Monograph

08 November 2002

Nicobifen

Alternative common name proposed to ISO in August 2002:
Boscalid

Volume 1

Report and
Proposed Decision

Rapporteur Member State: Germany

Contents

Level 1

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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)

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

As BASF is the only notifier, this point is not relevant.

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)

Applicant:

BASF Aktiengesellschaft
Agricultural Center
Product Registration Management
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D-67114 Limburgerhof

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1.3.2 Common name and synonyms (Annex IIA 1.3)

Nicobifen (ISO, proposed)

1.3.3 Chemical name (Annex IIA 1.4)

IUPAC: 2-Chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide

CAS: 2-Chloro-*N*-(4'-chloro[1,1'-biphenyl]-2-yl)-3-pyridinecarboxamide

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

BAS 510 F, Reg. No. 300355, PS 300355

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

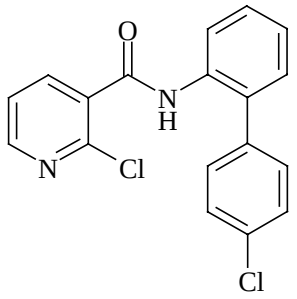
CAS: 188425-85-6
CIPAC: 673
EEC: not assigned
EINECS: not assigned

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

Molecular formular: $C_{18}H_{12}Cl_2N_2O$

Molecular mass: 343.21 g/mol

Structural formula:



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

Manufacturer:

BASF Aktiengesellschaft
Crop Protection Division
P.O. Box 120
D-67114 Limburgerhof

Person to contact: Dr. Wolfgang Türk
Production Crop Protection
Telephone: +49 (0) 621 60-79145
Telefax: +49 (0) 621 60-79519

Manufacturing site:
Pilot plant at BASF AG, Ludwigshafen

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)

≥ 960 g/kg (minimum purity)

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 510 01 F" (preliminary designator) (country specific alternatives are under consideration)	
Code Number:	Plant Protection Product:	BAS 510 01 F
	Active Substance:	BAS 510 F (proposed common name: nicobifen)
	BASF internal No.	Reg. No. 300355

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)

Water dispersible granule (WG)

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)

Nicobifen is envisaged to be used as a fungicide under field conditions in several agricultural and horticultural, ornamentals and viticulture.

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

Nicobifen controls several fungal pathogens belonging to the four major classes of plant pathogenic fungi.
Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound. Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.

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

Uses supported by available data are on grapes: *Botrytis cinerea* and *Uncinula necator*; on oilseed rape: *Sclerotinia sclerotiorum, Alternaria brassiceae, Phoma lingam* (*Leptoshaeria maculans*); on peas: *Sclerotinia sclerotiorum, Botrytis cinerea*; on beans (*Vicia*): *Botrytis cinerea, Sclerotinia sclerotiorum*.

Further uses envisaged are on oilseed rape: *Botrytis cinerea*; on beans (French): *Botrytis cinerea, Sclerotinia sclerotiorum*; on lettuce: *Botrytis cinerea, Sclerotinia sclerotiorum*; on potato: *Alternaria* spp.; on cabbages: *Alternaria* spp., *Botrytis cinerea, Sclerotinia sclerotiorum*; on tomato: *Botrytis cinerea, Leveillula taurica*; on peppers: *Botrytis cinerea, Leveillula taurica*; on winter leeks: *Stemphylium botryosum*; on stonefruit: *Monilinia fructigena, Monilinia laxa*; on pomefruit: *Podosphaera leucotricha, Venturia inaequalis*; on strawberry: *Botrytis cinerea*; on hops: *Sphaerotheca humuli*; on cucumber: *Erysiphe cichoracearum, Sphaerotheca fuliginea*; on melon: *Erysiphe cichoracearum, Sphaerotheca fuliginea*; on roses: *Sphaerotheca pannosa*; on lilies: *Botrytis* spp.; on bulb ornamentals: *Botrytis* spp.

Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound. Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.

The envisaged application rates in grapes against *Botrytis cinerea* are 0.6 kg as/ha active one time per year depending on the developmental stage of the plant; in oilseed rape against *Sclerotinia sclerotiorum, Alternaria brassicae* and *Phoma lingam* 0.25 kg as/ha two times per year; in beans and peas against *Botrytis cinerea* and *Sclerotinia sclerotiorum* 0.5 kg as/ha in two applications per year. In all cases the application method is spraying.

Summary of uses supported by available data Nicobifen (BAS 510 01 F)

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grape	**EU (North & South)**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 - 81	1	-	0.038 - 0.060	1000 - 1600	0.600	28	
Oil seed rape	**EU (North)**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63 - 65	2	4-6 weeks	0.062 - 0.125	200 - 400	0.250	-	
Oil seed rape	**EU (South)**	BAS 510 01 F	F	*Alternaria Sclerotinia, Phoma*	WG	500	spraying	30, 63 - 65	2	4-6 weeks	0.100 - 0.050	200 - 400	0.200	-	
Peas	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 - 10	0.125	400	0.500	7	
Beans	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 – 10	0.166	300	0.500	7	

(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

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

There is no registration of a product containing nicobifen in the EU.

Level 2

Nicobifen

Alternative common name proposed to ISO in August 2002:
Boscalid

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

Nicobifen (pure and technical active substance) is a white solid. A melting point of 143 – 145 °C was determined for PAS. The as decompose from 300 °C. The relative density determined at 20 °C is 1.38. The water solubility is 4.64 mg/L (20 °C) and the log $P_{o/w}$ is 2.96. The test substance is soluble in acetone, acetonitrile, dichloromethane, ethyl acetate and methanol (40 – > 250 g/L). Lowest solubility are observed in *n*-heptane (< 10 g/L). The substance is not highly flammable or autoflammable, not explosive and without oxidising properties.

BAS 510 01 F is a grey brown, free flowing water dispersible granule with a faint aromatic odour. It has neither explosive nor oxidising properties and it is not highly flammable. Its pH-value of 5.65 ± 0.15 lies within the naturally occurring acidic range. Although the shelf life test has not finished yet due to the accelerated storage stability test one can expect that the results will confirm its stability allowing storage at least for 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

2.1.4 Function

Nicobifen is envisaged to be used as a fungicide.
Nicobifen controls several fungal pathogens belonging to the four major classes of plant pathogenic fungi.
Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound. Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.

2.1.5 Field of use envisaged

Nicobifen will be used under field conditions in several agricultural and horticultural, ornamentals and viticulture.

2.1.6 Harmful organisms controlled and crops protected

Uses supported by available data are on grapes: *Botrytis cinerea* and *Uncinula necator*; on oilseed rape: *Sclerotinia sclerotiorum, Alternaria brassiceae, Phoma lingam* (*Leptoshaeria maculans*); on peas: *Sclerotinia sclerotiorum, Botrytis cinerea*; on beans (*Vicia*): *Botrytis cinerea, Sclerotinia sclerotiorum.*

Further uses envisaged are on oilseed rape: *Botrytis cinerea*; on beans (French): *Botrytis cinerea, Sclerotinia sclerotiorum*; on lettuce: *Botrytis cinerea, Sclerotinia sclerotiorum*; on potato: *Alternaria* spp.; on cabbages: *Alternaria* spp., *Botrytis cinerea, Sclerotinia sclerotiorum*; on tomato: *Botrytis cinerea, Leveillula taurica*; on peppers: *Botrytis cinerea, Leveillula taurica*; on winter leeks: *Stemphylium botryosum*; on stonefruit: *Monilinia fructigena, Monilinia laxa*; on pomefruit: *Podosphaera leucotricha, Venturia inaequalis*; on strawberry: *Botrytis cinerea*; on hops: *Sphaerotheca humuli*; on cucumber: *Erysiphe cichoracearum, Sphaerotheca fuliginea*; on melon: *Erysiphe cichoracearum, Sphaerotheca fuliginea*; on roses: *Sphaerotheca pannosa*; on lilies: *Botrytis* spp.; on bulb ornamentals: *Botrytis* spp.

2.1.7 Application rate

The envisaged application rates in grapes against *Botrytis cinerea* are 0.6 kg as/ha one time per year depending on the developmental stage of the plant; in oilseed rape against *Sclerotinia sclerotiorum, Alternaria brassicae* and *Phoma lingam* 0.25 kg as/ha two times per year; in beans and peas against *Botrytis cinerea* and *Sclerotinia sclerotiorum* 0.5 kg as/ha in two applications per year.
The application rate of the existing uses of BAS 510 01 F in grape vines, oilseed rape, peas, and beans are:

Crop	Rate of BAS 510 01 F	Spray (water) volume (max)
Grape vines	1.2 kg/ha	1600 L/ha
Oilseed rape	0.5 kg/ha	300 L/ha
Peas	1.0 kg/ha	400 L/ha
Beans	1.0 kg/ha	300 L/ha

2.1.8 Concentration of the active substance in material used (diluted spray)

The envisaged concentrations in grapes against *Botrytis cinerea* are 0.6 kg as/ha in 400-1600 L depending on the developmental stage of the plant; in oilseed rape against *Sclerotinia sclerotiorum, Alternaria brassicae* and *Phoma lingam* 0.25 kg as/ha in 200-400 L; in beans and peas against *Botrytis cinerea* and *Sclerotinia sclerotiorum* 0.5 kg as/ha in 300 L.
Depending on the figures on application rate and spray volume (see above) the concentrations in ready-to use spray of existing uses are as follows:

Crop	Concentration of formulation (BAS 510 01 F, 50 % WG in the spray	Concentration of a.s. nicobifen in the spray
Grape vines	0.075 %	0.38 g/L
Oilseed rape	0.17 %	0.83 g/L
Peas	0.25 %	1.25 g/L
Beans	0.33 %	1.67 g/L

2.1.9 Method of application

In all cases the application method is spraying by means of each type of spraying equipment which is normally used for applying fungicides in practical viticulture/agriculture. The diluent is water. The water volumes required are outlined above.

2.1.10 Number and timing of applications

The products are intended to be used one to two times per year depending on the crop and the developmental stage of the plant. For the existing uses the data are the following:

Crop	Max number of application	Timing of the first application	Timing of the second application
Grape vines	1	4 weeks before harvest	-
Oilseed rape	2	GS 51-55	GS 65
Peas	2	2 weeks before harvest	1 week before harvest
Beans	2	2 weeks before harvest	1 week before harvest

2.1.11 Necessary waiting periods or other precautions to avoid phytopathogenic effects on succeeding crops

It is to assume that there are no phytotoxic effects on succeeding crops by the use of the product.

2.1.12 Mode of action

Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound. Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.

2.1.12.1 Active substance

Nicobifen is a member of the class of carboxin fungicides. Nicobifen effectively controls several fungal pathogens belonging to the four major classes of plant pathogenic fungi.
The mode of action of nicobifen at the molecular level is the inhibition of the mitochondrial succinate dehydrogenase (SDH, complex II). This enzyme is part of tricarboxylic acid cycle (citrate cycle, Krebs cycle). It belongs also to a class of flavoproteins, which enter electrons into the mitochondrial respiration chain. Therefore, inhibition of succinate dehydrogenase by nicobifen affects both the carbon flow into crucial metabolites and the yield of ATP. The shortage of building blocks for amino acids and sugars together with the reduced energy yield severely interferes with basic principles of growth and maintenance of a living cell.
The efficacy of inhibition of SDH strongly depends on the species. In phytopathogenic fungi as *Botrytis cinerea*, SDH is strongly inhibited by a low concentration of nicobifen. In the yeast *Saccharomyces cerevisiae*, inhibition is still observed but a higher concentration is necessary and the mammalian enzyme from pig liver is almost resistant to nicobifen.
Nicobifen has excellent preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development).

2.1.12.2 Active metabolites

Not relevant here, since the fungicidal effect is directly caused by the parent active substance.

2.1.12.3 Formation of active metabolites

Not relevant here, since the fungicidal effect is directly caused by the parent active substance.

