		3.5	0.9	20.9	94.7
14	0.6	48.9	9.3	3.8		2.9	0.9	35.7	102.1
33	1.4	24.1	9.7	5.8		1.3	0.9	47.3	90.5
60	2.9	16.7	10.0	5.1		0.6	0.9	55.9	92.1
87	4.0	10.6	10.6	5.1		1.3	1.2	54.3	87.1
180	5.6	7.7	11.6	5.1		0.6	0.6	57.9	89.1
270	6.2	5.5	10.3	5.5		0.0	1.9	57.9	87.3
360	8.0	4.5	10.9	4.8		0.0	1.3	59.2	88.7
chlorophenyl-label									
0	ns	100.4	0.0	0.0	0.0	1.9	0.0	0.6	102.9
1	ns	91.0	1.9	0.6	0.0	0.9	0.0	7.2	101.6
3	ns	80.7	3.1	0.0	0.0	1.6	0.0	12.8	98.2
7	0.3	69.8	6.9	2.8	1.5	1.2	0.0	23.1	105.6
14	0.6	49.2	8.1	2.2	2.8	0.9	1.2	34.6	99.6
31	2.2	28.3	11.1	4.0	2.8	0.9	2.5	53.0	104.8
59	4.0	15.9	13.1	5.6	2.5	0.6	3.1	57.6	102.4
91	5.0	12.8	15.8	6.8	0.9	0.3	2.2	56.1	99.9
180	7.8	8.1	15.9	6.2	1.5	0.3	1.6	58.9	100.3
270	9.7	5.9	15.2	5.6	0.3	0.0	1.8	58.9	97.4
360	10.9	4.3	14.2	5.3	0.0	0.0	1.8	65.4	101.9

ns no sample

- sum of cis- and trans-isomer

Figure B.8.1-1: Proposed route of degradation of pyraclostrobin in soil



B.8.1.1.2 Bound residues

Ebert D, 1999, BOD2000-644

Since the degradation of pyraclostrobin in soil leads to the formation of high amounts of bound residues (max. 65% TAR within 360 days), a study was designed to investigate the possible release of soil-bound residues by earthworms and the potential toxicity of the bound residues for these soil-living animals.

GLP: yes
Guidelines: none

For this study, the same soil type as used in the metabolism study was treated with [^{14}C]-tolyl-labelled pyraclostrobin to form high amounts of bound residues. The sandy loam had an organic carbon content of 1.7%, a pH of 7.5, a maximum water holding capacity of 39 g H_2O/100 g dry soil, and a microbial biomass of 63.9 mgC/100g dry soil. The specific radioactivity of the active substance was 4.62 MBq/mg, the radiochemical purity was >98%. The soil was treated with a concentration of 0.6 mg/kg, which corresponds to the twofold of the maximum single recommended field application rate. After 132 days, about 60%TAR were bound to the soil matrix. The soil was extracted to remove the remaining, still extractable pyraclostrobin and metabolites. The extracted soil was mixed with fresh viable soil and adjusted to 70% MWC to make it palatable for earthworms. Then earthworms (*Eisenia foetida*, adult, minimum weight 250 mg, less than 1 year old) were kept up to 42 days in this soil. Soil samples (1 control without earthworm, 2 replicates with earthworms) were investigated for extractable and bound radioactivity. The extracts were analysed by HPLC.

Results:
The results of the soil analysis are shown in Table B.8.1-3. A release of soil-bound residues of pyraclostrobin by earthworms could not be observed. Also the peak pattern of chromatograms of the soil extracts was almost identical in soils with and without earthworms. This shows that pyraclostrobin is bound in the soil very tightly to the humic substances and cannot be released even by digestive processes of soil-eating animals.

Table B.8.1-3: Concentration of ^{14}C-pyraclostrobin equivalents in soil with and without earthworms after various sampling times

days		total radioactivity		extractable radioactivity		bound residues		material balance	
		[mg/kg] [1]	[%] [2]	[mg/kg] [1]	[%] [2]	[mg/kg] [1]	[%] [2]	[mg/kg] [1]	[%] [2]
0		0.285	100.0	0.011	3.9	0.277	97.2	0.288	101.1
7	control I	0.272	95.4	0.013	4.6	0.272	95.4	0.285	100.0
	E. foetida I	0.275	96.5	0.014	4.9	0.272	95.4	0.286	100.4
	E. foetida II	0.278	97.5	0.014	4.9	0.271	95.1	0.285	100.0
14	control I	0.274	96.1	0.011	3.9	0.263	92.3	0.274	96.1
	E. foetida I	0.278	97.5	0.011	3.9	0.258	90.5	0.269	94.4
	E. foetida II	0.278	97.5	0.011	3.9	0.262	91.9	0.273	95.8
21	control I	0.276	96.8	0.011	3.9	0.263	92.3	0.274	96.1
	E. foetida I	0.263	92.3	0.013	4.6	0.243	85.3	0.256	89.8
	E. foetida II	0.287	100.7	0.013	4.6	0.263	92.3	0.276	96.8
28	control I	0.276	96.8	0.012	4.2	0.259	90.9	0.271	95.1
	E. foetida I	0.268	94.0	0.014	4.9	0.271	95.1	0.285	100.0
	E. foetida II	0.273	95.8	0.014	4.9	0.260	91.2	0.274	96.1
42	control I	0.274	96.1	0.014	4.9	0.258	90.5	0.272	95.4
	E. foetida I	0.269	94.4	0.016	5.6	0.249	87.4	0.265	93.0
	E. foetida II	0.271	95.1	0.015	5.3	0.253	88.8	0.268	94.0

[1] mg/kg dry weight
[2] 100% = 0.285 mg/kg

B.8.1.1.3 Supplementary studies

B.8.1.1.3.1 Anaerobic soil metabolism

Kellner O, 1999, BOD2000-641 and BOD2000-642

GLP: yes
Guidelines: SETAC Europe, BBA, IV, 4-1

Although anaerobic conditions are not to be expected to exist under application conditions for pyraclostrobin, results from anaerobic soil metabolism studies are submitted as supporting information.

The anaerobic degradation was investigated with the tolyl- and with the chlorophenyl-labelled test compound. The application rate was 0.33 mg as/kg dry soil. The specific radioactivity was 4.46 MBq/mg with a radiochemical purity > 98% for the tolyl-label and 4.32 MBq/mg with a radiochemical purity of >96% for the chlorophenyl-label.

The soil was incubated in a test apparatus flushed continuously with nitrogen. A trapping system for volatiles was connected.The incubation conditions were: anaerobic (soil flooded with water), in the dark, temperature 20 °C.

Table B.8.1-4: Soils used to investigate the metabolism and degradation of pyraclostrobin under anaerobic conditions

	tolyl-label	chlorophenyl-label
Soil designation	Bruch West 97/060/02	Bruch West 98/060/01
Textural class (German scheme)	loamy sand	loamy sand/silty sand
Textural class (USDA scheme)	sandy loam	loamy sand
Origin	Limburgerhof, Germany	Limburgerhof, Germany
Particle size distribution [%] (German scheme): 0.063 – 2 mm 0.002 – 0.063 mm < 0.002 mm	 72 18 10	 78 17 5
Organic C [%]	1.5	1.4
Microbial biomass [mg C/100g dry soil]	38.3	40.3
CEC [mVal/100g]	12.1	13
pH[$CaCl_2$]	7.5	7.2
MWC [g H_2O/100g dry soil]	40	44
FC [g H_2O/100g dry soil], 0.33 bar	16.3	16.1

Results:

The metabolic profile of pyraclostrobin under anaerobic conditions is shown in Table B.8.1-5. Concerning low mineralisation to CO_2 and formation of high amounts of bound residues, the degradation pathway under anaerobic conditions seems to be similar to the degradation pathway under aerobic conditions. However, the reduced amount of oxygen in the soil seems to slow down the reactions at the de-methoxylated carbamate group of the tolyl-ring. Therefore, the de-methoxylated pyraclostrobin (BF 500-3) was produced in the anaerobic soil in high amounts. BF 500-3 is formed immediately after applying the pyraclostrobin to the anaerobic soil. Then the further reaction to BF 500-4 ("aniline") and its isomers leads slowly to the formation of bound residues. It is supposed that the pathway which leads to bound residues via the "aniline" BF 500-4 is the same as under aerobic conditions. However, under aerobic conditions the binding reaction occurs too fast to be able to detect this short-lived intermediate.

The cleavage of the ether bond results in the formation of the metabolite BF 500-5 (chlorophenyl-label). The corresponding tolyl-moiety could not be detected, obviously due to the fast binding to the soil matrix. This also explains the higher amounts of bound residues with the tolyl label compared to the chlorophenyl label.

Table B.8.1-5: Recovery of radioactivity in %TAR and distribution of metabolites after application of [^{14}C]-pyraclostrobin to soil and incubation under anaerobic conditions. HPLC results.

DAT	CO_2	pyraclostrobin	BF 500-3 (500M07)	BF 500-4 (500M73)	BF 500-5 (500M04)	500M74 *	500M75 *	others	bound residues	total
tolyl-label										
0	0.0	95.4	2.9	0.0		0.0	0.0	1.4	0.3	100.0
7	0.0	8.7	95.8	0.0		0.0	0.0	0.0	7.0	111.5
14	0.0	3.8	84.8	5.6		1.1	0.0	1.2	10.1	106.6
28	0.0	1.2	73.5	5.4		2.4	2.9	2.7	18.5	106.6
62	1.0	0.0	40.4	11.1		5.4	11.4	0.6	40.6	110.5
90	1.7	0.0	37.1	7.6		4.4	10.5	0.5	46.2	108.0
120	2.1	0.0	31.7	1.8		2.8	9.0	0.2	60.8	108.4
chlorophenyl-label										
0	0.0	95.2	2.5	1.1	0.0	0.0	0.0	0.9	0.3	100.0
3	0.0	39.4	52.6	3.0	0.9	0.0	0.0	1.4	2.7	100.0
7	0.0	13.1	68.7	4.9	1.3	0.0	0.6	1.1	5.3	95.0
14	0.0	3.6	79.9	7.0	0.9	0.9	0.7	0.5	9.2	102.7
28	0.0	0.2	63.0	6.9	4.4	2.6	2.0	0.1	20.8	100.0
63	0.0	0.0	45.2	5.7	7.5	5.5	3.9	1.0	30.3	99.1
88	0.0	0.0	39.2	4.4	7.1	7.3	3.6	1.9	37.4	100.9
120	0.0	0.0	36.4	3.4	7.7	6.9	3.1	0.0	37.4	94.9

- isomers of BF 500-4; aminogroup at tolyl ring in meta or para position: exact position of aminogroup could not be assigned to one of the two peaks

B.8.1.1.3.2 Soil photolysis

Scharf J, 1999, BOD2000-640

GLP: yes
Guidelines: SETAC Europe, US-EPA

For the soil photolysis the same soil type was used as for the aerobic soil metabolism. During the study, three different batches of this soil type were used.

The soil photolytic degradation of ^{14}C-pyraclostrobin was studied using ^{14}C-pyraclostrobin labeled in the chlorophenyl ring and the tolyl ring. To examine the influence of soil moisture, studies were conducted at 40% maximum water holding capacity (MWC) and 80% MWC. The study at 40% MWC was conducted using both ^{14}C-labels and the study at 80% MWC was conducted using only the tolyl label. Dark control samples were incubated at identical soil moisture levels. The specific radioactivity of the test substance was 4.62 MBq/mg with a radiochemical purity > 98% for the tolyl-label and 4.32 MBq/mg with a radioachemical purity of >96% for the chlorophenyl-label.

The soil was filled into small metal dishes with a surface area of 34 cm^2. Each soil layer had a thickness of 1 cm. Ten dishes were arranged in a thermostated bowl. The bowl had an air inlet and an air outlet, was airtight covered with a quartz glass cap and was continuously flushed

with air. A trapping system for volatiles was connected to the air outlet of the bowl. The soil of every dish was treated with about 50 µg [^{14}C]-pyraclostrobin/g dry soil. This corresponds to a field application rate of 250 g as/ha assuming an equal distribution in the top 1 cm soil layer.

The bowl was placed under a xenon lamp (SUNTEST apparatus) with a light intensity of about 3 mW/cm^2. Wavelengths below 290 nm were filtered off to simulate natural sunlight. The duration of the experiment was 15 days with continuous irradiation. A dark control was treated in the same way but without irradiation.

Results:

In soil incubated at 40% MWC, no major metabolites could be found (Table B.8.1-6 and Table B.8.1-7). In the irradiated soil samples, the dimeric metabolites BF 500-6 and BF 500-7 were formed in trace amounts (maximum 2% TAR). The concentration of BF 500-3 (desmethoxy metabolite) increased from 2 to 6% TAR (average of both labels). The same metabolites were formed in the dark control soil but their amounts were different. BF 500-6 and BF 500-7 amounted to maximum concentrations of 6.4% TAR (15 DAT), and 5% TAR (12 DAT), respectively (average of both labels). BF 500-3 was already present in the 0 DAT-samples and its concentration did not change significantly (average about 2% TAR). It can be assumed that the dimeric metabolites are primarily formed microbially, whereas the de-methoxylation to BF 500-3 mainly occurs through abiotic reactions which seem to be enhanced in the irradiated soil.

The formation of bound residues was comparable with and without irradiation (average 12% TAR). The mineralisation rate was generally low (maximum 2% TAR).

The higher soil moisture of 80% MWC (Table B.8.1-8) led to an accelerated degradation of pyraclostrobin and, therefore, to an accelerated formation of the known metabolites and bound residues. In the irradiated soil, BF 500-6 and BF 500-7 amounted to 5.2 and 4.8% TAR, respectively, after 15 days of continuous irradiation. BF 500-3 reached a maximum amount of 9% TAR after 12 days. In the dark control soil, BF 500-6 and BF 500-7 were formed with concentrations of 15.5% TAR (BF 500-6) and 8.3% TAR (BF 500-7). Only under this incubation conditions (high soil moisture, dark), BF 500-6 reached more than 10% TAR during the whole study. BF 500-3 was present in traces in all samples (< 2% TAR). Bound residues were formed in rather high concentrations of 34.9% TAR (with light), and 24.3% TAR (without light).

Table B.8.1-6: Recovery and distribution of radioactivity in % TAR during soil photolysis of [^{14}C]-pyraclostrobin, tolyl-label, 40 % MWC

DAT	pyraclostrobin	BF 500-3 (500M07)	BF 500-6 (500M01)	BF 500-7 (500M02)	bound residues	CO_2
irradiation						
0	91.8	1.8	0.0	0.0	0.1	n.a.
2	89.2	2.3	0.0	0.0	3.1	0.2
6	81.0	3.9	0.7	0.2	8.5	0.6
9	69.6	4.0	2.0	1.9	11.3	0.8
12	76.4	4.2	0.0	0.1	10.5	1.1
15	74.4	4.1	0.3	0.3	12.2	1.3
B.8.1.1.3.2.1.1	dark control					
0	91.8	1.8	0.0	0.0	0.1	n.a.
2	93.1	1.4	0.8	0.6	2.7	0.1
6	84.4	1.5	2.4	2.0	6.1	0.2
9	78.9	1.5	4.2	2.6	7.5	0.3
12	73.8	1.2	4.1	3.7	9.9	0.4
15	74.8	1.0	4.3	2.6	10.5	0.4

n.a. not analysed

Table B.8.1-7: Recovery and distribution of radioactivity in % TAR during soil photolysis of [^{14}C]-pyraclostrobin, chlorophenyl-label, 40 % MWC

DAT	pyraclostrobin	BF 500-3 (500M07)	BF 500-6 (500M01)	BF 500-7 (500M02)	bound residues	CO_2
irradiation						
0	93.2	2.3	0.0	0.0	0.2	n.a.
2	81.2	4.6	0.0	0.0	3.9	0.4
6	71.7	5.5	0.3	0.2	8.5	1.0
9	67.9	4.9	0.5	0.4	9.8	1.1
12	73.7	5.5	0.5	0.4	9.2	1.5
15	63.6	8.0	0.5	0.4	12.3	1.8
B.8.1.1.3.2.1.2	dark control					
0	93.2	2.3	0.0	0.0	0.2	n.a.
2	82.4	3.1	0.6	1.0	1.9	0.0
6	73.6	3.0	4.6	2.5	7.1	0.1
9	65.4	2.3	6.9	4.0	8.5	0.2
12	57.1	2.7	9.7	6.3	9.1	0.2
15	63.0	2.0	8.5	4.7	13.3	0.2

n.a. not analysed

Table B.8.1-8: Recovery and distribution of radioactivity in % TAR during soil photolysis of [^{14}C]-pyraclostrobin, tolyl-label, 80 % MWC

DAT	pyraclostrobin	BF 500-3 (500M07)	BF 500-6 (500M01)	BF 500-7 (500M02)	bound residues	CO_2
irradiation						
0	97.6	1.8	0.0	0.0	0.1	n.a.
2	72.9	7.3	1.8	1.7	8.9	0.3
6	47.5	7.7	3.3	2.9	21.1	0.9
9	42.9	8.7	3.8	4.3	26.0	2.7
12	44.8	9.0	2.5	3.6	24.0	1.9
15	29.2	6.1	5.2	4.8	34.9	2.3
dark control						
0	97.6	1.8	0.0	0.0	0.1	n.a.
2	82.6	1.7	2.9	1.7	4.8	0.1
6	53.8	1.3	9.1	5.4	13.3	0.3
9	55.3	1.3	10.1	6.9	16.8	0.4
12	42.5	0.7	14.1	7.5	20.6	0.6
15	38.7	0.7	15.5	8.3	24.3	0.9

n.a. not analysed

Summary and conclusions (route):

The behaviour of pyraclostrobin after application to soils is characterised by a rather low mineralisation rate and the formation of high amounts of bound residues.

A first step during degradation is the de-methoxylation of the methoxy-carbamate group. The des-methoxy-pyraclostrobin (BF 500-3) is transformed to the corresponding aniline (BF 500-4). In presence of oxygen, BF 500-4 reacts very fast either with another pyraclostrobin molecule (forming the metabolites BF 500-6 or BF 500-7) or with the humic substances (leading to high amounts of bound residues). Under natural aerobic conditions, these reactions are that fast that neither BF 500-3 nor BF 500-4 can be detected in soil. Under strong anaerobic conditions, the reactions are slowed down and BF 500-3 and BF 500-4 become detectable in soil extracts.

Since the methoxy-carbamate group at the tolyl ring is completely degraded before binding to the humic substances, a possible release of the active substance itself from the bound residues by degradation processes of the humic substances or by activity of soil eating animals (earthworms) can be excluded.

A further (minor) degradation reaction is the cleavage of the ether bond which results in the formation of the metabolite BF 500-5. This metabolite degrades further to CO_2 or reacts also with the humic soil matrix.

Under the influence of light (soil photolysis), the same metabolites could be detected as in the metabolism studies performed in the dark, however, only in amounts < 10% TAR.

B.8.1.2 Rate of degradation

B.8.1.2.1 Laboratory conditions

Ebert D, 1999, BOD2000-638

GLP: yes
Guidelines: BBA IV, SETAC Europe

Four different soils were treated with a concentration of 0.33 mg [^{14}C]-tolyl-pyraclostrobin/kg dry soil. This corresponds to a field application rate of 250 g as/ha, assuming an equal distribution in the top 5 cm soil layer and a soil density of 1.5 g/cm^3. The specific radioactivity of the test substance was 4.46 MBq/mg, the radiochemical purity was > 98%. The soil characteristics are given in Table B.8.1-9.

The incubation conditions were: aerobic, in the dark, at 20 °C, 40% maximum water holding capacity. The soil extracts were analysed by radio-HPLC.

Table B.8.1-9: Soils used to investigate the degradation rate of pyraclostrobin

Soil designation	Lufa 2.2 96/736/04	Li35 b 96/145/03	US 771-15	Canadian soil
Textural class (German scheme)	loamy sand	loamy sand	n.d.	n.d.
Textural class (USDA scheme)	loamy sand	loamy sand	loamy sand	loam
Origin	Speyer, RP, Germany	Limburgerhof, RP, Germany	Holly Springs, NC, USA	Minto, Manitoba, Canada
Particle size distribution [%] (German scheme)				
0.063 – 2 mm	85	82	n.d.	n.d.
0.002 – 0.063 mm	10	10		n.d.
< 0.002 mm	5	8	n.d.	n.d.
			n.d.	
(USDA scheme)				
0.05 – 2 mm	86	84		49
0.002 – 0.05 mm	9	8	83	36
< 0.002 mm	5	8	5	15
			12	
Organic C [%]	2.1	1.0	0.5	3.0
Microbial biomass [mg C/100g dry soil]	45.9	32.0	17.3	51.0
CEC [mVal/100g]	12.2	3.6	4.5	33
pH[$CaCl_2$]	5.4	6.5	5.6	7.7
MWC [g H_2O/100g dry soil]	36	30	35	43
FC [g H_2O/100g dry soil], 0.33 bar	13.6	9.5	6.6	33

n.d. not determined

Results:
The findings are summarised in Table B.8.1-10. The metabolites found in all four soils are the same as also identified in the metabolism studies. BF 500-3 never exceeded 2% TAR in any of the soil types. BF 500-6 appeared in all soils as a major metabolite. In three soils it acounted to 14-17% TAR. Only in one German soil it reached a maximum of 31% TAR. BF 500-7 was < 10% TAR in three soils and slightly exceeded 10% TAR only in the US-soil.

Table B.8.1-10: Distribution of pyraclostrobin and metabolites during soil degradation of [^{14}C]-tolyl-labelled pyraclostrobin, concentrations according HPLC-results (values in % TAR)

	DAT	pyraclostrobin	BF 500-6 (500M01)	BF 500-7 (500M02)	BF 500-3 (500M07)	total extractable residues
Lufa 2.2 *	0	101.5	0.0	0.0	0.0	101.5
	3	99.2	0.0	0.0	0.5	99.7
	7	97.1	0.0	0.0	0.0	97.1
	14	85.7	3.1	0.2	0.5	89.5
	34	72.0	7.3	0.6	0.9	80.8
	63	56.5	13.2	1.3	1.5	72.5
	92	53.1	13.9	0.8	1.4	69.2
	120	47.2	15.3	1.1	1.2	64.8
Li 35 b *	0	100.8	0.0	0.0	0.0	100.8
	3	97.9	0.0	0.0	0.0	97.9
	7	91.4	1.3	0.8	0.5	94.0
	14	89.2	0.0	0.0	0.6	89.8
	30	59.0	14.8	5.5	0.3	79.6
	62	42.3	23.2	4.5	0.6	70.6
	90	50.5	12.9	3.1	0.0	66.5
	120	21.6	30.9	7.2	0.6	60.3
US 771-15 *	0	101.0	0.0	0.0	0.0	101.0
	3	89.3	2.8	2.4	0.6	95.1
	7	77.6	4.4	5.3	0.8	88.1
	14	70.9	5.4	8.1	0.5	84.9
	30	50.9	10.1	11.5	0.7	73.2
	62	40.0	12.8	12.5	0.4	65.7
	90	39.3	11.7	10.9	0.5	62.4
	120	26.6	13.8	11.3	0.4	52.1
Canadian soil **	0	99.0	0.0	0.0	0.7	99.7
	3	84.5	0.0	0.0	0.3	84.8
	7	86.9	1.7	0.4	0.2	89.2
	14	79.0	2.5	0.9	0.0	82.4
	30	70.7	3.0	0.0	0.0	73.7
	60	61.5	4.2	0.5	0.0	66.2
	90	48.3	13.3	1.4	0.0	63.0
	120	38.3	16.7	4.0	0.0	59.0

* the values for BF 500-6 (sum of both isomers) include the values for the cis-isomer of BF 500-7 (maximum <1%TAR), which could not be separated from the trans-isomer of BF 500-6

** BF 500-6 and BF 500-7 = sum of both isomers; separation of cis BF 500-7 and trans BF 500-6 could be achieved

Summary and conclusions (rate):

The degradation rates for pyraclostrobin in various soils and under different incubation conditions are shown in Table B.8.1-11.

The DT_{50}-values for pyraclostrobin under standard incubation conditions (20°C, 40% MWC) were in the range of 12 – 101 days. Higher soil moisture generally accelerates the degradation, whereas lower soil moisture slows it down. Photolysis does not have significant influence on the disappearance time. At cold temperatures (5°C) and under sterile conditions, almost no degradation took place, most likely due to the reduced soil microbial activity under these conditions.
Anaerobic soil conditions lead to a fast de-methoxylation of pyraclostrobin, resulting in DT_{50}-values of 2 - 3 days and DT_{90}-values of 5 - 9 days.

Table B.8.1-11: DT50/DT90 values for pyraclostrobin in laboratory soil studies

Doc.#	label	soil	study duration [days]	temp. [°C]	moisture [%MWC]	DT_{50} [days]	DT_{90} [days]
aerobic soil metabolism							
BOD2000-636	tolyl	Bruch West	360	20	40	12	143
BOD2000-637	chlorophenyl	Bruch West	360	20	40	14	152
aerobic soil degradation (DT_{50}/DT_{90})							
BOD2000-638	tolyl	Lufa 2.2	120	20	40	101	n.c.
		Li35b	120	20	40	50	163
		US 771-15	120	20	40	38	n.c.
		Canadian	120	20	40	85	n.c.
		Lufa 2.2	120	20	20	137	n.c.
			120	5	40	n.c.	n.c.
			120	30	40	86	n.c.
		Lufa 2.2 (sterile)	129	20	40	n.c.	n.c.
soil photolysis							
BOD2000-640 irradiated	tolyl	Bruch West	15	22	40	42	n.c.
			15	22	80	9	n.c.
	chlorophenyl	Bruch West	15	22	40	32	n.c.
Dark control	tolyl	Bruch West	15	22	40	41	n.c.
			15	22	80	10	n.c.
	chlorophenyl	Bruch West	15	22	40	22	n.c.
anaerobic soil metabolism							
BOD2000-641	tolyl	Bruch West	120	20	flooded	2	5
BOD2000-642	chlorophenyl	Bruch West	120	20	flooded	3	9

n.c. not calculated (degradation time > twofold of study duration)

The DT_{50}-values for metabolites are calculated from data obtained from the study with the active substance. They are shown in Table B.8.1-12. Where possible, also degradation values of metabolites which were formed in amounts <10% TAR were calculated.

Table B.8.1-12: DT50/DT90 of pyraclostrobin soil metabolites in laboratory studies (incubation conditions 20°C, 40 % WMC if not stated otherwise)

metabolite	Doc #	study	label	soil	maximum % TAR	DT_{50} [days]	DT_{90} [days]
BF 500-6	BOD2000-636	aerobic soil metabolism	tolyl	Bruch West	12	129	428
	BOD2000-637	aerobic soil metabolism	chlorophenyl	Bruch West	16	166	552
	BOD2000-638	aerobic soil degradation (DT_{50}/DT_{90})	tolyl	Lufa 2.2 Li35b US 771-15 Canadian	15 31 14 17	131 n.c. 70 n.c.	n.c. n.c. 231 n.c.
				Lufa 2.2, 5°C Lufa 2.2, 30°C Lufa 2.2, 20%MWC	< 3 < 10 11	n.c. 69 n.c.	n.c. 229 n.c.
BF 500-7	BOD2000-636	aerobic soil metabolism	tolyl	Bruch West	6	112	372
	BOD2000-637	aerobic soil metabolism	chlorophenyl	Bruch West	7	159	529
	BOD2000-638	aerobic soil degradation (DT_{50}/DT_{90})	tolyl	Lufa 2.2 Li35b US 771-15 Canadian	1 7 14 4	n.c. n.c. 38 n.c.	n.c. n.c. 126 n.c.
				Lufa 2.2, 5°C Lufa 2.2, 30°C Lufa 2.2, 20%MWC	< 1 < 3 < 2	n.c. n.c. n.c.	n.c. n.c. n.c.
BF 500-3	BOD2000-641	anaerobic soil	tolyl	Bruch West	96	60	200
	BOD2000-642	metabolism	chlorophenyl	Bruch West	80	70	231
BF 500-4	BOD2000-641	anaerobic soil	tolyl	Bruch West	11	8	26
	BOD2000-642	metabolism	chlorophenyl	Bruch West	7	8	25
500M74	BOD2000-641	anaerobic soil	tolyl	Bruch West	5	20	65
	BOD2000-642	metabolism	chlorophenyl	Bruch West	7	53	177
500M75	BOD2000-641	anaerobic soil	tolyl	Bruch West	11	44	146
	BOD2000-642	metabolism	chlorophenyl	Bruch West	4	22	71
BF 500-5	BOD2000-642	anaerobic soil metabolsim	chlorophenyl	Bruch West	8	12	39

MWC maximum water holding capacity
n.c. not calculated, no parameter estimation possible, see original values in Table B.8.1-10
TAR total applied radioactivity

B.8.1.2.2 Field conditions

The laboratory rate studies showed that under certain conditions the DT_{50}-value of pyraclostrobin in soil can be greater than 60 days. This is the trigger value given by EEC Directive 91/414 amended by EC Directive 95/36 that requires the performance of field soil dissipation studies.

Kellner O., Zangmeister W, 1999, BOD2000-647
Kellner O, Zangmeister W, 1999, BOD2000-648

GLP: yes

Guidelines: BBA IV, 4-1, Setac Europe

Two field soil dissipation studies have been performed to investigate the degradation and dissipation of pyraclostrobin in soil and to determine the concentrations of the metabolites BF 500-3, BF 500-6 and BF 500-7. In total, 6 trials were conducted: 3 trials at locations in Germany, 2 trials in Spain and 1 trial in Sweden.

The geographical distribution of the trial locations and the soil parameters of the fields are given in Table B.8.1-13.

Table B.8.1-13: Characterisation of fields in field soil dissipation studies with pyraclostrobin

Doc.#	trial no.	location (postal code)	soil type	soil properties	
				% organic C	pH
BOD2000-647	D05/02/97	Germany Großharrie (24625)	loamy sand	1.1	6.2
BOD2000-647	D08/01/97	Germany Bad Sassendorf Lohne (59505)	loamy silt	2.5	6.8
BOD2000-647	DU2/02/97	Germany Meckenheim (67149)	loamy sand	0.8	5.6
BOD2000-648	ALO/01/98	Spain Manzanilla (21890)	sandy loam/ loamy sand	0.6	7.6
BOD2000-648	ALO/02/98	Spain Alcala del Rio (41200)	sandy loam	0.9	7.6
BOD2000-648	HUS/02/98	Sweden Bjärred (23791)	loamy sand	1.4	5.8

The six locations were distributed over Northern, Central and Southern Europe. A range of soils with organic carbon contents from 0.6 to 2.5% with a pH range from 5.6 to 7.6 was covered. Nevertheless, hydrolytical degradation of pyraclostrobin within this pH range is of no importance.

The trials were performed using the formulated product BAS 500 01 F, a typical formulation for the end use product of pyraclostrobin in cereals (concentration of active substance 250 g/l) with the highest single application rate of 250 g as/ha. The applied product, the nominal application rates and the verified recoveries with an application verification method are given in Table B.8.1-14.

Table B.8.1-14: Application rate verification for pyraclostrobin in field soil dissipation studies

Doc.#	trial no.	formulation	target rate [g as/ha]	recovery [% of applied amount]
BOD2000-647	D05/02/97	BAS 500 01 F	250	81
BOD2000-647	D08/01/97	BAS 500 01 F	250	88
BOD2000-647	DU2/02/97	BAS 500 01 F	250	83
BOD2000-648	ALO/01/98	BAS 500 01 F	250	67
BOD2000-648	ALO/02/98	BAS 500 01 F	250	76
BOD2000-648	HUS/02/98	BAS 500 01 F	250	77

The mean value is close to 80%, although the values are not corrected for the analytical recoveries of the method.

The formulated product was always broadcast sprayed onto uncropped (bare) soil with knapsack sprayers and an attached sprayboom. Samples (soil cores) were taken up to about 1 year after application with at least 7 samplings.

The soil cores were analysed with BASF method no. 432 which determines soil residues of pyraclostrobin and the soil metabolites BF 500-3, BF 500-6 and BF 500-7 down to a limit of determination of 0.01 mg/kg dry soil.

Before compiling the field soil dissipation rates of pyraclostrobin in a summary table (Table B.8.1-14), one detailed residue table (Table B.8.1-15Table B.8.1-15) for trial ALO/01/98 in Spain is presented. The general characteristics of the degradation of pyraclostrobin in field soils can be demonstrated with these results as an example.

Table B.8.1-15: Field soil dissipation results from one trial in Spain; Study EU/FA/049/98, trial ALO/01/98: summary results

soil depth [cm]	days after application	pyraclostrobin [mg/kg]
0 – 10	0	0.167
10 – 25	0	0
0 – 10	14	0.041
10 – 25	14	0
0 – 10	30	0.024
10 – 25	30	0
0 – 10	60	0.024
10 – 25	60	0
0 – 10	98	0.016
10 – 25	98	0
25 – 50	98	0
0 – 10	182	0
10 – 25	182	0
25 – 50	182	0
0 – 10	349	0
10 – 25	349	0
25 – 50	349	0

(0 means below limit of determination)

The results for the other five trials are similar. All trials show that pyraclostrobin degrades fast, with an initially very high degradation rate. Furthermore, pyraclostrobin does not show any tendency to move into deeper layers of soil. It was only detected in the top 10 cm soil layer. Almost no metabolites of pyraclostrobin were found in soil above the limit of quantification. Only in one trial, BF 500-6 was found sporadically.
The transformation parameters of pyraclostrobin presented here were estimated with the mathematical program ModelMaker (A. Walker, N. Crout 1997: Modelmaker User Manual, Version 3.03/3.0.4, Cherwell Scientifc Publishing Limited, Oxford). The DT_{50} and DT_{90}-values were determined by the use of compartment models.* They are summarised in Table B.8.1-16.

Table B.8.1-16: Degradation rates of pyraclostrobin in field dissipation studies

Reference BASF Reg.Doc.#	trial no.	location	DT_{50} [days]	DT_{90} [days]	r^2
BOD2000-647	D05/02/97	Germany	25	83	0.997
BOD2000-647	D08/01/97	Germany	37	122	0.999
BOD2000-647	DU2/02/97	Germany	26	85	0.91
BOD2000-648	ALO/01/98	Spain	8	117	0.99
BOD2000-648	ALO/02/98	Spain	2	230	0.99
BOD2000-648	HUS/02/98	Sweden	31	103	0.92

The DT_{50}-values were always much less than 3 months and the DT_{90}-values were much less than 1 year. Therefore, the degradation behaviour of pyraclostrobin is sufficiently characterised by the field soil dissipation studies. No additional soil accumulation studies are required.

*** Rapporteur's comment:**
The above listed DT50 and DT90 values were recalculated by the Rapporteur using the Timme and Frehse Modell considering the 1st order kinetics.

Results:

Table B.8.1-17: Pyraclostrobin, DT50 and DT50 values calculated according to 1st order kinetics

		1st order		
Reference	trial	DT-50 (d)	DT-90 (d)	r^2
BOD2000-647 BASF Reg. Doc. #1999/11292	DO5/02/97	30.59	101.6	0.7900
	DO8/01/97	**34.39**	114.2	0.9367
	DU2/02/97	14.64	48.6	0.8403
BOD2000-648 BASF Reg. Doc. #1999/11301	ALO/01/98	19.52	64.8	0.8153
	ALO/02/98	85.59	not extr.	0.8165
	HUS/02/98	31.22	103.7	0.8352

With an exception of trial ALO/02/98, the DT50 and DT90 values represent the best fit.

DT50 of 34.4 d can therefore be considered as reasonable worst case value for further calculations (e.g. PEC).

B.8.1.2.3 Storage stability of soil residues

Bayer H, 1999, BOD2000-650

GLP:yes
Guidelines: IVA-Guideline for residue chemistry part II, US-EPA

Ebert D, 1999, BOD2000-637
GLP: yes
Guidelines: SETAC Europe, US-EPA

Soil samples were stored generally at less than – 18°C in a freezer. Therefore, two approaches were used to determine the storage stability of pyraclostrobin residues in frozen soil. The first approach was to fortify control samples of soil with pyraclostrobin and the second approach was to reanalyse radioactive samples from the soil metabolism study.

In the first approach, the stability of pyraclostrobin in fortified and frozen soil samples was shown for up to 2 years.

In the second approach, the stability of pyraclostrobin and its soil metabolites BF 500-6 and BF 500-7 was demonstrated over a period of 15 months. The extractablility and the peak pattern for the freshly worked up sample and the stored sample proved to be the same.

Additionally, storage stability studies with the soil metabolites BF 500-3, BF 500-6 and BF 500-7 with fortified control samples are in progress.

Summary and conclusions (rate, field):
Pyraclostrobin degraded in soil under laboratory conditions with DT_{50}-values from 12 to 137 days. The DT_{50}-values under outdoor field conditions are considerably shorter with a mea value of 26.1 days (1^{st} order). DT_{90}-values of pyraclostrobin in some of the laboratory studies could not be determined, because the incubation period did not cover the DT_{90} range. Under field conditions, the DT_{90}-values were determined with values from 49 to 114 days (mean of 86.6 d). Soil accumulation studies are not required, because the DT_{90}-values of the field dissipation studies was much less than 1 year.
The degradation rates of the soil metabolites of pyraclostrobin could be sufficiently determined by laboratory metabolism and rate studies. Under field conditions, the metabolites BF 500-3 and BF 500-7 were never detected and BF 500-6 was only sporadically found in minor quantities.
The field soil dissipation studies further demonstrate that pyraclostrobin does not have any leaching potential into deeper soil layers.

B.8.2 Adsorption, desorption and mobility in soil (Annex IIA 7.1.2, 7.1.3; Annex IIIA 9.1.2)

B.8.2.1 Adsorption and desorption

Ziegler G, 1998, BOD2000-651

GLP: yes
Guidelines: OECD 106, US-EPA, Subdivision N, 163-1

Adsorption and desorption of **pyraclostrobin** were measured using a batch equilibrium procedure to determine the K_d- and K_{oc}-values of [chlorophenyl-U-^{14}C]-labelled pyraclostrobin in three German soils, two US soils and one Canadian soil. Details of the soils used are provided in Table B.8.2-1. The soil water ratio was 1:5. The test temperature was 22 °C.

Table B.8.2-1: Soils used to investigate adsorption and desorption of [chlorophenyl-U-^{14}C]labelled pyraclostrobin

Soil designation	type (German scheme)	organic carbon [%]	pH value ($CaCl_2$)	particle size distribution			CEC [MVal/ 100 g soil]	MWC [g H_2O/ 100 g dry soil]	FC* [g H_2O/ 100 g dry soil]
				< 2 µm (clay)	2 – 63 µm (silt)	63-2000 µm (sand)			
Li 35 b Limburgerhof, Germany	sand	0.8	6.4	5	7	88	5.3	27	8.6
LUFA 2.2 Speyer, Germany	loamy sand	1.9	5.6	3	11	86	8.3	38	14.1
Bruch West Limburgerhof Germany	sandy loam	1.8	7.3	10	28	62	13.9	43	19.0
USA 538-30-5	loamy sand	0.5	5.9	5	10	85	3.1	23	n.d.
USA 538-31-2	sandy loam	0.6	5.3	9	45	46	10.2	34	n.d.
CAN-95024/ RCN 95012	sandy loam	3.9	7.6	18	29	53	39.1	47	n.d.

n.d. not determined
* determined at 0.33 bar

Results:

A summary of the results obtained can be found in Table B.8.2-2. The adsorption process for pyraclostrobin in the investigated concentration range of 0.008 – 1.0 µg/ml can be described with a high degree of accuracy using the Freundlich equation. The adsorption constants K_d calculated from the Freundlich isotherms for the six test soils range from 30 to 368. K_{oc}-values of 6000 – 16000 were obtained.

A subsequent determination of the desorption in two steps with 0.01 M $CaCl_2$-solution resulted for six soils in K_{desI}-values from 49 to 800 and in K_{desII} values from 51 to 681.

Table B.8.2-2: Adsorption and desorption of [chlorophenyl-U-^{14}C] pyraclostrobin in different soil types

soil designation	textural class	adsorption K_d [ml/g]	adsorption 1/n	adsorption K_{oc} [ml/g]	desorption K_{des} [ml/g]	
Li 35 b	sand	60	0.896	7500	87*	96**
LUFA 2.2	loamy sand	304	1.025	16000	160*	374**
Bruch West	sandy loam	142	1.012	7889	136*	278**
USA 538-30-5	loamy sand	30	0.861	6000	49*	51**
USA 538-31-2	sandy loam	54	0.873	9000	97*	83**
CAN-95024/ RCN 95012	sandy loam	368	1.005	9436	800*	681**

* desorption step 1
** desorption step 2

Seher A, 1999, BOD2000-652

GLP: yes
Guidelines: OECD 106, US-EPA, Subdivision N, 163-1

Adsorption and desorption of the **metabolite BF 500-3** were measured using a batch equilibrium procedure to determine the K_d- and K_{oc}-values of [tolyl-U^{14}C]-labelled BF 500-3 in six soils (three German soils, one Canadian soil and two US soils). Details of the soils which were used are provided in Table B.8.2-3.
The soil waterratio was 1:10. The test temperature was 23 °C +/- 2 °C.

Table B.8.2-3: Soils used to investigate adsorption and desorption of [tolyl-^{14}C]-labelled BF 500-3

soil designation	type (USDA scheme)	org. carbon [%]	pH value ($CaCl_2$)	soil type			CEC [MVal/ 100 g soil]	MWC [g H_2O/ 100 g dry soil]	FC* [g H_2O/ 100 g dry soil]
				<2 µm [clay]	2-63 µm [silt]	63-2000 µm [sand]			
Li 35 b Limburgerhof, Germany	loamy sand	1.1	6.5	7	12	81	7.2	33	10.2
LUFA 2.2 Speyer, Germany	sand/loamy sand	2.5	5.8	5	10	85	11.2	43	14.7
Bruch West Limburgerhof Germany	sandy loam	1.5	7.5	10	18	72	12.1	40	16.3
USA 538-30-5	loamy sand	0.4	5.8	8	11	81	4.0	22	7.3
USA 538-31-2	loam	0.5	5.2	13	47	40	10.0	31	19.6
CAN 95024/ RCN 95012	sandy clay loam	3.4	7.5	23	31	46	26.0	46	32.5

* determined at 0.33 bar

Results:
A summary of the results obtained can be found in Table B.8.2-4. The adsorption process for BF 500-3 in the studied concentration range (2.5 – 20 ng/ml) can be described with a high degree of accuracy using the Freundlich equation. The adsorption constants K_d calculated from the Freundlich isotherms for the six test soils range from 47 to 354.
K_{oc}-values of 4240 to 12000 were obtained. A subsequent desorption determination in two steps with 0.01 M $CaCl_2$-solution resulted in K_{desI} values in the range of 205 to 3417 for the first desorption step and in K_{desII} values in the range of 280 to 4787 for the second desorption step.

Table B.8.2-4: Adsorption and desorption of [tolyl-U-^{14}C] BF 500-3 in different soil types

soil designation	textural class	Adsorption K_d [ml/g]	Adsorption 1/n	Adsorption K_{oc} [ml/g]	Desorption K_{des} [ml/g]	
Li 35 b	loamy sand	74	0.802	6750	367*	434**
LUFA 2.2	sand/ loamy sand	268	0.942	10700	1317*	1143**
Bruch West	sandy loam	64	0.688	4240	494*	415**
USA 538-30-5	loamy sand	47	0.942	11800	205*	280**
USA 538-31-2	loam	60	0.773	12000	275*	382**
CAN-95024/ RCN 95012	sandy clayey loam	354	0.831	10400	3417*	4787**

* desorption step 1
** desorption step 2

Seher A, 1999, BOD2000-653

GLP: yes
Guidelines: OECD 106, US-EPA, Subdivision N, 163-1

Adsorption and desorption of the **metabolite BF 500-6** were measured using a batch equilibrium procedure to determine the K_d- and K_{oc}-values of [tolyl-U^{14}C]-labelled BF 500-6 in six soils (three German soils, one Canadian soil and two US soils). Details of the soils which were used are provided in Table B.8.2-3.

The soil water ratio was 1:10. The test temperature was 21 °C +/- 1 °C.

Results:
A summary of the results obtained can be found in Table B.8.2-5.
Because of the low solubility of the test substance in water (3 µg/L) the Freundlich isotherm test for the adsorption, resp. desorption was not performed. The distribution coefficients K_d for the adsorption and K_{des} for the desorption were measured with only one concentration of the test substance in water. The calculated adsorption constants K_d range from 84 to 634.
A subsequent desorption determination in two steps with 0.01 M $CaCl_2$-solution resulted in K_{desI} values in a range of 4950 to 9800 for the first step and in K_{desII} values of 1300 to 1900 for the second desorption step.

Table B.8.2-5: Adsorption and desorption of [tolylU^{14}C] BF 5000-6 in different soil types

soil designation	textural class	Adsorption K_d [ml/g] 1)	Adsorption K_{oc} [ml/g] 1)	Desorption K_{des} [ml/g] 1)	
Li 35 b	loamy sand	350	31830	9800*	1500**
LUFA 2.2	sand/ loamy sand	84	3360	2)*	1900**
Bruch West	sandy loam	248	16550	9750*	1900**
USA (538-30-5)	loamy sand	366	91650	-2)*	1500**
USA (538-31-2)	loam	634	126800	4950*	1600**
CAN-95024/ RCN 95012	sandy clayey loam	630	18500	-2)*	1300**

* desorption step 1
** desorption step 2
1) average of two replicates
2) - means that no desorption could be measured

Seher A, 1999, BOD2000-654

GLP: yes
Guidelines: OECD 106, US-EPA, Subdivision N, 163-1

Adsorption and desorption of the **metabolite BF 500-7** were measured using a batch equilibrium procedure to determine the K_d- and K_{oc}-values of [tolyl-U^{14}C]-labelled BF 500-7 in six soils (three German soils, one Canadian soil and two US soils). Details of the soils which were used are provided in Table B.8.2-3.
The soil water ratio was 1:10. The test temperature was 21 °C +/- 1 °C.

Results:

A summary of the results obtained can be found in Table B.8.2-6.

Because of the low solubility of the test substance in water (5 µg/L) the Freundlich isotherm test for the adsorption and desorption was not performed. The distribution coefficients K_d for the adsorption and K_{des} for the desorption were measured with only one concentration of the test substance in water. The calculated adsorption constants K_d range from 101 to 750.

A subsequent desorption determination in two steps with 0.01 M $CaCl_2$ solution resulted in K_{desI} values in a range of 2605 to 8289 for the first step and in K_{desII} values of 2676 to 12815 for the second desorption step.

Table B.8.2-6: Adsorption and desorption of [tolyl-U-14C] BF 500-7 in different soil types.

soil designation	textural class	Adsorption K_d [ml/g] [1)]	Adsorption K_{oc} [ml/g] [1)]	Desorption K_{des} [ml/g]	
Li 35 b	loamy sand	418	37950	5114[1)] *	4634[1)] **
LUFA 2.2	sand/ loamy sand	101	4020	3255[1)] *	2676[1)] **
Bruch West	sandy loam	450	29950	8289 *	- [2)] **
USA 538-30-5	loamy sand	544	135900	5534[1)] *	10241[1)] **
USA 538-31-2	loam	750	149900	2605[1)] *	8307[1)] **
CAN-95024/ RCN 95012	sandy clayey loam	543	15950	6499 *	12815[1)] **

* desorption step 1
** desorption step 2
1) average of two replicates
2) - means that no desorption could be measured

Conclusion:
On the basis of these findings, pyraclostrobin as well as its metabolites BF 500-3, BF 500-6 and BF 500-7 can be classified as non-mobile in soil.

B.8.2.2 Mobility in soil

B.8.2.2.1 Column leaching studies

Ziegler G, 1998, BOD2000-656

GLP: yes
Guidelines: BBA IV 4-2, SETAC Europe

The leaching characteristics of [chlorophenyl-U-^{14}C]-labelled pyraclostrobin was studied using 4 soils. The experiments were performed with two treated soil columns for each of the four soils. Details of the soils used are provided in Table B.8.2-7. Air-dried soil, sieved to particle size < 2 mm was filled into glass columns (column dimensions: length 30 cm, segmented into 5 x 6 cm, internal diameter 5 cm). The packed soil columns were infiltrated from the bottom with 0.01 M calcium chloride solution until the liquid level reached the soil surface. Thereafter, 50 µg pyraclostrobin incorporated into 100 g soil (corresponding to an application rate of 250 g active substance/ha) was applied onto the top of each soil column. The columns were eluted with 393 ml (= 200 mm rain) of 0.01 M aqueous calcium chloride solution. Four eluate fractions of approx. 100 ml each were collected during 2 days. The different leachate fractions and the individual soil segments were analysed for [^{14}C]-residues.

Table B.8.2-7: Characteristics of soils used for column leaching

soil designation origin	Lufa 2.1 Speyer Germany	Lufa 2.2 Speyer Germany	Li 35 b Limburgerhof Germany	Bruch West Limburgerhof Germany
textural class (USDA scheme) textural class (German scheme)	sand sand	loamy sand sand/ loamy sand	loamy sand silty sand/ loamy sand	sandy loam loamy sand
particle size distribution [%]				
(USDA scheme):				
0.050 mm – 2 mm sand	93	86	84	69
0.002 mm – 0.050 mm silt	4	9	11	18
< 0.02 mm clay	3	5	5	13
(German scheme):				
0.063 mm – 2 mm sand	92	85	83	66
0.002 mm – 0.063 mm silt	5	10	12	21
< 0.002 mm clay	3	5	5	13
Organic C [%]	0.5	2.1	1.0	2.0
CEC [meq/100 g]	4.3	12.2	6.8	15.6
pH value (0.01 M $CaCl_2$)	5.4	5.4	6.5	7.5
MWC [g H2O/100 g dry soil]	24	36	30	44
FC* [g H_2O/100 g dry soil]	5.1	13.6	9.3	19.0

* determined at 0.33 bar

Results:
The results obtained for all tests are summerised in Table B.8.2-8.

Table B.8.2-8: Leaching behaviour of [chlorophenyl-U-14C]-labelled FAS 500 F in different soils (values in % TAR)

Soil designation	Lufa 2.1	Lufa 2.2	Li 35 b	Bruch West
1. soil (total)	100.3 / 104.1	98.8 / 104.8	96.3 / 98.4	96.6 / 100.8
segment 0 – 6 cm	93.1 / 95.4	96.2 / 103.0	93.3 / 95.1	94.4 / 99.0
segment 6 – 12 cm	7.2 / 8.7	2.6 / 1.8	3.1 / 3.3	2.2 / 1.8
segment 12 – 18 cm	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
segment 18 – 24 cm	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
segment 24 – 30 cm	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
quartz sand	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
2. leachate (total, in 393 ml)	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
(1 + 2) total	100.3 / 104.1	98.8 / 104.8	96.3 / 98.4	96.6 / 100.8

The results clearly showed that pyraclostrobin does not leach even under worst case laboratory conditions. All radioactivity remained in the top two soil layers. No radioactivity could be found in the leachates or in soil layers below 12 cm.

B.8.2.2.2 Aged residue column leaching

Ziegler G, 1998, BOD2000-657

GLP: yes
Guidelines: BBA IV, 4-2, SETAC Europe

The leaching characteristics of aged pyraclostrobin soil residues were studied in a German soil using [chlorophenyl-U-^{14}C]-labelled pyraclostrobin. Details of the soil used are provided in Table B.8.2-9. Soil samples containing labelled pyraclostrobin (at rates equivalent to about 250 g as/ha, resp. 50 µg per column) were incubated in the dark for 30 days at 20 °C +/- 2 °C at 40% water holding capacity. After ageing, the incubated soil was transfered to the top of a column (length 30 cm, inner diameter 5 cm, 2 replicates) containing untreated soil. Water was applied to simulate 200 mm rainfall (393 ml in two days). The leachate was collected in four fractions of about 100 ml and analysed for radioactive residues.

Table B.8.2-9: Soil used to investigate aged soil leaching of pyraclostrobin

soil designation	LUFA 2.1 Speyer, Germany
textural class (German scheme)	loamy sand
particle size distribution [%] (German scheme): 0.063 – 2 mm 0.002 – 0.063 mm < 0.002 mm	 92 5 3
organic C [%]	0.5
microbial biomass [mg C/100 g dry soil]	6.5
CEC [meq/100 g]	4.3
pH value (0.01 M $CaCl_2$)	5.4
MWC [g H_2O/100 g dry soil]	24
FC [g H_2O/100 g dry soil], 0.33 bar	5.1

Results:
The results obtained for all tests are summarised in Table B.8.2-10.

Table B.8.2-10: Aged soil leaching behaviour of [chlorophenyl-U-^{14}C]-labelled pyraclostrobin (values in % of applied radioactivity)

amount applied	50 µg (250 g as/ha)	
ageing [days]	30	
individual tests	A	B
1. volatile compounds		
^{14}C-CO_2	0.0	0.0
2. soil (total)	89.9	96.3
segment I	87.7	94.1
segment II	2.1	2.2
segment III	0.0	0.0
segment IV	0.0	0.0
segment V	0.0	0.0
quartz sand	0.0	0.0
3. leachate (total)	0.0	0.0
fraction a	0.0	0.0
fraction b	0.0	0.0
fraction c	0.0	0.0
fraction d	0.0	0.0
(1 – 3) total	89.9	96.3

In this experiment, no radioactivity was found in the leachates. The first (= upper, 0 – 6 cm) soil segment of the columns contained more than 87% of the total radiactive residues (TRR).

Analysis of methanol and methanol/water extracts of the upper soil segments (radio-HPLC) showed that most of the radioactivity consists of unchanged pyraclostrobin, however, small amounts of the metabolites BF 500-6 and BF 500-7 could also be detected.

Conclusion:
The column leaching experiments (aged and non-aged) clearly show that pyraclostrobin is not mobile in soil. There is no risk of displacement of pyraclostrobin into deeper soil layers.

B.8.2.2.3 Lysimeter studies or field leaching studies

Under the worst case conditions of laboratory leaching experiments (aged and unaged) no movement of active substance or soil metabolites within the soil columns could be observed. The adsorption/desorption studies revealed very high adsorption coefficient values. PELMO- and PESTRAS-simulations with the most unfavorable scenarios (lowest K_{oc}-values, longest half lives of active substance and metabolites in soil, wet climate) clearly showed that there is no risk of displacement of pyraclostrobin or of its metabolites into deeper soil layers or groundwater (see PEC_{GW}, point 8.06). Therefore, neither a lysimeter study nor a field leaching study was necessary.

B.8.3 Predicted environmental concentrations in soil (Annex IIIA 9.1.3)

B.8.3.1 Notifier's calculation

Van de Veen J, 1999, BOD2000-622 (active substance in vineyard)
Van de Veen J, 1999, BOD2000-623 (active substance in cereals)
Van de Veen J, 1999, BOD2000-624 (metabolites in vineyards and cereals)

Guidelines: FOCUS recommendations

The studies are not reported due to reasons given under B.8.3.2.

B.8.3.2 Rapporteur's calculation

The predicted environmental concentrations of pyraclostrobin was recalculated for following reasons:

- the Notifier considered uses or use patterns, which differ from the supported or intended uses
- the DT50 values for the parent compound (field) were recalculated according to Timme and Frehse Modell (1st order kinetics)

Table B.8.3-1: Input parameter for PEC_{soil} calculation

Parameter	Vine	Turf
Application rate (g a.s./ha)	100	250
Number of applications (n)	3	2
Interval (d)	12	14
DT50 in soil field (d)	34.4	34.4
DT50 in soil at10°C (d)	51	51
Depth of the soil layer (cm)	5	5
Soil density g/cm³	1.5	1.5
Plant interception factor (%)	70/70/85	90
g of a.s per application	30/30/15	25/25
Initial conc. (g a.s./kg soil)	0.04/0.04/0.02	0.03/0.03

The PECsoil were recalculated for the active substance **pyraclostrobin** using recalculated DT50 values derived from the field studies as shown in the following table:

Table B.8.3-2: DT50 and DT90 (field) for pyraclostrobin calculated according to 1st order using Timme and Frehse Model

		1st order		
Reference	trial	DT-50 (d)	DT-90 (d)	r^2
BOD2000-647 BASF Reg. Doc. #1999/11292	DO5/02/97	30.59	101.6	0.7900
	DO8/01/97	**34.39**	114.2	0.9367
	DU2/02/97	14.64	48.6	0.8403
BOD2000-648 BASF Reg. Doc. #1999/11301	ALO/01/98	19.52	64.8	0.8153
	ALO/02/98	85.59	not extr.	0.8165
	HUS/02/98	31.22	103.7	0.8352

With an exception of trial ALO/02/98, the DT50 and DT90 values represent the best fit.

DT50 of **34.4 d** can therefore be considered as reasonable worst case value for further calculations (e.g. PEC). Additionally PEC_{soil} were calculated also using the DT50 value of **51** d which was established by the Notifier for cooler regions (10°C).
The PEC_{soil} were calculated as actual and time weighted average values. Two kindes of time weighted average values were considered:
I = concentration calculated (weighted) since first application
II = concentration calculated since the highest concentration point (after the last application)

Results:

Table B.8.3-3: PEC_{soil} for pyraclostrobin, vineyard scenario, DT50 = 34.4 d

Days after last appl.	Days after 1st appl.	Concentration (mg/kg)		
		Actual	Time Weighted Average	
			I	**II**
	24 (ini)	0.076	0.049	0.076
1	25	0.075	0.051	0.075
2	26	0.073	0.051	0.075
4	28	0.070	0.053	0.073
7	31	0.066	0.054	0.071
28	52	0.043	0.054	0.058
50	74	0.028	0.048	0.048
100	124	0.010	0.036	0.033

Table B.8.3-4: PEC_{soil} for pyraclostrobin, vineyard scenario, DT50 = 51 d

Days after last appl.	Days after 1st appl.	Concentration (mg/kg)		
		Actual	Time Weighted Average	
			I	II
	24 (ini)	0.083	0.053	0.083
1	25	0.082	0.054	0.082
2	26	0.081	0.055	0.082
4	28	0.078	0.057	0.081
7	31	0.075	0.059	0.079
28	52	0.057	0.061	0.069
50	74	0.042	0.058	0.060
100	124	0.021	0.047	0.045

Table B.8.3-5: PEC_{soil} for pyraclostrobin, turf scenario, DT50 = 34.4 d

Days after last appl.	Days after 1st appl.	Concentration (mg/kg)		
		Actual	Time Weighted Average	
			I	II
	14 (ini)	0.053	0.026	0.053
1	15	0.052	0.028	0.052
2	16	0.051	0.029	0.052
4	18	0.049	0.032	0.051
7	21	0.046	0.034	0.049
28	42	0.030	0.036	0.040
50	64	0.019	0.032	0.033
100	114	0.007	0.023	0.023

Table B.8.3-6: PEC_{soil} for pyraclostrobin, turf scenario, DT50 = 51 d

Days after last appl.	Days after 1st appl.	Concentration (mg/kg)		
		Actual	Time Weighted Average	
			I	II
	14 (ini)	0.055	0.027	0.055
1	15	0.054	0.029	0.054
2	16	0.053	0.031	0.054
4	18	0.052	0.033	0.053
7	21	0.050	0.036	0.052
28	42	0.037	0.039	0.046
50	64	0.028	0.037	0.040
100	114	0.014	0.030	0.030

B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2.1, 9.2.3)

B.8.4.1 Rate and route of degradation in aquatic systems

B.8.4.1.1 Hydrolytic degradation

Scharf J, 1999, WAS2000-352

GLP: yes
Guidelines: EC Method C7, US-EPA, Subdivision N, 161-1

Hydrolysis of pyraclostrobin was tested in aqueous buffer solutions at 50 °C at four different pH-values (pH 4, 5, 7, and 9), and at 25°C at three different pH-values (pH 5, 7, 9). Duplicate samples were taken at each sampling time and analysed by radio-HPLC. The specific radioactivity of the tolyl-labelled substance was 4.50 MBq/mg (radiochemical purity 94-95%). The specific radioactivity of the chlorophenyl-labelled substance was 4.34 MBq/mg (radiochemical purity 94-95%). Both labels contained a defined impurity (BF 500-3; de-methoxylated BAS 500F) which did not influence the outcome of the tests. The concentration of pyraclostrobin in the buffer solutions was 0.5 mg/L and 1.0 mg/L for the tests at 25 °C and 50 °C, respectively. The solutions were incubated in the dark under sterile conditions. Sampling times for the test at 50 °C were 0, 1, 2, 3, 4, and 5 DAT, and for the test at 25°C 0, 1, 3, 7, 15, 21, 24, and 30 DAT.

Results:
The results of the hydrolysis study at 25 °C are summarised in Table B.8.4-1 for the chloro-phenyl labelled active substance. During hydrolysis at pH 5 and 7, the active substance was stable. At pH 9, a very slow decrease to 78% TAR within 30 days could be observed. At all pH's, very small amounts of BF 500-5, BF 500-6 and BF 500-7 were produced sporadically.

At 50°C and pH 9, the same degradation products were detected. Because of the higher temperature, their concentrations were accordingly higher. After 5 days, BF 500-5 amounted to 13.3% TAR. BF 500-6 and BF 500-7 reached concentrations of 4.3% TAR and 12.8% TAR, respectively, within the same time. The results for the tolyl labelled active substance were comparable. In all tests, BF 500-3 was present as impurity already at 0 DAT.

No DT_{50}-values were calculated neither for acidic nor for alkaline conditions because they will exceed the period of reliable extrapolation (twice the duration of the studies).

Table B.8.4-1: Recovery of radioactivity in % TAR and distribution of metabolites during hydrolysis of [^{14}C]-chlorophenyl-labelled pyraclostrobin at 25 °C

pH	DAT	pyraclostrobin	BF 500-3 (500M07)	BF 500-5 (500M04)	BF 500-6 (500M01)	BF 500-7 (500M02)	others [1)]	sum
5	0	97.1	2.9					100.0
	1	90.5	4.7					95.2
	3	95.2	3.5					98.7
	7	92.5	4.5					97.0
	15	93.4	4.3		1.7	2.3	1.6	101.7
	21	84.5	3.7				5.0	93.2
	24	91.2	4.2				1.4	96.8
	30	88.7	3.6				1.4	95.4
7	0	90.7	6.4			1.3	1.6	100.0
	1	96.6	3.7				3.2	103.5
	3	95.3	6.9					102.2
	7	95.4	3.9					99.3
	15	94.6	4.3				1.0	99.8
	21	90.8	1.3	4.0			7.1	103.2
	24	91.1	4.9				4.7	100.7
	30	97.1	5.1				5.0	107.2
9	0	91.4	4.6	4.0				100.0
	1	83.0	5.4			2.9	6.2	97.6
	3	95.3	4.8					100.1
	7	89.7	5.1			0.9		95.8
	15	92.0	3.1					95.1
	21	95.8	4.0					99.8
	24	91.8	4.7					96.5
	30	78.4	5.6		1.9	5.4		91.2

[1)] each < 4% TAR

Conclusion:
The hydrolytic degradation of pyraclostrobin depends on the pH-value. At pH 5 and 7, the active substance is stable. Under alkaline conditions (pH 9) a very slow degradation of the parent compound was observed at 25 °C. Only at high temperatures (50 °C) and under alkaline conditions (pH 9) a comparatively faster degradation was observed, but this does not represent common environmental conditions.

B.8.4.1.2 Photochemical degradation

Scharf J, 1998, LUF2000-251

GLP: yes
Guidelines: BBA IV, 6-1, OECD Draft Test Guideline "Phototransformation of Chemicals in Water" Part A

Scharf J, 1999, LUF2000-249

GLP: yes

Guidelines: FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides, Revision 3, US-EPA, Subdivision N, 161-2

The direct photolysis was performed because the absorption coefficients of pyraclostrobin for wavelengths above 290 nm were > 10 l/(mol x cm). The absorption coefficients were used for the determination of the quantum yield and to calculate the photolytical half life in the top layer of aqueous systems.

The direct photolysis was performed with both labels of the active substance. The specific radioactivity of the tolyl-labelled substance was 4.62 MBq/mg (radiochemical purity > 98%). The specific radioactivity of the chlorophenyl-labelled substance was 4.32 MBq/mg (radiochemical purity > 96 %). The study was performed at pH 5 (acetate buffer) because the active substance is hydrolytically stable at this pH-value. The concentration of the active substance in the sterile aqueous buffer solution was about 0.5 mg/L.

For each label a separate experiment was performed. Sterilised glass vessels with quartz glass caps containing 20 ml test solution were irradiated in a thermostated block. Each vessel had an air inlet and an air outlet. The incoming air was moistened, sterilised, and the CO_2 was removed. A trapping system for volatiles was connected to each vessel. The thermostated vessels were located under a xenon lamp with a light intensity of about 3 mW/cm^2 and a cut-off for wavelengths < 290 nm to simulate natural sunlight. The duration of the experiment was 25 days with continuous irradiation.

Appropriate volumes of each test solution were stored in a climatic chamber to be used as dark control. The temperature was 22 ± 1 °C during the experiments.

For the determination of the quantum yield of pyraclostrobin, a mixture of p-nitroanisole and pyridine was used as chemical actinometer according to DULIN and MILL (D.Dulin and T.Mill (1982), Development and Evaluation of Sunlight Actinometers, Environ.Sci.Technol. 16, 815-820). During each irradiation experiment, two vessels with the actinometer solution were irradiated simultaneously with the test solutions.

Results:
During direct photolysis, a very fast degradation of the active substance was observed. A large number of degradation and rearrangement products occured, only some of them being stable under simulated environmental conditions. The molar mass and/or structure of 33 metabolites could be determined ("sum of minor peaks identified" in Table B.8.4-2 and Table B.8.4-3). Five of the metabolites occured once or several times with amounts >10% TAR. These substances were: BF 500-11, BF 500-13, BF 500-14, BF 500-15, and 500M58. With the chlorophenyl-label, about 22% TAR was mineralised after 15 days.
In the dark control, no degradation was observed. This finding corresponds to the results of the hydrolysis study. The results of the aqueous photolysis study are summarised in Table B.8.4-2 and Table B.8.4-3.
The proposed degradation pathway for pyraclostrobin during aqueous photolysis is shown in Figure B.8.4-1.
The half-lifes of the active substance and the main metabolites were calculated with the ModelMaker computer program. The results are shown in Table B.8.4-4.

Table B.8.4-2: Recovery of radioactivity in % and distribution of metabolites after application of ^{14}C-pyraclostrobin during aqueous photolysis-chlorophenyl label

time after treatment	% TAR							
	pyraclostrobin	BF500-14 (500M76)	BF 500-15 (500M78)	500M58	CO_2	sum of minor peaks identified	others*	sum
0 h	94.1				n.m.	5.9	0.0	100.0
3 h	10.4	20.7	6.2	13.2	0.1	43.5	6.0	100.1
6 h	2.4	12.8	15.6	22.7	0.1	35.6	15.3	104.5
9 h	0.6	11.8	17.8	21.2	0.3	33.2	13.9	98.8
1 d	0.5	5.6	26.6	23.4	1.8	22.5	20.4	100.8
3 d	0.0	4.0	25.3	19.5	6.0	19.2	26.2	100.2
6 d	0.0	4.5	24.2	19.0	13.1	18.0	20.4	99.1
10 d	1.3	0.0	3.0	2.2	13.2	14.6	59.8	94.2
15 d	0.0	0.0	7.9	0.3	15.2	17.5	49.9	90.9
18 d	0.0	0.0	8.5	6.3	18.0	12.0	51.6	96.4
21 d	0.0	0.0	8.0	1.1	21.7	16.8	50.9	98.5
25 d	0.0	0.0	5.2	3.7	21.9	10.5	55.8	97.1

n.m.: not measured
* each peak <7% TAR at any sampling time

Table B.8.4-3: Recovery of radioactivity in % and distribution of metabolites after application of ^{14}C-pyraclostrobin during aqueous photolysis-tolyl label

time after treatment	% TAR								
	pyraclostrobin	BF500-11 (500M60)	BF500-13 (500M62)	BF500-14 (500M76)	500M58	CO_2	sum of minor peaks identified	others*	sum
0 h	100.0					n.m.			100.0
3 h	39.7	12.5	3.5	14.1	4.4	0.0	23.4	0.4	98.0
6 h	3.8	22.8	12.3	14.8	12.8	0.0	29.4	2.5	98.5
9 h	4.7	21.7	12.1	13.4	12.9	0.0	29.0	3.3	96.9
1 d	0.0	27.6	13.9	3.7	20.3	0.1	27.3	2.4	95.2
3 d	0.0	27.9	14.6	3.3	17.5	0.3	28.1	1.9	93.5
6 d	0.0	31.1	16.8	1.7	11.7	1.3	29.3	5.2	97.0
10 d	0.0	38.3	11.7	0.6	4.7	3.0	32.7	8.1	99.1
15 d	0.0	39.6	12.9	1.5	9.0	1.6	25.1	7.2	96.9
18 d	0.0	38.3	9.5	0.4	2.7	3.7	24.5	20.1	99.0
21 d	0.0	44.5	3.9	0.0	3.0	4.5	24.7	16.2	96.8
25 d	0.0	37.4	8.0	0.0	2.8	3.7	27.6	19.1	98.6

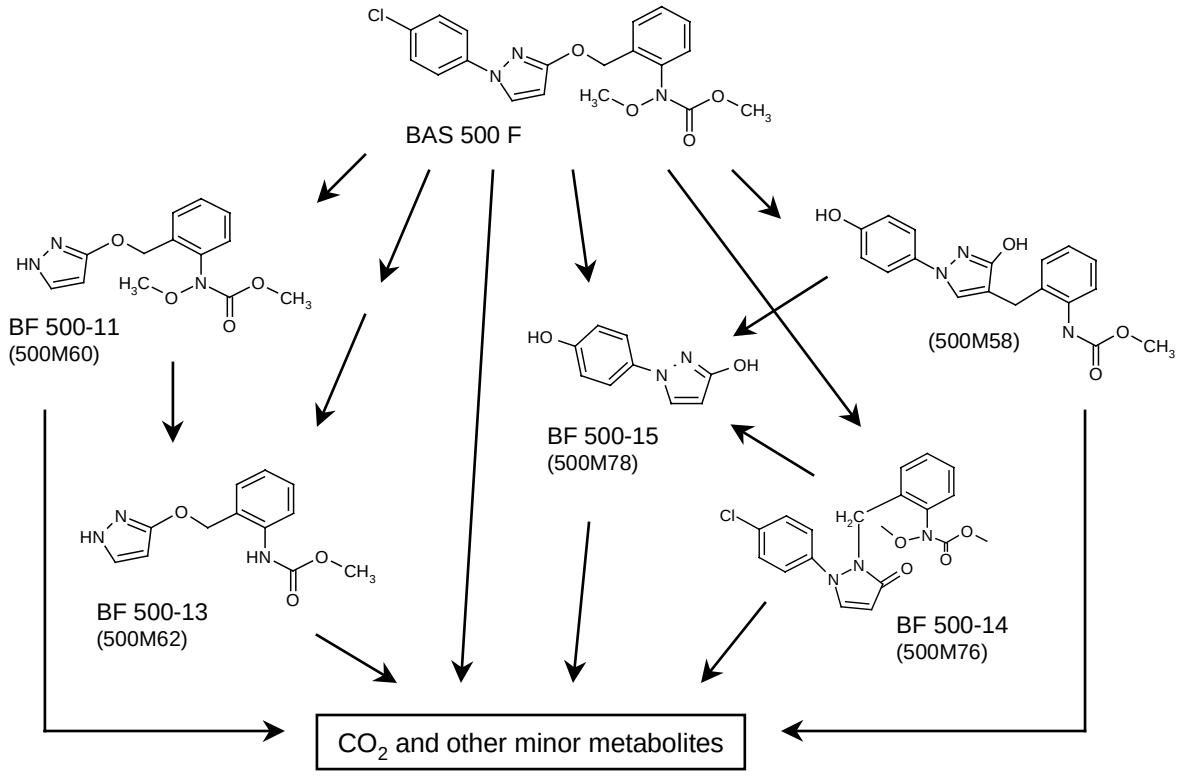
n.m.: not measured
* each peak <7% TAR at any sampling time

Table B.8.4-4: ModelMaker calculation of the DT50 values of pyraclostrobin and major metabolites during aqueous photolysis (continuous irradiation)

substance		half-life [d]		
		chlorophenyl label	tolyl label	mean value
pyraclostrobin		0.04	0.08	0.06
BF 500-14	(500M76)	0.22	0.34	0.28
500M58		7.14	10.14	8.64
BF 500-15	(500M78)	4.62		-
BF 500-11	(500M60)		-*	
BF 500-13	(500M62)		30.67	-

* no calculation possible

Figure B.8.4-1: Proposed route of degradation of pyraclostrobin during aqueous photolysis



The determination of the quantum yield was based on the following equation:

$$\Phi_{ts} = \frac{\Phi_{ac} * \Sigma(\varepsilon_{(\lambda)ac} I_{(\lambda)ac}) * DT50_{ac}}{\Sigma(\varepsilon_{(\lambda)ts} I_{(\lambda)ts}) * DT50_{ts}}$$

Φ_{ts}: Quantum yield of the test substance
Φ_{ac}: Quantum yield of the actinometer
$\varepsilon_{(\lambda)ts}$: absorption coefficient of the test substance
$\varepsilon_{(\lambda)ac}$: absorption coefficient of the actinometer
$I_{(\lambda)ts}$: light intensity of the used irradiation source during irradiation of the test substance
$I_{(\lambda)ac}$: light intensity of the used irradiation source during irradiation of the actinometer
$DT50_{ts}$: half life of the test substance
$DT50_{ac}$: half life of the actinometer

The quantum yield of pyraclostrobin was estimated to be 2.17 . 10-1.
With quantum yield, absorption spectrum, and with the help of a program which uses the algorithms developed by FRANK and KLÖPFFER for the direct photochemical transformation of chemicals in water (Frank, R, and Klöpffer, W. (1985), Ermittlung von Strahlungsdaten und Entwicklung eines Programms zur Abschätzung der abiotischen Transformation von Chemikalien in natürlichen Gewässern, Forschungsbericht Nr. 106 020 46), the theoretical photolytical half-life of pyraclostrobin in the top layer of aqueous systems was calculated for the main application periods. The values are given in Table B.8.4-5.