2.1.13 Information on the occurrence or possible occurrence of the development of resistance of the target organisms

2.1.13.1 Mechanism of resistance

No resistant field isolates of the intended target pathogens for nicobifen have so far been found. There is, however, published information on the putative mechanism of resistance in some fungal pathogens to carboxin which is also a carboxanilide product. It is currently thought that mutations which lead to amino acid substitutions in the iron-sulphur protein sub-unit of succinate dehydrogenase confer resistance to carboxin in *Ustilago maydis* (Keon et al., 1991) and *Mycosphaerella graminicola* (*Septoria tritici*) (Skinner et al., 1998).

2.1.13.2 Evidence of resistance

There is no evidence of field resistance to nicobifen in any of the target pathogens. Evidence of resistance comes mainly from resistance to other carboxanilides shown by mutant fungal strains. Carboxin-resistant mutant stains or laboratory strains have been studied in *Ustilago maydis* (Keon et al., 1991), *Ustilago hordei* (Ben-Yephet et al., 1975), *Aspergillus nidulans* (Gunatilleke et al., 1976), *Sclerotinia sclerotiorum*, *Fusarium solani pisi* (Bochow et al., 1971), and *Mycosphaerella graminicola* (Skinner et al., 1998).
Reports of naturally occurring field resistance to carboxanilides are restricted to *Ustilago nuda* (Leroux and Berthier, 1988), (Newcombe and Thomas, 1991) and *Puccinia horiana* (Grouet et al., 1981).

2.1.13.3 Cross resistance

There is no indication yet of cross-resistance between nicobifen and the other carboxanilide fungicides mentioned in the above section on evidence of resistance. Whether such cross-resistance exists has not yet been tested - because there is no activity on the pathogens in question.

2.1.13.4 Resistance management strategy

The management strategies will depend on the products containing nicobifen as an active ingredient, the target pathogens for those products and the resistance risk assessments for the pathogens which are outlined in the above table.
In very general terms it can be stated here that a maximum of two applications of nicobifen are to be recommended for the solo product. For read-mix products containing nicobifen as one of the active ingredients the notifier recommends the maximum number of three applications with the exception of pomefruits (Max. four applications) and various ornamentals where the total number of fungicide applications per year is relatively high. For the target pathogens where an increased or high risk of resistance is assessed (see above

table), the notifier plans to include further modifiers and to outline these in the dossiers submitted for the individual products.

2.1.13.5 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 plant protection product have been supplied and are acceptable.

2.1.14 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):

Nicobifen

Hazard symbol:	N
Indication of danger:	Dangerous to the (aquatic) environment
Risk phrases:	R51/53 Toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment

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 510 01 F

Hazard symbol:	N
Indication of danger:	Dangerous to the (aquatic) environment
Risk phrases:	R51/53 Toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment

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 .

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

Impurities in the technical active substance are determined by a HPLC method on a reversed phase column with UV detection. Trace amounts of some other organic impurities are quantified by capillary gas chromatography/mass spectrometry (GC/MS). Quantification is achieved by external calibration.

The 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 formulation.

Nicobifen in the formulation is determined by a GC internal standard method on a DB-1 capillary column with flame ionisation detection an calibration using an internal standard.

The method is fully validated.

2.2.3 Analytical methods for residue analysis

For the assessment of the analytical methods for the determination of nicobifen 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	0.02	mg/kg	proposed MRL
meat, fat, liver, kidney, eggs	0.05	mg/kg	proposed MRL (lowest MRL for poultry)
soil	0.05	mg/kg	general limit according to Directive 96/46/EC
drinking water	0.1	µg/L	EU drinking water limit
surface water	125	µg/L	NOEC of Oncorhynchus mykiss as most sensitive species
air	30	µg/m³	based on a proposed systemic AOEL of 0.1 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 only needed for nicobifen in food of animal origin. The metabolite M510F01 including its conjugates can be analysed according to Class (2001), Kampke-Thiel (2001) and Großhans (2001).

According to these criteria adequate analytical methods are available for the determination of nicobifen in plant material, food of animal origin, soil, drinking water, surface water and air and for the determination of M510F01 including its conjugates in food of animal origin. Additional validation data are required for the methods to determine the active substance in water and soil.

Analytical methods for body fluids are not submitted. Because of the classification of the active substance a method is not necessary according to Directive 96/46/EC.

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

Matrix		Method	Limit of quantification		Reference
crops	tomato, lemon, grain oilrape seed	GC-MS	0.01 0.02	mg/kg mg/kg	Weeren and Pelz, 1999
	apple, sour cherry, grapes, strawberry, carrot, onion, tomato, broccoli, cabbage, leek, dwarf beans, oilrape seed	LC-MS-MS	0.05	mg/kg	Funk and Mackenroth, 2001
	white cabbage, lettuce oilrape seed hops	GC-MS	0.01 0.02 0.05	mg/kg mg/kg mg/kg***	Reichert, 2001
animal matrices	milk muscle, liver, kidney, fat, eggs	GC-ECD	0.01 0.025	mg/kg* mg/kg*	Class, 2001
	milk liver	GC-ECD	0.01 0.025	mg/kg* mg/kg*	Kampke-Thiel, 2001
	milk, cream, eggs muscle, liver, kidney, fat	LC-MS-MS	0.01 0.025	mg/kg* mg/kg*	Grosshans, 2001
	milk liver	GC-MS	0.01 0.05	mg/kg** mg/kg**	Fabian, 2001
soil		GC-MS	0.01	mg/kg	Keller, 1998
water	drinking, leaching	GC-MS	0.05	µg/L	Keller, 1998
	surface	GC-MS	0.5	µg/L	Grote, 2001
air		GC-MS	1.5	$\mu g/m^3$	Zangmeister, 2000

* identical for M510F01
** only M510F53 (due to bound residues in liver and minor metabolites in milk)
*** average recovery only 63 %

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 to rats, radiolabelled nicobifen was rapidly but incompletely absorbed from the gastrointestinal tract, widely distributed and rapidly eliminated from the body. Based on recovery of the radiolabel in bile from bile-duct cannulated rats within 48 h and in urine from non-cannulated rats within 12 h of application, gastrointestinal absorption of

an administered low and high dose was estimated to be approx. 44 % and 12 %, respectively. Blood/plasma kinetics revealed initial half-lives of approx. 8 h and terminal half-lives ranging between 20 and 40 h. AUC values of both dose levels indicated a sublinear kinetics. Tissue distribution determined 8 h after administration revealed highest amounts of radioactivity in the GI tract, liver and adipose tissue in low-dose rats. In the high-dose group, a similar distribution was observed in males, while in females, highest concentrations were found in the GI-tract, liver, thyroid and kidney. There was no evidence of a cumulative potential of nicobifen. The administered low dose was completely recovered in excreta within 2 days (approx. 20 % via urine and 80 % via faeces). At the high dose level of 500 mg/kg bw, total excretion within 7 days was in the range of 93–106 % AD), while only 3–5 % AD was eliminated via the urine. There were no significant differences in the excretory pattern with regard to sex, radiolabel used or frequency of application.

The systemically available portion of nicobifen was rapidly and intensively metabolised to a large number of biotransformation products. The hydroxylation of the diphenyl moiety was the quantitatively most important pathway. Second important was the substitution of the Cl in the 2-chloropyridine moiety against SH by conjugation with glutathione. Partial cleavage of the glutathione moiety afforded the cysteine conjugate and finally the SH-compound, which was subsequently methylated or oxidised. In addition, the introduction of glutathione and a second hydroxy group into the diphenyl part of the molecule was observed. Combinations of these reactions and the conjugation of the OH-groups with glucuronic acid or sulphate and the conjugation of the SH-group with glucuronic acid led to the large number of metabolites. The cleavage of the amide bond is negligible because the 2-chloronicotinic acid was detected only in trace amounts. No major differences were observed with regard to label, sex, and dose level.

For estimation of *in vivo* human dermal absorption of nicobifen contained in the commercial formulation BAS 510 01 F, an *in vivo* dermal absorption study in rats and an *in vitro* comparison of the penetration of radiolabelled nicobifen through rat and human epidermal membranes was performed using different test concentrations. On the basis of worst-case assumptions, an overall dermal absorption value of 7 % was derived from the results of the rat *in vivo* study. In *in vitro* investigations with rat and human epidermal membranes exposed to radiolabelled nicobifen for 24 h, the comparison of total radiolabel recovery in receptor fluid and epidermal membranes did not provide evidence for a notable species difference in the extent of bioavailability at concentration levels relevant for human exposure settings. In conclusion, *in vivo* human dermal absorption was estimated to be approx. 7 %. It is proposed to use this dermal absorption estimate for risk assessment calculation purposes.

2.3.1.2 Acute toxicity studies, local irritation and skin sensitising properties

Nicobifen is characterised by a very low acute oral (LD_{50} > 5000 mg/kg bw) dermal (LD_{50} > 2000 mg/kg bw) and inhalation (LC_{50} > 6.7 mg/L) toxicity. The substance is neither irritating to the skin nor to the eyes. It is not a skin sensitiser in the Maximisation Test.

2.3.1.3 Short-term toxicity

The short-term toxicity of nicobifen was studied in dietary 3-month studies in rats and mice, and in 3- and 12-month studies in dogs. In addition, the short-term toxicity following dermal exposure was determined in a 28-day study in rats. The short-term studies are summarised in Table 2.3-1.

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

Study **Dose levels** **Nicobifen purity**	**NOAEL** **males/females** **mg/kg bw/d**	**LOAEL** **males/females** **mg/kg bw/d**	**Effects**
Rat 90-day oral 0–100–500–2000–5000–15000 ppm purity: 95.3 %	34 / 40 (500 ppm)	137 / 159 (2000 ppm)	≥ 2000 ppm: altered clin.-chem. & haematological parameters; ↑ thyroid wt., follicular cell hypertrophy & hyperplasia (males) ≥ 5000 ppm: ↑ liver wt & centrilob. hypertrophy; ↑ thyroid wt. (both sexes)
Mouse 90-day oral 0–150–1000–4000–8000 ppm purity: 95.3 %	29 / 42 (150 ppm)	197 / 277 (1000 ppm)	≥ 1000 ppm: ↑ cholesterol; ↑ liver wt (both sexes), 4000 ppm: altered clin.-chem. parameters, fatty liver change
Dog 90-day oral 0–250–2500–25000 ppm purity: 94.4 %	7.6 / 8.1 (250 ppm)	78.1 / 81.7 (2500 ppm)	≥ 2500 ppm: ↑ liver wt (both sexes) ↑ AP, ↓ ALAT, ASAT (females) ↑ triglycerides (both sexes) 25000 ppm: initial ↓ bw, subsequently retarded bw gain (females) ↓ red blood cell parameters (females) ↑ AP, ↓ ALAT, ASAT (both sexes) ↑ thyroid wt (females)
Dog 1-year oral 0–200–800–2000–20000 ppm purity: 94.4 %	22 / 22 (800 ppm)	57.4 / 58.3 (2000 ppm)	≥ 2000 ppm: vomitus, ↓ bw & food efficiency, altered clinical chemical parameters ↑ thyroid wt & liver wt.
Rat 28-day dermal 0–10–250–1000 mg/kg bw/d purity: 96.3 %	1000	–	No systemic adverse effects; no signs of local irritation

Nicobifen has a very low toxic potential as demonstrated by the high dose levels which were administered. For all studies the high dose level was in the range of 1000 mg/kg bw/d. Even at this dose level, clinical signs of toxicity or adverse effects on food consumption or body weight gain were very rarely seen. The signs of toxicity observed in the three species tested were overall similar and consisted mainly of altered clinical-chemical changes. Main target organ was the liver. Weight increases of the liver were observed in all three species. Histopathological changes, however, were minor (hypertrophy and fatty change) and suggested an adaptation of this organ to increased functional demand.

In rats and dogs the thyroids were identified as a second target organ as evidenced by weight increases (rats and dogs) and histopathologically by follicular cell hyperplasia (only rats).

In a 4-week dermal toxicity study in rats no substance-related systemic adverse effects were detected up to the highest dose level tested of 1000 mg/kg bw/d. There were no signs of local irritation in this study.

A particular species sensitivity was not clearly evident from comparison of LOAELs and NOAELs obtained in oral short-term toxicity studies with different species. The fact that the studies conducted with dogs yielded the lowest NOAEL and LOAEL values might just have well resulted from the choice of different dose levels tested in rats, mice and dogs, respectively. The 12-month oral dog study was identified as the most relevant short-term toxicity study for risk assessment purposes.

2.3.1.4 Genotoxicity studies

The potential genotoxicity of nicobifen was investigated in a series of both in vitro and in vivo studies. All regular end points for genetic damage (point mutations, chromosome damage and DNA-damage and repair) were assessed: In *in vitro* investigations, nicobifen was evaluated for its potential genotoxicity using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis 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 negative test result of this study corroborated the evidence obtained *in vitro* that nicobifen has no chromosome-damaging potential. In conclusion, there is no evidence from the available *in vitro* and *in vivo* data to assume mutagenic or genotoxic properties of nicobifen.

2.3.1.5 Long-term toxicity / carcinogenicity studies

The results of long-term studies conducted with rats and mice are shown in Table 2.3-2.

In a combined chronic toxicity / carcinogenicity study in rats, dietary exposure to a concentration of 15000 ppm resulted in overt toxicity indicating the maximum tolerated dose had been exceeded. Accordingly, treatment at the top dose level was discontinued. At the next lower concentration (2500 ppm), however, there were still sufficient signs of toxicity (reduced body weight in females, indications of an anaemic effect in females, clinical chemical changes indicating liver toxicity in both sexes as well as pathological changes to the liver and the thyroid).

In the carcinogenicity study there was a slight increase in the incidence of thyroid follicular cell adenomas at the high dose. A similar increase was not observed in the chronic toxicity study, possibly because the effect is so weak that the number of animals were not high enough to detect it. The concomitant changes to the thyroid (hypertrophy and hyperplasia), however, suggest that the slight increase in the carcinogenicity study is likely to be treatment related.

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

Study **Dose levels** **Nicobifen purity**	**NOAEL** **males/females** **mg/kg bw/d**	**LOAEL** **males/females** **mg/kg bw/d**	**Effects**
Rat 24-mo oral diet (combined oral chronic and carcinogenicity) 0–100–500–2500–15000 ppm purity: 94.4 %	4.4 / 5.9 (100 ppm)	22 / 30 (500 ppm)	<u>≥ 500 ppm:</u> ↑ γ-GT, ↓ total bilirubin (males), ↑ hepatocell. hypertrophy <u>≥ 2500 ppm:</u> ↓ bw (females, slight) signs of anaemia (females) clin-chem. changes indicative of liver toxicity, ↑ path. changes in thyroid and liver (both sexes) ↑ thyroid follicular cell adenomas (slight) <u>15000 ppm:</u> general toxicity, treatment discontinued
Mouse 18-mo oral diet (carcinogenicity) 0–80–400–2000–8000 ppm purity: 94.4 %	13 / 90 (80 / 400 ppm)	65 / 443 (400 / 2000 ppm)	<u>≥ 400 ppm:</u> ↓ bw (males) ↑ relative liver weight (males) <u>≥ 2000 ppm:</u> ↓ bw (both sexes) ↑ abs. liver wt, hepatocell. hypertrophy (females) <u>8000 ppm:</u> ↑ liver wt, hepatocell. hypertrophy (both sexes)

From a mechanistic point of view the thyroid changes can be linked to an increased metabolism of thyroid hormones (T3 and T4) due to increased phase II (conjugation) hepatic activity. The reduced thyroid hormone levels trigger, by means of a feedback mechanism, the release of increased amounts of TSH, in an attempt to restore homeostatic conditions. Due to continued treatment with nicobifen, the metabolic activity of the liver, however, remains elevated resulting in a continuously increased breakdown of thyroid hormone and continuously increased TSH levels. Chronic stimulation of the thyroid due to increased TSH levels is well known to result in follicular cell hypertrophy, hyperplasia and ultimately in benign thyroid tumours in rats. According to various publications the rat is particularly sensitive to this secondary mechanism.

Nicobifen does not need to be classified with respect to its tumourigenic potential in rats of the following reasons:

- Nicobifen is clearly non-genotoxic.
- For non-genotoxic agents, the mechanism of action must be determined. Nicobifen was shown to enhance the metabolism of thyroid hormones.
- Nicobifen has a very low potency of the tumourigenic effect, as indicated by the marginal increase of (thyroid follicular cell) adenomas in rats. There was no increase in the incidence of carcinomas. There was no tumourigenic response in the thyroid in mice.

In conclusion, the marginal increase of thyroid follicular cell adenomas in rats is not considered to be relevant to man.

A carcinogenicity study in mice was conducted up to a maximum tolerated dose as evidenced by significant body weight depression (8–10 % in both sexes) at 8000 ppm. Liver weights were increased at the high dose level, histopathology revealed hypertrophy. Moderate effects on body weights were seen at 2000 ppm (both sexes) and in 400 ppm males. Liver weights were also increased in 2000 ppm females, histopathology revealed hypertrophy. There was no evidence of a carcinogenic effect of nicobifen in mice at any dose level.

2.3.1.6 Reproductive toxicity / developmental (teratogenicity) studies

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

Nicobifen had no adverse effects on reproductive performance or fertility of the F0 or F1 parental animals of all substance-treated groups up to a dose of 10000 ppm (667 mg/kg bw/d). Signs of general toxicity/systemic effects occurred in both parental generations at 1000 and 10000 ppm. The effects at 10000 ppm were characterised by decreased food consumption and reduced body weights during parts of the administration period. Pathology showed statistically significantly increased liver weights, centrilobular hypertrophy of liver cells and centrilobular liver cell degeneration in single or all male and/or female animals. Systemic effects at 1000 ppm were confined to an increased incidence of centrilobular hepatocellular hypertrophy, which occurred in few F0 and F1 parental animals. No substance-related effects were noted at 100 ppm. Substance-induced signs of developmental toxicity were observed in progeny of the F0 and F1 parents at 1000 and 10000 ppm. At 10000 ppm a slightly increased pup mortality of the F2 litters was noted between days 0 and 4 post partum only. Pup body weight development was impaired in both F1 and F2 litters. At 1000 ppm, slightly decreased body weight gains were recorded for the male F2 pups only. 100 ppm did not induce any indication of developmental toxicity. The NOAEL for parental toxicity of the test substance was established at 100 ppm (11 mg/kg bw/d) for the F0 and F1 parental males and females. The NOAEL for developmental toxicity was 1000 ppm (67 mg/kg bw/d) for the male and female F1 and female F2 progeny and 100 ppm (6.7 mg/kg bw/d) for the male F2 progeny.

In the developmental toxicity study in rats, incomplete ossification of the thoracic centrum was observed at the highest dose tested (1000 mg/kg bw/d) in the absence of overt maternal toxicity. At this limit dose level there were also no signs of maternal toxicity. However, results from the 90-day oral feed study in rats indicate that liver toxicity would have been detected in dams at 1000 mg/kg bw/d. The NOAEL for developmental toxicity in rats was established at 300 mg/kg bw/d.

In the rabbit developmental toxicity study, incomplete ossification of the thoracic centrum was also observed at significantly increased incidences at the highest dose level (1000 mg/kg bw/d). At this dose level there was overt maternal toxicity (clinical signs of toxicity, reduced body weight and body weight gain). At 300 mg/kg bw/d clinical signs (abortion and discoloured/reduced faeces) were observed in a single animal only. Thus, the NOAELs for maternal and for developmental toxicity were 100 mg/kg bw/d and 300 mg/kg bw/d, respectively.

Results of all reproduction toxicity studies are summarised in Table 2.3-3.

Table 2.3-3: Summary of reproductive toxicity studies with nicobifen

Study dose levels purity	**Target**	**NOAEL mg/kg bw/d**	**LOAEL mg/kg bw/d**	**Effects**
Rat 2-generation study 0–100–1000–10000 ppm purity: 94.4 %	Parental tox.	6.7 (100 ppm)	67 (1000 ppm)	≥ 1000 ppm: ↑ hepatocell. hypertrophy 10000 ppm: ↓ bw gain & feed intake ↑ liver wt & hepatocyte degeneration
	Fertility	667 (10000 ppm)	–	No effects observed
	Offspring tox.	6.7 (100 ppm)	67 (1000 ppm)	≥ 1000 ppm: ↓ bw gain 10000 ppm: ↑ Male F2 pup mortality during days 0–4 p.p.
Rat teratogenicity 0–100–300–1000 mg/kg bw/d purity: 94.4 %	Maternal tox.	1000	–	No effects observed
	Developmental tox.	300	1000	1000 mg/kg bw/d: ↑ Incomplete ossification of the thoracic centrum
Rabbit teratogenicity 0–100–300–1000 mg/kg bw/d purity: 94.4 %	Maternal tox.	100	300	300 mg/kg bw/d: 1 doe with abortion and reduced / discoloured faeces 1000 mg/kg bw/d: 4 does with abortion ↓ feed intake, bw & bw gain
	Developmental tox.	300	1000	1000 mg/kg bw/d: ↑ Incomplete ossification of the thoracic centrum

2.3.1.7 Neurotoxicity / delayed neurotoxicity studies

Three oral neurotoxicity studies with nicobifen were conducted in rats (see in Table 2.3-4). In the acute neurotoxicity study, piloerection observed on the day of treatment in 2 of 20 rats was the only clinical sign of toxicity to be seen at the top dose level (2000 mg/kg bw). This finding was considered to reflect an unspecific reaction of the animals to excessive dosing and was therefore not regarded as a substance-related effect. No adverse reaction to treatment was observed in any animals at 1000 mg/kg bw or lower dose levels. No signs of neurotoxicity were observed at any dose level.

In the 90 day oral neurotoxicity study in rats, there were no test substance related adverse effects at any dose level and there were no signs of neurotoxicity at any dose level. The no observed effect level was 15000 ppm, i.e. 1050 mg/kg bw/d in males and 1272 mg/kg bw/d in females.

In a developmental neurotoxicity study, the slight reduction on pup body weight (at 10000 and 1000 ppm) were in line with similar effects seen in the 2-generation study in rats, where

parental toxicity was noted in form of hepatotoxicity at these dose levels. No signs of developmental neurotoxicity were noted up to the highest concentration of 10000 ppm (1442 mg/kg bw/d), which was clearly above a recommended limit dose of 1000 mg/kg bw/d.

In conclusion, nicobifen is neither neurotoxic to adult rats nor to the developing rat. As there were no neurotoxic effects observed in any of the studies with nicobifen, studies on the delayed neurotoxicity in hens were not triggered.

Table 2.3-4: Summary of neurotoxicity studies

Study dose levels purity	Target	NOAEL (males / females) mg/kg bw/d	LOAEL (males / females) mg/kg bw/d	Effects
Rat acute oral neurotoxicity 0–500–1000–2000 mg/kg bw purity: 96.3 %	Neurotox.	2000	–	No specific neurotoxic effects.
	General tox.	1000	2000	2000 mg/kg bw: Piloerection in 2/10 females on Day 0
Rat 90-day oral neurotoxicity 0–150–1500–15000 ppm purity: 96.3 %	Neurotox.	1050 / 1273 (15000 ppm)	–	No effects observed
Rat developmental neurotoxicity 0–100–1000–10000 ppm purity: 96.3 %	Maternal tox.	1442 (10000 ppm)	–	No effects observed
	General tox. in offspring	14 (100 ppm)	147 (1000 ppm)	1000 ppm: ↓ bw and bw gains (Days 1-4 p.p.) 10000 ppm: ↓ bw and bw gains until weaning ↓ abs. brain wt, brain length (Day 11 p.p. male pups only)
	Neurotox. in offspring	1442 (10000 ppm)	–	No effects observed

2.3.1.8 Further toxicological studies

Para-chlorobenzoic acid (CAS No. 74-11-3) was identified in the aquatic environment as degradation product of nicobifen. Results from a literature survey indicate that para-chlorobenzoic acid is more toxic than nicobifen after acute oral intake (LD_{50} 4170 mg/kg bw vs. > 5000 mg/kg bw). No adverse effects were reported in oral subchronic toxicity studies in rabbits (NOAEL: 1500 mg/rabbit/d) and rats (NOAELs: 26 mg/rat/d and 0.3 mg/kg bw/d). Limited *in-vitro* genotoxicity data indicate no concern. The feeding of 13 or 26 mg/rat/d over a period of five months did not induce adverse effects on the number of offspring and did not induce developmental toxicity. In view of the transient nature of this compound no further need for a toxicological investigation was identified.