Table B.8.4-5: Theoretical photolytical half-life of pPraclostrobin in the top layer of aqueous systems

month of application	environmental half-life [calendar days]
April	1.7
May	0.9
June	0.7
July	0.7
August	0.8

B.8.4.1.3 Biological degradation

B.8.4.1.3.1 Ready biodegradability (active substance)

Reuschenbach P, 1999, WAS2000-349

GLP: yes
Guidelines: EEC 92/69, OECD 301 F, ISO 9408

The aerobic biodegradability of pyraclostrobin was evaluated in the "Manometric Respirometry Test". Mixtures of the test substance at a concentration of 100 mg/L, a defined inorganic medium and a non-preadapted inoculum were incubated in a respirometer (Sapromat). The inoculum was activated sludge from laboratory wastewater treatment plants which were fed with municipal and synthetic sewage. The test vessels and appropriate controls were incubated and aerated at room temperature for up to 28 days. The oxygen used

for the biodegradation of the test substance (biochemical oxygen demand, BOD) was continuously produced and measured by the test apparatus. For evaluation the measured BOD is compared to the calculated theoretical oxygen demand (ThOD).

Results:
After 28 days a degree of biodegradation of 0 – 10% (BOD of ThOD) was measured. The test substance was considered as poorly biodegradable and not readily biodegradable in this test.

B.8.4.1.3.2 Water/Sediment Study

Staudenmaier H, 1999, WAS2000-350

GLP: yes
Guidelines: SETAC Europe, BBA IV, 5-1, US-EPA, Subdivision N, 162-4

The distribution and degradation of pyraclostrobin was studied in two natural systems of water and sediment. The water/sediment systems were taken from a pond (System A) and a pond-like side arm of a river (System B), respectively, both in Rhineland-Palatinate, Germany.
Both radiolabelled forms of pyraclostrobin, chlorophenyl-[^{14}C] and tolyl-[^{14}C], were used and applied separately to the test systems. The specific radioactivity of the active substance was 4.14 MBq/mg for the chlorophenyl-[^{14}C] label and 4.46 MBq/mg for the tolyl-[^{14}C] label, both with a radiochemical purity of >98%.

Characteristics of the water/sediment systems are given in Table B.8.4-6. pyraclostrobin was applied to the water at a rate of 30 µg a.s. per test vessel which corresponded to approx. 125% of the maximum recommended rate of 250 g as/ha when related to a 30 cm deep water body. Experiments under sterile conditions were also carried out in both water/ sediment systems. For the isolation and identification of degradation products, some water/sediment systems were additionally treated at an application rate of 300 µg a.s. per test vessel. The test vessels were incubated in the dark at a temperature of 20 ± 2 ^{o}C for up to 100 days. Aeration was achieved by a stream of air over the water surface.

Table B.8.4-6: Characterisation of the water/sediment systems

Designation Origin		System A Kastenbergheide Rhineland-Palatinate, FRG	System B Berghäuser Altrhein Rhineland-Palatinate, FRG
Sediment	sand [%]	78	38
	silt [%]	4	42
	clay [%]	18	20
	textural class (German scheme)	clayey sand	silty clayey loam
	pH	7.1	7.3
	organic C [%]	0.8	8.3
	total N [%]	0.07	0.46
	total P [%]	0.01	0.11
	CEC [mVal/100g]	13.4	32.0
	ATP [µg/kg]	69.5	1568
	plate counts [cfu/g]		
	bacteria	4.5×10^7	2.6×10^8
	actinomycetes	4.0×10^5	5.3×10^6
	fungi	4.0×10^4	1.7×10^6
Water	pH	8.4	8.1
	hardness [mmol/l]	1.52	1.90
	TOC [mg/L]	10.0	6.4
	total N [mg/L]	1	1
	total P [mg/L]	2	<3

The results from the two different radiolabels revealed no significant differences; therefore the differently radiolabelled replicates were averaged. The distribution and recovery of radioactivity from water/sediment system A is shown in Table B.8.4-7, the corresponding results from system B are presented in Table B.8.4-8.

The radioactivity moved quite fast from the water to the sediment. The radioactivity in the water decreased to less than 25% TAR within 7 days in system A and within 2 days in system B. A further decrease to less than 3% TAR after 100 days was observed in both systems. In the sediment a corresponding increase was seen which accounted for more than 90% TAR at the end of the incubation period. Mineralisation was low in both systems with 4.6% TAR and no other volatile degradates were detected.

High amounts of bound residues were formed in the sediment which accounted for up to 61.8% TAR in system A and 54.1 %TAR in system B. These residues were fractionated into humins, humic acids and fulvic acids. No detectable amounts of pyraclostrobin were released from the bound residues.

Table B.8.4-7: Material balance and distribution of radioactivity after application of [^{14}C]-pyraclostrobin to water/sediment system A (% TAR)

DAT	water	Sediment					CO_2	balance
		extractable residues			Bound residues	total		
		ACN/H_2O	ACN	total				
0	87.1	7.4	1.8	9.2	0.4	9.6	n.d.	96.7
0.25	74.4	15.5	4.2	19.7	1.0	20.7	0.0	95.1
1	60.2	27.1	7.2	34.2	3.4	37.6	0.0	97.9
2	50.0	21.6	12.8	34.4	11.2	45.6	0.0	95.7
7	24.6	32.4	20.2	52.6	16.8	69.5	0.0	94.1
14	15.6	44.3	15.4	59.7	22.4	82.2	0.0	97.8
30	6.9	32.3	15.9	48.2	37.2	85.5	0.5	92.8
61	3.5	19.6	16.8	36.4	53.1	89.4	4.6	97.5
100	2.1	15.5	14.0	29.5	61.8	91.3	4.1	97.5
103 s	4.1	57.8	21.4	79.2	15.0	94.2	n.d.	98.3

s = sterilised
n.d. = not determined

Table B.8.4-8: Material balance and distribution of radioactivity after application of [^{14}C]-pyraclostrobin to water/sediment system B (% TAR)

DAT	water	sediment					CO_2	balance
		extractable residues			bound residues	total		
		ACN/H_2O	ACN	total				
0	87.2	5.5	2.2	7.7	0.3	8.0	n.d.	95.3
0.25	63.3	22.2	9.3	31.4	1.2	32.7	0.0	95.9
1	38.5	40.4	16.5	56.9	2.7	59.6	0.0	98.1
2	24.8	45.0	19.7	65.1	4.2	69.4	0.0	94.1
7	8.9	55.7	21.4	77.1	8.0	85.2	0.0	94.1
14	3.7	52.6	27.9	80.5	14.2	94.7	0.0	98.3
30	2.7	38.7	33.3	72.0	20.3	92.3	0.3	95.3
61	3.2	28.6	29.2	57.9	33.7	91.6	0.8	95.6
100	2.8	20.7	17.4	38.1	54.1	92.2	4.6	99.6
103 s	2.8	58.2	31.7	90.0	4.9	94.9	n.d.	97.6

s = sterilised
n.d. = not determined

A comprehensive overview on the results of the HPLC analysis of the water and of the extracts of sediment is shown in Table B.8.4-9 for system A and in Table B.8.4-10 for system B.

Although the degradation seemed to proceed mainly in the sediment in both systems, some differences were observed between the two water/sediment systems: In system A significant degradation of the test substance was detectable from 2 DAT on, which proceeded continuously down to 6.5% TAR at 100 DAT. Metabolite BF 500-3 was formed in moderate amounts up to 11.6% TAR and BF 500-6 and BF 500-7 were formed up to approx. 6.5% TAR each. In contrast, in system B a pronounced decrease of the active substance was detected from 7 DAT on which was accompanied by a corresponding increase of BF 500-3 up to

67.7% TAR. This metabolite was degraded again and amounted to 28.5% TAR after 100 days. BF 500-6 and BF 500-7 were not detected in system B. Other metabolites were only detected in trace amounts in both systems.
In the water of both water/sediment systems, the active substance was found to be the only radiolabelled compound except trace amounts of BF 500-3. All other metabolites were detected only in the sediment. They were formed soon after significant amounts of the active substance had moved into the sediment.

Table B.8.4-9: HPLC analysis of the water and the extracts of the sediment of system A after application of [^{14}C]pyraclostrobin (% TAR)

DAT Rt [min]	pyraclostrobin 54.5	BF 500-3 (500M07) 54.0	BF 500-6 (500M01) 63.5	BF 500-7 (500M02) 65.5	others	total
water						
0	87.1					87.1
0.25	74.2	0.2				74.4
1	60.2					60.2
2	50.0					50.0
7	24.6					24.6
14	15.5	0.1				15.6
30	6.9					6.9
61	3.5*					3.5*
100	not analysed					(2.1)
103 s	4.1					4.1
sediment						
0	8.8	0.4				9.2
0.25	19.0	0.7				19.7
1	32.8	1.4				34.2
2	32.1	2.3				34.4
7	39.5	11.5	0.7	0.9		52.6
14	52.5	3.4	2.5	1.4		59.7
30	32.5	5.5	5.7	4.5		48.2
61	17.0	6.4	6.5	6.3		36.4
100	6.5	11.6	6.5	4.3	0.5	29.5
103 s	79.2					79.2

s = sterilised
* data from one radiolabel only

Table B.8.4-10: HPLC analysis of the water and the extracts of the sediment of system B after application of [^{14}C]-pyraclostrobin (% TAR)

DAT Rt [min]	pyraclostrobin 54.5	BF 500-3 (500M07) 54.0	BF 500-6 (500M01) 63.5	others	total
water					
0	87.0	0.3			87.2
0.25	63.1	0.1			63.3
1	38.5				38.5
2	24.7	0.1			24.8
7	8.8	0.1			8.9
14	1.7	1.9			3.7
30	0.8	1.3		0.6	2.7
61	1.1*	2.3*			3.4*
100	not analysed				(2.8)
103 s	2.8				2.8
sediment					
0	7.5	0.2			7.7
0.25	30.2	1.2			31.4
1	55.3	1.6			56.9
2	62.1	3.1			65.1
7	47.7	29.0	0.5		77.1
14	14.8	65.7			80.5
30	8.9	63.2			72.0
61	8.3	49.6			57.9
100	9.6	28.5			38.1
103 s	90.0				90.0

s = sterilised
* data from one radiolabel only

Degradation of the test substance in the sterilised test vessels was much slower than in the viable samples. After 103 days, 83.3 %TAR (system A) and 92.8 %TAR (system B) was still unchanged test substance. Metabolites were not detected at all and final degradation to CO_2 and bound residues was much reduced indicating the involvement of microbial processes in the degradation of pyraclostrobin.

Disappearance times of the active substance and of the metabolites were calculated separately for the water and for the sediment. For this purpose compartment models were established for the two water/sediment systems which were used for parameter estimation by the computer program ModelMaker. In the calculations the results from the two radiolabels were treated as replicates.
For the complete models very good coefficients of determination of $r^2 = 0.97$ and $r^2 = 0.94$ were achieved. Due to the complex model structure the DT_{50} values for the active substance had to be determined graphically instead by a 1st order estimation. Table B.8.4-11 gives an overview on the disappearance times of the active substance and the metabolites in water and sediment.

Table B.8.4-11: Disappearance times of pyraclostrobin and ist metabolites in water and sediment calculated using Model Maker or graphical estimation

compound	1st order estimation		graphical determination ("best fit")	
	half-life [d]	DT_{90} [d]	DT_{50} [d]	DT_{90} [d]
system A				
pyraclostrobin, water			3	41
pyraclostrobin, sediment			33	105
BF 500-3, sediment	n.r.*	n.r.*		
BF 500-6, sediment	116.3	n.r.*		
BF 500-7, sediment	80.0	n.r.*		
system B				
pyraclostrobin, water			1	9
pyraclostrobin, sediment			9	n.r.**
BF 500-3, sediment	54.8	181.9		

n.r. = not reported
* calculated value extrapolated too far beyond the period of investigation
** measured values deviated from the predicted values

For the parent compound DT50 values in water were recalculated by the Rapporteur using the Timme and Frehse Model. The results are presented in Table B.8.4-12.

Table B.8.4-12: Calculation of DT50 values for pyraclostrobine in the water phase of the water/sediment systems using Timme/Frehse Model

System A

Labeling position	best fit			1st order		
	DT50 (d)	DT90 (d)	r^2	DT50 (d)	DT90 (d)	r^2
^{14}C-chlorophenyl	2.1	23.3	0.9818	**8.7**	28.9	0.8166
^{14}C-tolyl	2.6	28.7	0.9938	13.9	46.3	0.6887

System B

Labeling position	best fit			1st order		
	DT50 (d)	DT90 (d)	r^2	DT50 (d)	DT90 (d)	r^2
^{14}C-chlorophenyl	0.6	6.9	0.9928	*	*	
^{14}C-tolyl	1.2	12.9	0.7783	*	*	

* = not extrap.

The DT50 value of 8.7 days will be used for further calculations (e.g. PEC).
This value does not represent the best fit but is more reliable for calculations of PEC_{sw} according to 1st order.

Ebert D, 1999, WAS2000-351

GLP:yes
Guidelines: US-EPA, Subdivision N, 162-4

This study was initiated after it became obvious that the degradation of pyraclostrobin in water is strongly dependent on **light conditions**. The aqueous photolysis study showed that pyraclostrobin is quickly degraded under irradiated conditions forming numerous rearrangement and breakdown products. In the water/sediment study, however, it could be shown that pyraclostrobin quickly binds to the sediment. Since in natural water/sediment systems (rivers, lakes etc.) both factors, photolysis and sediment adsorption, will influence the degradation of pyraclostrobin simultaneously, this additional study was designed where both factors were combined.
The water/sediment system taken for this study was of the same origin as one of the systems used for the aerobic aquatic metabolism (Kellmetschweiher, also named Kastenbergheide). The water/sediment characteristics are summarised in Table B.8.4-13. Test vessels were filled with about 1.5 cm sediment and a water layer of about 15 cm height. Both radiolabels of pyraclostrobin, chlorophenyl-[^{14}C] and tolyl-[^{14}C], were applied separately to the test vessels. The specific radioactivity of the test substance was 4.32 MBq/mg for the chlorophenyl-label and 4.62 MBq/mg for the tolyl-label, the radiochemical purity was >97% for both labels.
pyraclostrobin was applied at a rate of 244 µg per test vessel for the chlorophenyl-label and 217 µg for the tolyl-label. This roughly corresponds to twofold the maximum recommended application rate of 250 g active substance/ha, when assuming direct overspray of a 30 cm deep water body.
The treated water/sediment systems were incubated in a climatic chamber (phytotron), where light (simulated sun light) and temperature conditions of Central Europe were simulated (daily exposure and temperature cycles in the period of May 17th – July 18th). This period represents the main application period for pyraclostrobin within the year.
Water samples were taken at 0 h, 3 h, 6 h, 9 h, 1 d, 2 d, 3 d, 7 d, 10 d, 14 d, 21 d, 30 d, 45 d, and 62 days after treatment. Sediment samples were worked up 1, 3, 7, 14, 30, 45, and 62 days after treatment.

Table B.8.4-13: Characterisation of the water/sediment Kellmetschweiher

water/sediment designation origin		Kellmetschweiher Schifferstadt, Rhineland Palatinate, Germany
water	pH at site of sampling	8.6
sediment	textural class (German scheme)	sand / clayey sand
	clay [%]	5
	silt [%]	2
	sand [%]	93
	organic C [%]	0.4
	pH ($CaCl_2$)	7.5

Results:
The distribution of radioactivity and material balance in the water/sediment system is shown in Table B.8.4-14 for both labels.

Table B.8.4-14: Distribution of radioactivity and material balance in the water/sediment system after application of ^{14}C-pyraclostrobin and incubation under realistic light and temperature conditions

time after treatment	% TAR				
	water	sediment extractable residues	sediment bound residues	sediment total	material balance (water + sediment)
chlorophenyl-label					
0 h	88.0	n.s.	n.s.	n.s.	n.s.
3 h	89.4	n.s.	n.s.	n.s.	n.s.
6 h	87.1	n.s.	n.s.	n.s.	n.s.
9 h	83.7	n.s.	n.s.	n.s.	n.s.
1 d	82.8	10.6	0.5	11.1	93.9
2 d	76.7	n.s.	n.s.	n.s.	n.s.
3 d	72.0	18.6	3.5	22.1	94.1
7 d	61.1	24.6	8.5	33.1	94.2
10 d	55.8	n.s.	n.s.	n.s.	n.s.
14 d	50.2	24.6	21.1	45.7	95.9
21 d	45.1	n.s.	n.s.	n.s.	n.s.
30 d	42.0	22.5	23.1	45.7	87.7
45 d	37.5	19.7	25.9	45.6	83.1
62 d	31.4	18.1	27.6	45.7	77.1
tolyl label					
0 h	89.4	n.s.	n.s.	n.s.	n.s.
3 h	91.4	n.s.	n.s.	n.s.	n.s.
6 h	90.6	n.s.	n.s.	n.s.	n.s.
9 h	83.8	n.s.	n.s.	n.s.	n.s.
1 d	81.2	9.9	0.4	10.3	91.4
2 d	80.9	n.s.	n.s.	n.s.	n.s.
3 d	78.4	18.1	1.8	19.9	98.3
7 d	69.1	25.6	4.5	30.1	99.1
10 d	63.4	n.s.	n.s.	n.s.	n.s.
14 d	59.6	24.7	13.6	38.2	97.8
21 d	57.3	n.s.	n.s.	n.s.	n.s.
30 d	55.9	26.6	14.3	40.9	96.9
45 d	51.5	24.2	19.7	43.9	95.3
62 d	46.2	21.5	25.5	47.0	93.2

n.s. = not sampled

The results of this study clearly show that pyraclostrobin follows two major dissipation and degradation pathways in a natural water system. When reaching water, pyraclostrobin undergoes a very fast photolytical transformation forming many breakdown products and polar degradates (Table B.8.4-15 and Table B.8.4-16), and simultaneously, it adsorbes very fast to the sediment where it is finally bound to the sediment matrix.

HPTLC analysis revealed that three major metabolites (>10% TAR) were formed in the water phase (BF 500-11, BF 500-13, BF 500-14). All three metabolites are already known from the aqueous photolysis study. Two metabolites which occured during the aqueous photolysis study also in amounts > 10% (BF 500-15, 500M58) could not be detected at any sampling time.

In the sediment, pyraclostrobin is quickly de-methoxylated forming the metabolite BF 500-3 which reached a maximum of 17% TAR. Because of the low water solubility and high K_{oc}-

value BF 500-3 is not supposed to move from the sediment into the water. It is degraded further in the sediment and finally, the radioactivity is bound to the sediment matrix. The water metabolites are found in the sediment only in very low amounts.
In contrast to the tolyl-label, the chlorophenyl-label shows some very polar unidentified components in the water phase, reaching up to 10-13% TAR. However, when analysing the samples by HPLC with a special column for polar substances, this polar region is separated into several components each below 10% TAR. Since these polar components could not be detected with the tolyl-label, it can be concluded that they are various breakdown products derived from the chlorophenyl moiety which was split off from pyraclostrobin. With the chlorophenyl-label, the sum of radioactivity in water and sediment declined to 77% TAR after 62 days which indicates a mineralization of about 23% TAR. This finding is in agreement with the results of the aqueous photolysis study, where also a significant higher mineralization rate was observed with the chlorophenyl-label than with the tolyl-label.

Table B.8.4-15: HPLC analysis of the water samples and sediment extracts after application of ^{14}C-pyraclostrobin to a water/sediment system and incubation under realistic light and temperature conditions (chlorophenyl-label)

time after treatment	% TAR							
	total	unknown polars Rf 0.00	unknown polars Rf 0.01	BF 500-14 (500M76) Rf 0.18	unknown Rf 0.27	pyraclostrobin Rf 0.80	BF 500-3 (500M07) Rf 0.89	others*
water								
0 h	88.0	0.2	0.1	0.3		82.7	2.2	2.5
3 h	89.4	0.8	0.1	1.5		80.7	2.6	3.7
6 h	87.1	0.5		1.5		79.3	2.6	3.2
9 h	83.7	0.6		2.2		75.0	2.6	3.3
1 d	82.8	1.6		3.9	0.3	69.1	2.9	5.0
2 d	76.7	1.6		5.9	0.2	58.2	3.0	7.7
3 d	72.0	3.0		9.0	0.5	46.2	2.9	10.4
7 d	61.1	5.1	4.6	10.4	0.8	28.3	2.5	9.3
10 d	55.8	7.8	9.9	11.1	1.2	14.9	2.1	8.9
14 d	50.2	6.6	7.2	11.4	1.7	12.5	1.9	9.0
21 d	45.1	9.3	9.2	8.1	3.3	3.8	3.0	8.3
30 d	42.0	10.2	12.5	4.2	4.5	0.7	3.3	6.6
45 d	37.5	7.3	10.5	2.8	5.8		3.1	8.0
60 d	31.4	5.1	9.4	1.6	5.4		3.5	6.4
sediment								
1 d	10.6			0.1		9.5	0.7	0.3
3 d	18.6	0.1		0.4	0.1	15.6	1.6	0.9
7 d	24.6	0.1		0.7	0.4	17.5	4.1	1.7
14 d	24.6	0.5		0.7	0.9	9.7	10.0	2.8
30 d	22.5	0.6		0.5	1.5	0.8	15.9	3.1
45 d	19.7	0.6	0.2	0.7	1.7	0.4	13.2	3.0
62 d	18.1	0.5	0.1	0.5	1.6	0.3	12.2	2.9

* sum of up to 13 peaks, each of them ≤ 3% TAR

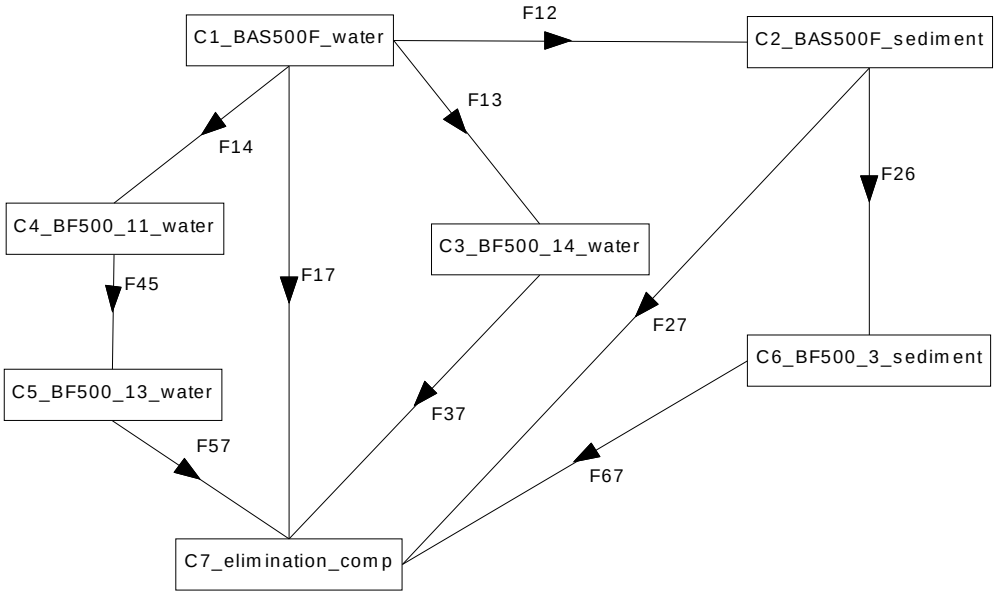
Table B.8.4-16: HPLC analysis of the water samples and sediment extracts after application of ^{14}C-pyraclostrobin to a water/sediment system and incubation under realistic light and temperature conditions (tolyl-label)

time after treatment	% TAR								
	total	BF500-14 (500M76) Rf 0.18	unknown Rf 0.26	BF 500-11 (500M60) Rf 0.31	BF 500-13 (500M62) Rf 0.44	BF 500-12 (500M59) Rf 0.27	pyraclostrobin Rf 0.80	BF 500-3 (500M07) Rf 0.89	others*
water									
0 h	89.4	0.1	0.1	0.2			85.2	2.4	1.5
3 h	91.4	1.3	0.1	0.9		0.8	84.0	2.7	1.6
6 h	90.6	1.4	0.1	1.1		0.9	82.4	2.8	1.9
9 h	83.8	1.7	0.2	1.2		1.2	75.5	2.6	1.5
1 d	81.2	2.7	0.2	2.1	0.4	1.7	68.7	2.6	2.8
2 d	80.9	4.7	0.3	3.7	0.6	3.1	61.0	3.0	4.4
3 d	78.4	6.6	0.5	5.7	1.2	3.9	51.0	3.0	6.5
7 d	69.1	8.5	0.7	7.8	2.2	3.3	34.2	2.7	9.7
10 d	63.4	10.8	1.3	10.4	3.7	2.2	17.3	2.3	15.4
14 d	59.6	9.7	1.6	10.3	4.1	1.8	14.0	2.4	15.1
21 d	57.3	8.6	3.0	11.4	7.0	1.0	5.4	3.3	17.6
30 d	55.9	5.6	4.7	10.5	10.5		2.1	5.0	17.7
45 d	51.5	2.3	5.9	5.5	14.0	0.6	0.8	4.7	17.6
62 d	46.2	1.7	5.9	3.9	15.7	0.9	0.9	4.1	13.1
Sediment									
1 d	9.9	0.1				0.1	8.9	0.6	0.2
3 d	18.1	0.3	0.1		0.1	0.2	15.0	1.4	0.9
7 d	25.6	0.5	0.3	0.1	0.4	0.3	18.3	4.0	1.7
14 d	24.7	0.6	0.8	0.2	0.8	0.2	6.4	12.4	3.2
30 d	26.6	0.4	1.7	0.3	1.8		0.9	16.9	4.7
45 d	24.2	0.6	1.9	0.5	2.1		0.5	14.3	4.3
62 d	21.5	0.5	1.8	0.6	1.9		0.3	12.7	3.8

* sum of up to 15 peaks, each of them <5% TAR

The half-lives for pyraclostrobin and the major metabolites were calculated by the computer program ModelMaker using a compartment model (Figure B.8.4-2) which reflects the degradation pathway of the active substance in a water/sediment system.

Figure B.8.4-2: Compartment model used for determination of the degradation rates of pyraclostrobin and ist metabolites in a natural water/sediment system



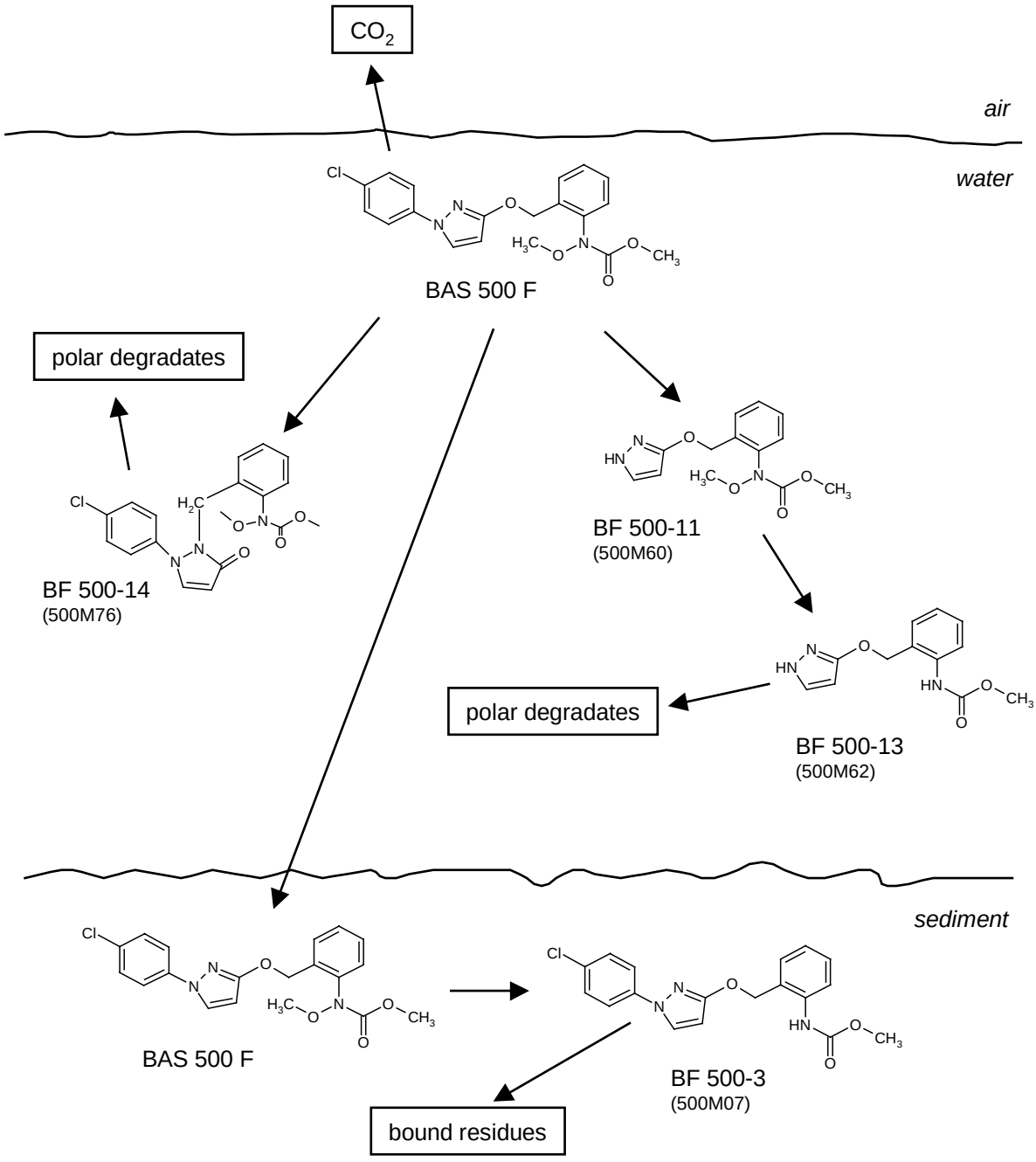
The coefficient of determination for the whole model was r^2 = 0.994. The half-lives of pyraclostrobin and metabolites are shown in Table B.8.4-17. For the metabolite BF 500-13, no reasonable half-life could be calculated in this study, however, the results of the aqueous photolysis study (LUF2000-249) clearly showed that also this metabolite is degradable in water under irradiated conditions.

Table B.8.4-17: DT50 values of pyraclostrobin and metabolites in the water/sediment study under irradiated conditions

Substance		DT_{50} [days] (first order)
pyraclostrobin	(water)	5
BF 500-11	(water)	20
BF 500-13	(water)	-*
BF 500-14	(water)	14
pyraclostrobin	(sediment)	4
BF 500-3	(sediment)	99

* no reasonable calculation possible

Figure B.8.4-3: Proposed route of degradation of pyraclostrobin in water and sediment under realistic outdoor conditions



Conclusion:
The water/sediment study conducted under light conditions is helpful to understand the influence of light on the degradation and distribution of pyraclostrobin in aquatic systems. The entire evaluation of the active substance will be however done on the base of the study conducted in the dark, according to the current guideline (Study WAS2000-350).

B.8.4.1.4 Degradation in the saturated zone

In the adsorption/desorption studies with pyraclostrobin very high adsorption coefficients were determined. Accordingly, even under the worst case conditions of laboratory soil column leaching experiments no movement of the active substance or soil metabolites could be observed. PELMO- and PESTRAS-simulations with the most unfavourable scenarios clearly showed that there is no risk of displacement of pyraclostrobin or its metabolites into deeper soil layers or into the ground-water. Therefore, investigations on the degradation in the saturated zone are considered not to be necessary.