Mechanistic studies with nicobifen have shown that the test substance induces a liver weight increase with concomitant histopathological changes in zone 3 hepatocytes (proliferation/accumulation of smooth endoplasmatic reticulum (SER) and glycogen depletion). These changes are considered to be related to the induction of hepatic metabolising

enzymes. Nicobifen induced both phase I (oxidative) enzymes, as demonstrated by increased cytochrome P450 content, as well as phase II (conjugation) enzymes. The latter was shown by increased activities of p-nitrophenol-glucuronyltransferase, 4-methylumbelliferone-glucuronyltransferase, and 4-hydroxybiphenyl-glucuronyltransferase. The increases in phase II (conjugation) hepatic activity were accompanied by the reduction of thyroid hormone T3 and T4 levels and by a concomitant increase in TSH. While the decrease in serum T3 and T4 concentrations appear to have resulted from increased elimination via nicobifen-mediated induction of phase II hepatic enzyme activities, the increased TSH levels were obviously the result of a feedback mechanism induced by decreased in serum T3 and T4 levels.

2.3.1.9 Human Data

Since industrial production has not yet commenced no data on medical surveillance of the manufacturing personnel is available. However, the personnel which is handling developmental compounds is surveyed by regular medical examinations.

2.3.2 ADI

Chronic feeding studies with nicobifen in dogs, rats and mice demonstrated that liver and the thyroid are the target organs. The long-term toxicity is rather similar to the short-term effects.

Table 2.3-5: Summary of NOAELs and LOAELs relevant for deriving the ADI

Study	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d
Dog 12-month oral	22 (800 ppm)	57 (2000 ppm)
Rat 24- month oral	4.4 (100 ppm)	22 (500 ppm))
Mouse 18-month oral	13 (80 ppm)	65 (400 ppm)

The lowest NOAEL in long-term studies was approx. 4 mg/kg bw/d in the chronic rat study.

The slight increase in the incidence of thyroid follicular adenomas at a dose of 2500 ppm in the carcinogenicity study in rats is considered to be a rat specific phenomenon and is not relevant for humans. Nicobifen was not carcinogenic in mice.

Nicobifen has been investigated in a series of studies both in vitro and in vivo designed to measure the major endpoints of genotoxicity. The results of these studies clearly demonstrate that nicobifen has no genotoxic potential.

Nicobifen has no reproduction toxicity potential, and the NOAELs obtained were higher than the 4 mg/kg bw from the long-term rat study.

A full and current toxicology database evaluating all major endpoints of toxicity has been developed for nicobifen, and clear no effect levels have been determined for all treatment-related effects. Therefore, the standard assessment factor of 100 is considered appropriate.

The proposed ADI is:

$$\frac{4\ \text{mg/kg bw}}{100} = \quad \textbf{0.04 mg/kg bw}$$

2.3.3 AOEL

2.3.3.1 AOEL (systemic)

For the definition of the AOEL and the risk assessment to be made thereof the results of the short term toxicity and reproduction/developmental toxicity studies are considered to be of relevance. The corresponding NOAEL and LOAEL values are shown in Table 2.3-6.

Table 2.3-6: Summary of NOAEL and LOAEL values relevant for deriving the systemic AOEL

Study	NOAEL mg/kg bw/d		LOAEL mg/kg bw/d	
Rat 90-day oral	34	(500 ppm)	137	(2000 ppm)
Rat 2-gen. study, parental toxicity	6.7	(100 ppm)	67	(1000 ppm)
Rat teratogenicity	300		1000	
Mouse 90-day oral	29	(150 ppm)	197	(1000 ppm)
Dog 90-day oral	7.6	(250 ppm)	78.1	(2500 ppm)
Dog 1-year oral	22	(800 ppm)	57.4	(2000 ppm)
Rabbit maternal tox., teratogenicity	100		300	
Rat 28-day dermal	1000		–	

From the above studies, those with the overall highest NOAELs were determined for each species and subsequently compared. By this approach, the 90-day studies in rats and mice, and the 1-year dog study were identified to be the most relevant. The corresponding NOAELs were in the same range (22–34 mg/kg bw/d), the dog appearing to be the most sensitive species with both the lowest NOAEL and LOAEL. However, it can not be excluded that the "sensitivity" of the dog was only a consequence of the choice of doses used in the different studies. Nevertheless, for setting the AOEL the most relevant study was considered to be the dog 1-year oral feed study (NOAEL of 22 mg/kg bw/d, corresponding to 800 ppm).

The systemic AOEL ($AOEL_{(SYS)}$) is derived from the NOAEL of the dog 1-year oral feed study by applying a standard safety factor of 100. Since gastrointestinal absorption was estimated to be approx. 44 % based on toxicokinetic investigations with rats, an additional correction factor was employed for calculation of the systemic AOEL:

$$\mathbf{AOEL}_{\mathbf{(SYS)}} = \frac{22\ \text{mg / kg bw / d}}{100} \times 44\ \% = \mathbf{0.1\ mg/kg\ bw/d}$$

2.3.3.2 AOEL (dermal)

A 28 -day dermal toxicity study in rats was employed in the assessment. No systemic toxicity was detected up to the highest dose level tested of 1000 mg/kg bw. A 90-day dermal study, which could be compared with the oral 90-day study, was not submitted. A dermal AOEL was therefore not established.

2.3.4 ARfD (acute reference dose)

From the evaluation of the available toxicological database of nicobifen, there is no need to establish an ARfD. Acute oral studies demonstrate the low toxicity of nicobifen. No adverse clinical signs were observed early in repeated-dose studies at dose levels that were relevant for human exposure. No developmental toxicity was induced by nicobifen treatment. Slight

alterations of thyroid hormone levels and induction of liver enzymes were observed within several days after repeated administration of 15000 ppm (approx. 1000 mg/kg bw/d). However, these effects are not considered relevant since the expected human exposure is lower by several orders of magnitude. In conclusion, due to low toxicity of nicobifen, it is not necessary to derive an ARfD.

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 89/778/EEC.

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

According to the toxicological profile of nicobifen, harmful effects on the health of operators, bystanders, workers or consumers are not to be expected when the plant protection product is used in accordance with good plant protection practice. The available data for nicobifen does not support evidence of genotoxic, carcinogenic and the fertility or development damaging properties of the active substance.

The potential operator exposure was estimated for the intended uses. Using the German model, the operator exposure to BAS 510 01 F does never exceed the proposed systemic AOEL for nicobifen even if no protective clothing is worn (exposures < 66.6 % of the systemic AOEL). Also the always higher exposure values derived on the basis of the UK-POEM are acceptable if gloves are used.

Thus, the estimated exposures does not present an undue risk. In view of a single rather than a repeated scenario as it is the situation for field applicators, it unlikely that the potential exposure of bystanders will exceed the AOELs.

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

2.4.1.1 Plants

The metabolism of nicobifen was investigated in grapes, lettuce and beans. Unchanged parent compound formed the major part of the residue in these studies. The cleavage products M510F62 (chlorophenylaminobenzene) and M510F47 (chloronicotinic acid) and in addition hydroxy-parent and sugar conjugates were identified in beans. All metabolites were of minor importance. Therefore parent only is included in the residue definition.

Residue definition plant: **Nicobifen**

2.4.1.2 Animals

Metabolism studies performed on goats and hens show that residues in products of animal origin derive from the parent compound as well as from the hydroxylated metabolite M510F01 including its conjugates. Further metabolites result from a substitution of the chlorine of the 2-chloropyridine moiety by the thiol group of glutathione to create metabolites as the cysteine conjugate. Nicobifen derived residues were also bound in liver based on this substitution (most likely SH-groups from cysteine containing protein). The amide bond of nicobifen was very stable under metabolic conditions in goats and hens.
The results of the goat and hen metabolism studies selects the following compounds that are listed differentiated in residues for monitoring purposes and residues for risk assessment:

Residue definition for monitoring:	**Nicobifen, M510F01 (including its conjugates)**
Residue definition for risk assessment:	**Nicobifen, M510F01 (including its conjugates), M510F53 (for bound residues in liver and minor metabolites in milk)**

2.4.2 Residues relevant to consumer safety

Chronic dietary intake levels were estimated using the proposed MRL values derived from supervised residue trials and from the livestock feeding study. The results obtained on the basis of the German and WHO European regional diet were compared with the ADI value of 0.04 mg/kg. A chronic dietary consumer risk is unlikely.

TMDI (WHO European diet 1998):	0.0112 mg/kg bw/day – 28.1 % of the ADI
TMDI (German diet, 4-6 years old girl):	0.0056 mg/kg bw/day – 14.1 % of the ADI
Additional (woman, 36 – 50 years old):	0.0081 mg/kg bw/day – 20.4 % of the ADI

2.4.3 Residues relevant to worker safety

The intended use of BAS 510 01 F is as a fungicide in grapes and in field crops such like oilseed rape, peas and field beans. Applications will be primarily performed by using vehicle-mounted or trailed sprayers, however, uses with knapsack sprayers may also occur in grapes.
The results of the operator exposure estimation have shown that operators are not exposed to critical levels when handling the product under the recommended conditions of use (see Volume 3, Annex B.6.14.1.1: operator exposure < systemic AOEL; German model: without PPE and UK-POEM: with PPE). Using the re-entry situation in grapes as a worst case scenario, the results of the risk assessment indicates that re-entry of treated vineyards and other field crops is possible after the spray solution has dried up (see Volume 3, Annex B.6.14.2.1).

2.4.4 Proposed EU MRLs and compliance with existing MRLs

The proposed MRL´s for plants are based on an assessment of the submitted residue data. The MRL´s for food of animal origin are derived from the livestock feeding study and are given as sum of nicobifen and metabolite M510F01 and its conjugates.

Table 2.4-1: Proposed MRL´s for nicobifen

	Proposed MRL [mg/kg]	**Remarks**
Grapes	5	-
Rape	0.05*	-
Beans	2	Further trials required for Southern Europe and for indoor applications
Peas without pods	0.3	Further trials required for Southern Europe
Other food of plant origin	0.05*	-
Milk	0.02*	No residues of nicobifen above the LOQ in the 1 x dose group in milk, but in cream at 0.04 mg/kg
Fat	0.1	Except for poultry (0.05 mg/kg)
Liver	0.1	Except for poultry (0.05 mg/kg)
Kidney	0.1	Except for poultry (0.05 mg/kg)
Other food of animal origin	0.05*	

* limit of quantification

2.4.5 Proposed EU import tolerances and compliance with existing import tolerances

No import tolerances have been proposed in the EU or applied for in any EU Member State.

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

Not applicable since no Codex MRLs have been established yet.

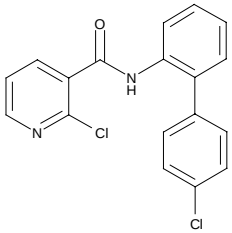
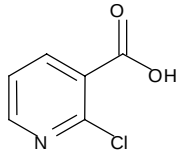
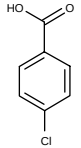
2.5 Fate and behaviour in the environment

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. A characteristic feature of BAS 510 F are the low amounts of intermediary metabolites that are formed in the environment. Only one environmental metabolite approached, but did not exceed 10 % TAR. This compound is of transient nature and in addition is of no toxicological or ecotoxicological relevance.

For more detailed information see Table 2.5-1:

Table 2.5-1: Metabolites found in soil, water and sediment

Code	Active substance	Residue definition relevant to the environment			
BAS 510 F		The active substance (nicobifen) 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**
M510F47		Soil: anaerobic conditions. 2.6 % after 3 d, 6 % after 62 d, 5.9 % after 90 d, 6.7 % after 120 d	Non-relevant metabolite	Non-relevant metabolite	Non-relevant metabolite
M510F64		Sediment: under outdoor condit. 7.3 % after 7 d 9 % after 14 d 9.4 % after 30 d 1.9 % after 120 d	Non-relevant metabolite	Non-relevant metabolite	Non-relevant metabolite

2.5.2 Fate and behaviour in soil

The degradation of nicobifen in aerobic soil is characterised by rather slow but substantial mineralisaton (up to 15 % TAR within 119 days) and the formation of moderate to high amounts of bound residues (up to 49 % TAR within 119 days). By far the major portion of the bound radioactivity was associated with the insoluble humin fraction.
The mineralisation rate indicates that ring cleavage and further metabolism to CO_2 occurs from both the pyridine and the diphenyl rings although almost no intermediary breakdown products could be identified. The initial steps in the degradation are slow and rate limiting. The subsequent steps are faster resulting in extremely low levels of intermediary metabolites. However, certain metabolites e.g. M510F47 become detectable under anaerobic conditions, in which degradation is slowed.

Half-lives of nicobifen in aerobic soil ranged from 108 days to 384 days under standard laboratory conditions (20 °C, 40 % MWC). Under the influence of light, degradation of nicobifen on soil may be gradually enhanced (DT_{50} of 135 d) whereas anaerobic conditions lead to longer half-lives, probably due to reduced microbial activity. Adaption of soils towards a faster degradation may occur at least in certain soils.

In field soil dissipation studies, DT_{50} values were shorter and ranged from 27 days in Spain to 208 days in Germany. However, the DT_{90} was not reached within one year after application. Therefore, field soil accumulation studies were initiated at two sites with different cropping: a vineyard and a field site with a vegetables crop rotation.
After three years of repeated application, the results of these studies were in good agreement with the accompanying modelling results based on half-lives from field soil dissipation studies. In the submitted accumulation studies no plateau concentration was reached after two years of application. Since the beginning of the studies the intended uses were changed by the notifier and therefore a definitive assessment is not possible yet. However, the studies are going on and the results need to be considered further. The notifier has submitted an additional modelling approach using the FOCUS scenarios.This modelling approach shows that the accumulation plateau is reached within the first years, depending on the actual scenario. Predicted maximum plateau residue levels range from 2.48 kg as/ha for the worst case scenario (beans at Jokioinen, Finland without any interception) down to 0.11 kg as/ha for the vines scenario at Sevilla, Spain under consideration of the crop interception.

Adsorption/desorption and aged residue column leaching studies clearly show that there is no risk of displacement of nicobifen into deeper soil layers or into the groundwater. This is supported by the calculation of the predicted environmental concentration in the groundwater using current models.

2.5.3 Fate and behaviour in water

Nicobifen is hydrolytically stable between pH 4 and pH 9. Also under the rather artificial conditions of the direct aqueous photolysis, nicobifen is not significantly degraded.
Nicobifen is not readily biodegradable.
In the water/sediment study performed in the dark, the major elimination pathway for nicobifen in the water was binding to the sediment. The amount increased from 20 % after one

day to 53 % after 14 days and 69 % after 100 days. In addition to that around 13 % bound residues were formed. The dissipation of nicobifen into sediment is described by the DT_{50} for the water phase of 9 days. The DT_{50} extrapolated to the total system is approximately 3300 days.

However, the degradation of nicobifen in natural aqueous systems is insufficiently described by the basic laboratory studies (hydrolysis, aqueous photolysis and water/sediment). Nicobifen has a low water solubility and a high adsorption coefficient, which leads to a fast movement into the sediment. As can be deduced from the soil photolysis study, the compound is more susceptible to degradation under the influence of light. Therefore, an additional study was designed where all relevant mechanisms (sorption to the sediment, photolysis in natural water and biological degradation) should be integrated. The results of this study are not useful to overrule the results of the standard laboratory study because the information concerning the recovery was insufficient and the illumination was not realistic. In this study a DT_{50} for nicobifen in a water phase was established to be 21 days. The maximum amount of bound residues was 48 % after 103 days. An additional metabolite (M510F64), that was not detected in the other studies, was found, however this metabolite remained below 10 % TAR and was of transient nature.

2.5.4 Fate and behaviour in air

Volatilisation studies from plant and soil surfaces showed that nicobifen has a very low volatilisation potential. Even if small amounts of nicobifen reach the troposphere, they will be degraded fast by photochemical processes.

2.6 Effects on non-target species

2.6.1 Effects on terrestrial vertebrates

The toxicity of nicobifen 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 100 ppm (NOAEL from rat multi-gen study)
Acute toxicity to birds:	LD_{50} > 2000 mg/kg bw
Dietary toxicity to birds:	LC_{50} > 5000 ppm
Reproductive toxicity to birds:	NOEL 300 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 for the active substance and the formulated product has been submitted. The data are sufficient for a final risk assessment.

With the active substance nicobifen an $EC_{50\ (96\ h,\ static)}$ of 1.34 mg as/L was determinded for the green algae *Pseudokirchneriella subcapitata*. Fish (*Oncorhynchus mykiss*, $LC_{50\ (96\ h,\ semistatic)}$ > 2.7 mg as/L) and daphniae (*Daphnia magna*, $EC_{50\ (48\ h,\ static)}$ 5,3 mg as/L) were less sensitive. The NOEC levels determined in chronic tests with *Oncoryhnchus mykiss* were 1.0 mg as/L (NOEC, prolonged study, 28 d, flow-through) and 0.125 mg as/L (NOEC, ELS-test, 97 d, flow-through). In a reproduction study using *Daphnia magna* a $NOEC_{(21\ d,\ semistatic)}$ of 1.31 mg as/L was observed. Since nicobifen is expected to dissipate into the sediment in considerable amounts, the effects on the sediment-dwelling species *Chironomus riparius* were tested. The NOEC determined in a static 28 d study was 2.0 mg as/L.

The formulated product is less toxic than could be predicted from the active substance. From the data submitted for fish, daphnia, algae and sediment dwelling insects the NOEC of 0.125 mg as/L from a 97 d-ELS-study with *Oncorhynchus mykiss* is considered most relevant for the overall risk assessment. As this toxicity value is derived from a flow-through test, time-weighted average PEC-values should be used for calculation of TER-values.

All relevant trigger values according to Annex VI of Directive 91/414/EEC are met. No metabolites of ecotoxicological relevance have been observed. With a log P_{ow} of 2.96 nicobifen shows a potential for bioaccumulation. The bioconcentration in fish was tested with *Oncorhynchus mykiss*. A maximum BCF (whole fish) of 125 was determined at the steady state between uptake and elimination. After the fish had been transfered into uncontaminated water the test substance was eliminated with a CT_{50} of 1 d and a CT_{90} of 3.3 d without considerable residues. The risk of bioaccumulation is considered to be acceptable. The overall risk to aquatic organisms arising from the uses described above is considered acceptable.

According to Directive 67/548/EEC nicobifen should be labelled with N, R51 and R53.

2.6.3 Effects on bees and other arthropod species

2.6.3.1 Effects on bees

Two laboratory studies have been performed to determine the possible side effects of nicobifen to honeybees, one with the active substance and one with the performed product. Both studies were performed according to GLP prescriptions. The LD_{50} values for oral and contact toxicity were > 100 µg as/bee. This indicates that the active substance is not toxic for honeybees. The hazard quotients calculated on the basis of the highest amount of the active substance per ha and the LD_{50} values were all below the threshold of 50. This indicates that honeybees will not be set at risk by practical use of nicobifen containing products.

2.6.3.2 Effects on other arthropod species

Details of use patterns
The fungicide BAS 510 01 F (50 % nicobifen) is a water dispersible granule formulation (WG) containing 500 g/kg nicobifen and is used in agriculture (production of field crops and grapevines). The maximum recommended field rate of BAS 510 01 F in vineyards is 1.2 kg/ha and in field crops 2 x 1 kg/ha.

Risk assessment
Non-target arthropods are likely to be exposed to formulated nicobifen 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.

The field rates tested correspond to the intended uses given above. According to the data submitted a low toxicity (effects < 30 %) was demonstrated in basic laboratory tests on a number of species (i.e. *Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Chrysopa carnea*, *Pardosa* spp., *Poecilus cupreus)* and in field tests with *Typhlodromus pyri.*

It is therefore concluded, that use of nicobifen as outlined above 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

Earthworms
The studies on the acute toxicity of technical nicobifen and a formulation containing nicobifen indicate that the acute risk for earthworms is low. The TER values are above the relevant triggers. Thus, the acute toxicity risk for earthworms is expected to be acceptable.

One study with the formulation BAS 510 01 F on reproduction has been submitted. The long term risk is assessed using a NOEC of 3.6 kg product/ha, corrected to 1.8 kg product/ha. Since log Pow is > 2, the toxicity data are divided by a factor of 2 (see EPPO risk assessment scheme for soil organisms). The NOEC converted into a soil concentration corresponds to 1.2 mg as/kg soil. For long term risk, concern is raised due to the TER_{lt} value for reproduction of 0.9 (bean scenario, 2 x 0,5 kg as/ha, no interception, worst case) being below the trigger value of 5.

Two field tests on the formulation BAS 510 01 F were conducted with 3 x 0.6 kg/ha and 3 x 1.2 kg/ha. One year after the last application there was a non-significant reduction in abundance and biomass of earthworms of about 30 % in case of the higher application rate. No long-lasting effects on overall abundance and biomass of earthworms were observed for the lower test concentration. However for both test concentrations effects on single species of about 30 % in comparison to control still exist one year after the last application.

Acute toxicity for earthworms:	LC_{50} > 500 mg as/kg (nicobifen) LC_{50} > 250 mg/kg (formulation BAS 510 01 F containing 500 g/kg nicobifen)
Reproductive toxicity to earthworms:	NOEC 0.9 kg as/ha (formulation BAS 510 01 F containing 500 g/L nicobifen)

The laboratory data submitted have shown that a longterm risk to earthworms can not be excluded. However, on the basis of the additional field data submitted a potential risk to earthworms can be excluded only for the lower treatment tested in the field tests (3 x 0.6 kg BAS 510 01 F/ha respectively 0.9 kg as/ha). As the highest application rate according to the intended uses is 2 x 0.5 kg as/ha in beans which is only slightly higher than 0.9 kg as/ha, this

is considered acceptable too. Higher application rates than 1 kg as/ha and year should not be applied on the basis of the data submitted.

Other soil macro-organisms
In two field studies the effects of BAS 510 01 F (max. 3.6 kg/ha) on organic matter degradation were not more than 15 %. Therefore no unacceptable risk to the organic matter breakdown is given.

2.6.5 Effects on soil micro-organisms

The influence of the formulation BAS 510 01 F (500 g nicobifen/kg) on the soil respiration and the nitrogen turnover was evaluated in a loamy sand soil and a loamy silt soil. The presented results (< 25 % effect in comparison to untreated control) show that when applying nicobifen containing plant protection products no lasting effects on microbial activities are to be expected at application rates up to 6 kg as/ha.

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

Greenhouse studies on vegetative vigour were conducted with BAS 510 01 F with 3 mono- and 3 dicotyledonous species. The application rates tested corresponded to 600 g and 1800 g as/ha. 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.