B.8.5 Impact on water treatment procedures (Annex IIIA 9.2.2)

According to the laboratory studies and calculations presented, it is expected that the very small pyraclostrobin concentrations in the water can not affect water treatment procedures. Therefore, no data regarding the impact of pyraclostrobin on water treatment procedures have been generated.

B.8.6 Predicted environmental concentrations in surface water and in ground water (Annex IIIA 9.2.1, 9.2.3)

B.8.6.1 Predicted environmental concentration in groundwater (PEGgw)

Predicted environmental concentrations in groundwater (PEC_{gw}) of the fungicidal active ingredient pyraclostrobin, the aerobic degradation products BF 500-6 and BF 500-7 and the anaerobic metabolite BF 500-3 were calculated for different European Scenarios using the simulation model FOCUS-PELMO 1.1.1.
The PEC_{gw}-calculations were made for the cereal application scenario with 2 applications of 250 g as/ha reaching the soil. In the cereal scenario, the rate of pyraclostrobin reaching the soil represents the worst case covering also the vineyard scenario when considering the crop interception of grapevine.

Scenarios:
Different FOCUS scenarios on the European level were used for the simulation: Hamburg, Jokionen, Kremsmünster, Okehampton and Porto.
The upper ground water level was assumed to be constant at 1 m depth.

Table B.8.6-1: Management parameters used for the simulation of PECgw of pyraclostrobin, BF 500-3, BF 500-6 and BF 500-7.

Crop	Application dates	Amount reaching soil
Summer wheat	15/04 + 15/05	0.25 kg/ha per application*

* no interception considered

Table B.8.6-2: Pesticide parameters used for the simulation of PECgw of pyraclostrobin, BF 500-3, BF 500-6 and BF 500-7

	Molecular weight [g/mol]	Half-life [d]	Koc-value [dm³/kg]	1/n
pyraclostrobin	387.8	100 (max.)	7500	0.9
BF500-3	357.8	65	4240	0.69
BF 500-6	611.5	166 (max.)	3160	1.0
BF 500-7	595.5	159 (max.)	3920	1.0

As a worst case it is assumed for illustrative purposes that the parent pyraclostrobin is converted completely to BF 500-3, for which a degradation to BF 500-6 or BF 500-7 is assumed.

Results:
The PECgw for pyraclostrobin and metabolites BF 500-3, BF 500-6 and BF 500-7 are far below the 0.1 µg/l-level in all scenarios. The results are in line with the experimental findings of the column leaching studies and can be deduced also from the very high sorption values of the substances. Therefore, it can be concluded that pyraclostrobin and ist metabolites BF 500-3, BF 500-6 and BF 500-7 do not leach into groundwater even under worst case scenarios.
All metabolites have been shown to be not relevant with regard to ecotoxicology, toxicology and biological activity.

Studies submitted by the Notifier:
Van de Veen J, 1999, WAS2000-495
Van de Veen J, 1999, WAS2000-496
These studies were not considered because the calculations were not conducted using currently valid models and scenarios.

B.8.6.2 Predicted environmental concentration in surface water (PECsw)

B.8.6.2.1 Notifiers calculation

Because of reasons given under B.8.6.4 the studies submitted by the Notifier were not considered.
Submitted studies:
Platz K, Gottesbueren B, 1999, WAS2000-340
Gottesbuehren B, 1999, WAS2000-341
Platz K, Gottesbueren B, 1999, WAS2000-342
Platz K, Gottesbueren B, 1999, WAS2000-343
Platz K, Gottesbueren B, 1999, WAS2000-344
Platz K, Gottesbueren B, 1999, WAS2000-345
Platz K, Gottesbueren B, 1999, WAS2000-346

Supporting studies:
Hauck, T. and Gottesbueren, B., 2000, WAS2000-671
GLP: not required
Hollis, J. M., 2000, WAS2000-672
GLP: not required

Van de Veen, J. R., WAS2000-669
GLP: not required

B.8.6.2.2 Rapporteur's PECsw calculations

The new calculation was necessary because
- The calculations submitted with a dossier consider the use in vine and cereals but the use patterns are slightly different from those listed in Volume 1
- The spray drift values represent for the use in vine the 75.percentil, which is not accepded in the EU registration process yet (95.percentil has to be considered)
- The calculatios for moving water scenario were done using the model TOXSWA which is still not fully accepted.

Table B.8.6-3: Assumptions used for recalculation of PEC_{sw} values for pyraclostrobin

Parameter	Vine	Turf
Application rate (g a.s./ha)	100	250
Number of applications (n)	3	2
Interval (d)	12	14
DT50 inwater (d)	8.7	8.7
Depth of the water (cm)	30	30
Drift values represent	95. percentil	95. percentil

The **initial** concentration (after the 1st application) to **vine** was 33.33 µg a.s./L, assuming 0 m distance.
The **initial** concentration (after the 1st application) to **turf** was 83.33 µg a.s./L, assuming 0 m distance.

Results:

Table B.8.6-4: PEC_{sw} actual for pyraclostrobin, vineyard scenario

Days after last applic.	Days after first applic.	µg/L				
		Distance				
		0 m	**5 m**	**10 m**	**20 m**	**50 m**
0	24 (ini.)	51.021	2.551	0.765	0.204	0.102
1	25	47.114	2.356	0.707	0.188	0.094
2	26	43.506	2.175	0.653	0.174	0.087
4	28	37.098	1.855	0.556	0.148	0.074
7	31	29.211	1.461	0.438	0.117	0.058
14	38	16.724	0.836	0.251	0.067	0.033
21	45	9.575	0.479	0.144	0.038	0.019
28	52	5.482	0.274	0.082	0.022	0.011
42	66	1.797	0.090	0.027	0.007	0.004

Table B.8.6-5: PEC_{sw} time weighted average for pyraclostrobin, calculated for the time period since 1st application, vineyard scenario

Days after last applic.	Days after first applic.	(µg/L)				
		Distance				
		0 m	**5 m**	**10 m**	**20 m**	**50 m**
0	24 (ini.)	25.562	1.278	0.383	0.102	0.051
1	25	26.502	1.325	0.398	0.106	0.053
2	26	27.224	1.361	0.408	0.109	0.054
4	28	28.152	1.408	0.422	0.113	0.056
7	31	28.621	1.431	0.429	0.114	0.057
14	38	27.473	1.374	0.412	0.110	0.055
21	45	25.194	1.260	0.378	0.101	0.050
28	52	22.790	1.140	0.342	0.091	0.046
42	66	18.657	0.933	0.280	0.075	0.037

Table B.8.6-6: PEC_{sw} time weighted average for pyraclostrobin, calculated since the maximum concentration (after last application), vineyard scenario

Days after last applic.	Days after first applic.	(µg/L)				
		Distance				
		0 m	**5 m**	**10 m**	**20 m**	**50 m**
0	24 (ini.)	51.021	2.551	0.765	0.204	0.102
1	25	49.042	2.452	0.736	0.196	0.098
2	26	47.164	2.358	0.707	0.189	0.094
4	28	43.690	2.185	0.655	0.175	0.087
7	31	39.108	1.955	0.587	0.156	0.078
14	38	30.749	1.537	0.461	0.123	0.061
21	45	24.772	1.239	0.372	0.099	0.050
28	52	20.414	1.021	0.306	0.082	0.041
42	66	14.710	0.736	0.221	0.059	0.029

Table B.8.6-7: PEC_{sw} actual values for pyraclostrobin, turf scenario

Days after last applic.	Days after first applic.	(µg/L)			
		Distance			
		0 m	**5 m**	**10 m**	**20 m**
0	14 (ini.)	110.604	0.664	0.442	0.111
1	15	102.134	0.613	0.409	0.102
2	16	94.312	0.566	0.377	0.094
4	18	80.421	0.483	0.322	0.080
7	21	63.323	0.380	0.253	0.063
14	28	36.254	0.218	0.145	0.036
21	35	20.756	0.125	0.083	0.021
28	42	11.883	0.071	0.048	0.012
42	56	3.895	0.023	0.016	0.004

Table B.8.6-8: PEC_{sw} time weighted values for pyraclostrobin, calculated for the time period since 1st application, turf scenario

Days after last applic.	Days after first applic.	(µg/L)			
		Distance			
		0 m	**5 m**	**10 m**	**20 m**
0	14 (ini.)	50.202	0.301	0.201	0.050
1	15	53.943	0.324	0.216	0.054
2	16	56.707	0.340	0.227	0.057
4	18	60.093	0.361	0.240	0.060
7	21	61.727	0.370	0.247	0.062
14	28	58.430	0.351	0.234	0.058
21	35	52.301	0.314	0.209	0.052
28	42	46.236	0.277	0.185	0.046
42	56	36.468	0.219	0.146	0.036

Table B.8.6-9: PEC_{sw} time weighted average for pyraclostrobin, calculated since the maximum concentration (after last application), turf scenario

Days after last applic.	Days after first applic.	(µg/L)			
		Distance			
		0 m	**5 m**	**10 m**	**20 m**
0	14 (ini.)	110.604	0.664	0.442	0.111
1	15	106.313	0.638	0.425	0.106
2	16	102.242	0.613	0.409	0.102
4	18	94.712	0.568	0.379	0.095
7	21	84.778	0.509	0.339	0.085
14	28	66.657	0.400	0.267	0.067
21	35	53.701	0.322	0.215	0.054
28	42	44.253	0.266	0.177	0.044
42	56	31.889	0.191	0.128	0.032

B.8.6.3 Predicted environmental concentrations in sediment PECsed

Submitted studies:
Platz K, Gottesbueren B, 1999, WAS2000-347 (for the active substance)
Platz K, Gottesbueren B, 1999, WAS2000-348 (for the metabolite BF 5000-3)
Not considered for evaluation

PECsediment, Rapporteur's calculation

Table B.8.6-10: Assumptions used for calculation of PEC values in sediment

Parameter	Value	
Scenario	Vine	Turf
Initial conc. in water after last application, **overspray**	51.0 µg/L	110.6
% max. portion of a.s. in sediment after x days	70 %	70 %
Depth of sediment layer	1 cm	1 cm
Bulk density (g/cm³)	1.3 g/cm³	1.3 g/cm³

The calculation of the concentration of pyraclostrobin in Sediment was done according to the equation:

$$PEC_{sed}(t) = \frac{PEC_{ini,sw} \cdot V_{sw} \cdot P_{sed}(t)}{V_{sed} \cdot bd_{sed} \cdot 100}$$

where $PEC_{ini,sw}$ = initial PEC in the surface water [µg/L]

V_{sw} = water volume [300 l]

$P_{sed}(t)$ = % portion of the active substance in sediment at time t (from water/sediment study)

V_{sed} = volume of sediment for a given depth of the sediment layer

bd_{sed} = bulk density

For the calculation of $PEC_{ini,sw}$ the elimination of pyraclostrobin (including adsorption to sediment) between the times of application has been considered here. Therefore this approach does not represent a worst case approach generally, which should consider the cumulative adsorption for multiple application.

Result:
The maximum expected concentration of pyraclostrobin is calculated to reach 1.79 µg/g wet sediment for the application to turf. The concentration decreases with the distance to the surface water as shown in the following table.

Table B.8.6-11: Maximum PEC_{sed} for pyraclostrobin after application to vine and turf (µg/g wet sediment) due to the initial PEC in surface water after the last application

Distance (m)	0	5	10	20	50
VINE					
$PEC_{ini,\ act.}$ in water (µg/L)	51.0	2.55	0.765	0.204	0.102
PEC_{sed} (µg/g)	0.83	0.041	0.012	0.003	0.002
TURF					
$PEC_{ini.,act.}$ in water (µg/L)	110.6	0.66	0.44	0.11	-
PEC_{sed} (µg/g)	1.79	0.011	0.007	0.002	-

B.8.7 Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3)

Ohnsorge U, 2000, LUF2000-248

GLP: no (calculation)
Guidelines: not relevant (Calculation)

The volatilisation from water was determined by calculating the Henry constant according to the equation:

$H = p \times MW/c$ (kPa m^3/mol).

p:	vapor pressure	($2.6 \cdot 10^{-11}$ kPa at 20 °C)
MW:	molecular weight	(387.8 g/mol)
c:	water solubility	($1.9 \cdot$ g/m^3 at 20 °C)

The resulting Henry constant of the active substance is $H = 5.307 \times 10^{-9}$ kPa m^3/mol.

Scharf J, 1999, LUF2000-250

GLP: yes
Guidelines: BBA IV, 6-1

The volatilisation study was performed with the formulation BAS 500 00 F (containing nominal 250 g active substance/l) based on a field application rate of 250 g active substance/ha. The formulation was mixed with 2-6% chlorophenyl-[^{14}C]-labelled active substance to enable a total balance. The specific radioactivity of the labelled pyraclostrobin was 248 400 dpm/µg, the radiochemical purity was > 97%. Soil and plant were treated in a special glass container. The formulation was applied via a nozzle (1.2 bar) to a small dish filled with soil (first experiment), and to a dish with a plant (bush bean, soil covered; second experiment). The soil characteristics were: 89% sand, 5% silt, 6% clay, organic C 0.6%, pH 5.7, MWC 23 g/100 g dry soil. Application losses were determined by rinsing the glass container and all equipment with methanol. The treated soil/plant was kept in a special volatilization chamber which allowed an air flow rate to be controlled (200 l/h) and the temperature of the air to be measured (20 – 21 °C). The wind speed was adjusted to 1 m/s. The radioactive volatiles was determined with the help of charcoal traps. The charcoal traps were sampled 1, 3, 6, and 24 h after application. At the end of the study, the remaining radioactivity in soil and plant was determined.

Results:
The total recovery of radioactivity was 102% both for the plant and the soil experiment. The volatilisation rates were about 3% from the plant surface and <1% from the soil surface.

Scharf J, 1999, LUF2000-246

GLP: no
Guidelines: not relevant (calculation)

The rate constant for reactions of pyraclostrobin with OH radicals in the atmosphere was calculated using the AOPWIN Program (Atmospheric Oxidation Program for Microsoft

Windows 3.1, Version 1.88, Syracuse Research Corp. 1988-97) based on ATKINSON's increment method (Atkinson, R. (1987) A Structure-Activity Relationship for the Estimation of Rate Constants for the Gas-Phase Reactions of OH Radicals with Organic Compounds, Int.J.Chem.Kin. 19, 799).

At first, the rate constant k_{OH} of the active substance was estimated based on the chemical structure. The resulting value was

k_{OH} = 206.3747 10^{-12} cm^3/molecule · s.

Because of a constant average OH radical concentration in the troposphere, the degradation of the active substance follows pseudo-first order kinetics with the rate constant k' = k_{OH} · [OH radicals]:

-d[pyraclostrobin]/dt = k' · [pyraclostrobin]

The half-life of this process was calculated by the following equation:

$t_{1/2}$ = ln2/k' = ln2/k_{OH} · [OH radicals].

The diurnally and seasonally averaged tropospheric OH radical concentration ([OH radicals]) for the northern hemisphere is 5 · 10^5 cm^{-3} (Crutzen,P.J. (1982) The Global Distribution of Hydroxyl, in Goldberg, E.D. (editor), Atmospheric Chemistry, Springer Verlag Berlin).

The half life of pyraclostrobin was calculated to be

$t_{1/2}$ = 1.87 h (0.08 d, 24h day).

Conclusion:
pyraclostrobin has a very low volatilisation potential and, if reaching the troposphere, is degraded very fast by photochemical processes.

B.8.8 Predicted environmental concentrations in air (Annex IIIA 9.3)

The volatilisation behaviour of pyraclostrobin from plant and soil surfaces is reported in the section 8.07. Since only about 3 % of pyraclostrobin volatilise within 24 h after application, the active substance has, according to the today valid criteria, no relevant tendency to enter the air. Furthermore, the DT50 of the photochemical-oxidative degradation is lower than 2 h.
Until the criteria and procedure for assessment of short and long range atmospheric transport are not finalised, the calculation of PEC_{air} for pyraclostrobin is not obligatory.

B.8.9 Definition of the residue (Annex IIA 7.3)

B.8.9.1 Soil

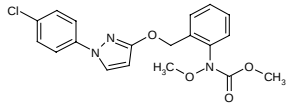
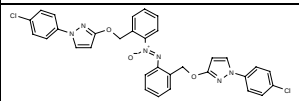
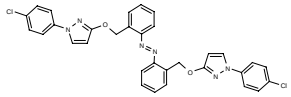
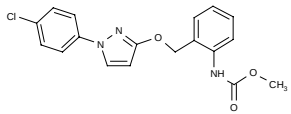
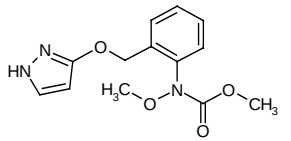
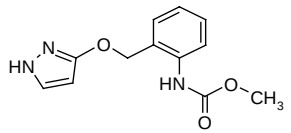
According to the presented results, the parent compound pyraclostrobin is the only relevant residue for quantitation in soil. Although the metabolites BF 500-6 (azoxy-dimer) and BF 500-7 (azo-dimer) did appear > 10% in one or several aerobic soils in the laboratory studies, the results of the field dissipation study showed that those metabolites are detected if at all only in trace amounts close to the determination limit. The soil photolysis revealed that the formation of both metabolites is reduced under irradiated conditions which can explain the results from the field studies. Ecotoxicity studies showed that the metabolites do not have any

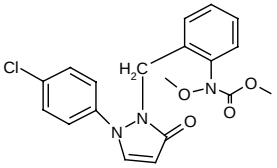
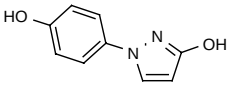
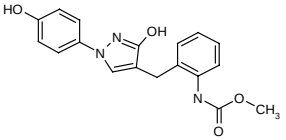
effect on earthworms or on the microbial activity in soil. Furthermore, both metabolites are not biologically active.
The metabolite BF 500-3 (des-methoxy-pyraclostrobin) occured in amounts significantly > 10% only under strong anaerobic conditions in soil. The metabolite BF 500-4 ("aniline") and its structural isomers (500M74/ 500M75) only hardly reached 10% during the anaerobic soil metabolism study and were quickly degraded again.
Since such anaerobic soil conditions will never occur during application of pyraclostrobin according to common agricultural practice, both metabolites have no environmental significance in soil. BF 500-3 could not be detected in the field studies. The aerobic soil metabolism studies and the field soil dissipation studies showed that under natural outdoor conditions, BF 500-3 and BF 500-4 are very short-lived intermediates, which are immediately degraded further and will never reach relevant amounts.
Although no metabolite could be detected in the field studies, PEC groundwater calculations were performed for BF 500-3, BF 500-6 and BF 500-7. The calculations clearly show that even if the metabolites are formed in soil, they do not have any leaching potential into deeper soil layers or groundwater.
Therefore, the parent pyraclostrobin is the only relevant residue in soil.

B.8.9.2 Water

According to the presented results, the parent compound pyraclostrobin is the only relevant residue for quantitation in water. A water/sediment study performed under realistic light and temperature conditions showed that three metabolites (BF 500-11, BF 500-13, and BF 500-14) temporarily exceeded 10% of the total applied radioactivity. However, ecotoxicity tests with rainbow trout, daphnia, and algea as well as biological efficacy tests (see Doc MII, 3.4) show that all metabolites are considerably less toxic and less biologically active than the parent. They are therefore considered as non-relevant residues.
The metabolite BF 500-3, which was formed in amounts > 10% only in the sediment, did not have any effect on sediment dwelling animals (Chironomids). Since it has a very high K_{oc}-value and a low water solubility, there is no risk of movement from the sediment into the water. It is also considered as non-relevant.
Therefore, the parent pyraclostrobin is proposed as the only relevant residue in water.

Table B.8.9-1: Major metabolites found in soil, water and sediment

Code	Active substance	Residue definition relevant to the environment			
BAS 500 F		The active substance pyraclostrobin is relevant for the compartments soil, water (including ground- and surface water), sediment and air			
Metabolites		**Occurrence in**	**Assessment of the relevance**		
Code	**Structural formula**	**Soil/Water/Sediment**	**Toxicology**	**Ecotoxicology**	**Biological activity**
BF 500-6		Soil:max. 31 % after 120 d	Not found in rats*	No unacceptable effects observed in soil micro-flora and earthworms	Not relevant
BF 500-7		Soil: max. 13 % after 62 d	Not found in rats	No unacceptable effects observed in soil micro-flora and earthworms	Not relevant
BF 500-3		Soil: max. 85 % after 14 d (tolyl-label), anaerobic conditions Sediment: 12 % after 100 d (pond system), 66 % after 14 d (river system)	Occurring in rat faeces (< 6 %) = 500M07	Detectable in significant amounts only under anaerobic conditions	Not relevant
BF 500-11		Water/photolysis study: 44.5 % after 21 d (tolyl label)	Not found in rats, no indication of point mutations *in vitro*	Considerably less toxic than parent compound to fish, daphnia and algae	Not relevant
BF 500-13		Water/photolysis study: 16.8 % after 6 d (tolyl label)	Not found in rats, no indication of point mutations *in vitro*	Considerably less toxic than parent compound to fish, daphnia and algae	Not relevant

BF 500-14		Water/photolysis study: 14.8 % after 6 h (tolyl label)	Not found in rats, no indication of point mutations *in vitro*	Considerably less toxic than parent compound to fish, daphnia and algae	Not relevant
BF 500-15		Water/photolysis study: 27 % after 1 day	Not found in rats	Not found in water/ sediment study (i.e. under more realistic conditions)	Not relevant
500 M 58		Water/photolysis study: 20.3 % after 1 d (tolyl label), 22.7 % after 6 h (chlorophenyl label)	Not found in rats	Not found in water/ sediment study (i.e. under more realistic conditions)	Not relevant

B.8.10 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-7.1.1.1.2	Scharf, J.	1999	Soil Photolysis of BAS 500 F. 1999/11300, 35876 GLP, unpublished BOD2000-640	Y	BAS
AIIA-7.1.1.2.1	Bayer, H.	1999	Storage Stability of BAS 500 F in Terrestrial Soil Samples. 1999/11288, 35881 GLP, unpublished BOD2000-650	Y	BAS
AIIA-7.1.1.2.1	Ebert, D.	1999	Investigations on the release of soil-bound residues of 14C-BAS 500 F by earthworms. 1999/11289, 53058 GLP, unpublished BOD2000-644	Y	BAS
AIIA-7.1.1.2.1	Ebert, D.	1999	The degradation Behaviour of 14C-BAS 500 F in Different Soils (DT50/DT90). 1999/11091, 35877 GLP, unpublished BOD2000-638	Y	BAS
AIIA-7.1.1.1.1; AIIA-7.1.1.2.1	Ebert, D.	1999	The Aerobic Soil Metabolism of 14C-BAS 500 F. 1999/10090, 35644 GLP, unpublished BOD2000-637	Y	BAS
AIIA-7.1.1.1.1; AIIA-7.1.1.2.1	Ebert, D.	1998	The aerobic soil metabolism of 14C-BAS 500 F. 98/11201, 35643 GLP, unpublished BOD2000-636	Y	BAS
AIIA-7.1.1.1.2; AIIA-7.1.1.2.1	Kellner, O.	1999	The Anaerobic Soil Metabolism of BAS 500 F (14C-Chlorophenyl). 1999/11103, 35875 GLP, unpublished BOD2000-642	Y	BAS

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-7.1.1.1.2; AIIA-7.1.1.2.1	Kellner, O.	1999	The Anaerobic Soil Metabolism of BAS 500 F (14C-Tolyl). 1999/10079, 35645 GLP, unpublished BOD2000-641	Y	BAS
AIIA-7.1.1.2.2	Kellner, O. and Zangmeister, W.	1999	Field soil dissipation of BAS 500 F (304428) in formulation BAS 500 01 F (1998 - 1999). 1999/11301, EU/FA/049/98 GLP, unpublished BOD2000-648	Y	BAS
AIIA-7.1.1.2.2	Kellner, O. and Zangmeister, W.	1999	Field soil dissipation of BAS 500 F (304428) in formulation BAS 500 01 F. 1999/11292, DE/FA/045/97 GLP, unpublished BOD2000-647	Y	BAS
AIIA-7.1.2	Seher, A.	1999	Soil Adsorption/Desorption Study of 369315 (BF 500-7). 1999/10684, PI990009 GLP, unpublished BOD2000-654	Y	BAS
AIIA-7.1.2	Seher, A.	1999	Soil Adsorption/Desorption Study of 364380 (BF 500-6). 1999/10686, PI990002 GLP, unpublished BOD2000-653	Y	BAS
AIIA-7.1.2	Seher, A.	1999	Soil Adsorption/Desorption Study of 340266 (BF 500-3) on Soils. 1999/10695, PI990019 GLP, unpublished BOD2000-652	Y	BAS
AIIA-7.1.2	Ziegler, G.	1998	Soil Adsorption/Desorption Study of 304428 (BAS 500 F). 98/10650, 35882 GLP, unpublished BOD2000-651	Y	BAS
AIIA-7.1.3.1	Ziegler, G.	1998	Leaching Behaviour of 14C-BAS 500 F in four soils under laboratory conditions. 98/11350, 42375 GLP, unpublished BOD2000-656	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-7.1.3.2	Ziegler, G.	1998	Leaching Behaviour of 14C-BAS 500 F after Aerobic Aging for 30 Days. 98/11202, 35648 GLP, unpublished BOD2000-657	Y	BAS
AIIA-2.9; AIIA-7.2.1.2	Scharf, J.	1999	Aqueous Photolysis of BAS 500 F. 1999/11286 GLP, unpublished LUF2000-249	Y	BAS
AIIA-7.2.1.3.2	Ebert, D.	1999	Degradation of BAS 500 F in Aerobic Aquatic Environment Under Irradiated Conditions. 1999/11791, 52978 GLP, unpublished WAS2000-351	Y	BAS
AIIA-7.2.1.3.1	Reuschenbach	1999	Determination of the Biodegradability of BAS 500 F in the Manometric Respirometry Test according to GLP, EN 45001 and ISO 9002. 99/10655, 99/0164/26/1 GLP, unpublished WAS2000-349	Y	BAS
AIIA-7.2.1.3.2	Staudenmaier, H.	1999	Degradation of BAS 500 F in Aerobic Aquatic Environment. 1999/11241, 35642 GLP, unpublished WAS2000-350	Y	BAS
AIIA-7.2.2	Scharf, J.	1999	Volatilization of BAS 500 F after Application of BAS 500 00 F on Soil and on Plant Surfaces. 1999/11093, 36008 GLP, unpublished LUF2000-250	Y	BAS
AIIA-2.10; AIIA-7.2.2	Scharf, J.	1999	Photochemical Oxidative Degradation Of BAS 500 F (QSAR Estimates). 99/10086, JS-99-04 GLP, unpublished LUF2000-246	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-9.1.3; AIIIA-11	Regenstein, H.	1999	Document M III - Tier II summeries and assessments of individual tests and studies and groups of tests and studies. 1999/11982 not GLP, unpublished BOD2000-660	Y	BAS
AIIIA-9.1.3	van de Veen, J.R.	1999	Calcuation of Predicted Environmental Concentrations for BF 500-6 and BF 500-7 in Soil (PECs) for a Vineyard and a Cereals Scenario. 1999/11802, CALC-152 not GLP, unpublished BOD2000-624	Y	BAS
AIIIA-9.1.3	van de Veen, J.R.	1999	Calcuation of Predicted Environmental Concentrations for BAS 5000 F in Soil (PECs) on a European Level in Cereals. 1999/11801, CALC-151 not GLP, unpublished BOD2000-623	Y	BAS
AIIIA-9.1.3	van de Veen, J.R.	1999	Calcuation of Predicted Environmental Concentrations for BAS 5000 F in Soil (PECs) on a European Level for a Vineyard Scenario. 1999/11800, CALC-150 not GLP, unpublished BOD2000-622	Y	BAS
AIIIA-9.2.1	van de Veen, J.R.	1999	Calcuation of Predicted Environmental Concentrations (PECgw) of BF 500-3 in Groundwater. 1999/11803, CALC-153 not GLP, unpublished WAS2000-496	Y	BAS
AIIIA-9.2.1	van de Veen, J.R.	1999	Calcuation of Predicted Environmental Concentrations (PECgw) of BAS 500 F and its Metabolites BF 500-6 and BF 500-7 in Groundwater on a European Level. 1999/11799, CALC-141 not GLP, unpublished WAS2000-495	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-9.2.3	Becker, F.A., Klein, A.W., Winkler, R., Jung, B., Bleiholder, H. and Schmider, F.	1999	The degree of ground coverage by arable crops in estimating the amount of spray solution intercepted by the plants. GLP, published Nachrichtenbl. Deut. Pflanzenschutzd., 51, 9, 1999, 237-242 WAS2001-52	N	-
AIIIA-9.2.3	Gottesbueren, B.	2000	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Moving Surface Water (PECsw) for a Vineyard Scenario. 99/11798, CALC-149 not GLP, unpublished WAS2000-341	Y	BAS
AIIIA-9.2.3	Hauck, T. and Gottesbueren, B.	2000	Derivation of low flow rates for standard surface waters (slow moving water bodies). DocID 2000/1014985, CALC-189 not GLP, unpublished WAS2000-671	Y	BAS
AIIIA-9.2.3	Hollis, J.M.	2000	The Derivation of Flow Statistics for UK Rivers. DocID 2000/1004087 not GLP, unpublished WAS2000-672	Y	BAS
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	2000	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Moving Surface Water (PECsw) for a Cereal Scenario. 99/11805, CALC-154 not GLP, unpublished WAS2000-343	Y	BAS
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	1999	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Static Surface Water (PECsw) for a Cereal Scenario. 1999/11806, CALC-155 not GLP, unpublished WAS2000-342	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	1999	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Static Surface Water (PECsw) for a Vineyard Scenario. 1999/11792, CALC-142 not GLP, unpublished WAS2000-340	Y	BAS
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	1999	Calcuation of Predicted Environmental Concentrations for BF 500-3 in Sediment (PECsed) for a Vineyard Scenario. 1999/11797, CALC-148 not GLP, unpublished WAS2000-348	Y	BAS
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	1999	Calcuation of Predicted Environmental Concentrations for BAS 500 F in Sediment (PECsed) for a Vineyard Scenario. 1999/11796, CALC-147 not GLP, unpublished WAS2000-347	Y	BAS
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	1999	Calcuation of Predicted Environmental Concentrations for BF 500-14 in Surface Water (PECsw) for a Vineyard Scenario. 1999/11795, CALC-145 not GLP, unpublished WAS2000-346	Y	BAS
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	1999	Calcuation of Predicted Environmental Concentrations for BF 500-13 in Surface Water (PECsw) for a Vineyard Scenario. 1999/11794, CALC-144 not GLP, unpublished WAS2000-345	Y	BAS
AIIIA-9.2.3	Platz, K. and Gottesbueren, B.	1999	Calcuation of Predicted Environmental Concentrations for BF 500-11 in Surface Water (PECsw) for a Vineyard Scenario. 1999/11793, CALC-143 not GLP, unpublished WAS2000-344	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-9.2.1; AIIIA-9.2.3; AIIIA-11	Regenstein, H.	1999	Document M III - Tier II summeries and assessments of individual tests and studies and groups of tests and studies. 1999/11982 not GLP, unpublished WAS2000-355	Y	BAS
AIIIA-9.2.3	van de Veen, J.R.	2000	Estimation of the Drainage Entry Route Percentages of BAS 500 F and its Soil Metabolites BF 500-6 and BF 500-7 into Surface Waters. ID 2000/1014991, CALC-204 not GLP, unpublished WAS2000-669	Y	BAS
AIIIA-9.3; AIIIA-11	Regenstein, H.	1999	Document M III - Tier II summeries and assessments of individual tests and studies and groups of tests and studies. 1999/11982 not GLP, unpublished LUF2000-253	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Pyraclostrobin

B-9: Ecotoxicology

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.9 Ecotoxicology 413

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1) 413
B.9.1.1 Acute Oral Toxicity (Annex IIA 8.1, Annex IIIA 10.1.1) 413
B.9.1.2 Dietary toxicity (Annex IIA 8.1.2) 414
B.9.1.3 Effects on reproduction (Annex IIA 8.1.3) 415
B.9.1.4 Other studies (Annex IIIA 10.1.2, 10.1.3, 10.1.4) 417
B.9.1.5 Risk assessment for birds 417
B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2) 418
B.9.2.1 Acute toxicity to fish (Annex IIA 8.2.1, Annex IIIA 10.2.1) 418
B.9.2.1.1 Active Substance 418
B.9.2.1.2 Major metabolites BF 500-11, BF 500-13 and BF 500-14 420
B.9.2.1.3 Formulated product (BAS 500 00 F) 422
B.9.2.2 Chronic toxicity to fish (Annex IIA 8.2.2) 426
B.9.2.2.1 Active Substance 426
B.9.2.3 Bioconcentration in fish (Annex IIA 8.2.3) 428
B.9.2.3.1 Active substance 428
B.9.2.3.2 Metabolites 430
B.9.2.4 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4) 430
B.9.2.4.1 Active substance 430
B.9.2.4.2 Major metabolites BF 500-11, BF 500-13 and BF 500-13 431
B.9.2.4.3 Formulated product (BAS 500 00 F) 432
B.9.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5) 433
B.9.2.5.1 Active substance 433
B.9.2.6 Effects on algal growth (Annex IIA 8.2.6) 434
B.9.2.6.1 Active substance 434
B.9.2.6.2 Major metabolites BF 500-11, BF 500-13 and BF 500-14 434
B.9.2.6.3 Formulated product (BAS 500 00 F) 435
B.9.2.7 Effects on sediment-dwelling organisms (Annex IIA 8.2.7) 436
B.9.2.7.1 Active substance 436
B.9.2.8 Aquatic mesocosm (Annex III A 10.2.2) 437
B.9.2.9 Risk assessment for aquatic organisms 441
B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3) 446
B.9.3.1 Toxicity to mammals (Annex IIIA 10.3) 446
B.9.3.2 Risk assessment for mammals 447
B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.4) 448
B.9.4.1 Acute toxicity (Annex IIA 8.3.1, Annex IIIA 10.4) 448
B.9.4.1.1 Contact and oral toxicity of pyraclostrobin (Reg. No. 304428) to honeybees 448
B.9.1.1.2 Contact and oral toxicity of BAS 500 00 F 448
B.9.1.2 Bee brood feeding test (Annex IIA 8.3.1.2) 449
B.9.1.3 Residue test (Annex IIIA 10.4.2) 449
B.9.1.4 Cage test (Annex IIIA 10.4.3) 449
B.9.1.5 Field test (Annex IIIA 10.4.4) 449
B.9.1.6 Tunnel test (Annex IIIA 10.5.5) 449
B.9.1.7 Risk assessment for honeybees 450