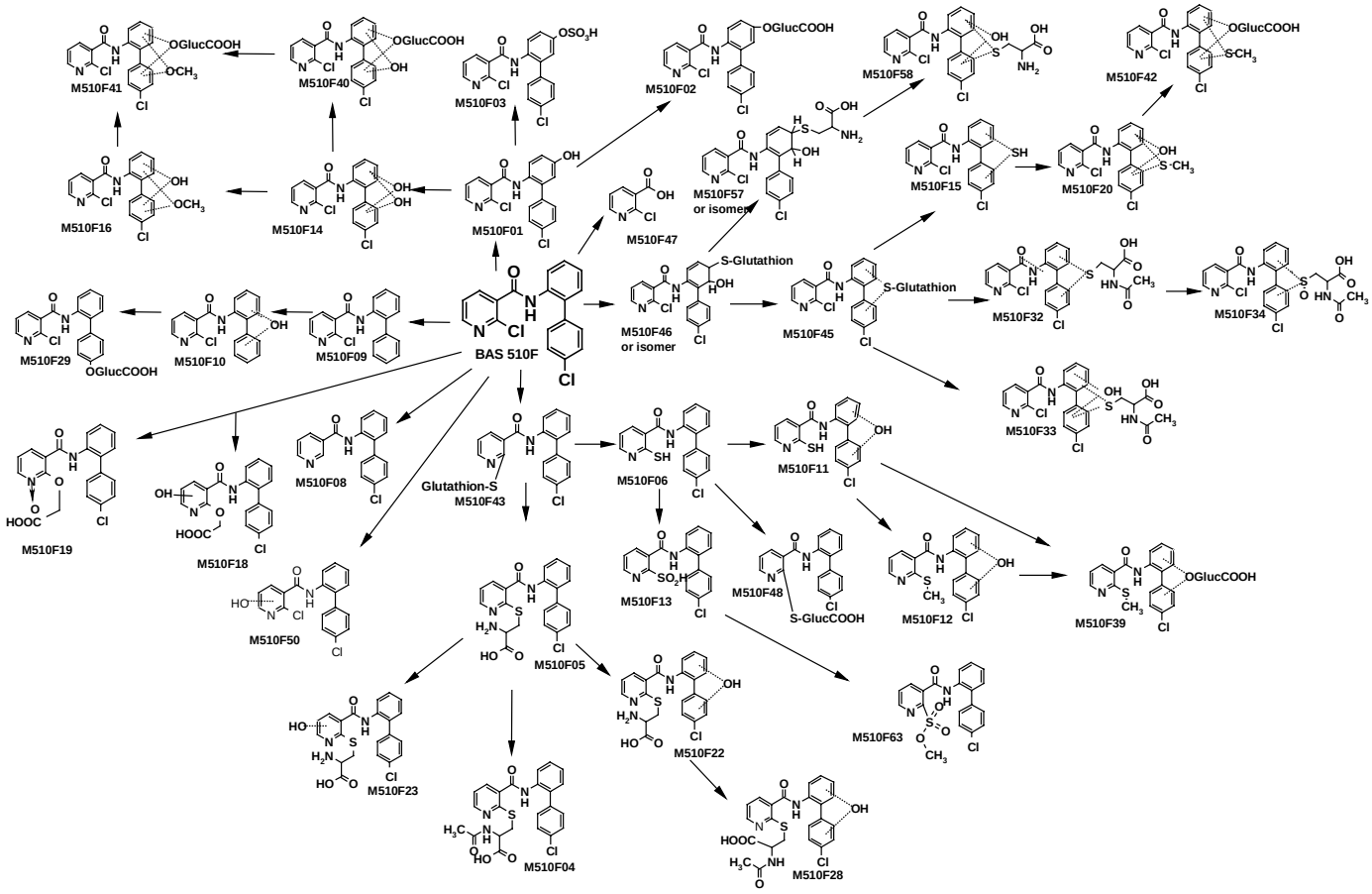
2.6.7 Effects on biological methods of sewage treatment

A significant inhibition of respiration was not observed up to the highest teste 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: Proposed metabolic pathway of nicobifen in rats



2.7.2 Residues (plant, plant products, livestock animals)

Figure 2.7-2: Metabolic pathway of nicobifen in beans

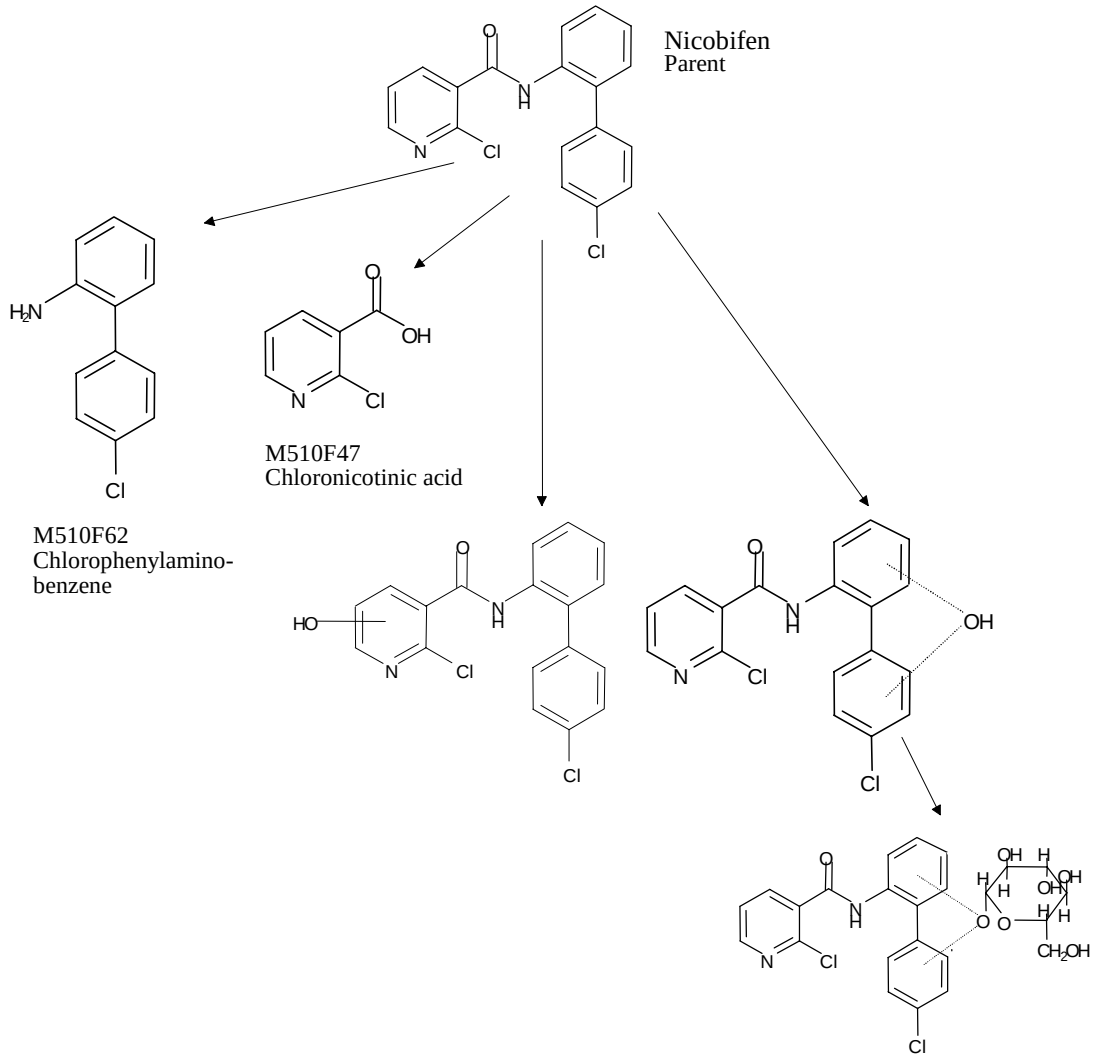


Figure 2.7-3: Metabolic pathway of nicobifen in succeeding crops

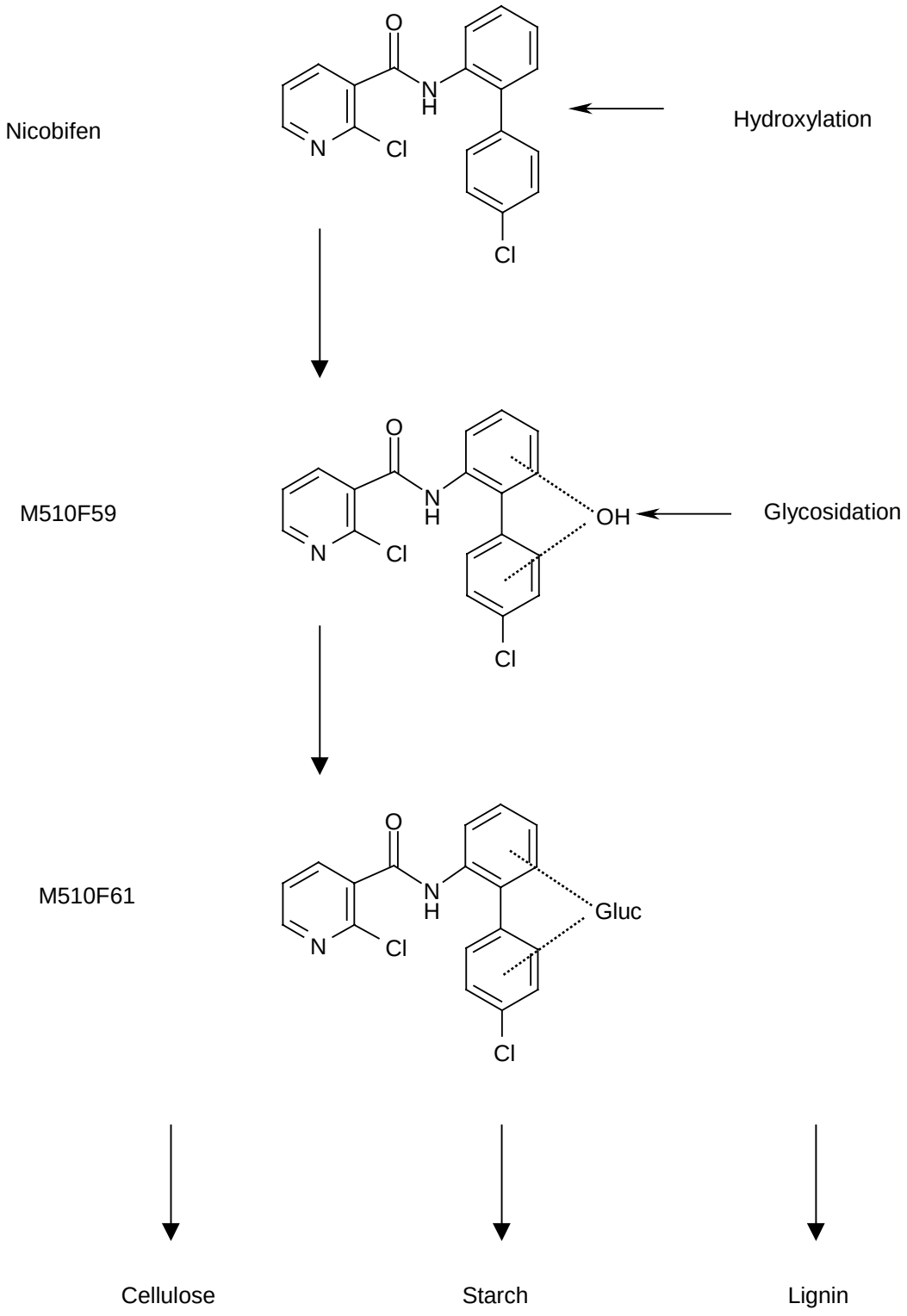


Figure 2.7-4: Metabolic pathway of nicobifen in the goat

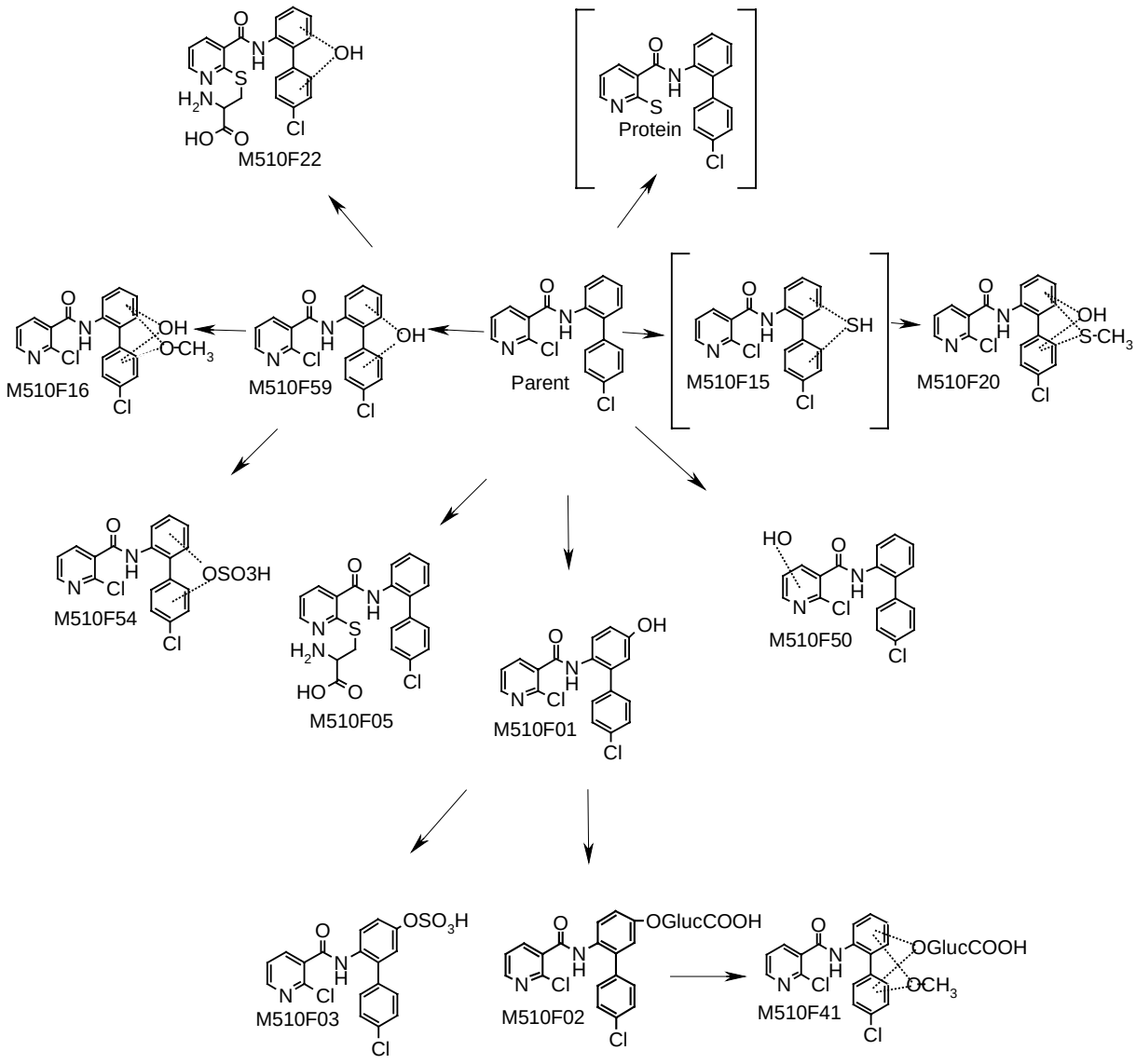
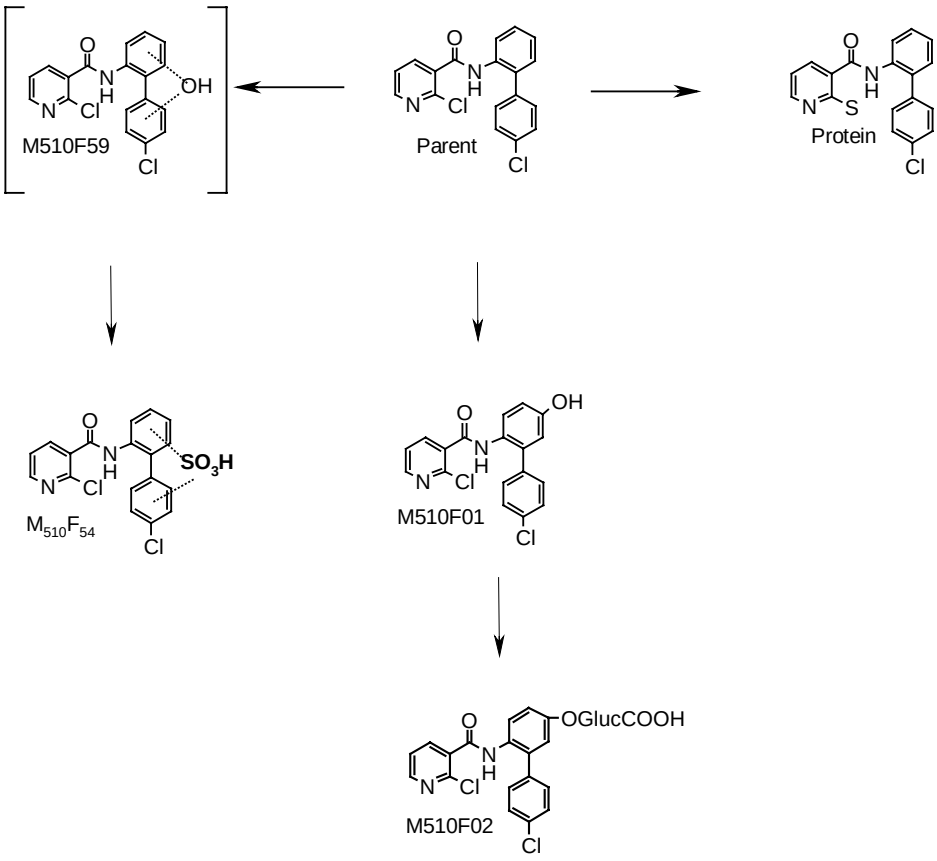


Figure 2.7-5: Metabolic pathway of nicobifen in the hen



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

Figure 2.7-6: Proposed route of degradation of BAS 510 F in soil

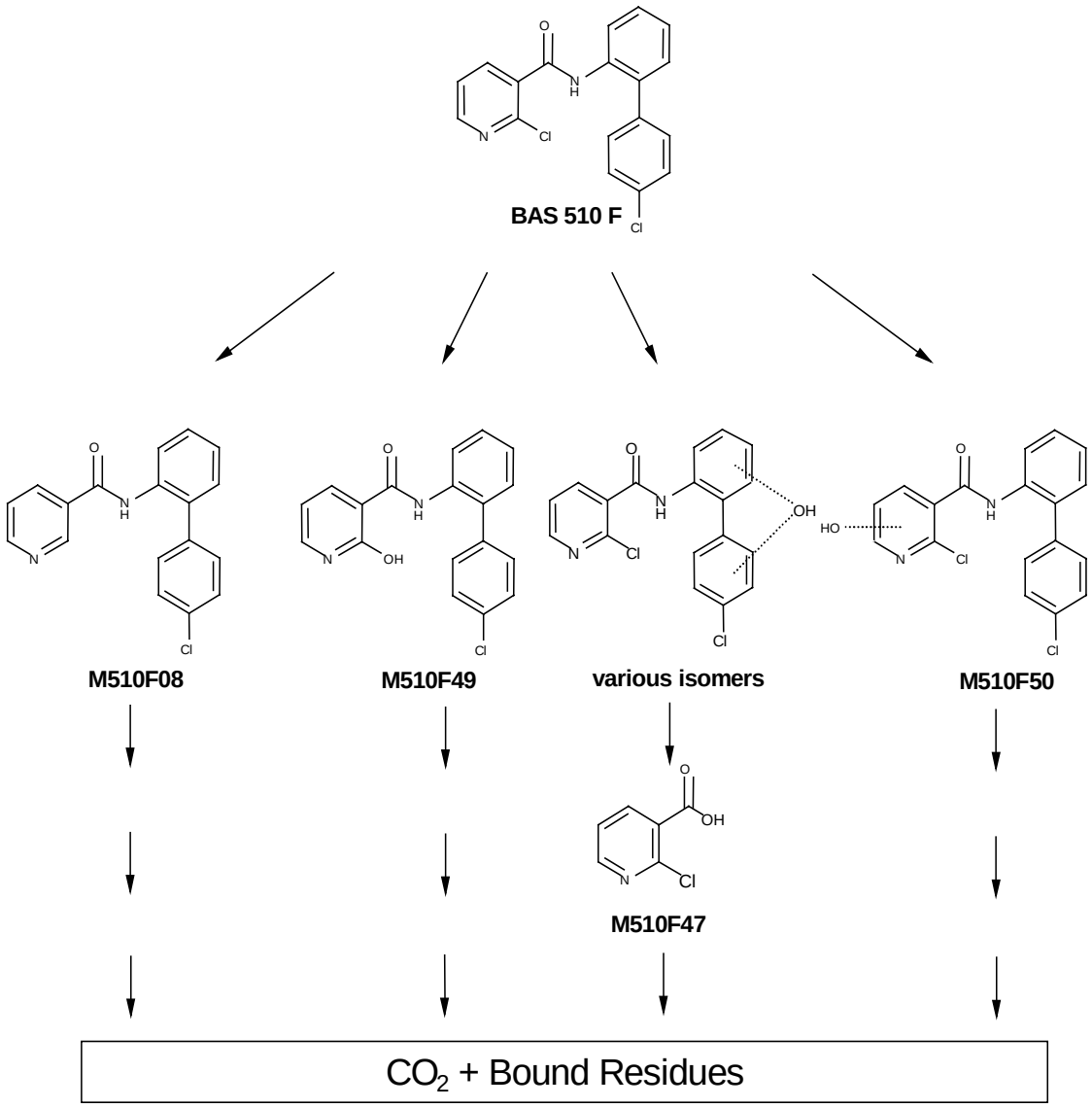
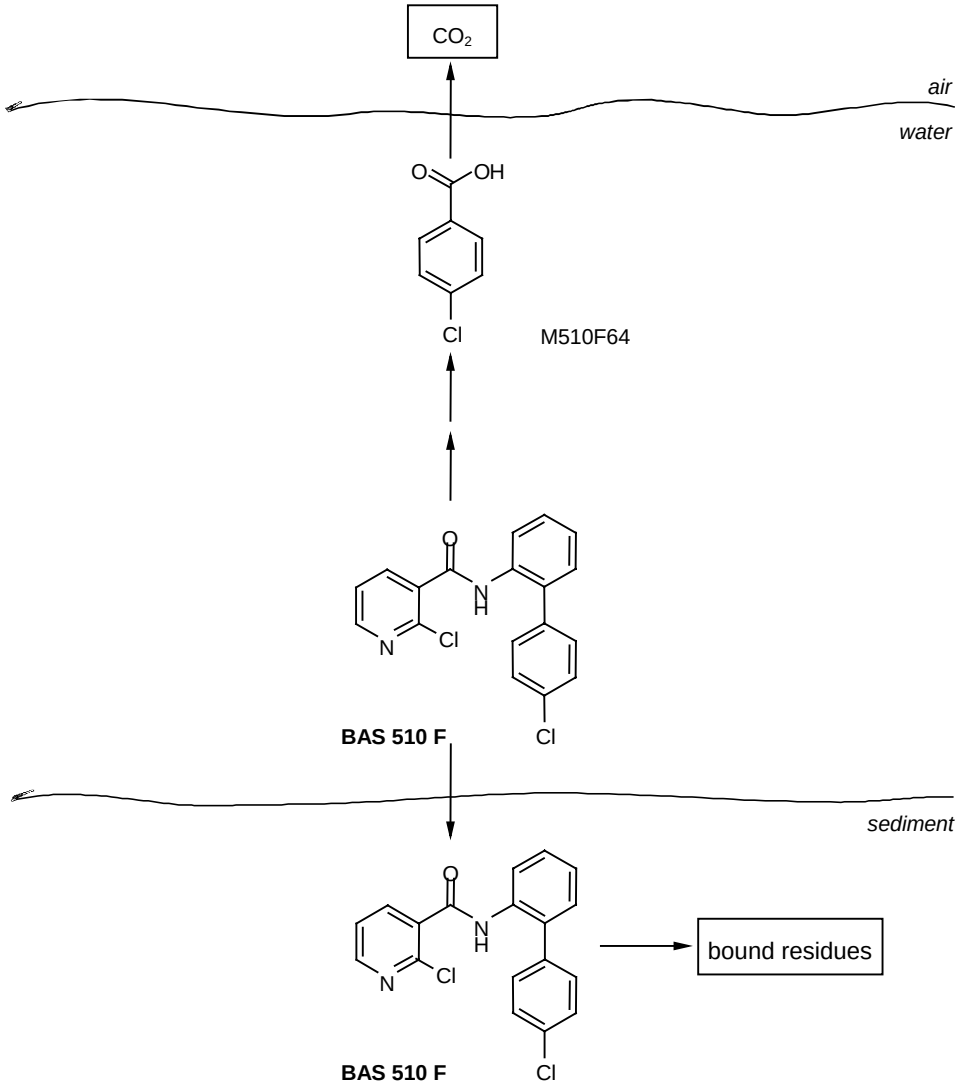