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5) 450
B.9.5.1 Acute toxicity (Annex IIA 8.3.2, Annex IIIA 10.5.1) 450
B.9.5.2 Field tests (Annex III 10.5.2) 457
B.9.5.3 Risk assessment for non-target terrestrial arthropods 459
B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1) 460
B.9.6.1 Acute toxicity (Annex IIA 8.4.1, Annex IIIA 10.6.1.1) 460
B.9.6.2 Other studies (Annex IIA 8.4.2, Annex IIIA 10.6.1.2, Annex IIIA 10.6.1.3) 462
B.9.6.2.1 Effect on reproduction 462
B.9.6.2.2 Effect of soil bound residues of BAS 500-F 463
B.9.6.2.3 Effects in the field 463
B.9.6.3 Risk assessment for earthworms 469
B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2) 470
B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7) 472
B.9.8.1 Carbon conversion (Annex IIA 8.5; Annex IIIA 10.7) 472
B.9.8.2 Nitrogen conversion (Annex IIA 8.5; Annex IIIA 10.7) 473
B.9.8.3 Risk assessment for soil micro-organisms 473
B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6) 474
B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7) 474
B.9.10.1 Risk assessment 475
B.9.11 References relied on 475

B.9 Ecotoxicology

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.1 Acute Oral Toxicity (Annex IIA 8.1, Annex IIIA 10.1.1)

Title:	**Report BAS 500 F (Reg.No.304 428) - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus);**
Author:	Munk, R. (1997)
BBA-Ref.-No.:	AVS2000-60
Test substance:	Technical Pyraclostrobin
Purity:	97.09 %
Guideline:	EPA 71-1
Test species:	Bobwhite quail (Colinus virginianus)
Age:	5.5 months
Birds per treatment:	5 M + 5 F
Administration:	Intubation
Solvent / vehicle:	Olive oil
Dose levels:	0/500/1000/2000 mg/kg
Findings:	There were no mortalities; diarrhea and/or soft feces in all groups including the control were attributed to the solvent (olive oil). Body weight and feed consumption were reduced in the two highest dose groups.
LD50:	>2000 mg/kg bw
Lowest lethal dose:	>2000 mg/kg bw
NOED:	500 mg/kg bw
valid:	yes
GLP compliance:	yes

Title:	**Report BAS 500 00 F - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	AVS2000-65
Test substance:	Formulation BAS 500 00 F Pyraclostrobin 247.83 g/l
Guideline:	EPA 71-1
Test species:	Bobwhite quail (Colinus virginianus)
Age:	ca. 14 months
Birds per treatment:	5 M + 5 F

Administration:	Intubation
Solvent / vehicle:	none
Dose levels:	0/250/500/1000/2000 mg/kg
Findings:	A single mortality was observed in the top dose group; soft faeces occurred in all groups including the control, however more pronounced at doses of 1000 and 2000 mg/kg bw; food uptake was reduced at doses of 500 mg/kg bw and higher; body weight was slightly reduced at 2000 mg/kg bw.
LD50:	>2000 mg/kg bw
Lowest lethal dose:	2000 mg/kg bw
NOED:	250 mg/kg bw
valid:	yes
GLP compliance:	yes

B.9.1.2 Dietary toxicity (Annex IIA 8.1.2)

Title:	**Test Report BAS 500 F - Avian dietary LC50 test in chicks of the bobwhite quail (Colinus virginianus);**
Author:	Munk, R. (1998)
BBA-Ref.-No.:	AVS2000-61
Test substance:	Technical Pyraclostrobin
Purity	98.7 %
Guideline:	EPA 71-2 / OECD 205
Test species:	Bobwhite quail (Colinus virginianus)
Age:	12 days
Birds per treatment:	10 (unsexed)
Solvent / vehicle:	Acetone
Exposure period:	5 d
Conc. levels (nom.):	0/313/625/1250/2500/5000 ppm
Conc. levels (meas.):	0/304/609/1247/2529/5177 ppm
Findings:	In the 313 ppm group 5 out of 10 chicks died due to a technical failure; apart from that there were no mortalities and no signs of intoxication. Feed consumption was slightly reduced in the two highest test groups (2500 and 5000 ppm) during the first day of the study, however, feed consumption for the entire exposure period as well as body weight were not deviant from the control.
LC50:	>5000 ppm
Lowest lethal conc.:	>5000 ppm
NOEC:	5000 ppm
valid:	yes
GLP compliance:	yes

Title:	**Test Report BAS 500 F - Avian dietary LC50 test in chicks of the mallard duck (Anas platyrhynchos L.)**
Author:	Munk, R. (1998)
BBA-Ref.-No.:	AVS2000-62
Test substance:	Technical Pyraclostrobin
Purity	97.09 %
Guideline:	EPA 71-2 / OECD 205
Test species:	Mallard duck (Anas platyrhynchos)
Age:	9 days
Birds per treatment:	10 (unsexed)
Solvent / vehicle:	Acetone
Exposure period:	5 d
Conc. levels (nom.):	0/157/313/625/1250/2500/5000 ppm
Conc. levels (meas.):	0/162/322/644/1287/2575/5150 ppm
Findings:	There were observed no mortalities and no signs of intoxication. Feed consumption was reduced at concentrations of 313 ppm and higher. Body weight gain was reduced at concentrations of 1250 ppm and higher.
LC50:	>5000 ppm
Lowest lethal conc.:	>5000 ppm
NOEC:	157 ppm
valid:	yes; the lowest concentration (157 ppm) was tested in a supplementary study.
GLP compliance:	yes

B.9.1.3 Effects on reproduction (Annex IIA 8.1.3)

Title:	**BAS 500 F: A reproduction study with the northern bobwhite**
Author:	Frey, L.T., Beavers, J.B. and Jaber, M. (1999)
BBA-Ref.-No.:	AVS2000-64
Test substance:	Technical Pyraclostrobin
Purity:	98.7 %
Guideline:	EPA 71-4 / OECD 206
Test species:	Bobwhite quail (Colinus virginianus)
Age:	25 weeks
Birds per treatment:	16 pairs
Solvent / vehicle:	Acetone
Exposure period:	23 w
Conc. levels (nom.):	0/100/300/1000 ppm

Findings:	There were no treatment-related effects on adult birds nor on any of the reproductive parameters.
NOEC:	1000 ppm
valid:	yes
GLP compliance:	yes

Title:	**BAS 500 F: A reproduction study with the mallard**
Author:	Frey, L.T., Beavers, J.B. and Jaber, M. (1999)
BBA-Ref.-No.:	AVS2000-63
Test substance:	Technical Pyraclostrobin
Purity:	98.7 %
Guideline:	EPA 71-4 / OECD 206
Test species:	Mallard duck (Anas platyrhynchos)
Age:	22 w
Birds per treatment:	16 P
Solvent / vehicle:	Acetone
Exposure period:	20 w
Conc. levels (nom.):	0/50/100/300/1000 ppm
Conc. levels (meas.):	0/84.3-91.4/89.3-107.5/104-104.3/97.6-110.1 %
Findings:	There were no treatment-related effects on adults nor on any of the reproductive parameters
NOEC:	1000 ppm
valid:	yes
GLP compliance:	yes

Table 9.1-1: Summary of avivan toxicity data

Test material	Species	Test	NOEL	LD50/LC50	Unit
Pyraclostrobin	Bobwhite quail	Acute	500	>2000	mg/kg bw
BAS 500 00 F	Bobwhite quail	Acute	250	>2000	mg/kg bw
Pyraclostrobin	Bobwhite quail	5-day-dietary	5000	>5000	ppm
Pyraclostrobin	Mallard duck	5-day-dietary	157	>5000	ppm
Pyraclostrobin	Bobwhite quail	Reproduction	1000		ppm
Pyraclostrobin	Mallard duck	Reproduction	1000		ppm

B.9.1.4 Other studies (Annex IIIA 10.1.2, 10.1.3, 10.1.4)

Supervised field trials were not conducted due to the favourable toxicity/exposure ratios (see below).

Acceptance of bait, granules, or treated seeds by birds is not applicable, because pyraclostrobin formulations are to be applied exclusively as sprays.

B.9.1.5 Risk assessment for birds

Birds may be exposed to pyraclostrobin mainly by the consumption of contaminated feed. Depending on species this may be insects, grape fruits or green plant material. The risk assessment will be based on a maximum rate of 0.16 kg as/ha in grapes and 0.25 kg as/ha on turf.

Exposure assessment:
- Residues in insects are estimated according to Hoerger and Kenaga
- Residues in grapes were in the range of 0.42-2.0 mg/kg (measured data, see chapter 7.6)
- With regard to grass measured data from cereals are taken as a surrogate which were in the range of 2.7-14.0 mg/kg (see chapter 7.6)

In order to consider the worst-case condition it is assumed that birds feed exclusively on contaminated material and that herbivorous birds have a daily feed demand of 25 % of their body weight and insectivorous and frugivorous birds of 40 % of their body weight. Then the maximum daily intake is 3.8 mg/kg bw.

Table 9.1-2: Exposure assessment for birds

Use	Maximum application rate (kg/ha)	Feed	Typical maximum residue[1] (mg/kg)	Initial residue (mg/kg)	Relative feed demand (%)	Maximum daily intake (mg/kg bw)
Grape	0.16	Insects	29*R	4.6	40	1.9
Grape	0.16	Fruit		2.0[2]	40	0.8
Turf	0.25	Insects	29*R	7.3	40	2.9
Turf	0.25	Grass		15[2]	25	3.8

[1] according to Hoerger and Kenaga (1972); R = application rate in kg/ha
[2] based on measured residues

Toxicity/exposure ratios: For the acute TER the LD_{50} is related to the maximum daily intake; for the short-term TER the LC_{50} is related to the initial residue; for the long-term TER the NOEC from the reproduction test is related to the initial residue. All TER-values are well above the Annex-VI-triggers; so the risk to birds is considered as low.

Table 9.1-3: Toxicity/exposore ratios for birds

Use	Feed	Time-scale	Toxicity/Exposure ratio
Turf	Insects	acute	TERa = >2000/2.9 = >690
Turf	Insects	short-term	TERst = >5000/7.3 = >690
Turf	Insects	long-term	TERlt = 1000/7.3 = 136
Turf	Grass	acute	TERa = >2000/3.8 = >530
Turf	Grass	short-term	TERst = >5000/15 = >330
Turf	Grass	long-term	TERlt = 1000/15 = 67

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

The following data were generated in accordance with adopted international guidelines and are considered valid. Analytical concentration controls have been performed in all aquatic test systems. The risk assessment is based on the use pattern and application rates outlined in this monograph.

B.9.2.1 Acute toxicity to fish (Annex IIA 8.2.1, Annex IIIA 10.2.1)

B.9.2.1.1 Active Substance

Title: BAS 500 F - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours)
Author: Zok, S. (1999)
BBA-Ref.-No.: WAT2000-232

Test substance: BAS 500 F; purity: 99.0 %

Guideline: EPA 72-1, EEC 92/69, OECD 203
Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792),
mean body length 6.25 (5.9 – 6.9) cm;
mean body weight 2.15 (1.7 – 3.1) g.
Animals per treatment: 10 fish per aquarium (loading about 0.2 g fish/L) and per concentration
Dose levels: Control, 0.00316, 0.00464, 0.00681, 0.01, 0.0147 and 0.0215 mg as/L (nominal), static system (96 h).

Findings: BAS 500 F caused mortality to the rainbow trout at concentrations ≥ 0.01 mg as/L. Behavioural symptoms such as apathy, convulsions, narcotic-like state and swimming near the bottom were monitored in the 0.00681 mg as/L concentration and above during the study.
LC_{50}: 0.00616 mg as/L (mean measured concentration)
NOEC: 0.00330 mg as/L (mean measured concentration)

GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 F - Acute toxicity study on the bluegill (*Lepomis macrochirus* RAF.) in a static system (96 hours)**
Author:	Munk, R. (1998)
BBA-Ref.-No.:	WAT2000-233
Test substance:	BAS 500 F; purity: 97.1 %
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Bluegill (*Lepomis macrochirus* Raf.), mean body length 6.31 (5.5 – 7.0) cm; mean body weight 4.02 (2.5 – 5.4) g.
Animals per treatment:	10 fish per aquarium (loading about 0.4 g fish/l) and per concentration.
Dose levels:	Control, solvent control acetone, 0.00464, 0.00681, 0.01, 0.0147, 0.0215, 0.0316, 0.0464 mg as/L (nominal), stock solution in acetone, static system (96 h)
Findings:	BAS 500 F caused mortality to the bluegill at nominal concentrations ≥ 0.0316 mg as/L. Behavioural symptoms such as tumbling were monitored in the highest concentration tested (0.0464 mg as/L) before the fish died.
LC_{50}:	> 0.0196 and < 0.0335 mg as/L (mean measured concentration)
NOEC:	0.0109 mg as/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

Title:	**Acute toxicity study on the common carp (*Cyprinus carpio* L.) in a static system (96 hours)**
Author:	Munk, R. (1998)
BBA-Ref.-No.:	WAT2000-234
Test substance:	BAS 500 F; purity: 97.1 %
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Common carp (*Cyprinus carpio* L.) scaly variety, mean body length 7.23 (4.5 – 7.7) cm; mean body weight 6.42 (4.6 – 7.2) g.
Animals per treatment:	10 fish per aquarium (loading about 0.6 g fish/L) and per concentration.
Dose levels:	Control, solvent control, 0.01, 0.0147, 0.0215, 0.0316, 0.0464 and 0.0681 mg as/L (nominal), stock solution in acetone, static system (96 h)

Findings:	BAS 500 F caused mortality to the common carp at nominal concentrations ≥ 0.0464 mg as/L. Behavioural symptoms such as apathy, tumbling and narcosis-like state were monitored at the test concentrations 0.0464 mg as/L and 0.0681 mg as/L before the fish died.
LC_{50}:	> 0.0121 and < 0.0258 mg as/L (mean measured concentrations)
NOEC:	0.0121 mg as/L (mean measured concentrations)
GLP compliance:	yes
Valid:	yes

B.9.2.1.2 Major metabolites BF 500-11, BF 500-13 and BF 500-14

Title:	**Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-235
Test substance:	BF 500-11; purity: 98.9 %
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), mean body length 6.71 (5.9 – 7.7) cm; mean body weight 2.8 (2.1 – 3.8) g.
Animals per treatment:	10 fish per aquarium (loading about 0.6 g fish/L) and per concentration; 3 x 10 fish at 100 mg/L
Dose levels:	Control, 1.0, 10.0, 100.0 mg/L (nominal), static system (96 h).
Findings:	BF 500-11 caused no mortality to the rainbow trout at any of the concentrations tested. Behavioural symptoms such as swimming near the bottom and apathy were observed in several fish of the 100 mg/L dose groups.
LC_{50}:	> 100 mg/L (nominal concentration)
NOEC:	10 mg/L (nominal concentration)
GLP compliance:	yes
Valid:	yes

Title:	**Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours)**
Author:	Zok S. (1999)
BBA-Ref.-No.:	WAT2000-236
Test substance:	BF 500-13; purity: 99.6 %
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), mean body length 6.715 (6.2 – 7.4) cm;

	mean body weight 2.735 (2.1 – 3.5) g.
Animals per treatment:	10 fish per aquarium (loading about 0.5 g fish/L) and per concentration
Dose levels:	Control, 5, 10, 22, 50, 100 mg/L (nominal), static system (96 h).
Findings:	BF 500-13 caused mortality to the rainbow trout at 100 mg/L. Behavioural symptoms such as convulsions, narcotic-like state, tumbling, swimming near the water surface and swimming near the bottom were observed at 22 mg/L and higher concentrations.
LC_{50}:	> 50 mg/L and < 100 mg/L (nominal concentration)
NOEC:	10 mg/L (nominal concentration)
GLP compliance:	yes
Valid:	yes

Title:	**Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours)**
Author:	Zok S. (1999)
BBA-Ref.-No.:	WAT2000-237
Test substance:	BF 500-14; purity: 96.1 %
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), mean body length 6.08 (5.5 – 6.8) cm; mean body weight 2.11 (1.6 – 2.6) g.
Animals per treatment:	10 fish per aquarium (loading about 0.8 g fish/L) and per concentration
Dose levels:	Control, solvent control ,5, 10, 22, 50, 100 mg/L (nominal), static system (96 h).
Findings:	BF 500-14 caused no mortality to the rainbow trout up to concentrations of 50 mg/L. In the highest concentration tested (100 mg/L) all fish were dead at the end of the study. No behavioural symptoms were observed in surviving fish.
LC50:	> 39.4 mg/L and < 82.6 mg/L (mean measured concentration)
NOEC:	39.4 mg/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

B.9.2.1.3 Formulated product (BAS 500 00 F)

Title:	**BAS 500 00 F - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-257
Test substance:	BAS 500 00 F, 247.83 g as/L
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1972), mean body length 6.1 (5.4 – 6.8) cm; mean body weight 2.8 (2.0 – 3.6) g.
Animals per treatment:	10 fish per aquarium (loading about 0.3 g fish/L) and per concentration.
Dose levels:	Control, 0.01, 0.0147, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1 mg/L (nominal), static system (96 h).
Findings:	BAS 500 00 F caused mortality at concentrations ≥ 0.0215 mg/L. Other substance-related effects included symptoms like apathy, convulsions, narcotic-like state, swimming near the bottom and discoloration.
LC_{50}:	0.0169 mg/L (mean measured concentration)
NOEC:	0.0123 mg/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 00 F - Acute toxicity study on the bluegill (*Lepomis macrochirus* RAF.) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-258
Test substance:	BAS 500 00 F, 247.83 g ai/L
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Bluegill (*Lepomis macrochirus* RAF.), mean body length 7.03 (6.1 – 7.9) cm; mean body weight 3.79 (2.7 – 5.1) g.
Animals per treatment:	10 fish per aquarium (loading about 0.4 g fish/L) and per concentration.
Dose levels:	Control, 0.0032, 0.0058, 0.01, 0.018, 0.032, 0.058 mg as/L (nominal), static system (96 h).
Findings:	BAS 500 00 F caused mortality at concentrations ≥ 0.018 mg as/L. Other substance-related effects included symptoms like apathy and narcotic-like state.
LC_{50}:	> 0.0146 and < 0.0299 mg as/L (mean measured concentration)

NOEC:	0.008 mg as/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 00 F: Acute toxicity study on the common carp (*Cyprinus carpio* L.) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-259
Test substance:	BAS 500 00 F, 247.83 g as/L
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Common carp (*Cyprinus carpio* L.), mean body length 5.77 (4.9 7.0) cm; mean body weight 3.36 (2.1 – 5.8) g.
Animals per treatment:	10 fish per aquarium (loading about 0.3 g fish/L) and per concentration.
Dose levels:	Control, 0.0032, 0.0058, 0.01, 0.018, 0.032, 0.058 mg as/L (nominal), static system (96 h)
Findings:	BAS 500 00 F caused mortality at concentrations ≥ 0.058 mg as/L. No substance-related effects were observed at lower concentrations.
LC_{50}:	> 0.0209 and < 0.0497 mg as/L (mean measured concentration)
NOEC:	0.0209 mg as/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 00 F - Acute toxicity study on the orange red killifish (*Oryzias latipes* SCHLEGEL) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-260
Test substance:	BAS 500 00 F, 247.83 g as/L
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Orange red killifish (*Oryzias latipes* SCHLEGEL), mean body length 3.79 (3.6 – 4.2) cm; mean body weight 0.523 (0.41 – 0.69) g.
Animals per treatment:	10 fish per aquarium (loading about 0.5 g fish/L) and per concentration.
Dose levels:	Control, Control*, 0.0032, 0.0058, 0.01, 0.018, 0.032, 0.058, 0.1* mg as/L (nominal), static system (96 h) *added concentrations.

Findings:	BAS 500 00 F caused mortality at concentrations ≥ 0.058 mg as/L. Other substance-related effects included symptoms like apathy, narcotic-like state, tumbling and swimming near the bottom.
LC_{50}:	> 0.0325 and < 0.0885 mg as/L. (mean measured concentration)
NOEC:	0.0165 mg as/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 00 F - Acute toxicity study on the fathead minnow (*Pimephales promelas* RAF.) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-261
Test substance:	BAS 500 00 F, 247.83 g as/L
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Fathead minnow (*Pimephales promelas* RAF.), body length 5.05 (4.6 – 5.7) cm; body weight 1.19 (0.9 – 1.7) g.
Animals per treatment:	10 fish per aquarium (loading about 0.1 g fish/L) and per concentration.
Dose levels:	Control, 0.0032, 0.0058, 0.01, 0.018, 0.032, 0.058 mg as/L (nominal), static system (96 h).
Findings:	BAS 500 00 F caused mortality at concentrations ≥ 0.018 mg as/L. No substance-related effects were observed at lower concentrations.
LC_{50}:	> 0.012 and < 0.0235 mg as/L (mean measured concentration)
NOEC:	0.007 mg as/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 00 F - Acute toxicity study on the zebra fish (*Brachydanio rerio* HAM. and BUCH.) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-262
Test substance:	BAS 500 00 F, 247.83 g as/L
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Zebra fish (*Brachdanio rerio* HAM. and BUCH.), mean body length 3.39 (3.1 – 3.7) cm; mean body weight 0.335 (0.25 – 0.46) g.
Animals per treatment:	10 fish per aquarium (loading about 0.3 g fish/L) and per concentration.

Dose levels:	Control, Control*, 0.0032, 0.0058, 0.01, 0.018, 0.032, 0.058, 0.1*, 0.18* mg as/L (nominal), static system (96 h), *added concentrations.
Findings:	BAS 500 00 F caused mortality at concentrations ≥ 0.058 mg as/L. No substance-related effects were observed at lower concentrations.
LC_{50}:	> 0.0417 and < 0.0887 mg as/L (mean measured concentration)
NOEC:	0.0234 mg as/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 00 F - Acute toxicity study on the golden orfe (*Leuciscus idus melanotus*) in a static system (96 hours)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-263
Test substance:	BAS 500 00 F, 247.83 g as/L
Guideline:	EPA 72-1, EEC 92/69, OECD 203
Test species:	Golden orfe (*Leuciscus idus melanotus*), mean body length 7.48 (7.0 – 7.8) cm; mean body weight 3.43 (2.8 – 4.2) g.
Animals per treatment:	10 fish per aquarium (loading about 0.3 g fish/L) and per concentration.
Dose levels:	Control, 0.0032, 0.0058, 0.01, 0.018, 0.032, 0.058 mg as/L (nominal), static system (96 h).
Findings:	BAS 500 00 F caused mortality at concentrations ≥ 0.032 mg as/L. No other substance-related effects could be observed at lower concentrations.
LC_{50}:	> 0.0135 and < 0.027 mg as/L (mean measured concentration)
NOEC:	0.014 mg/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

B.9.2.2 Chronic toxicity to fish (Annex IIA 8.2.2)

B.9.2.2.1 Active Substance

B.9.2.2.1.1 Chronic toxicity test on juvenile fish (Annex IIA 8.2.2.1)

Title:	**BAS 500 F - Sublethal toxic effects on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a flow-through system (28 days)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-238
Test substance:	BAS 500 F, purity: 99.0%
Guideline:	OECD 204
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), mean body length 3.9 (3.5 – 4.3) cm, mean body weight 0.6 (0.5 – 0.65) g.
Animals per treatment:	20 fish per aquarium
Dose levels:	control, 0.00215, 0.00464, 0.01, 0.0215 mg as/L (nominal). Flow-through system (28 days), flow rate of the test solution 10 l/h/aquarium.
Findings:	Compound-related mortality occurred in the two highest test concentrations 0.01 and 0.0215 mg as/L.. Compound-related toxic signs were observed only in the second highest concentration starting on day 1 in the form of reduced or no food uptake, convulsions and narcotic-like state. The one surviving fish showed sporadically apathy, reduced or no food uptake and swimming near the bottom.
NOEC:	0.00464 mg as/L (nominal)
GLP compliance:	yes
Valid:	yes

B.9.2.2.1.2 Fish early life stage toxicity test (Annex IIA 8.2.2.2)

Title:	**BAS 500 F - Early life-stage toxicity test on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-239
Test substance:	BAS 500 F, purity: 99.0%
Guideline:	OECD 210, EPA 72-4 (a)
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), embryos (appr. 90 – 120 min after fertilization)
Animals per treatment:	4 replicates of 25 embryos per test vessel and per concentration.
Dose levels:	Control, 0.0001, 0.000316, 0.001, 0.00316, 0.01 mg as/L (nominal),

	flow-through system. The study was terminated on day 98, 60 days after completion of hatch (day 38).
Findings:	In the highest test concentration 0.01 mg as/L all fish died until day 44. This was considered to be a clear substance related effect. No significant deviation in survival from the control group was seen in the dose groups 0.001 and 0.00316 mg as/L until the end of the study. Therefore the significant decrease in survival for the time period 0-56 days observed in the concentration group 0.000316 mg as/L was considered to be not test compound related. The larvae of the highest concentration group (0.01 mg as/L) showed apathy and partly a narcotic state and a distended yolk-sac for a period of 6 days after the end of hatch until all larvae of this group had died. In the other concentration groups effects were limited to single individuals and are judged not to be compound-related. No external abnormalities were observed in the surviving animals at the end of the study. Due to an infection of the control group, a second control group of another study conducted parallely with eggs from the same hatch was used additionally.
NOEC:	0.00316 mg as/L (nominal) 0.00230 mg as/L (measured)
GLP compliance:	yes
Valid:	yes

Title:	**BAS 500 F - Early life-stage toxicity test on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a flow through system with variable concentrations**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2000-240
Test substance:	BAS 500 F, purity: 99.0%
Guideline:	EPA 72-4 (a), OECD 210
Test species:	Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), embryos (appr. 60 min after fertilization)
Animals per treatment:	4 replicates of 50 embryos per test vessel and per concentration
Dose levels:	Control, 0.0025, 0.005, 0.01 and 0.02 mg as/L (nominal), flow-through system; flow rate: 10 L/hour/test group. The study was terminated on day 97, 60 days after completion of hatch (day 37).
Findings:	Compared to the control group the survival up to the start of hatch was statistically significantly decreased in the 0.01 mg as/L and in the 0.02 mg as/L dose groups to 72.2 and 62% of the control survival, respectively. Statistically significant decrease in survival was also observed in the 0.0025 mg as/L dose group but not in the 0.005 mg as/L group. In comparison to the control, survival from start of the hatch to termination of the hatch was not impaired in any of the dose groups. Survival from the end of the hatch to the end of swim-up was significantly decreased in the 0.02 mg as/L dose group,

the highest concentration tested as all larvae died until day 48. From the end of swim-up to the end of the study no substance-related statistically significant effect was observed in the surviving dose groups. Over the whole study period the survival in the 0.0025, 0.01 and the 0.02 mg as/L dose groups were statistically significantly decreased in comparison to the control group, however in the 0.0025 and the 0.01 mg as/L the survival was 97.2% of the control group. As there was no significant decrease in survival in the 0.005 mg as/L dose group the decrease in the 0.0025 mg as/L dose group was considered to be not treatment-related and that in the 0.01 mg as/L dose group was considered to be questionably attributed to the test compound.

NOEC: 0.005 mg as/L (nominal concentration).

GLP compliance: yes
Valid: no (due to high control mortality)

B.9.2.3 Bioconcentration in fish (Annex IIA 8.2.3)

B.9.2.3.1 Active substance

Title: **Bioaccumulation and metabolism of (14C)-BAS 500 F in bluegill sunfish**
Author: Chapleo, S. (1999)
BBA-Ref.-No.: WAT2000-241

Test substance: [Tolyl-U-^{14}C]-BAS 500 F, radiochemical purity > 97 %
[Chlorophenyl-U-^{14}C]-BAS 500 F, radiochemical purity > 97 %

Guideline: OECD 305, EPA 165-4
Test species: Bluegill Sunfish (*Lepomis macrochirus*)
Animals per treatment: *ca*. 150 of *ca.* 1 g size for each label
Dose levels: 300 ng a. i./L (nominal concentration). Flow-through system
The active substance was dissolved in N,N-dimethyl formamide (DMF) and this stock solution was pumped into a mixing vessel supplied with a diluent stream of water. From there, the treated water flowed through the tanks at rates of 12.1 – 13.0 L/hour. Test fish were exposed for 37 days. Subsequently fish were exposed to a continuous flow of dilution water alone for either 14 days (chlorophenyl label) or 21 days (tolyl label). A control experiment with *ca.* 150 fish was conducted where fish were exposed to DMF and diluent water alone.

Findings: **Chlorophenyl label**
During the uptake phase the actual concentration of total radioactivity in water was in the range of 263 - 344 ng/L with a mean concentration of 305 ng/l. Water sampled at the first day of depuration contained 14% of the nominal concentration used in the uptake phase and thereafter the concentration dropped to levels

below the limit of determination. The only radioactive component in water was unchanged BAS 500 F.
Mean concentrations of radioactivity in the total fish reached an apparent steady state of 184 – 235 ng/g after 4 days of exposure. Mean concentrations of radioactivity in the fillet increased from 47 ng/g on Day 1 of the exposure period to a plateau of 66 – 71 ng/g. Mean concentrations of radioactivity in the inedible fraction (viscera) increased from 180 ng/g on Day 1 to a plateau of 314 - 404 ng/g during Days 4 - 35 of the exposure period.
The bioconcentration factors and kinetic parameters based on total radioactivity concentrations were derived from Non-Linear-Regression Analysis using a 2-Compartment Model. The depuration half-life in whole fish was 0.9 day. Accordingly, the time to reach 90% depuration is 3.0 days. The bioconcentration factor calculated directly from the ratio of the ^{14}C-concentrations in water and tissue fractions (mean of Days 4 – 35) was 673 for whole fish, 232 for edible tissues and 1169 for viscera. The values were in good accordance with those obtained by kinetic modelling.
The BCF values for unchanged BAS 500 F were 379 for whole fish, 191 for edibles and 574 for inedibles.

Tolyl label
During the uptake phase the actual concentration of total radioactivity in water was in the range of 278 - 336 ng/l with a mean concentration of 300 ng/l. Water sampled at the first day of depuration contained 22% of the nominal concentration used in the uptake phase and thereafter the concentration dropped to levels below the limit of determination. The only radioactive component in water was unchanged BAS 500 F.

Mean concentrations of radioactivity in the total fish reached an apparent steady state of 192 - 243 ng/g after 4 days of exposure. Mean concentrations of radioactivity in the fillet increased from 70 ng/g on Day 1 of the exposure period to a plateau of 74 – 84 ng/g. Mean concentrations of radioactivity in the inedible fraction (viscera) increased from 197 ng/g on Day 1 to a plateau of 331 - 425 ng/g during Days 4 - 35 of the exposure period.
The bioconcentration factors and kinetic parameters based on total radioactivity concentrations were derived from Non-Linear-Regression Analysis using a 2-Compartment Model. The depuration half-life in whole fish was 0.9 days. Accordingly, the time to reach 90% depuration is 2.8 days. The bioconcentration factor calculated directly from the ratio of the ^{14}C-concentrations in water and tissue fractions (mean of Days 4 – 35) was 719 for whole fish, 262 for edible tissues and 1221 for viscera. The values were in good accordance with those obtained by kinetic modelling.
The BCF values for unchanged BAS 500 F were 507 for whole fish, 178 for edibles and 853 for inedibles.

BCF: After exposure of fish to BAS 500 F at a nominal exposure level of 300 ng/l, apparent steady state was reached after 2 - 4 days. After

termination of the exposure, radioactivity levels in fish tissues decreased rapidly with a half-life of *ca.* 0.7 – 1.0 days. Bioconcentration factors based on total radioactivity were relatively low in edibles (232 - 262) and relatively high in inedibles (1169 - 1221). For unchanged parent compound, the BCF values were considerably lower in all tissues. This is an indication for an intensive metabolic clearance of BAS 500 F. Only minor differences were observed between the two labelled forms of the test compound with regard to the kinetic parameters.

GLP compliance: yes
Valid: yes

B.9.2.3.2 Metabolites

Major degradation products of BAS 500 F in surface water are BF 500-11, BF 500-13, and BF 500-14. These degradation products have log P_{ow} values of less than 3 and thus are unlikely to accumulate in fish.

BF 500-11	log Pow = 1.87
BF 500-13	log Pow = 1.71
BF 500-14	log Pow = 2.54

B.9.2.4 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4)

B.9.2.4.1 Active substance

Title: **Effect of BAS 500 F on *Daphnia magna* STRAUS in a 48 hours acute toxicity test**
Author: Dohmen, G.P. (1999)
BBA-Ref.-No.: WAT2000-247

Test substance: BAS 500 F, purity: 97.1 %

Guideline: OECD 202, EEC 92/69
Test species: Waterflea (*Daphnia magna* STRAUS),
neonates with age at test initiation less than 24 hours.
Animals per treatment: 4 replicates with 5 daphnids in each
Dose levels: Control, 0.005, 0.0076, 0.0115, 0.0174, 0.0264 and 0.04 mg as/L (nominal), static test (48 h).

Findings: The dose-response curve for BAS 500 F is steep. No significant immobility (one Daphnia after 48 h) was observed at 0.0115 mg as/L. At 0.0174 mg as/L more than half of the daphnids were immobile after 48 hours and all daphnids were immobile at 0.0264 mg as/L. No other effects were observed.
EC_{50} (48 h): 0.0157 mg as/L (nominal)
NOEC (48 h): 0.0115 mg as/L (nominal)

GLP compliance: yes
Valid: yes

B.9.2.4.2 Major metabolites BF 500-11, BF 500-13 and BF 500-13

Title: **Determination of the acute effect of BF 500-11 on the swimming ability of the water flea *Daphnia ma*gna STRAUS according to OECD 202 and GLP, EN 45001 and ISO 9002**
Author: Jatzek, J. (1999)
BBA-Ref.-No.: WAT2000-248

Test substance: BF 500-11, purity: 98.9 %

Guideline: OECD 202, EEC 92/32, EPA 850.1010, ISO 6341, ISO/DIS 10706
Test species: Waterflea (*Daphnia magna* STRAUS),
neonates with age at test initiation less than 24 hours
Animals per treatment: 4 replicates with 5 daphnids in each
Dose levels: Control, 6.25, 12.5, 25, 50 and 100 mg/L (nominal), static test (48 h)

Findings: BF 500-11 caused no mortality in the concentrations tested with the exception of the highest test concentration of 100 mg/L where one *Daphnia* proved immobile after 48 hours. No other effects were observed.
EC_{50} (48 h): > 100 mg/L (nominal)
NOEC (48 h): > 100 mg/L (nominal)

GLP compliance: yes
Valid: yes

Title: **Determination of the acute effect of BF 500-13 on the swimming ability of the water flea *Daphnia magna* STRAUS according to OECD 202 and GLP, EN 45001 and ISO 9002**
Author: Jatzek, J. (1999)
BBA-Ref.-No.: WAT2000-249

Test substance: BF 500-13, purity: 99.6 %

Guideline: OECD 202, EEC 92/32, EPA 850.1010, ISO 6341, ISO/DIS 10706
Test species: Waterflea (*Daphnia magna* STRAUS),
neonates with age at test initiation less than 24 hours
Animals per treatment: 4 replicates with 5 daphnids in each
Dose levels: Control, 12.5, 25, 50 and 100 mg/L (nominal), static test (48 h)

Findings: BF 500-13 caused no mortality in the concentrations tested with the exception of the highest test concentration of 100 mg/L where 7 *Daphnia* proved immobile after 48 hours. No other effects were observed.
EC_{50} (48 h): > 100 mg/L (nominal)

NOEC (48 h):	50 mg/L (nominal)
GLP compliance:	yes
Valid:	yes

Title:	**Determination of the acute effect of BF 500-14 on the swimming ability of the water flea *Daphnia magna* STRAUS according to OECD 202 and GLP, EN 45001 and ISO 9002**
Author:	Jatzek, J. (1999)
BBA-Ref.-No.:	WAT2000-250
Test substance:	BF 500-14, purity: 96.1 %
Guideline:	OECD 202, EEC 92/32, EPA 850.1010, ISO 6341, ISO/DIS 10706
Test species:	Waterflea (*Daphnia magna* STRAUS), neonates with age at test initiation less than 24 hours
Animals per treatment:	4 replicates with 5 daphnids in each
Dose levels:	Control, 12.5, 25, 50 and 100 mg/L (nominal), static test (48 h)
Findings:	BF 500-14 caused no treatment-related mortality in any of the concentrations tested. No other effects were observed.
EC_{50} (48 h):	> 60.9 mg/L (mean measured concentration)
EC_0 (48 h):	≥ 60.9 mg/L (mean measured concentration)
GLP compliance:	yes
Valid:	yes

B.9.2.4.3 Formulated product (BAS 500 00 F)

Title:	**Effect of BAS 500 00 F on the immobility of *Daphnia magna* STRAUS in a 48 hours static, acute toxicity test**
Author:	Dohmen, G.P. (1999)
BBA-Ref.-No.:	WAT2000-264
Test substance:	BAS 500 00 F, 247.8 g as/L
Guideline:	OECD 202, EEC 92/69
Test species:	Waterflea (*Daphnia magna* STRAUS), neonates collected from in house culture, less than 24 hours old at test initiation.
Animals per treatment:	4 replicates with 5 daphnids in each
Dose levels:	Control, 0.01, 0.018, 0.032, 0.056 and 0.1 mg/L (nominal), static test (48 h).
Findings:	Significant mortality of the daphnids was only observed at the two highest concentrations 0.056 mg/L (20%) and 0.1 mg/L (100%).
EC_{50} (48 h):	0.0152 mg as/L (nominal concentration)
NOEC (48 h):	0.0075 mg as/L (nominal concentration)

GLP compliance: yes
Valid: yes

B.9.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

B.9.2.5.1 Active substance

Title:	**Effects of BAS 500 F on mortality and reproduction of *Daphnia magna***
Author:	Dohmen, G.P. (1999)
BBA-Ref.-No.:	WAT2000-251
Test substance:	BAS 500 F, purity: 97.1 %
Guideline:	OECD 202
Test species:	Waterflea (*Daphnia magna* STRAUS), neonates collected from in house culture, age at test initiation less than 24 hours.
Animals per treatment:	7 test concentrations plus control, 10 replicates per concentration with 1 daphnid in each.
Dose levels:	Control, solvent control, 0.00025, 0.0005, 0.001, 0.002, 0.004, 0.008 and 0.016 mg as/L (nominal), semi-static test (21 d).
Findings:	Some parent mortality was observed at the two highest test concentrations, two dead daphnids at 0.008 mg as/L and one dead daphnid at 0.016 mg as/L. However, the numbers of dead daphnids were very low and not concentration dependent. No significant difference between treatments was observed for the onset of reproduction. The mean number of offspring per parent in the controls was about 130. A significant reduction in the number of offspring was observed at 0.008 mg as/L and 0.016 mg as/L. No effect on reproduction was observed at concentrations of 0.004 mg as/L and less. The two highest concentrations, 0.008 and 0.016 mg as/L, caused slightly but statistically significantly reduced growth of parent daphnids.
NOEC (21 d):	0.004 mg as/L (nominal)
LOEC (21 d):	0.008 mg as/L (nominal)
EC_{50} (21 d):	for reproductive effects: 0.0112 mg as/L (nominal)
GLP compliance:	yes
Valid:	yes

B.9.2.6 Effects on algal growth (Annex IIA 8.2.6)

B.9.2.6.1 Active substance

Title:	**Effect of BAS 500 F on the growth of the green alga *Pseudokirchneriella subcapitata***
Author:	Dohmen, G.P. (1999)
BBA-Ref.-No.:	WAT2000-252
Test substance:	BAS 500 F, purity: 97.1%
Guideline:	OECD 201
Test species:	*Pseudokirchneriella subcapitata* (syn.: *Selenastrum capricornutum)*, SAG 61.81.
Animals per treatment:	5 replicates per test concentration plus a control with 10 replicates.
Dose levels:	Control, 0.008, 0.016, 0.031, 0.063, 0.125, 0.250, 0.5, 1.0 mg as/L (nominal), static system (96 h).
Findings:	No morphological effects on the algae could be observed.
E_rC_{50} (0-96 h):	> 0.843 mg as/L (measured concentration)
E_bC_{50} (0-96 h):	0.152 mg as/L (measured concentration)
GLP compliance:	yes
Valid:	yes

B.9.2.6.2 Major metabolites BF 500-11, BF 500-13 and BF 500-14

Title:	**Determination of the inhibitory effect of BF 500-11 on the cell multiplication of unicellular green algae according to OECD 201 and GLP, EN 45001 and ISO 9002**
Author:	Reuschenbach, P. (1999)
BBA-Ref.-No.:	WAT2000-253
Test substance:	BF 500-11, purity: 98.9%
Guideline:	EEC 92/69, OECD 201, EPA 850.5400
Test species:	*Scenedesmus subspicatus* CHODAT SAG 86.81
Animals per treatment:	3 replicates per concentration plus a control with 5 replicates
Dose levels:	Control, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L, static system (72 h).
Findings:	No morphological effects on the algae could be observed.
E_rC_{50} (72 h):	> 100 mg/L (nominal)
E_bC_{50} (72 h):	> 100 mg/L (nominal)
GLP compliance:	yes
Valid:	yes

Title:	**Determination of the inhibitory effect of BF 500-13 on the cell multiplication of unicellular green algae according to OECD 201 and GLP, EN 45001 and ISO 9002**
Author:	Reuschenbach P. (1999)
BBA-Ref.-No.:	WAT2000-254
Test substance:	BF 500-13, purity: 99.6%
Guideline:	EEC 92/69, OECD 201, EPA 850.5400
Test species:	*Scenedesmus subspicatus* CHODAT SAG 86.81
Animals per treatment:	3 replicates per concentration plus a control with 5 replicates
Dose levels:	Control, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L (nominal), static system (72 h)
Findings:	No morphological effects on the algae could be observed.
E_rC_{50} (72 h):	> 100 mg/L (nominal)
E_bC_{50} (72 h):	66.0 mg/L (nominal)
GLP compliance:	yes
Valid:	yes

Title:	**Determination of the inhibitory effect of BF 500-14 on the cell multiplication of unicellular green algae according to OECD 201 and GLP, EN 45001 and ISO 9002**
Author:	Reuschenbach P. (1999)
BBA-Ref.-No.:	WAT2000-255
Test substance:	BF 500-14, purity: 96.1%
Guideline:	EEC 92/69, OECD 201, EPA 850.5400
Test species:	*Scenedesmus subspicatus* CHODAT SAG 86.81
Animals per treatment:	3 replicates per concentration plus a control with 5 replicates
Dose levels:	Control, 12.5, 25, 50 and 100 mg/L (nominal), static system (72 h)
Findings:	No morphological effects on the algae could be observed.
E_rC_{50} (72 h)	> 100 mg/L (nominal)
E_bC_{50} (72 h)	46.6 mg/L (nominal)

B.9.2.6.3 Formulated product (BAS 500 00 F)

Title:	**Effect of BAS 500 00 F on the growth of the green alga *Pseudokirchneriella subcapitata***
Author:	Dohmen, G.P. (1999)
BBA-Ref.-No.:	WAT2000-265
Test substance:	BAS 500 00 F, 247.8 g as/L
Guideline:	OECD 201

Test species:	*Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz) SAG 61.81
Animals per treatment:	8 test concentrations, each with 5 replicates plus a control with 10 replicates
Dose levels:	Control, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L nominal, static system (72 h).
Findings:	No morphological effects were observed.
E_rC_{50} (0-72 h)	0.788 mg as/L (nominal concentration)
E_bC_{50} (0-72 h)	0.320 mg as/L (nominal concentration)
GLP compliance:	yes
Valid:	yes

B.9.2.7 Effects on sediment-dwelling organisms (Annex IIA 8.2.7)

B.9.2.7.1 Active substance

Title:	**Effects of BAS 500 F on the development of sediment dwelling larvae of *Chironomus riparius* in a water-sediment system**
Author:	Dohmen, G.P. (2000)
BBA-Ref.-No.:	WAT2000-256
Test substance:	BAS 500 F, unlabelled: purity: 97.1 % ^{14}C-labelled BAS 500 F, purity 98.0 %
Guideline:	BBA-guideline proposal: 'Effects of plant protection products on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system.", Mitteilungen aus der Biol. Bundesanstalt, Heft 315, Blackwell Berlin, 1995, pp 70-84.
Test species:	*Chironomus riparius* Meigen, egg masses obtained from in-house cultures, larvae $\leq$ 3 days at test initiation
Animals per treatment:	25 larvae per test vessel, 3 replicates per concentration plus a control with 2 and a solvent control with 4 replicates
Dose levels:	Control, solvent control, 0.020, 0.040, 0.080, 0.160, 0.320 mg as/L (nominal), static system containing standard sediment (according to OECD 207) and water (Elendt, M4-medium); test duration 28 days.
Findings:	The emergence rates were generally quite high. More than 70% - the required minimum for the untreated controls - of the chironomids emerged in all but the highest test concentration and more than 90% emergence was observed in the controls and concentrations up to 0.080 mg/L. Statistically significant effects on the emergence rate were only found at the highest test concentrations, 0.160 and 0.320 mg/L. The latter caused a nearly 50% reduction in emergence. Statistically significant differences between the overall development rates of the treatments and the controls were found at the two highest

	test concentrations, respectively the three highest concentrations depending on the statistical method used.
EC_{50}:	0.377 mg/L (nominal)
NOEC:	0.040 mg/L (nominal)
LOEC:	0.080 mg/L (nominal)
GLP compliance:	yes
Valid:	yes

B.9.2.8 Aquatic mesocosm (Annex III A 10.2.2)

Title:	**The effect of BAS 500 00 F on aquatic ecosystems - an outdoor mesocosm investigation**
Author:	Dohmen, G.P. (2000)
BBA-Ref.-No.:	WAT2000-231
Test substance:	BAS 500 00 F, 247.8 g as/L
Guideline:	SETAC, EWOFFT,CLASSIC, HARAP
Test system:	Outdoor mesocosm site consisting of 15 ponds, each with a diameter of 2.84 m, a water depth of 100 cm and an according water volume of 6.335 m^3. They are largely embedded in the ground, the top 10 cm are protruding. The bottom is covered by a 15 cm layer of sand, 5 cm of clay and 10 cm of natural sediment. Sediment and water in the ponds originated from a beta-mesosaprobic, species rich lake, "Neuhofener Altrhein" (in addition deionized water had been added and used, when replenishment was needed). The mesocosm site is located within BASF's Agricultural Center, Limburgerhof in Rheinland-Pfalz (southwest Germany). The system was equilabrated and homogenized via continuous mixing over several months. In April 1997, three pots with plants macrophytes were introduced into each pond. In addition, non-introduced plants started to grow from the sediment.
Test design:	A mixed, replicated anova- regression approach was used with four treatment levels plus control, each with three replicate ponds.
Dose levels:	A vineyard situation was simulated with eight applications of BAS 500 F in 14 day intervals and with rates increasing from 60 to 160 g as/ha during the season. To simulate spray drift, the test substance had been applied via spray boom and nozzle; it was therefore applied in the lead formulation BAS 500 00 F. For drift calculations the 95%ile values from Ganzelmeier et al. (1995) were used and a standard 30 cm shallow, standing surface water. For the highest application rate of 160 g as/ha and 5% drift at 5 m distance this corresponds to a theoretical concentration of 2.67 µg/L, constituting the 2nd test concentration ("II") in this study. The lowest test concentration, 0.9 µg/L ("I"), simulates a distance of about 10 m. In addition concentrations of 8.0 µg/L ("III") and 24 µg/L ("IV") were used, according to three and nine times the amount which might be expected at 5 m distance (correspondingly lower

concentrations resulted from the early applications when lower rate were used).

Findings:

A large number of different species (approximately 260 taxa) was observed in this mesocosm study at varying abundances during the course of the experiment. The important freshwater phytoplankton groups were present with c. 65 different taxa. BAS 500 F caused no significant, concentration related effect on the total density of phytoplankton or on the number of different taxa in the various treatments. In addition, there was neither a significant change in species composition. Accordingly, biodiversity and similarity indices and multivariate statistical methods (Principal Response Curves) did not indicate any treatment related effects.

In detailed evaluations of the single phytoplankton groups no significant effects were observed for Cyanophyta, Euglenophyta or Cryptophyta or for the dominant taxa in these groups. Population densities of Dinophyceae appeared to decrease following the two last applications with the highest rates of BAS 500 F in treatment "IV" and possibly following the last application in treatment "III". This decrease, however, was not statistically significant. In addition, the population decrease observed at the highest concentration was of limited duration and higher than control numbers were reached again in late summer/autumn.

No concentration related responses were observed for the whole group Chlorophyceae or the dominant family Chlorococcales; highest cell densities were observed in the controls and the highest treatment group. Statistical tests indicated significant effects only at the two highest treatment levels for the subgroup Ankistrodesmoidae. However, this may well be due to arbitrary variability since population levels remained low in all ponds of the two high treatments whereas increased abundances were found in some replicates (not in all) of the other treatments. At a later sample period, no significant differences were observed. Additionally, further biological information (standard laboratory tests) shows that green algae are not sensitive to BAS 500 F at the tested concentrations. Similarly, for 'Scenedesmaceae' cell densities were higher in the controls for a certain period; however, shortly after the last treatment a population outbreak of this group was observed in a pond of the high treatment, showing that differences observed in algal cell densities cannot be attributed to BAS 500 F treatments.

Conjugatophyceae abundances were transiently reduced in the high treatment group. However, it increased at the time of the highest application volumes showing that differences in population densities were not test substance related. No significant effects were observed for Bacillariophyceae (diatoms). In conclusion, as indicated by laboratory tests, BAS 500 F did not adversely affect phytoplankton communities at the tested concentrations. For taxa, for which effects could not be excluded, at least sufficient recovery was demonstrated.

Zooplankton, too, showed a large biodiversity and the important groups - Rotifera, Phyllopoda, Copepoda - where present in this study in large numbers. The analysis of the data showed no effect of BAS 500 F treatments on the number of species, total abundances, species biodiversity or similarity indices. Multivariate, statistical techniques did neither indicate significant treatment related effects.

Testaceae abundances appeared reduced at the time of the last two applications. However, shortly afterwards population densities in the highest treatment were the same or higher than in the controls.

Rotatoria showed large population fluctuations during the course of the experiment. The total number of rotifers was highest in the controls. This reflects basically a bloom of *Keratella sp*. in one of the control ponds (reaching densities of more than 600 individuals/L, whereas another control pond contained hardly any organism of that species). Second highest rotifer populations were observed in the highest treatment level. There were no test substance related effects visible on the group of Rotatoria as a whole nor on most of the many (36) different taxa within this group. Only for *Lecane lunaris* a (partly) statistically significant, concentration related effect was observed at the highest treatment levels. However, population densities had reached control levels again shortly after the applications.

Detailed assessments of the single groups pointed to possible effects only on few taxa. Phyllopoda as a group were not affected. However, within the group Daphniidae, the species *Daphnia longispina* appeared significantly inhibited during the time of the highest applications. Decreased populations were found in each treatment except the lowest one, however, not in a concentration dependent way (the highest treatment group was not significantly different according to a Dunnett's test). In the post treatment period no significant differences between treatments could be detected; population levels of *Daphnia longispina* reached the same levels in the high treatment group as the control group. In none of the other main Phyllopoda taxa (*Ceriodaphnia, Diaphanosoma, Simocephalus, Alona, Alonella, Chydorus*) significant, treatment related effects could be observed. For some of the taxa, control levels were higher in the initial part of the treatment series. However, during the time of the highest test substance applications, population levels in the highest treatment group were similar or higher as compared to the control group.

The same was true for Copepoda, which were dominated in numbers by nauplia larvae. Population levels were often higher in the control than in most treatment groups. However, the highest treatment group also showed higher densities than the other groups and was in the same range as the controls during the time of the highest test substance applications. Thus, occasional differences in nauplia population densities must be attributed to variability and was not caused by BAS 500 F treatments. Adult Calanoida occurred only in

the early phase of the experiment in very low and variable numbers. They disappeared from the system fairly soon. This disappearance was significantly faster in the high treatment groups than in the control. However, due to the low numbers, the low test substance applications during that phase of the study and the general population development pattern this is not considered to represent a treatment related effect. Cyclopoida, which reached their highest population levels later in the season, were found in higher numbers. Population densities in the highest treatment group were at the same level or higher than controls during and after the time of the highest test substance applications, indicating that differences observed during other times of the experiment were not BAS 500 F treatment related.

Population densities of Ostracoda were somewhat higher (not significantly) in the two highest treatment groups than in control at the time of the highest applications and afterwards.

Due to the adsorptive capacity of the test compound, effects on Aufwuchs could not be excluded and appropriate investigations were conducted. However, neither gross parameters like biomass or chlorophyll content nor species abundance were significantly affected by BAS 500 F treatments during the observation intervals.

The abundance of benthic organisms was determined in sediment and artificial substrate samples at three occasions during the course of the experiment. For most of the many taxa found in this study - Nematoda, Turbellaria, Oligochaeta, Crustacea, Insecta and Arachnida - no clear treatment related effects were observed. Only within the Molluscs two snail species (*Bithynia tentaculata* and *Valvata* sp.) and one mussel species (*Dreissena polymorpha*) appeared affected (partly statistically significant) at the highest treatment level only.

No treatment related effects were observed for aquatic insects as measured in emergence, plankton and benthos samples.

Parallel to the mesocosm study, the effects on fish under outdoor conditions were assessed using the same application scheme (omitting the first low application) in separate small ponds. Five ponds, each containing ca. 500 L water and sediment, were used for the control and the four BAS 500 F treatments. Seven young carps (*Cyprinus carpio*) were introduced initially into each of the ponds. Effects were only observed at the highest treatment level at the last application (during a period of high temperatures and low oxygen concentrations) causing fish mortality. No sublethal effects (behaviour, growth, gross pathological findings) were observed in any of the other treatments.

The results of a complex mesocosm study show, that BAS 500 F can have effects on few species at concentrations of 24 µg as/L (equivalent to the nine-fold of the 5% drift scenario) and higher. Fish and molluscs may be affected at this concentration, too. For all

planktonic species the effects were found to be reversible. No clear effects were observed at 8 µg as/L, which is equivalent to the three-fold of a 5% drift scenario in shallow, static water bodies. The multitude of endpoints and species and environmental conditions in this mesocosm study show clearly that at this (8 µg as/L and lower) concentration no adverse effects on aquatic communities can be expected even after multiple applications. The ecologically acceptable concentration (EAC) is thus > 8 µg as/L.

NOEC: 8 µg as/L.
LOEC: 24 µg/L
EAC: ≥ 8 µg as/L

GLP compliance: yes
Valid: yes

B.9.2.9 Risk assessment for aquatic organisms

A data package in accordance with the requirements of Annexes II and III of Directive 91/414/EEC for the active substance, relevant metabolites and the formulated product has been submitted. The effects of the active substance pyraclostrobin (BAS 500 F) and the major metabolites BF 500-11, BF 500-13 and BF 500-14 on aquatic organisms are summarized in Table B.9.2-1. The effects of the formulated product BAS 500 00 F on aquatic organisms are summarized in Table B.9.2-2.

Table B.9.2-1: Summary of aquatic toxicity data of pyraclostrobin (BAS 500 F)and the major metabolites BF 500-11, BF 500-13 and BF 500-14

Test species	Test system	Result [mg as/L] measured		Reference
		LC_{50}	NOEC	
Pyraclostrobin				
Oncorhynchus mykiss	static - 96 h	0.00616[1)]	0.0033[2)]	WAT2000-232
Lepomis macrochirus	static - 96 h	> 0.0196 < 0.0335[1)]	0.0109[2)]	WAT2000-233
Cyprinus carpio	static - 96 h	> 0.0121 < 0.0258[1)]	0.0121[2)]	WAT2000-234
Oncorhynchus mykiss	flow-through – 28 d	not determined	0.00464[3)]*	WAT2000-238
Oncorhynchus mykiss	ELS - 98 d	not determined	0.0023[3)]	WAT2000-239
Daphnia magna	static – 48 h	0.0157*	0.0115*	WAT2000-247
Daphnia magna	semi-static – 21 d	0.0112*	0.004*	WAT2000-251
Pseudokirchneriella subcapitata	static – 96 h	> 0.843[4)]	0.078[5)]	WAT2000-252
Chironomus riparius	static – 28 d	0.377	0.040	WAT2000-256
Metabolite BF 500-11				
Oncorhynchus mykiss	static - 96 h	100[1)]	10[2)]	WAT2000-235
Daphnia magna	static – 48 h	> 100*	≥ 100*	WAT2000-248
Scendedesmus subspicatus	static – 72 h	> 100[4)] *	40.9[5)]*	WAT2000-253
Metabolite BF 500-13				
Oncorhynchus mykiss	static - 96 h	> 50 < 100	10	WAT2000-236
Daphnia magna	static – 48 h	> 100*	50*	WAT2000-249
Scendedesmus subspicatus	static – 72 h	> 100[4)] *	> 100[5)] *	WAT2000-254
Metabolite BF 500-14				
Oncorhynchus mykiss	static - 96 h	> 39.4 < 82.6[1)]	39.4[2)]	WAT2000-237
Daphnia magna	static – 48 h	> 60.9	≥ 60.9	WAT2000-250
Scendedesmus subspicatus	static – 72 h	> 100[4)] *	30.6[5)] *	WAT2000-255

[1)] LC_{50} (1+96 h)
[2)] NOEC (1 + 98 h)
[3)] NOAEC,
[4)] = growth rate;
[5)] = E_rC_{10} ;
* measured values confirmed nominal values.

Table B.9.2-2: Summary of aquatic toxicity data of the formulated product BAS 500 00 F

Test species	Test system	Toxicity [mg as/L] measured [1)]	Reference
		LC/EC_{50}	
BAS 500 00 F			
Oncorhynchus mykiss	static - 96 h	0.0042	WAT2000-257
Lepomis macrochirus	static - 96 h	>0.0146 <0.0299	WAT2000-258
Cyprinus carpio	static - 96 h	>0.0209 <0.0497	WAT2000-259
Oryzias latipes	static - 96 h	>0.0325 <0.0885	WAT2000-260
Pimephales promelas	static - 96 h	>0.012 <0.0235	WAT2000-261
Brachydanio rerio	static - 96 h	>0.0417 <0.0887	WAT2000-262
Leuciscus idus melanotus	static - 96 h	>0.0135 <0.027	WAT2000-263
Daphnia magna	static - 48 h	0.0152[2)]	WAT2000-264
Pseudokirchneriella subcapitata	static - 72 h	0.788[2)]	WAT2000-265

[1)] LC/EC_{50} (1 + 96h), related to measured concentrations of pyraclostrobin
[2)] nominal, since measured confirmed nominal
[3)] E_rC_{10}

The active substance pyraclostrobin is highly toxic to fish and aquatic invertebrates, whereas green algae are less sensitive. Compared to the active substance the three major metabolites (BF 500-11, BF 500-13 and BF 500-14) proved to be far less toxic to *Oncorhynchus mykiss*, *Daphnia magna* and *Scenedesmus subspicatus*. Results with the formulated product BAS 500 00 F showed a comparable acute toxicity to *Oncorhynchus mykiss*, *Daphnia magna* and *Scenedesmus subspicatus* as compared to studies with the active substance (if data are based on the amount of the active substance present in the formulated product). *Oncorhynchus mykiss* was the most sensitive fish species out of the 7 species tested.

For multiple applications of 3 x 0.1 kg as/ha a $PEC_{sw,\ initial}$ of 51.021 µg as/L can be calculated according to the Draft Guidance Document on Aquatic Ecotoxicology (Doc. 8075/VI/97 rev. 7, 08.07.2000) and the Ganzelmeier spray drift values (overspray; DT_{50} water: 8.5 d; 12 d interval between applications; for more details on PEC-calculation see Chapter B.8.6). The respective surface water concentrations in distances of 3 m, 5 m, 10 m, 15 m and 20 m to a waterbody are 3.827, 2.551, 0.765, 0.408 and 0.204 µg as/L, respectively.

Fish can be identified as the most sensitive test organisms. Table B.9.2-3 summarises the acute TER-values calculated for *Oncorhynchus mykiss.*

Table B.9.2-3: Acute TER values for the most sensitive test organism (*Oncorhynchus mykiss;* $LC_{50\ (96\ h)}$: 6.16 µg as/L)

Application rate: 3 x 0.100 kg as/ha (12 d interval, DT_{50} water: 8.5 d) Scenario: Grapevines, late growth stage				
Distance	Drift rate [%]	$PEC_{sw\ initial}$ (µg as/L)	TER_{acute}	Annex VI Trigger
3 m	7.5	3.827	**1.6**	100
5 m	5.0	2.551	**2.4**	100
10 m	1.5	0.765	**8.1**	100
15 m	0.8	0.408	**15.1**	100
20 m	0.4	0.204	**30.2**	100

According to the standard risk assessment acute TER values are below the relevant Annex VI trigger of 100. The applicant however has provided further higher tier studies which allow a refined risk assessment.

In addition to the standard species four other fish species were tested using the formulated product BAS 500 00 F (see Table B.9.2-2). Under the assumption of a 20 m buffer zone only three of the test species met the Annex VI-trigger for acute effects of 100. For the remaining four test species acute TER-values were below the relevant trigger value (see Table B.9.2-4). The dose/response relationship observed in acute tests was very steep (e.g. *O. mykiss*: NOEC: 4.5 µg as/L, LC_{50} 6.16 µg as/L; *C. carpio*: NOEC: 12.1 µg as/L, LC_{50} 17.7 µg as/L).

Table B.9.2-4: Acute TER values for different fish species at a distance of 20 m (tested with the formulated product BAS 500 00 F)

Application rate: 3 x 0.100 kg as/ha (12 d interval, DT_{50} water: 8.5 d) Scenario: Grapevines, late growth stage, drift rate 0.4 %				
	LC_{50} (µg as/L)	$PEC_{sw\ initial}$ (µg as/L)	TER_{acute}	Annex VI Trigger
Oncorhynchus mykiss	4.2	0.204	**20.6**	100
Lepomis macrochirus	> 14.6 < 29.9	0.204	**71.6**	100
Cyprinus carpio	> 20.9 < 49.7	0.204	103	100
Oryzias latipes	> 32.5 < 88.5	0.204	159	100
Pimephales promelas	> 12.0 < 23.5	0.204	**58.8**	100
Brachydanio rerio	> 41.7 < 88.7	0.204	204	100
Leuciscus idus melanotus	> 13.5 < 27.0	0.204	**66.2**	100

Two early life-stage studies were conducted with *Oncorhynchus mykiss*, resulting in NOEC-values in the same order of magnitude. One study (97 d) was conducted with variable concentrations following a saw tooth shaped scheme derived from a realistic model of the

expected environmental concentration in the field. The resulting NOEC (5.0 µg as/L), however is disregarded here due to the high control mortality observed in this study.
The second study (98 d) is considered valid. As measured concentration levels varied between 60.7 to 80.6 % of nominal concentrations during the study, the NOEC is expressed in terms of the mean analytical determined concentration (i.e. 2.3 µg as/L).

Overall the NOEC-values from tests with *Oncorhynchus mykiss* were more or less in the same order of magnitude (28 d study: 4.64 µg as/L; 98 d ELS: 2.3 µg as/L; acute toxicity tests: 4.2 µg as/L when tested with the formulated product, 6.16 µg as/L when tested with the active substance).

From the results described above it can be concluded that the ratio of acute to chronic effects is almost 1. Long term effects can be attributed to the acute toxic mode of action. A risk assessment based on the higher tier chronic toxicity data which also reflect possible sublethal effects is considered more reliable than a risk assessment based on the acute toxicity data alone. For this reason the lowest NOEC from the early life-stage test with *Oncorhynchus mykiss* is considered relevant for the risk assessment. Due to the mode of action of the active substance this NOEC is compared with the $PEC_{sw,\ initial}$.

According to Annex VI of Directive 91/414/EEC the relevant trigger-value is 10. Adequate risk mitigation measures (i.e. buffer zones) should be set at Member State level in order to avoid unacceptable effects to aquatic organisms, especially fish.

The respective TER values are summarised in Table B.9.2-5.

Table B.9.2-5: TER values for the most sensitive test organism (*Oncorhynchus mykiss;* NOEC: 2.3 µg as/L, 98 d ELS-study)

Application rate: 3 x 0.100 kg as/ha (12 d interval, DT_{50} water: 8.5 d) Scenario: Grapevines, late growth stage				
Distance	Drift rate [%]	$PEC_{sw\ initial}$ (µg as/L)	TER	Annex VI Trigger
3 m	7.5	3.827	0.6	10
5 m	5.0	2.551	0.9	10
10 m	1.5	0.765	3.0	10
15 m	0.8	0.408	5.6	10
20 m	0.4	0.204	**11.2**	10

Mesocosm study
The effects of pyraclostrobin (applied as formulated product BAS 500 00 F) on the aquatic environment were investigated in an outdoor mesocosm study under more realistic conditions. A vineyard situation was simulated with eight applications in 14 day intervals and with rates increasing from 60 to 160 g as/ha. Four concentration levels ranging from 0.9 µg as/L to 24 µg as/L were investigated. Approximately 260 different taxa were determined in the study. For phytoplankton only insignificant transient effects were observed in some of the taxa with recovery taking place until the end of the study. No significant treatment related long lasting effects were observed on abundance and species diversity of the zooplankton. In case of

transient effects recovery was observed. Whereas for most benthic organisms no clear treatment related effects were observed, molluscs (*Bithynia tentaculata* and *Valvata* sp.) and a mussel species (*Dreissena polymorpha*) appeared affected at the highest treatment level (24 µg as/L). No treatment related effects appeared with aquatic insects. The overall NOEC was 8 µg as/L. The EAC (ecologically acceptable concentration) was determined to be > 8 µg as/L.

Probabilistic approach
The results of the acute toxicity tests obtained with 7 freshwater fish species (see Table B.9.2-2) were used in a probabilistic risk assessment. According to the calculations of the applicant 95 % of all NOEC-values are supposed to be above 4.6 µg as/L. However, this calculation is based on results from only one cold water fish species (*O. mykiss*), whereas all other test species are considered to be warm water species. *O. mykiss* also proved to be the most sensitive test species, whereas the other species could be grouped in a rather insensitive group (LC_{50} above 50 µg as/L) and a group with intermediate sensitivity (LC_{50} between 16 and 25 µg as/L). Due to these differences the calculated value of 4.6 µg as/L might be questionable. The respective NOEC-value from the chronic test with *O. mykiss* (98 d ELS: 2.3 µg as/L) is lower than the calculated $Probit_{5\%}$-value. The overall risk assessment is therefore based on this lower NOEC-value (see above).

Bioconcentration
Pyraclostrobin has a log P_{ow} of 3.99, indicating a potential risk of bioaccumulation. Therefore a bioconcentration study with Bluegill Sunfish (*Lepomis macrochirus*) has been conducted. BCFs (whole fish) of 673 (chlorophenyl label) and 736 (tolyl label) were determined after 37 d of exposure. An apparent steady state was reached after 2-4 d of exposure. Elimination rates were relatively high with half-lives for elimination ranging between 0.7 and 1.0 d. The time for elimination of 90 % of the accumulated net total radioactive residue varied between 2.3 and 3.2 d. Therefore the risk of bioaccumulation is considered acceptable.

Metabolites
In studies conducted with the metabolites BF 500-11, BF 500-13 and BF 500-14 it could be demonstrated that these metabolites are considerably less toxic to aquatic organisms than the active substance pyraclostrobin. A potential risk to the aquatic environment will be covered by the risk mitigation measures to be set due to the ecotoxicological properties of the active substance. It can be concluded that the metabolites are not of ecological relevance with respect to aquatic organisms.

Classification and labelling
According to Directive 67/548/EEC the active substance pyraclostrobin should be labelled with N, R50 and R53.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

B.9.3.1 Toxicity to mammals (Annex IIIA 10.3)

There were conducted no wild mammal toxicity studies nor field studies.

The acute oral LD_{50} of pyraclostrobin for rats is >5000 mg/kg body weight. Regarding the long-term risk the assessment will be based on 75 ppm that was the NOAEL for reproductive effects in a multi-generation study with rats for (see section B.06, Toxicology).

B.9.3.2 Risk assessment for mammals

Mammals may be exposed to pyraclostrobin mainly by the consumption of contaminated feed. Highest residues will be in vegetation, therefore herbivorous species are considered as worst-case. The risk assessment will be based on a maximum rate of 0.16 kg as/ha in grapes and 0.25 kg as/ha on turf.

Exposure assessment:
- Use in grapes: It is assumed that half of the applied amount reaches the vegetation on the ground; for this material residues are estimated to Hoerger and Kenage (category short grass)
- Use on turf: Measured data from cereals are taken as a surrogate which were in the range of 2.7-14.0 mg/kg (see chapter 7.6)

In order to consider the worst-case condition it is assumed that mammals feed exclusively on contaminated material and that they have a daily feed demand of 25 % of their body weight. Then the maximum daily intake is 3.8 mg/kg bw.

Exposure assessment for mammals:

Use	Maximum application rate (kg/ha)	Feed	Typical maximum residue[1] (mg/kg)	Initial residue (mg/kg)	Relative feed demand (%)	Maximum daily intake (mg/kg bw)
Grape	0.16	Grass	(112/2)*R	9.0	25	2.2
Turf	0.25	Grass		14[2]	25	3.8

[1] according to Hoerger and Kenaga (1972); R = application rate in kg/ha
[2] based on measured data

Toxicity/exposure ratios: For the acute TERa the LD_{50} is related to the maximum daily intake; for the long-term TER the NOAEL from the reproduction study is related to the initial residue.

Toxicity/exposure ratios for mammals:

Use	Feed	Time-scale	Toxicity/Exposure ratio
Grape	Grass	acute	TERa = >5000/2.2 = >2200
Grape	Grass	long-term	TERlt = 75/9.0 = 8.3
Grape	Grass	acute	TERa = >5000/3.8 = >1300
Grape	Grass	long-term	TERlt = 75/14 = 5.4

The acute TER-values are well above the Annex-VI-triggers; so the risk to mammals is considered as low.

The long-term TER-values are only just above the Annex-VI-trigger of 5. However, it has to be considered that the assessment is conservative in assuming maximum residues, no degradation and exclusive feeding on treated material over a long time period. Thus, also the long-term risk for mammals is acceptable.

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.4)

B.9.4.1 Acute toxicity (Annex IIA 8.3.1, Annex IIIA 10.4)

B.9.4.1.1 Contact and oral toxicity of pyraclostrobin (Reg. No. 304428) to honeybees

Reference:
Dagmar Sack, Reg. Doc. BASF 1999/11457, study code 35842.

Testguidelines:
EPPO No. 170 and guidelines for the testing of chemicals No 213 and 214.

GLP compliance:
yes

Method:
The test with the test substance (content of as 97.09 %) was performed as a "limit test", i.e. the test substance was applied only in one high dosage: 100 µg/bee for oral and contact toxicity. The test included a toxic standard (dimethoate) and the following control variants: for the oral toxicity test a sugar water control; for the contact toxicity test a control with solvent aceton and a control with deionised water. 5 replicates were used for the test substance and the control variants each. The toxic standard run with 5 concentrations. 10 worker honeybees were used for each replicate. Mortality was measured after 24h and 48h.

Results:

Test substance:	LD_{50} oral (48h) = 73.1 µg/bee
(pyraclostrobin)	LD_{50} cont (48h) ≥100 µg/bee
Reference substance:	LD_{50} oral (48h) = 0.36 µg/bee
(dimethoate)	LD_{50} cont (48h) = 0.22 µg/bee

B.9.4.1.2 Contact and oral toxicity of BAS 500 00 F

Reference
Dagmar Sack, Reg. Doc. BASF 1999/11455, study code 35865.

Test guideline:
EPPO No. 170 and guidelines for the testing of chemicals No 213 and 214.

Method:
The test product contained 247,83 g as/l. Tests were performed as a "limit test" and a "multiple dose test".

"Limit test": the test was performed only in one high dosage with five replicates. Dosage was 100 µg as/bee for oral and contact toxicity.
"Multiple dose" test: the test substance was applied in a range of 6 doses (6.25; 12.5; 25; 50; 75 and 100 µg as/bee) with 3 replicates/dose for oral and contact toxicity each. The test included a toxic standard (dimethoate) and the following control variants: for the oral toxicity a sugar water control, for the contact toxicity a control with the solvent acetone and a control with deionized water. 5 replicates were used for the test substance in the limit test and the control variants. 3 replicates were used for the multiple dose test and the toxic standard. For each replicate 10 adults worker honeybees were used. Mortality was measured after 24h and 48h.

Results:

Test substance BAS 500 00 F

Limit test: LD_{50} oral (48h) = 69.1 µg as/bee
LD_{50} cont (48h) ≥100 µg as/bee

Multiple dose test: LD_{50} oral (48h) = 76.9 µg as/bee
LD_{50} cont (48h) ≥100 µg as/bee

Reference substance (dimethoate)

LD_{50} oral (48h) = 0.37 µg as/bee
LD_{50} cont (48h) = 0.23 µg as/bee

B.9.4.2 Bee brood feeding test (Annex IIA 8.3.1.2)

No data submitted, as the test substance is not an IGR.

B.9.4.3 Residue test (Annex IIIA 10.4.2)

No data submitted, as the test substance is of low toxicity for bees.

B.9.4.4 Cage test (Annex IIIA 10.4.3)

No data submitted, as the test substance is of low toxicity for bees.

B.9.4.5 Field test (Annex IIIA 10.4.4)

No data submitted, as the test substance is of low toxicity for bees.

B.9.4.6 Tunnel test (Annex IIIA 10.5.5)

No data submitted, as the test substance is of low toxicity for bees.

B.9.4.7 Risk assessment for honeybees

Risk assessment is done according to the EPPO/Coe risk assessment scheme:

Hazard Quotient = LD_{50}^{-1} x g as/ha

The calculation is based on an amount of 250 g as/ha.

Active substance:

HQ oral = 3.4
HQ con = 2.5

Formulation BAS 500 00 F:

Limit test:

HQ oral = 3.6
HQ con = 2.5

Multiple dose test:

HQ oral = 3.2
HQ con = 2.5

All values are clearly below the threshold value of 50. This indicates a low risk for honeybees by the practicle use of pyraclostrobin containg products.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5)

The results presented below are considered valid (i.e. quality criteria are fulfilled). The risk assessment is based on the uses and nominal field rates outlined in this monograph. Investigations into the toxicity of pyraclostrobin are conducted using a representative formulation as suggested in the SETAC/ESCORT "*Guidance document on regulatory testing procedures for pesticides with non-target arthropods*" (Barrett et al., 1994).

B.9.5.1 Acute toxicity (Annex IIA 8.3.2, Annex IIIA 10.5.1)

Investigations into the acute toxicity of formulated pyraclostrobin in laboratory and extended laboratory tests:

Predatory mites

Title:	**Effect of BAS 500 00 F on the Predatory mite *Typhlodromus pyri* Scheuten in a laboratory trial.**
Author:	Ufer, A. (1999)
BBA-Ref.-No.:	ANA2000-719

Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l
Guideline: Typhlodromus (Bakker 1992)
Test species: *Typhlodromus pyri*
Developmental stage: Protonymphs
Substrate: Glass plates
Exposure route: deposit
Exposure duration: 14 d (7 + 7)
Results:

Appl. rate	Mortality	Sublethal effects
1.28 l/ha	47.3 %	98.5 % (Fertility)

valid: yes, acceptable, but number of eggs in untreated only 2
GLP compliance: yes

Parasitoids

Title: **Effect of BAS 500 00 F on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Aphidiidae) in a laboratory trial.**
Author: Ufer, A. (1998)
BBA-Ref.-No.: ANA2000-708
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l
Guideline: Aphidius, Lab (Mead-Briggs 1992)
Test species: *Aphidius rhopalosiphi*
Developmental stage: Adults
Substrate: Glass plates
Exposure route: deposit
Exposure duration: 48 h
Results:

Appl. rate	Mortality	Sublethal effects
1.28 l/ha	30 %	80 % (Parasitation)

Remarks: Data on mortality and fertility alone miss sublethal effects as 16 wasps were affected, 2 were moribund and only 9 were dead.
valid: yes
GLP compliance: yes

Title: **Effect of BAS 500 00 F on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in an extended laboratory trial.**
Author: Ufer, A. (1999)
BBA-Ref.-No.: ANA2000-711
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l

Guideline: Aphidius, extended Lab (Mead-Briggs 1997)
Test species: *Aphidius rhopalosiphi*
Developmental stage: Adults
Substrate: Natural substrate (barley seedlings)
Exposure route: deposit
Exposure duration: 48 h
Results:

Appl. rate	Mortality	Sublethal effects
1.28 l/ha	0 %	0 % (Parasitation)

valid: yes
GLP compliance: yes

Plant dwelling species

Title: **Effect of BAS 500 00 F on the Green Lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) in an laboratory trial.**
Author: Ufer, A. (1999)
BBA-Ref.-No.: ANA2000-712
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l
Guideline: Chrysopa (Bigler 1988)
Test species: *Chrysopa carnea*
Developmental stage: Larvae
Substrate: Glassplate
Exposure route: deposit
Exposure duration: 11-18 d (larvae and pupae)
Results:

Appl. rate	Mortality	Sublethal effects
1.28 l/ha	78.57 %	0 % (Fertility)

valid: yes
GLP compliance: yes

Title: **BAS 500 00 F: Toxicity to the Ladybird Beetle, *Coccinella septempunctata* L. (Coleoptera, Coccinellidae); Life Cycle Test (Extended Laboratory Test).**
Author: Kemmeter, F. (1999)
BBA-Ref.-No.: ANA2000-716
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 253.95 g/l
Guideline: Coccinella (Schmuck 1997)
Test species: *Coccinella septempunctata*
Developmental stage: LC
Substrate: Natural substrate (bean seedlings)

Exposure route: overspray: eggs, adults; deposit: larvae
Exposure duration: 63 d (Lifecycle)
Results:

Appl. rate	Stage	Mortality	Sublethal effects
0.032 l/ha	Eggs	0 %	
0.032 l/ha	Larvae	0 %	
0.032 l/ha	Adults	0 %	23.7 % (Fertility)
0.256 l/ha	Eggs	1.1 %	
0.256 l/ha	Larvae	0 %	
0.256 l/ha	Adults	0 %	3.1 % (Fertility)

Remarks: Life-Cycle-Test (LC). Each of the different growth stages is treated 1 time, starting with the eggs, and subsequently surviving larvae and adults are treated.
valid: yes
GLP compliance: yes

Title: **Acute Toxicity to the Lady Bird Beetle, *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) in the Laboratory.**
Author: Kühner, C.. (1999)
BBA-Ref.-No.: ANA2000-715

Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l

Guideline: Coccinella (Pinsdorf 1989)
Test species: *Coccinella septempunctata*
Developmental stage: Larvae
Substrate: Glass plates
Exposure route: deposit
Exposure duration: until pupation

Results:

Appl. rate	Mortality	Sublethal effects
1.28 l/ha	100 %	- (Fertility)

Remarks: Due to high mortality fertility could not be assessed.
valid: yes
GLP compliance: yes

Title: **Effect of BAS 500 00 F on reproduction of the leaf dwelling predator *Chrysoperla carnea* (Neuroptera: Chrysopidae) in an extended laboratory trial.**

Author: Ufer, A. (2000)
BBA-Ref.-No.: ANA2000-714
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l
Guideline: Chrysopa (Bigler 1988)
Test species: *Chrysopa carnea*
Developmental stage: Adults
Substrate: Natural substrate (cotton pads)
Exposure route: overspray
Exposure duration: few minutes (see remarks)
Results:

Appl. rate	Mortality	Sublethal effects
0.032 l/ha	0 %	0 % (Fertility)
0.160 l/ha	0 %	6.6 % (Fertility)

Remarks: Adult lace wings were sprayed directly and were transferred into reproduction cages a few minutes after application.
valid: yes
GLP compliance: yes

Title: **Effect of BAS 500 00 F on the Green Lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) in an extended laboratory trial.**

Author: Ufer, A. (1999)
BBA-Ref.-No.: ANA2000-713
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l
Guideline: Chrysopa (Bigler 1988)
Test species: *Chrysopa carnea*
Developmental stage: LC
Substrate: Natural substrate (bean leaves)
Exposure route: deposit: Larvae/Adults; overspray: Eggs
Exposure duration: 4-5 d (eggs), 14-19 d (larvae and pupae), 7 d (adults)
Results:

Appl. rate	Stage	Mortality	Sublethal effects
0.64 l/ha	Eggs	0 %	
0.64 l/ha	Larvae	0 %	
0.64 l/ha	Adults	6.4 %	82.5 % (Fertility)
0.64 l/ha	Adults[1)]	27.3 %	79.9 % (Fertility)

Remarks: Life-Cycle-Test (LC). Each of the different growth stages is treated 1 time, starting with the eggs, and subsequently surviving larvae and adults are treated, a) eggs (max. 24 h old), b) larvae (3 d old), c) adults (3-8 d old), adults[1)] taken from rearing, i.e. developmental stages not exposed.

valid: yes
GLP compliance: yes

Soil dwelling species

Title: **Acute toxicity to the Wolf Spider, *Pardosa* spp. (Araneae, Lycosidae) in the Laboratory.**
Author: Kühner, C. (1999)
BBA-Ref.-No.: ANA2000-718
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l
Guideline: Pardosa (Wehling 1998)
Test species: *Pardosa spp.*
Developmental stage: Adults
Substrate: Quartz sand
Exposure route: overspray
Exposure duration: 14 d
Results:

Appl. rate	Mortality	Sublethal effects
1.28 l/ha	0 %	9.9 % (Food uptake)

valid: yes
GLP compliance: yes

Title: **Effect of BAS 500 00 F on the Ground Dwelling Predator *Poecilus cupreus* in a Laboratory trial.**
Author: Ufer, A. (1999)
BBA-Ref.-No.: ANA2000-717
Test substance: Formulation BAS 500 00 F
Pyraclostrobin 247.83 g/l
Guideline: Poecilus (Heimbach 1992)
Test species: *Poecilus cupreus*
Developmental stage: Adults
Substrate: Quartz sand
Exposure route: overspray
Exposure duration: 14 d
Results:

Appl. rate	Mortality	Sublethal effects
1.28 l/ha	0 %	10.7 % (Food uptake)

valid: yes
GLP compliance: yes

Table B.9.5-1: Summary of arthropod toxicity data with the formulation effects of BAS 500 00 F (250 EC)

Test material	Species	Developm. stage	Sub-strate	Dosage L/ha	Effects % lethal	sublethal
basic laboratory tests						
Predatory mites						
BAS 500 00 F	*T. pyri*	Protonymphs	I	1.28	47.3	98.5
Parasitoids						
BAS 500 00 F	*A. rhopalosiphi*	Adults	I	1.28	30	80
Plant dwelling species						
BAS 500 00 F	*C. carnea*	Larvae	I	1.28	78.6	0
BAS 500 00 F	*C. septempunctata*	Larvae	I	1.28	100	
Soil dwelling species						
BAS 500 00 F	*P. cupreus*	Adults	I	1.28	0	10.7
BAS 500 00 F	*Pardosa spp.*	Adults	I	1.28	0	9.9
extended laboratory tests						
Parasitoids						
BAS 500 00 F	*A. rhopalosiphi*	Adults	N	1.28	0	0
Plant dwelling species						
BAS 500 00 F	*C. carnea*	LC(E)	N	0.64	0	
BAS 500 00 F	*C. carnea*	LC(L)	N	0.64	0	
BAS 500 00 F	*C. carnea*	LC(A)	N	0.64	6.4	82.5
BAS 500 00 F	*C. carnea*	LC(A)	N	0.64	27.3	79.9
BAS 500 00 F	*C. carnea*	Adults	N	0.032	0	0
BAS 500 00 F	*C. carnea*	Adults	N	0.160	0	6.6
BAS 500 00 F	*C. septempunctata*	LC(E)	N	0.032	0	
BAS 500 00 F	*C. septempunctata*	LC(L)	N	0.032	0	
BAS 500 00 F	*C. septempunctata*	LC(A)	N	0.032	0	23.7
BAS 500 00 F	*C. septempunctata*	LC(E)	N	0.256	1.1	
BAS 500 00 F	*C. septempunctata*	LC(L)	N	0.256	0	
BAS 500 00 F	*C. septempunctata*	LC(A)	N	0.256	0	3.1

I = Inert substrate, N = Natural substrate, LC = Life cycle, E = Eggs, L = Larvae, A = Adults

B.9.5.2 Field tests (Annex III 10.5.2)

Field and semi-field tests

Predatory mites

Title:	**A field toxicity test determine the effects of BAS 500 00 F on Predatory Mites (Acari: Phytoseiidae).**
Author:	Engelhardt, E.. (1998)
BBA-Ref.-No.:	ANA2000-722
Test substance:	Formulation BAS 500 00 F Pyraclostrobin 247.83 g/l
Guideline:	BBA VI/23-2.3.4
Species:	*Typhlodromus pyri*
Developmental stage:	Lifecycle(s)
Substrate(crop):	Natural substrate (grapes)
Exposure route:	overspray+oral
Exposure duration:	146 d
Calculation of effects:	Henderson and Tilton

Results:

DAT	Effects
Intermediate counting after 1st appl.	24.8 %
1st final counting (8 d days after 8th appl.)	0.0 %
2nd final counting (4 w after 8th appl.)	0.0 %

Remarks:	Number of applications: 8 Dosage per application: 0.16-0.64 l/ha Total dosage: 3.2 l/ha
valid:	yes
GLP compliance:	yes

Title:	**Effect of BAS 500 00 F on the Predatory mites *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in Vine (Field Experiment).**
Author:	Goßmann, A. (1999)
BBA-Ref.-No.:	ANA2000-721
Test substance:	Formulation BAS 500 00 F Pyraclostrobin 247.83 g/l
Guideline:	BBA VI/23-2.3.4
Species:	*Typhlodromus pyri*
Developmental stage:	Lifecycle(s)
Substrate(crop):	Natural substrate (grapes)
Exposure route:	overspray+oral
Exposure duration:	ca. 124 d
Calculation of effects:	Henderson and Tilton

Results:

DAT	Effects
Intermediate counting after 1st appl.	17 %
1st final counting (8 d days after 8th appl.)	0.0 %
2nd final counting (4 w after 8th appl.)	12 %

Remarks:	Number of applications:	8
	Dosage per application:	0.16-0.64 l/ha
	Total dosage:	3.12 l/ha
valid:	yes	
GLP compliance:	yes	

Title:	**Effect of BAS 500 00 F on populations of the Predatory mite *Typhlodromus pyri* Scheuten in a Field Study (Vineyard).**
Author:	Ufer, A. (1999)
BBA-Ref.-No.:	ANA2000-720
Test substance:	Formulation BAS 500 00 F Pyraclostrobin 247.83 g/l
Guideline:	BBA VI/23-2.3.4
Species:	*Typhlodromus pyri*
Developmental stage:	Lifecycle(s)
Substrate(crop):	Natural substrate (grapes)
Exposure route:	overspray+oral
Exposure duration:	139 d
Calculation of effects:	Henderson and Tilton

Results:

DAT	Effects
1st intermediate counting after 1st appl.	0.0 %
2nd intermediate counting after 3rd appl.	0,0 %
3rd intermediate counting after 5th appl.	9.6 %
4th intermediate counting after 7th appl.	0,0 %
5th intermediate counting after 8th appl.	0.0 %
1st final counting (4 w after 8th appl.):	58.1 %
2nd final counting (7 w after 8th appl.):	0.0 %

Remarks:	Number of applications:	8
	Dosage per application:	0.24-0.64 l/ha
	Total dosage:	3.12 l/ha
valid:	yes	
GLP compliance:	yes	

Table B.9.5-2: Summary of arthropod field testing with the formulation BAS 500 00 F (250 EC)

Test material	Species	Test	No. of appl.	Dosage L/ha per appl.	Dosage L/ha total	Effect % Final bonitur 1st/2nd
Predatory mites						
BAS 500 00 F	*T. pyri*	Field	8	0.16-0.64	3.2	0.0 / 0.0
BAS 500 00 F	*T. pyri*	Field	8	0.16-0.64	3.12	0.0 / 12
BAS 500 00 F	*T. pyri*	Field	8	0.24-0.64	3.12	58.1 / 0.0

B.9.5.3 Risk assessment for non-target terrestrial arthropods

Intended uses of plant protection products containing pyraclostrobin cover 3 applications per season of max. 0.16 kg as · ha^{-1} to control fungal diseases in grapes and 2 applications per season of max. 0.25 kg as · ha^{-1} to control fungal diseases in turf. Non-target arthropods are likely to be exposed to formulated pyraclostrobin by direct spray, contact on fresh or dry residues. Oral uptake of contaminated pollen, nectar and honey dew, prey or via host organisms is considered of minor importance. As a tier 1 worst-case exposure scenario, the predicted initial environmental exposure of non-target arthropods is assumed to be equivalent to the maximum nominal field rate.

The field rates tested given in Table B.9.5-1: Summary of arthropod toxicity data compared to the intended uses given above. According to the data submitted some inherent toxicity was demonstrated in basic laboratory tests on a number of species (i.e. *A. rhopalosiphi, T. pyri, C. septempunctata, Chrysoperla carnea)*, but was no longer apparent in extended lab tests or field tests. Some sublethal effects for plant dwelling species (e.g. *Chrysopa carnea* and *Typhlodromus pyri*) cannot be ruled out for the in-field situation. However, these effects are not unacceptable as lethal effects on all developmental stages were very low in *Chrysopa carnea* and recovery was demonstrated from field studies for *Typhlodromus pyri* (Table B.9.5-2: Summary of arthropod field testing). None of the species tested was adversely affected using rates covering relevant spray drift scenarios.

It is therefore established in the light of current scientific and technical knowledge and as laid down in the SETAC/ESCORT "*Guidance document on regulatory testing procedures for pesticides with non-target arthropods*" (Barrett et al., 1994), that the use of pyraclostrobin as outlined in this monograph has no unacceptable influence on non-target arthropods, represented by species of four ecological groups.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

B.9.6.1 Acute toxicity (Annex IIA 8.4.1, Annex IIIA 10.6.1.1)

Title:	**Effect of BAS 500 F on the mortality of the earthworm Eisenia foetida**
Author:	Krieg, W. (1999)
BBA-Ref.-No.:	ARW2000-83
Test substance:	Technical pyraclostrobin
Purity:	97.09 %
Guideline:	OECD 207
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	50; 87; 151.4; 263.4; 485.3; 797.5 mg/kg
Findings:	
LC50:	565.9 (501.9 - 638.0) mg/kg
Lowest lethal conc.:	263.4 mg/kg
NOEC:	151.4 mg/kg
valid:	yes
GLP compliance:	yes

Title:	**Effect of BF 500-6 on the mortality of the earthworm Eisenia foetida**
Author:	Krieg, W. (1999)
BBA-Ref.-No.:	ARW2000-84
Test substance:	Metabolite BF 500-6
Guideline:	OECD 207
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	100; 178; 317; 563; 1000 mg/kg
Findings:	
LC50:	>1000 mg/kg
Lowest lethal conc.:	>1000 mg/kg
NOEC:	1000 mg/kg
valid:	yes
GLP compliance:	yes

Title:	**Effect of BF 500-7 on the mortality of the earthworm Eisenia foetida**
Author:	Krieg, W. (1999)
BBA-Ref.-No.:	ARW2000-85
Test substance:	Metabolite BF 500-7
Guideline:	OECD 207
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	100; 178; 317; 563; 1000 mg/kg
Findings:	
LC50:	>1000 mg/kg
Lowest lethal conc.:	>1000 mg/kg
NOEC:	1000 mg/kg
valid:	yes
GLP compliance:	yes

Title:	**Effect of BAS 500 00 F on the mortality of the earthworm Eisenia foetida**
Author:	Krieg, W. (1998)
BBA-Ref.-No.:	ARW2000-87
Test substance:	Formulation BAS 500 00 F (250 g as/L)
Guideline:	OECD 207
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	25; 50; 100; 200; 400 mg/kg
Findings:	
LC50:	281.8 mg/kg
Lowest lethal conc.:	400 mg/kg
NOEC:	200 mg/kg
valid:	yes
GLP compliance:	yes

Table B.9.6-1: Summary of earthworm acute toxicity data

Test material	Species	Test	NOEC (mg/kg)	LC50 (mg/kg)
Pyraclostrobin	*Eisenia fetida*	Acute	151.4	565.9
BF 500-6 (metabolite)	*Eisenia fetida*	Acute	1000	>1000
BF 500-7 (metabolite)	*Eisenia fetida*	Acute	1000	>1000
BAS 500 00 F (formulated product)	*Eisenia fetida*	Acute	200	281.8 (product)

B.9.6.2 Other studies (Annex IIA 8.4.2, Annex IIIA 10.6.1.2, Annex IIIA 10.6.1.3)

B.9.6.2.1 Effect on reproduction

Title: **Effect of BAS 500 00 F on growth and reproduction of the earthworm Eisenia foetida**
Author: Krieg, W. (1999)
BBA-Ref.-No.: ARW2000-88

Test substance: Formulation BAS 500 00 F (pyraclostrobin 250.00 g/L)
Exposure: application on soil surface

Guideline: ISO/DIS 11268-2
Test species: *Eisenia fetida*
Exposure duration: 8 w
Worms per treatment: 4 x 10

Findings:

NOEC: 1 L/ha
valid: yes
GLP compliance: yes

Table B.9.6-2: Effects of BAS 500 00 F on reproduction and biomass of *Eisenia fetida*

Dose level	Adult mortality	Adult weight (% of initial weight)	Mean number of juveniles/test box
Control	0	118.04	145.3
1.0 L/ha	0	121.80	146.8
2.0 L/ha	0	125.40	128.5*
5.0 L/ha	0	116.99	103.0*
10.0 L/ha	2.5	108.64	83.3*

* sign. $\alpha = 0.05$, Dunett-test

B.9.6.2.2 Effect of soil bound residues of BAS 500-F

Investigation on the release of soil-bound residues of ^{14}C-BAS 500 F by earthworms, Ebert, D., 1999, BASF Reg.Doc. #1999/11289 (BBA Ref.-No. ARW 2000-86)

GLP: yes
valid: yes

A study was designed to investigate the possible release of soil-bound residues (max. 65% total applied radioactivity within 360 days) by earthworms and possible effects of the bound residues on earthworms.

Soil:
Sandy loam (organic carbon content 1.7 %, pH 7.5, max. water holding capacity 39 g H_2O/100 g dry soil, microbial biomass 63.9 mgC/100 g dry soil), same soil type as used in the metabolism study.

Treatment:
4.5 kg soil (dry weight) was treated with 0.6 mg/kg ^{14}C tolyl-labelled BAS 500 F (corresponding to the double maximum single recommended field rate). The double rate was chosen in order to produce sufficient bound residues for proper analysis.

Performance and findings:
After 132 days, about 60 % total applied radioactivity were bound to the soil matrix. The soil was extracted to remove the still extractable BAS 500 F and metabolites. The extracted soil was mixed with fresh soil (in the ratio 80/20) to achieve sufficient microbial activity and make the substrate palatable for earthworms. The soil was mixed with dried cattle manure (2.5 g/100 g dry soil) and adjusted to 70 % maximum water holding capacity. After 7 days earthworms were introduced in two replicates with 10 *Eisenia fetida/*200 g dry weight soil and were kept for 42 days in the soil. Two controls without earthworms were set up: one control with untreated soil and one control with ^{14}C BAS 500 F.

All earthworms survived, gained weight and showed normal behaviour. The radioactivity concentration found in earthworms was constant during the experiment and the concentration in the worms was lower than the total radioactive concentration in soil. No release of soil-bound residues by earthworms could be observed. The peak pattern of chromatograms of the soil extracts was almost identical in soil with and without earthworms.

B.9.6.2.3 Effects in the field

B.9.6.2.3.1 Field study to evaluate the effects of BAS 500 00 F on earthworms (grassland site), Krieg, W., 2000

Interim report BASF Reg.Doc.#2000/1000008 (BBA Ref-No. ARW 2000-89)
Final report BASF Reg.Doc.#2000/1012435 (BBA Ref.-No. ARW 2000-182)

Year: 1999/2000
Guideline: ISO/CD 11268-3 (draft 1997), BBA guideline part VI, 2-3(1994)

GLP: yes
valid: yes
Test substance: BAS 500 00 F
Application: 8 applications with increasing rates using recommended application rates (0.24 to 0.64 L form./ha, corresponding to 0.06 kg to 0.16 kg as/ha; sum applied is 0.78 kg as/ha) and 8 applications with increasing rates using half of the application rates (0.12 L/ha to 0.32 L form./ha, taking into account vegetation cover during application, corresponding to 0.03 to 0.08 kg as/ha; sum applied is 0.39 kg as/ha) with intervals of approx. 14 days
Site: Rheinland-Pfalz/Germany
grassland, 5 mulching dates/year
Soil: silty sand
Irrigation: Irrigation with 8000 L on 18.08. and 20.08.1999 each (corresponding to about 3.5 mm precipitation)
Test design: 4 replicates with 10 x 10 m plot size;
4 treatments: water treated control, reference substance Benomyl with 4 kg as/ha and two rates of BAS 500 00 F
Earthworm sampling: electrical octett method, 0.25 m^2 sample with 4 samples /sampling date and plot (= 16 samples/treatment and date)

Findings:

Table B.9.6-3: Abundance of earthworms (individuals/m^2 and % of untreated control)

Sampling date	**11.5.1999**		**15.06.1999**		**26.08.1999**		**19.10.1999**		**10.05.2000**	
	prior to application		**after 2nd appl.**		**after 7th appl.**		**about 7.5 weeks after 8th appl.**		**about 1 year after study start**	
Treatment	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**
BAS 500 F 0.78 kg as/ha	170	129.3	90.5	220.7	70.5	154.9	102.8	186.0	145.3	99.3
BAS 500 F 0.39 kg as/ha	149.8	113.9	56.5	137.8	44.3	97.3	81.8	148.0	131.8	90.1
Benomyl 4 kg as/ha	148	112.5	15.5	37.8 *	17.5	38.5*	37.5	67.9	71.8	49.1*
Control	131.5	100.0	41.0	100.0	45.5	100.0	55.3	100.0	146.3	100.0

* sign. $\alpha = 0.05$, Dunett-test

Table B.9.6-4: Biomass of earthworms (biomass in g/m^2, and % of untreated control)

Sampling date	**11.5.1999**		**15.06.1999**		**26.08.1999**		**19.10.1999**		**10.05.2000**	
	prior to appl.		**after 2nd appl.**		**after 7th appl.**		**about 7.5 weeks after 8th appl.**		**about 1 year after study start**	
Treatment	**g/m^2**	**% of contr.**	**g/m^2**	**% of contr.**	**g/m^2**	**% of contr.**	**g/m^2**	**% of contr.**	**g/m^2**	**% of contr.**
BAS 500 F 0.78 kg as/ha	55.6	123.3	37.7	234.0	16.5	149.9	29.5	204.8	63.6	121.6
BAS 500 F 0.39 kg as/ha	44.1	97.8	23.9	148.8	11.4	103.8	19.7	139.1	50.4	96.4
Benomyl 4 kg as/ha	49.4	109.5	7.0	43.6 *	2.9	26.1*	9.7	68.4	46.1	88.2
Control	45.1	100	16.1	100	11.0	100	14.2	100	52.3	100

* sign. α = 0 0.05, Dunett-test

B.9.6.2.3.2 Field study to evaluate the effects of BAS 500 00 F on earthworms, Ehlers H., 2000

Interim report: BASF RegDoc #2000/1000012 (BBA Ref-No. ARW 2000-90)
Final report: BASF RegDoc #2000/1012439 (BBA Ref-No. ARW 2000-184)

Year:	1999/2000
Guideline:	ISO/CD11268-3 (draft 1997), BBA guideline part VI, 2-3(1994)
GLP:	yes
valid:	yes
Test substance:	BAS 500 00 F
Application:	8 applications with increasing rates using recommended application rates (0.24 L to 0.64 L form./ha, corresponding to 0.06 to 0.16 kg as/ha, sum applied is 0.78 kg as/ha) and 8 applications with increasing rates using half of the application rates (0.12 to 0.32 L form./ha, taking into account vegetation cover during application, corresponding to 0.03 to 0.08 kg as/ha; sum applied is 0.39 kg as/ha) with intervals of approx. 14 days
Site:	Darmstadt/Germany grassland, 2 mulching dates/year
Soil:	silty loamy sand to sandy silty loam
Irrigation:	One week before third collection with 60.97 L/m^2
Test design:	4 replicates with 10 x 10 m plot size; 4 treatments: water treated control, reference substance Benomyl with 4 kg as/ha and two rates of BAS 500 00 F
Earthworm sampling:	electrical octett method, 0.25 m^2 sample with 4 samples /sampling date and plot (= 16 samples/treatment and date)

Findings:

Table B.9.6-5: Abundance of earthworms (individuals/m^2 and % of untreated control):

Sampling date	**18./19.5.1999**		**01./15.07.1999**		**21./22.09.1999**		**05./06.06.2000**	
	prior to appl.		**after 3nd appl.**		**2 weeks after the last appl.**		**one year after the first application**	
Treatment	**Ind/m^2**	**% of contr**	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**
BAS 500 F 0.78 kg as/ha	122.3	105	81.3	65.4 *	83.5	72.7	138.3	104.6
BAS 500 F 0.39 kg as/ha	121.5	104	91.8	73.9	115.8	100.9	133.3	100.8
Benomyl 4 kg as/ha	137.8	118	43.8	35.2 *	82.8	72.1	90.3	68.3
Control	116.3	100	124.3	100	114.8	100	132.2	100.0

* sign. $\alpha = 0.05$ (Dunett-test for data which were normally distributed and homogenous; Bonferroni U-test for data which were not normally distributed and homogenous)

Table B.9.6-6: Biomass of earthworms (g/m^2 and % of untreated control)

Sampling date	**18./19.5.1999**		**1./15.07. 1999**		**21./22.08.1999**		**05./06.06.2000**	
	prior to appl.		**after 3rd appl.**		**2 weeks after the last appl.**		**one year after the first application**	
Treatment	**g/m^2**	**% of contr.**	**g/m^2**	**% of contr.**	**g/m^2**	**% of contr.**	**g/m^2**	**% of contr.**
BAS 500 F 0.78 kg as/ha	28.2	98	14.6	68.5	20.9	84.3	29.8	116.9
BAS 500 F 0.39 kg as/ha	31.1	108	15.6	73.2	28.4	114.5	26.9	105.5
Benomyl 4 kg as/ha	26.1	90	9.3	43.7 *	20	80.6	26.9	105.5
Control	28.9	100	21.3	100	24.8	100	25.5	100.0

* sign. $\alpha = 0.05$ (Dunett-test)

B.9.6.2.3.3 Field study to evaluate the effects of BAS 500 01 F on earthworms, Krieg, W., 2000 Report BASF Reg.Doc.#2000/1012437 (BBA Ref.-No. ARW 2000-183)

Year: 1999/2000
Guideline: ISO/CD 11268-3 (draft 1997), BBA guideline part VI, 2-3(1994)
GLP: yes
valid: yes
Test substance: BAS 500 01 F
Application: 2 x 1 L form./ha (corresponds to 2 x 0.25 kg as/ha) with an interval of approx. one month
Site: Rheinland-Pfalz/Germany
crop (wheat)
additional application of 3 plant protection products in spring 1999
Soil: loamy sand
Test design: 4 replicates with 10 x 10 m plot size;
3 treatments: water treated control, reference substance Benomyl with 4 kg as/ha and one rate of BAS 500 01 F
Earthworm sampling: electrical octett method, 0.25 m^2 sample with 4 samples /sampling date and plot (= 16 samples/treatment and date)

Findings:

Table B.9.6-7: Abundance of earthworms (number of individuals; % of untreated control)

Sampling date	**10.5.1999**		**14.06.1999**		**25.08.1999**	
	prior to application		**about 1 month after first application**		**about 2 month after second application**	
Treatment	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**	**Ind/m^2**	**% of contr.**
BAS 500 01 F 2 x 0.25 kg as/ha	24.0	109.1	30.3	97.6	50.75	78.4
Benomyl 4 kg as/ha	27.5	125.0	8.8 *	28.2 *	31.5 *	48.6 *
Control	22.0	100.0	31.0	100.0	64.8	100.0

* sign. $\alpha = 0.05$, Dunett-test

Sampling date	**18.10.1999**		**11.05.2000**		**23.05.2000**	
	after 5 month after 2nd appl.		**about one year after first appl.**		**about 1 year after first application****	
Treatment	**Ind/m²**	**% of contr.**	**Ind/m²**	**% of contr.**	**Ind/m²**	**% of contr.**
BAS 500 01 F 2 x 0.25 kg as/ha	20.8	131.7	13.8	96.5	60.0	116.5
Benomyl 4 kg as/ha	11.8	74.6	8.3	57.9	32.0 *	62.1 *
Control	15.8	100.0	14.3	100.0	51.5	100.0

* sign. $\alpha = 0.05$, Dunett-test
** extraction by digging out and handsorting

Table B.9.6-8: Biomass of earthworms (biomass in g/m², % of untreated control)

Sampling date	**10.5.1999**		**14.06.1999**		**25.08.1999**	
	prior to application		**about 1 month after first application**		**about 2 month after second application**	
Treatment	**g/m²**	**% of contr.**	**g/m²**	**% of contr.**	**g/m²**	**% of contr.**
BAS 500 01 F 2 x 0.25 kg as/ha	4.6	77.4	5.1	67.7	13.7	74.6
Benomyl 4 kg as/ha	7.4	124.2	1.1 *	14.6 *	7.1 *	38.7 *
Control	6.0	100.0	7.5	100.0	18.4	100.0

* sign. $\alpha = 0.05$, Dunett-test

Sampling date	**18.10.1999**		**11.05.2000**		**23.05.2000**	
	after 5 month after 2nd appl.		**about 1 year after first appl.**		**about 1 year after first application**	
Treatment	**g/m²**	**% of contr.**	**g/m²**	**% of contr.**	**g/m²**	**% of contr.**
BAS 500 01 F 2 x 0.25 kg as/ha	5.9	115.0	9.2	112.2	16.9	98.2
Benomyl 4 kg as/ha	5.6	110.1	7.3	88.3	13.2	76.8
Control	5.1	100.0	8.2	100.0	17.2	100.0

* sign. $\alpha = 0.05$, Dunett-test

B.9.6.3 Risk assessment for earthworms

Since Log Pow is > 2, the toxicity data are divided by the factor of 2 (see EPPO risk assessment scheme for soil organisms). For estimation of risk the lowest toxicity data from Table B.9.6-1 are used.

Table B.9.6-9: TER_a and TER_{lt} for earthworms

Test material	Toxicity data (corrected) (mg as/kg substrate)	$PEC_{initial}$ (mg as/kg) *	Time scale	TER
Pyraclostrobin	282	0.10	Acute	2820
BAS 500 00 F	35.2	0.10	Acute	352
BAS 500-6	> 500	0.17	Acute	> 2941
BAS 500-7	> 500	0.15	Acute	> 3333
BAS 500 00 F	0.443	0.10	Longterm	4.43

* see chapter B.08.03

The acute TER values for the active substance, the product (calculated for the as content) and the metabolites BAS 500-6 and 500-7 are far above the relevant Annex VI trigger of 10 (see Table B.9.6-9). Therefore it is concluded that the active substance and the metabolites do not pose an acute risk to earthworms.

The longterm risk is assessed using a NOEC of 1 l product/ha, corrected to 0.5 l product/ha. The NOEC is converted into a soil concentration using the following calculation:

NOEC = 0.5 L/product/ha
= 0.528 kg product/ha (product density 1.055 g/cm^3)
= 0.0528 g/m^2
= 0.00106 g/200 cm^2 (surface area of test container)
= 1.06 mg/600 g dry mass substrate per container
= 1.77 mg product/kg

corresponds to 0.443 mg as/kg

The longterm TER therefore amounts to 4.43 (0.443/0.10) (for PEC see chapter B.08.03) for both cereals and vine (seeTable B.9.6-9). This trigger is below the relevant trigger of 5. Taking additionally into account that in the test the reduction of juveniles at 1 L/ha (corrected to 0.5 L/ha) was 12 % compared to the control, which is considered a small difference, there is sufficient safety to state that no longterm risk to earthworm populations is likely to occur.

The field tests did not show identical results. In all field tests benomyl yielded a significant reduction in abundance of earthworms, thus showing that an exposure of earthworms was given. Two field tests were identical concerning the product and the application rates tested (BBA-ref-no. ARW 2000-182 and ARW 2000-184). In the first field test (BBA-ref.-no. ARW 2000-182) no reduction of either abundance or biomass in time was observed in comparison to control. In most application dates an increase in numbers was observed in comparison to control. In the second field test (BBA-ref.-no. ARW 2000-184) there was a significant reduction in abundance of about 35 % compared to control after the third application of 0.06 kg as/ha. Two weeks after the last application there was still a reduction of about 28 %, which was not significant. One year after study start the population densities of this treatment were

comparable to the control values. The application rate of 0.03 kg as/also showed some reduction after the third application (about 24 %), which was not significant. Two weeks after the last application the abundance was comparable to control values. The differences between the outcome in the two field studies may be partly explained by a different precipitation regime. The second study yielded a higher precipitation, therefore exposure might have been higher. As with the lower rate - which takes into account that vegetation cover is given during application and that therefore not the whole applied amount will reach the soil surface - no longlasting effects occured in both studies, it is concluded that no long-lasting effect on earthworm populations will probably occur.
In the third field study (ARW 2000-183) BAS 500 01 F was tested with a rate of 2 x 1 L formulation (corresponding to 2 x 0.25 kg as/ha). As this test was done in a wheat field the numbers of earthworms were lower compared to the studies on grassland. No longlasting effects on earthworm populations were observed in this study.

The study on release of soil bound residues (BBA-ref.-no. ARW 2000-86) showed that no release of soil bound residues by earthworms could be observed and that the concentration in worms was lower than the total radioactive concentration in soil.

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

Bait lamina test to evaluate the activity of soil organisms following an exposure to BAS 500 00 F in the field (BASF Doc ID 2000/1000016) (BBA-Ref-No. ARW 2000-91)

A bait lamina study was conducted together with the earthworm field study (BBA Ref-No. ARW 2000-182), see chapter B.09.06.

Year:	1999/2000
Guideline:	ISO/CD 11268-3 (draft 1997), BBA guideline part VI, 2-3(1994)
GLP:	yes
valid:	yes
Test substance:	BAS 500 00 F
Application:	8 applications with increasing rates (0.24 to 0.64 l form./ha, recommended application rate, corresponding to 0.06 to 0.16 kg as/ha) and 8 applications with increasing rates (0.12 to 0.32 l form./ha, half application rate, taking into account vegetation cover during application, corresponding to 0.03 to 0.08 kg as/ha) with intervals of approx. 14 days
Site:	grassland, 5 mulching dates/year
Soil:	silty sand
Irrigation:	Irrigation with 8000 l on 18.08. and 20.08.1999 each (corresponding to about 3.5 mm precipitation)
Test design:	4 replicates with 10 x 10 m plot size; 4 treatments: water treated control, reference substance Benomyl with 4 kg as/ha and two rates of BAS 500 00 F
Sampling:	about 12 weeks after the last application (in november) soil cores of 20 cm length and 7 cm diameter were taken into the glasshouse (10 cores/plot and 40 cores/treatment) and kept at 16 to 26 °C and a constant moisture content of 14 to 18 %. Ten days after taking the cores

into the laboratory, three bait laimina sticks with 16 holes each, filled with a standard mixture of cellulose, bran flakes and active coal, were vertically exposed per soil core (i.e. 120 bait-lamina per treatment).

Evaluation: After 11 days the bait-lamina sticks were examined on a lighted bench using three steps: total bait eaten (> 50 %, light falls through the bait), bait eaten (< 50 %) and bait not eaten (0 %).

Findings: The main feeding activity per bait lamina was 17.3 % in the control, 24.8 % in the 0.03 kg as/ha (significant increase) and 19.4 % in the 0.06 kg as/ha treatment. The feeding activity distribution in dependence of the soil depth was in accordance with general findings and was similar for the control and the 0.06 kg as/ha treatment, showing a lower feeding activity in the deeper soil layers. In the 0.03 kg as/ha treatment the feeding activity distribution was not so clear depth related as in the other two variants.

Monitoring of collembola populations following an exposure to BAS 500 00 F in the field (grassland) BASF Doc ID 2000/1000020 (BBA Ref-No. ARW 2000-92)

A field monitoring to evaluate potential effects on collembola populations was conducted together with the earthworm field study (BBA Ref-No. ARW 2000-182), see chapter B.09.06.

Year: 1999/2000
Guideline: ISO/CD 11268-3 (draft 1997), BBA guideline part VI, 2-3(1994)
GLP: yes
valid: yes
Test substance: BAS 500 00 F
Application: 8 applications with increasing rates (0.24 to 0.64 l form./ha, recommended application rate, corresponding to 0.06 to 0.16 kg as/ha) and 8 applications with increasing rates (0.12 to 0.32 l form./ha, half application rate, taking into account vegetation cover during application, corresponding to 0.03 to 0.08 kg as/ha) with intervals of approx. 14 days
Site: grassland, 5 mulching dates/year
Soil: silty sand
Irrigation: Irrigation with 8000 l on 18.08. and 20.08.1999 each (corresponding to about 3.5 mm precipitation)
Test design: 4 replicates with 10 x 10 m plot size;
4 treatments: water treated control, reference substance Benomyl with 4 kg as/ha and two rates of BAS 500 00 F
Sampling: in july after the 5^{th} application and in october 5 weeks after the 8^{th} application 5 soil cores (8 cm length and 6.8 cm diameter) per plot (i.e. 20 cores/ variant) were taken
Evaluation: extraction with McFayden within 7 days, abundance/m^2 and differentiation up the family level
Findings: The composition of collembola families (*Sminthuridae, Entomobryidae*, *Isotomidae, Onychiuridae, Poduridae*) was comparable in the control and the treatments. The ratio of species was similar in october, but more different in the july evaluation. The mean abundance was comparable high in july in all treatments (control: 56793 individuals/m^2; 0.03 kg

as/ha: 57352 ind/m^2; 0.06 kg as/ha: 52316 ind/m^2). In october the abundance was lower, possibly due to dry wheather conditions and ranged from 19102 ind/m^2 in the control to 16848 ind/m^2 in the 0.06 kg as/ha treatment and 18278 Ind/m^2 in the 0.03 kg as/ha treatment. The family *Poduridae* was reduced to very low numbers in all treatments and control in october, possibly due to wheather conditions.
The variability of collembola abundance was high and no statistically significant differences were found between treatments.

Risk assessment
The study on feeding activity on bait lamina which was conducted on the site of one earthworm field study did not show any obvious adverse effect on feeding activity. Additionally the collembola numbers which were evaluated on this site did not yield a clear tendency or significant differences in numbers on the different plots.
It has to be noted that these additional studies have been done on the earthworms site where no effect on earthworms were observed (study no. ARW 2000-182).

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

Laboratory tests were performed to examine the effects of pyraclostrobin on microbial activities in soil. One test was carried out with an EC formulation (BAS 500 00 F) containing 25 % active substance. Effects of the two metabolites BF 500-6 and BF 500-7 were investigated in a second test.
In the test with the formulation the soil was exposed to concentrations of 1.33 and 13.33 µL BAS 500 00 F/kg dry weight soil (application rates were equivalent to 1.0 and 10 L BAS 500 00 F/ha respectively = 1x and 10x maximum field rate).
The two metabolites BF 500-6 and BF 500-7 were investigated in a mixture. The test concentrations were 0.1 mg (1x) and 1.0 mg (10x) BF 500-6 and 0.05 (1x) and 0.5 mg as (10x) BF 500-7 per kg dry weight soil, respectively. This corresponded to field application rates of 75 g/ha (1x) and 750 g/ha (10x) BF 500-6 and 37.5 g/ha (1x) and 375 g/ha (10x) BF 500-7, respectively.
All tests were conducted according to BBA-guideline 1-1, part VI, March 1990.

B.9.8.1 Carbon conversion (Annex IIA 8.5; Annex IIIA 10.7)

The effects on short term respiration were tested with the EC formulation BAS 500 00 F and with the mixture of the two metabolites (BAS 500-6 and BAS 500-7) in two different soil types.

Table B.9.8-1: Effects of the EC formulation BAS 500 00 F on carbon conversion

type of soil	application rate in L product/ha	in comparison with untreated in %	test duration in days	influence tolerable	ref.
silty sand	1	-3.9	28	yes	(1)
	10	0	28	yes	(1)
loamy sand	1	-2.8	28	yes	(1)
	10	-1.4	28	yes	(1)

(1) Krieg, W., 1998 (BMF2000-39)

Table B.9.8-2: Effects of the metabolites BAS 500-6 + BAS 500-7 on carbon conversion

type of soil	application rate in kg as/ha	in comparison with untreated in %	test duration in days	influence tolerable	ref.
loamy sand (72.4 % sand)	0.075 + 0.0375	-1.5	28	yes	(2)
	0.75 + 0.375	0	28	yes	(2)
loamy sand (57.7 % sand)	0.075 + 0.0375	-2.5	28	yes	(2)
	0.75 + 0.375	-3.7	28	yes	(2)

(2) Krieg, W., 1999, BMF2000-37

B.9.8.2 Nitrogen conversion (Annex IIA 8.5; Annex IIIA 10.7)

The effects on nitrogen mineralisation were tested with the EC formulation BAS 500 00 F and with the mixture of the two metabolites (BAS 500-6 and BAS 500-7) in two different soil types.

Table B.9.8-3: Effects of the EC formulation BAS 500 00 F on nitrate formation

type of soil	application rate in L product/ha	in comparison with untreated in %	test duration in days	influence tolerable	ref.
silty sand	1	+1.5	28	yes	(3)
	10	+1.9	28	yes	(3)
loamy silt	1	+5.9 / -0.7	28 / 49	yes	(3)
	10	+17.4 / +5.0	28 / 49	yes	(3)

(3) Krieg, W., 1998 (BMF2000-40)

Table B.9.8-4: Effects of the metabolites BAS 500-6 + BAS 500-7 on nitrate formation

type of soil	application rate in kg as/ha	in comparison with untreated in %	test duration in days	influence tolerable	ref.
loamy sand (72.4 % sand)	0.075 + 0.0375	-10.2	28	yes	(4)
	0.75 + 0.375	-9.8	28	yes	(4)
loamy sand (57.7 % sand)	0.075 + 0.0375	-17.6 / -10.8	28 / 77	yes	(4)
	0.75 + 0.375	-23.4 / -16.9	28 / 77	yes	(4)

(4) Krieg, W., 1998 (BMF2000-38)

B.9.8.3 Risk assessment for soil micro-organisms

The influence of the EC formulation BAS 500 00 F on the microbial activity is in case of one- and ten-fold application rate (1 and 10 l/ha) < 25 % in comparison with untreated soil. Also the two metabolites (BF 500-6 and BF 500-7) have no lasting effects on carbon- and nitrogen conversion.
When applying BAS 500 00 F containing plant protection products according to the recommended pattern of use no lasting effects on microbial activities are to be expected.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

The fungicide pyraclostrobin (BAS 500 00 F) is used in cereals and in grapes.

BAS 500 00 F was tested in the greenhouse in a limit test according to OECD-guideline no. 208 with 3 mono- and 3 dicotyledonous species in a vegetative vigour test in a loamy sand. The test substance was applied post-emergence 2 to 4 weeks after seedling at growth stage BBCH 12 to 14 depending on the plant species. Phytotoxicity was evaluated 7 and 14 days after application and the fresh weight of the plant biomass above ground was determined at termination of the study after 14 days. The application rates tested were corresponding to 160 and 480 g as/ha.

Table 9.9-1: Effects on non-target plants (phytotoxicity and fresh weight) after 14 days of exposure

	0.160 kg as/ha Mean phytotoxicity (% of control)	0.480 kg as/ha Mean phytotoxicity (% of control)	0.160 kg as/ha Mean fresh weight (% of control)	0.480 kg as/ha Mean fresh weight (% of control)
Daucus carota	0	5	120.3	111.1
Brassica napus	3	7	85.4	93.3
Pisum sativum	6	6	95.0	100.7
Zea mays	0	0	98.6	98.1
Avena sativa	0	0	95.6	97.8
Allium cepa	0	0	99.9	104.5

No significant effects on weight were observed (Dunett test, $\alpha < 0.05$). The maximum observed reduction in weight was 14 % in the single application rate. There were no effects on phytotoxicity of more than 10 %.

Risk assessment
From the data available it is concluded that no risk for terrestrial non-target plants is likely to occur.

B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

Title:	**Determination of the inhibition of oxygen consumption by activated sludge by BAS 500 F in the activated sludge respiration inhibition test according to GLP, EN 45001 and ISO 9002**
Author:	Reuschenbach, P. 1999
BBA-Ref.-No.:	WAT2000-230
Test substance:	BAS 500 F, purity: 97.1 %
Guideline:	EEC 88/302, OECD 209, ISO 8192
Test species:	Activated sludge from laboratory wastewater plants treating municipal sewage. Concentration of dry substance 1000 mg/L.

Animals per treatment: Not applicable
Dose levels: Control, 1000 mg/L, reference substance: 3,5-dichlorophenol

Findings: No significant inhibition of respiration was measured up to the highest tested concentration of 1000 mg/L (nominal). EC_{50} of the reference substance was about 20 mg/L.
EC_{20}: >1000 mg/L (nominal)

GLP compliance: yes
Valid: yes

B.9.10.1 Risk assessment

A significant inhibition of respiration was not observed up to the highest tested concentration of 1000 mg/L. An effect on the biodegradation process of activated slugde is not to be expected.

B.9.11 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-8.1.1	Munk, R.	1997	Report BAS 500 F (Reg.No.304 428) - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus);. 11W0494/96117 /BAS 97/11136 GLP, unpublished AVS2000-60	Y	BAS
AIIA-8.1.2	Munk, R.	1998	Test Report BAS 500 F - Avian dietary LC50 test in chicks of the mallard duck (Anas platyrhynchos L.). 31W0494/96123/BAS 98/10933 GLP, unpublished AVS2000-62	Y	BAS
AIIA-8.1.2	Munk, R.	1998	Test Report BAS 500 F - Avian dietary LC50 test in chicks of the bobwhite quail (Colinus virginianus);. 31W0494/96126/BAS 98/10932 GLP, unpublished AVS2000-61	Y	BAS

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-8.1.3	Frey, L.T., Beavers, J.B. and Jaber, M.	1999	BAS 500 F: A reproduction study with the northern bobwhite. 145-175 GLP, unpublished AVS2000-64	Y	BAS
AIIA-8.1.3	Frey, L.T., Beavers, J.B. and Jaber, M.	1999	BAS 500 F: A reproduction study with the mallard. 147-176 GLP, unpublished AVS2000-63	Y	BAS
AIIA-8.2.1	Dohmen, G-P	1999	Effect of BAS 500 F on Daphnia magna Straus in a 48 hours acute toxicity test. 35806 GLP, unpublished WAT2000-247	Y	BAS
AIIA-8.2.1	Munk, R.	1998	BAS 500 F Acute toxicity study on the common carp (Cyprinus carpio L.) in a static system (96 hours). 12F0494/965178 GLP, unpublished WAT2000-234	Y	BAS
AIIA-8.2.1	Munk, R.	1998	BAS 500 F Acute toxicity study on the bluegill (Lepomis macrochirus Raf.) in a static system (96 hours). 12F0494/965179 GLP, unpublished WAT2000-233	Y	BAS
AIIA-8.2.1	Zok, S.	1999	Reg.-Nr. 413038 Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0249/995035 GLP, unpublished WAT2000-237	Y	BAS
AIIA-8.2.1	Zok, S.	1999	Reg.-Nr. 412785 Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0252/995034 GLP, unpublished WAT2000-236	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-8.2.1	Zok, S.	1999	Reg.-Nr. 411847 Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0251/995037 GLP, unpublished WAT2000-235	Y	BAS
AIIA-8.2.1	Zok, S.	1999	BAS 500 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0494/965180 GLP, unpublished WAT2000-232	Y	BAS
AIIA-8.2.2	Zok, S.	1999	BAS 500 F Sublethal toxic effects on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a flow-through system (28 days). 42F0494/965177 GLP, unpublished WAT2000-238	Y	BAS
AIIA-8.2.2.1	Zok, S.	2000	BAS 500 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss)after short time exposure over 0,5, 2 and 8 hours in a flow-through system followed up by a post exposure period. 12F0494/965190 GLP, unpublished WAT2001-139	N	BAS
AIIA-8.2.2.1	Zok, S.	1999	BAS 500 F Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss Walbaum 1792). 52F0494/965141 GLP, unpublished WAT2000-239	Y	BAS
AIIA-8.2.3	Chapleo, S.	1999	Bioaccumulation and metabolism of [14C]-BAS 500 F in bluegill sunfish. 391491, 17015 GLP, unpublished WAT2000-241	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-8.2.3	Daum, A.	1999	Determination of the octanol/water-partition coefficient of Reg.-No. 413038 (BF 500-14) by HPLC. PCP05354 GLP, unpublished WAT2000-244	Y	BAS
AIIA-8.2.3	Daum, A.	1999	Determination of the octanol/water-partition coefficient of Reg.-No. 412785 (BF 500-13) by HPLC. PCP05339 GLP, unpublished WAT2000-243	Y	BAS
AIIA-8.2.3	Daum, A.	1999	Determination of the octanol/water-partition coefficient of Reg.-No. 411847 (BF 500-11) by HPLC. PCP05338 GLP, unpublished WAT2000-242	Y	BAS
AIIA-8.2.5	Dohmen, G-P	1999	Effects of BAS 500 F on mortality and reproduction of Daphnia magna. 35811 GLP, unpublished WAT2000-251	Y	BAS
AIIA-8.2.5	Jatzek	1999	Determination on the acute effect of BF 500-14 on the swimming ability of the water flea Daphnia magna Straus. 99/0519/50/1 GLP, unpublished WAT2000-250	Y	BAS
AIIA-8.2.5	Jatzek	1999	Determination on the acute effect of BF 500-13 on the swimming ability of the water flea Daphnia magna Straus. 99/0518/50/1 GLP, unpublished WAT2000-249	Y	BAS
AIIA-8.2.5	Jatzek	1999	Determination on the acute effect of BF 500-11 on the swimming ability of the water flea Daphnia magna Straus. 99/0517/50/1 GLP, unpublished WAT2000-248	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-8.2.6	Dohmen, G-P	1999	Effect of BAS 500 F on the growth of the green algae Pseudokirchneriella subcapitata. 35803 GLP, unpublished WAT2000-252	Y	BAS
AIIA-8.2.6	Reuschenbach	1999	Determination of the inhibitory effect of BF 500-14 on the cell multiplication of unicellular green algae. 99/0519/60/1 GLP, unpublished WAT2000-255	Y	BAS
AIIA-8.2.6	Reuschenbach	1999	Determination of the inhibitory effect of BF 500-13 on the cell multiplication of unicellular green algae. 99/0518/60/1 GLP, unpublished WAT2000-254	Y	BAS
AIIA-8.2.6	Reuschenbach	1999	Determination of the inhibitory effect of BF 500-11 on the cell multiplication of unicellular green algae. 99/0517/60/1 GLP, unpublished WAT2000-253	Y	BAS
AIIA-8.2.7	Dohmen, G-P	2000	Effects of BAS 500 F on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system. 35966 GLP, unpublished WAT2000-256	Y	BAS
AIIA-8.3	Sack, D.	1999	Effects of Reg.No. 304 428 on the Honeybee (Apis mellifera L.) in Laboratory Trials. 11457 GLP, unpublished BIE2000-12	Y	BAS
AIIA-8.4.1	Ebert, D.	1999	Investigations on the release of soil-bound residues of 14C-BAS 500 F by earthworms. Std:: 53058 ! BASF 1999/11289 GLP, unpublished ARW2000-86	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-8.4.1	Krieg, W.	1999	Effect of BF 500-7 on the mortality of the earthworm Eisenia foetida. Std:: 54484 ! BASF 1999/11309 GLP, unpublished ARW2000-85	Y	BAS
AIIA-8.4.1	Krieg, W.	1999	Effect of BF 500-6 on the mortality of the earthworm Eisenia foetida. Std.: 35987 ! BASF 1999/11308 GLP, unpublished ARW2000-84	Y	BAS
AIIA-8.4.1	Krieg, W.	1999	Effect of BAS 500 F on the mortality of the earthworm Eisenia foetida. Std.: 35801 ! BASF 1999/10708 GLP, unpublished ARW2000-83	Y	BAS
AIIA-8.5	Krieg, W.	1999	Effect of BF 500-6 and BF 500-7 on the nitrogen turnover in soil. 54482 ! BASF DocID 1999/11311 GLP, unpublished BMF2000-38	Y	BAS
AIIA-8.5	Krieg, W.	1999	Effect of BF 500-6 and BF 500-7 on soil respiration. 54483 ! BASF DocID 1999/11120 GLP, unpublished BMF2000-37	Y	BAS
AIIIA-10.1.1	Zok, S.	1999	Report BAS 500 00 F - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus). 11W0185/97164 /BAS 1999/11838 GLP, unpublished AVS2000-65	Y	BAS
AIIA-8.7; AIIIA-10.2	Reuschenbach	1999	Determination of the inhibition of oxygen consumption by activated sludge by BAS 500 F in the activated sludge respiration inhibition test. 99/0164/08/1 GLP, unpublished WAT2000-230	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-10.2.1	Dohmen, G-P	1999	Effect of BAS 500 00 F on the growth of the green alga Pseudokirchneriella subcapitata. 35848 GLP, unpublished WAT2000-265	Y	BAS
AIIIA-10.2.1	Dohmen, G-P	1999	Effect of BAS 500 00 F on the immobility of Daphnia magna Straus in a 48 hour static, acute toxicity test. 35851 GLP, unpublished WAT2000-264	Y	BAS
AIIIA-10.2.1	Zok, S.	1999	BAS 500 00 F Acute toxicity study on the fathead minnow (Pimephales promelas RAF.) in a static system (96 hours). 15F0185/975117 GLP, unpublished WAT2000-261	Y	BAS
AIIIA-10.2.1	Zok, S.	1999	BAS 500 00 F Acute toxicity study on the golden orfe (Leuciscus idus melanotus) in a static system (96 hours). 10F0185/975121 GLP, unpublished WAT2000-263	Y	BAS
AIIIA-10.2.1	Zok, S.	1999	BAS 500 00 F Acute toxicity study on the zebra fish (Brachidanio rerio HAM. and BUCH.) in a static system (96 hours). 17F0185/975120 GLP, unpublished WAT2000-262	Y	BAS
AIIIA-10.2.1	Zok, S.	1999	BAS 500 00 F Acute toxicity study on the common carp (Cyprinus carpio L.) in a static system (96 hours). 11F0185/975119 GLP, unpublished WAT2000-259	Y	BAS
AIIIA-10.2.1	Zok, S.	1999	BAS 500 00 F Acute toxicity study on the orange red killifish (Oryzias latipes Schlegel) in a static system (96 hours). 13F0185/975122 GLP, unpublished WAT2000-260	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-10.2.1	Zok, S.	1999	BAS 500 00 F Acute toxicity study on the bluegill (Lepomis macrochirus RAF.) in a static system (96 hours). 14F0185/975118 GLP, unpublished WAT2000-258	Y	BAS
AIIIA-10.2.1	Zok, S.	1999	BAS 500 00 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 12F0185/975052 GLP, unpublished WAT2000-257	Y	BAS
AIIIA-10.2.2	Dohmen, G-P	2000	The effect of BAS 500 00 F on aquatic ecosystems - an outdoor mesocosm investigation. 35980 GLP, unpublished WAT2000-231	Y	BAS
AIIIA-10.4	Sack, D.	1999	Effects of BAS 500 00 F on the Honeybee (Apis mellifera L.) in Laboratory Trials. 11455 GLP, unpublished BIE2000-11	Y	BAS
AIIIA-10.6.1.1	Krieg, W.	1998	Effect of BAS 500 00 F on the mortality of the earthworm Eisenia foetida. Std.: 35846 ! BASF 1998/11395 GLP, unpublished ARW2000-87	Y	BAS
AIIIA-10.6.1.2	Krieg, W.	1999	Effect of BAS 500 00 F on growth and reproduction of the earthworm Eisenia foetida. Std.: 56987 ! BASF 1999/10650 GLP, unpublished ARW2000-88	Y	BAS
AIIIA-10.6.1.3	Ehlers, H. A.	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms. Proj.: 6180023 ! BASF 2000/1000012 GLP, unpublished ARW2000-90	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-10.6.1.3	Ehlers, H. A.	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms. BASF 2000/1012439 GLP, unpublished ARW2000-184	Y	BAS
AIIIA-10.6.1.3	Krieg, W.	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms (grassland site). Std.: 60996 ! BASF 2000/1000008 GLP, unpublished ARW2000-89	Y	BAS
AIIIA-10.6.1.3	Krieg, W.	2000	Field study to evaluate the effects of BAS 500 01 F on earthworms. BASF 2000/1012437 GLP, unpublished ARW2000-183	Y	BAS
AIIIA-10.6.1.3	Krieg, W.	2000	Field study to evaluate the effects of BAS 500 00 F on earthworms (grassland site). BASF 2000/1012435 GLP, unpublished ARW2000-182	Y	BAS
AIIIA-10.6.2	Krieg, W.	2000	Monitoring of Collembola populations following an exposure to BAS 500 00 F in the field (grassland). Proj.: 60997 ! BASF 2000/1000020 GLP, unpublished ARW2000-92	Y	BAS
AIIIA-10.6.2	Krieg, W.	2000	Bait-lamina test to evaluate the activity of soil organisms following an exposure to BAS 500 00 F in the field. Std.: 69959 ! BASF 2000/1000016 GLP, unpublished ARW2000-91	Y	BAS
AIIIA-10.7.1	Krieg, W.	1998	Effects of BAS 500 00 F on the nitrogen turnover in soil. 35845 ! BASF 98/11260 GLP, unpublished BMF2000-40	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIIA-10.7.1	Krieg, W.	1998	Effects of BAS 500 00 F on soil respiration. 35844 ! BASF 98/11252 GLP, unpublished BMF2000-39	Y	BAS
AIIA-8.6; AIIIA-10.8	Oberwalder, C. and Schmidt, O.	2000	BAS 500 00 F: Effects on non-target plants in the greenhouse - A limit test. Std.: 67673 ! BASF 2000/1000024 GLP, unpublished PFL2000-99	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Appendix 1

Pyraclostrobin

Standard Terms and Abbreviations

Contents

B Rapporteur Member State summary, evaluation and assessment of the data and information

B.10 Appendices 487

B.10.1 Appendix I: Standard terms and abbreviations 487
B.10.2 Appendix II: Specific terms and abbreviations 499

B.10 Appendices

B.10.1 Appendix I: Standard terms and abbreviations

Part 1 Technical Terms

A	ampere
ACH	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosine diphosphate
AE	acid equivalent
AFID	alkali flame-ionisation detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50 %
ALT	alanine amitrotransferase (SGPT)
AMD	automatic multiple development
ANOVA	analysis of variance
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathy
BSP	bromosulfophthalein
Bt	*Bacillus thuringiensis*
Bti	*Bacillus thuringiensis israelensis*
Btk	*Bacillus thuringiensis kurstaki*
Btt	*Bacillus thuringiensis tenebrionis*
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degree Celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela
CDA	controlled drop(let) application

cDNA	complementary DNA
CEC	cation exchange capacity
cf	confer, compare to
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coefficient of variation
Cv	ceiling value
CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days past inoculation
DRES	dietary risk evaluation system
DT_{50}	period required for 50 percent dissipation (define method of estimation)
DT_{90}	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
EC_{50}	effective concentration
ECD	electron capture detector
ECU	European currency unit
ED_{50}	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immunosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
ERL	extraneous residue limit
F	field
F_0	parental generation
F_1	filial generation, first
F_2	filial generation, second
FIA	fluorescence immuno assay
FID	flame ionisation detector
FOB	functional observation battery
fp	freezing point
FPD	flame photometric detector

FPLC	fast protein liquid chromatography
g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography
GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulose virus
h	hour(s)
H	Henry's Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high energy electron diffraction
HID	helium ionisation detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HRGC	high resolution gas chromatography
Hs	Shannon-Weaver index
Ht	haematocrit
I	indoor
I_{50}	inhibitory dose, 50 %
IC_{50}	median immobilisation concentration
ICM	integrated crop management
ID	ionisation detector

IEDI	international estimated daily intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilisation
k	kilo
K	Kelvin or Henry's Law constant (in atmospheres per cubic meter per mole) (see also H)13
K_{ads}	adsorption constant
K_{des}	apparent desorption coefficient
K_{oc}	organic carbon adsorption coefficient
K_{om}	organic matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network
LASER	light amplification by stimulated emission
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC_{50}	lethal concentration, median
LCA	life cycle analysis
LCLo	lethal concentration low
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDLo	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometer (micron)
MC	moisture content
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration

MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mol
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue limit or level
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration)
NAEL	no adverse effect level
nd	not detected
NEDI	no effect daily intake (mg/kg body wt/day)
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intent to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorus pesticide
Pa	Pascal
PAD	pulsed amperometric detection

2-PAM	2-pralidoxime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PEC_A	predicted environmental concentration in air
PEC_S	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
PEC_{GW}	predicted environmental concentration in ground water
PED	plasma-emissions-detector
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibition capacity
PIXE	proton induced X-ray emission
pK_a	negative logarithm (to the base 10) of the dissociation constant
PNEC	predicted no effect concentration
po	by mouth (per os)
P_{ow}	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10^{-9})
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
ppq	parts per quadrillion (10^{-24})
ppt	parts per trillion (10^{-12})
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r^2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval
R_f	ratio of fronts
RfD	reference dose
RH	relative humidity
RL_{50}	residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	reversed phase material
rRNA	ribosomal ribonucleic acid
RRT	relative retention time
RSD	relative standard deviation
s	second

SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedure
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogen free
spp	subspecies
sq	square
SSD	sulphur specific detector
SSMS	spark source mass spectrometry
STEL	short term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
$t_{1/2}$	half-life (define method of estimation)
T_3	tri-iodothyroxine
T_4	thyroxine
TADI	temporary acceptable daily intake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TCLo	toxic concentration low
TID	thermionic detector, alkali flame detector
TDLo	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ratio
TER_I	toxicity exposure ratio for initial exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution
TMRL	temporary maximum residue limit

TOC	total organic chlorine
Tremcard	Transport emergency card
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Part 2 Organisations and Publications

ACPA	American Crop Protection Association
ASTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
CA	Chemical Abstracts
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe
CIPAC	Collaborative International Pesticides Analytical Council Ltd
COREPER	Comité des Representants Permanents
EC	European Commission
ECB	European Chemical Bureau
ECCA	European Crop Care Association
ECDIN	Environmental Chemicals Data and Information of the European Communities
ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe

ECETOC	European Chemical Industry Ecology and Toxicology Centre
ECLO	Emergency Centre for Locust Operations
ECMWF	European Centre for Medium Range Weather Forecasting
ECPA	European Crop Protection Association
EDEXIM	European Database on Export an Import of Dangerous Chemicals
EHC (number)	Environment Health Criteria (number)
EHCD	Environmental Health Criteria Document
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organisation
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive Operator Exposure Model
FAO	Food and Agriculture Organisation of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monitoring System
GIEWS	Global Information and Early Warning System for Food and Agriculture
GIFAP	Groupement International des Association Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GRIN	Germplasm Resources Information Network
HRAC	Herbicide Resistance Action Committee
IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
IBT	Industrial Bio-Test Laboratories
ICBB	International Commission of Bee Botany
ICBP	International Council for Bird Preservation
ICES	International Council for the Exploration of the Seas
ICPBR	International Commission for Plant-Bee Relationships
ILO	International Labour Organisation
IMO	International Maritime Organisation
IOBC	International Organisation for Biological Control of noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Resistance Action Committee
IRC	International Rice Commission
ISCO	International Soil Conservation Organisation
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JECFA	FAO/WHO Joint Expert Committee on Food Additives

JFCMP	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticides (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
NATO	North Atlantic Treaty Organisation
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Centre for Toxicological Research (USA)
NGO	non-governmental organisation
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Co-operation and Development
OLIS	On-line Information Service of OECD
PAN	Pesticides Action Network
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SCPH	Standing Committee on Plant Health
SETAC	Society of Environmental Toxicology and Chemistry
SI	Systeme International d'Unites
SITC	Standard International Trade Classification
TOXLINE	Toxicology Information On-line
UN	United Nations
UNEP	United Nations Environment Programme
WCDP	World Climate Data Programme
WCP	World Climate Programme
WCRP	World Climate Research Programme
WFP	World Food Programme
WHO	World Health Organisation
WTO	World Trade Organisation
WWF	World Wide Fund for Nature

Appendix 2

Pyraclostrobin

Specific Terms and Abbreviations

B.10.2 Appendix II: Specific terms and abbreviations

PAS	pure active substance
TAS	technical active substance
DFR	dislodgeable foliar residue
TAR	total applied radioactivity