Figure 2.7-7: Proposed route of degradation of BAS 510 F in aqueous systems under outdoor conditions



Appendix 1

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

Specific Terms and Abbreviations

2.8.2 Appendix II: Specific terms and abbreviations

PAS	pure active substance
TAS	technical active substance
eq.	Equivalents
TRR	Total radioactive residue
ERR	Extractable radioactive residue
RRR	Residual radioactive residue
DMSO	Dimethylsulfoxide
DAT	Days after treatment
DAP	Days after planting
DALT	Days after last treatment
DALA	Days after last application
GS	Growth stage
TAR	Total applied radioactivity

Appendix 3

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

List of End Points

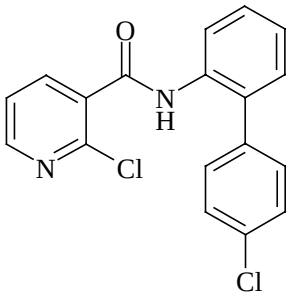
2.8.3 Appendix III: Listing of end points

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)	Nicobifen
Function (*e.g.* fungicide)	Fungicide

Rapporteur Member State	Germany

Identity (Annex IIA, point 1)

Chemical name (IUPAC)	2-Chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide
Chemical name (CA)	2-Chloro-*N*-(4'-chloro[1,1'-biphenyl]-2-yl)-3-pyridinecarboxamide,
CIPAC No	673
CAS No	188425-85-6
EEC No (EINECS or ELINCS)	not assigned
FAO Specification (including year of publication)	not assigned
Minimum purity of the active substance as manufactured (g/kg)	960
Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	–
Molecular formula	$C_{18}H_{12}Cl_2N_2O$
Molecular mass	343.21 g/mol
Structural formula	

Physical-chemical properties (Annex IIA, point 2)

Melting point (state purity)	143 – 144 °C (capillary method, 99.7 %) 145 °C (DSC method, 99.7 %)
Boiling point (state purity)	–
Temperature of decomposition	ca 300 °C
Appearance (state purity)	white crystalline solid, odourless (min 99.4 %)
Relative density (state purity)	d_4^{20} = 1.381 (99.7 %)
Surface tension	66.0 mN/m 0.5 % (w/w) and 61.7 mN/m 1.0 % (w/w) (98.16 %, both at 20 °C) 72.1 mN/m 0.5 % (w/w) and 72.4 mN/m 1.0 % (w/w) (99.7 %, both at 20 °C)
Vapour pressure (in Pa, state temperature)	7.2×10^{-7} Pa at 20 °C
Henry's law constant (Pa m^3 mol^{-1})	5.178×10^{-5} Pa m³/mol
Solubility in water (mg/l, 20 °C)	4.6
Solubility in organic solvents (in g/l, 20 °C)	*n*-Heptane < 10 g/L Toluene 20-25 g/L Dichloromethane 200-250 g/L Methanol 40-50 g/L Acetone 160-200 g/L Ethyl acetate 67-80 g/L N,N-Dimethylformamide > 250 g/L Acetonitrile 40-50 g/L 1-Octanol < 10 g/L 2-Propanol < 10 g/L olive oil < 10 g/L
Partition co-efficient (log $P_{O/W}$) (state pH and temperature)	2.96 (pH 7.1, 21 °C) No dissociation in water, therefore no pH dependency
Hydrolytic stability (DT_{50}) (state pH and temperature)	Stable between pH 4 and pH 9
Dissociation constant	No dissociation in water
UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength)	207 nm (ε 31534) 228 nm (ε 19834) 290 nm (ε 1529) 300 nm (ε 531)
Photostability (DT_{50}) (aqueous, sunlight, state pH)	Stable, no DT_{50} calculated because of exceeding at least twice the duration of the experiment
Quantum yield of direct phototransformation in water at λ > 290 nm	Smaller than 2.45×10^{-4}
Flammability	not highly flammable
Explosive properties	none

Summary of uses supported by available data Nicobifen (BAS 510 01 F)

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grape	**EU (North & South)**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 - 81	1	-	0.038 - 0.060	1000 - 1600	0.600	28	
Oil seed rape	**EU (North)**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63 - 65	2	4-6 weeks	0.062 - 0.125	200 - 400	0.250	-	
Oil seed rape	**EU (South)**	BAS 510 01 F	F	*Alternaria Sclerotinia, Phoma*	WG	500	spraying	30, 63 - 65	2	4-6 weeks	0.100 - 0.050	200 - 400	0.200	-	
Peas	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 - 10	0.125	400	0.500	7	
Beans	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 – 10	0.166	300	0.500	7	

(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	none
with regard to fate and behaviour data	none
with regard to ecotoxicological data	N, R51/53

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
Impurities in technical as (principle of method)	HPLC-UV; GC/MS
Plant protection product (principle of method)	GC-FID

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	GC-MS	0.01 mg/kg (wheat, lemon, tomato, cabbage, lettuce) 0.02 mg/kg (oilrape seed) 0.05 mg/kg (hops, recovery = 63 %)
	LC-MS-MS	0.05 mg/kg (apple, cherry, grapes, strawberry, carrot, onions, tomato, broccoli, cabbage, leek, dwarf beans, oilseed rape)
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	GC-ECD	0.01 mg/kg (milk) 0.025 mg/kg (muscle, liver, kidney, fat, egg)
	confirmation: GC-MS Methods for nicobifen and metabolite M510F01	
Soil (principle of method and LOQ)	GC-MS	0.01 mg/kg
Water (principle of method and LOQ)	GC-MS	0.05 µg/L (drinking water) 0.5 µg/L (surface water)
Air (principle of method and LOQ)	GC-MS	1.5 µg/m^3
Body fluids and tissues (principle of method and LOQ)	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	Approx. 44 % (based on bile excretion within 48 h and urinary exretion within 6 h, low dose)
Distribution	Widely distributed. Highest residues in liver and adipose tissue (8-h, low dose) In high-dose females, highest residues were observed in thyroid and kidney
Potential for accumulation	No evidence
Rate and extent of excretion	Complete excretion of low dose within 48 h (approx. 20 % via urine and 80 % via faeces)
Metabolism in animals	Extensive (< 1 % of absorbed dose excreted as parent via urine or bile), 38 metabolites identified in rat matrices. Major pathway was hydroxylation at the diphenyl moiety and subsequent O-glucuronidation
Toxicologically significant compounds (animals, plants and environment)	Parent and metabolites

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral	> 5000 mg/kg bw
Rat LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} inhalation	> 6.7 mg/l air (nose-only dust exposure)
Skin irritation	Non-irritant
Eye irritation	Non-irritant
Skin sensitization (test method used and result)	Not a skin sensitiser (M&K test)

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect	Liver, thyroid
Lowest relevant oral NOAEL / NOEL	Dog 1-yr: 800 ppm (22 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL	Rat 28-day: 1000 mg/kg bw/d
Lowest relevant inhalation NOAEL / NOEL	No studies submitted, not required.

Genotoxicity (Annex IIA, point 5.4)

No evidence

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

Target / critical effect	Liver, thyroid
Lowest relevant NOAEL / NOEL	Rat 2-yr: 100 ppm (4.4 mg/kg bw/d)
Carcinogenicity	Slight increase of thyroid follicular cell adenomas; not relevant to man. No classification and labelling necessary.

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction target / critical effect	Slightly reduced viability and decreased pup wt during lactation in the presence of parental adverse effects
Lowest relevant reproductive NOAEL / NOEL	100 ppm (6.7 mg/kg bw/d[1])
Developmental target / critical effect	Delayed ossification in rabbits and rats in the presence of maternal toxicity at the limit dose
Lowest relevant developmental NOAEL / NOEL	Rat & rabbit: 300 mg/kg bw/d

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

No evidence from oral acute and 90-d neurotoxicity studies. No evidence from developmental neurotoxicity study

Other toxicological studies (Annex IIA, point 5.8)

Toxic effects of metabolites	Para-chlorobenzoic acid (degradation product in aquatic environment): literature survey data indicates that para-chlorobenzoic acid exhibits higher acute oral toxicity than nicobifen. No concern from limited in-vitro genotoxicity data
Mechanistic studies	Nicobifen is an inducer of cytochrome P450; T3 and T4 levels are decreased and TSH is increased. The increased metabolism of T4 via hepatic enzyme conjugation appeared to be responsible for the increased TSH.

Medical data (Annex IIA, point 5.9)

No data (new compound)

[1] based on default conversion factor of 15 proposed by JMPR (WHO) to be used for rat multi-generation studies

Summary (Annex IIA, point 5.10)

	Value	Study	Safety factor
ADI	0.04 mg/kg bw	Rat 2-yr oral feed	100
AOEL systemic	0.1 mg/kg bw/d	Dog 1-yr oral feed; corrected for 44 % oral absorption	100 x [44 %]
ARfD (acute reference dose)	Not allocated	Not necessary, based on low acute toxicity and lack of developmental toxicity concerns	

Dermal absorption (Annex IIIA, point 7.3)

Rat in vivo: 7 %; rat/human in-vitro dermal penetration ratio: 1 => 7 % human dermal absorption proposed for use in exposure calculations

Acceptable exposure scenarios (including method of calculation)

Operator	Intended uses acceptable (operator exposure < systemic AOEL; German model: without PPE and UK-POEM: with PPE)
Workers	Intended uses acceptable
Bystanders	Intended uses 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	grapes (fruit), lettuce (leaf vegetables), beans (pulses)
Rotational crops	radish, lettuce, wheat
Plant residue definition for monitoring	Nicobifen
Plant residue definition for risk assessment	Nicobifen
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	Goat, hen
Animal residue definition for monitoring	Nicobifen and M510F01 (including its conjugate)
Animal residue definition for risk assessment	Nicobifen and M510F01 (including its conjugate) Bound residues in liver and minor metabolites in milk (as M510F53)
Conversion factor (monitoring to risk assessment)	Not applicable
Metabolism in rat and ruminant similar (yes/no)	Yes
Fat soluble residue: (yes/no)	Yes (log P_{ow} = 2.96), in livestock feeding study residues in fat and cream at 1x dose

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

30, 120, 270, 365 days plant back interval after application of 2.1 kg as/ha to soil. With the exception of wheat grain the major residue was parent nicobifen. Residues of parent: lettuce: 0.014 - 0.146 mg/kg; radish leaf: 0.09 - 0.30 mg/kg, radish root: 0.01 - 0.09 mg/kg, wheat grain: 0.005 - 0.028 mg/kg , wheat forage: 0.19 - 1.47 mg/kg, wheat straw: 0.81 - 7.99 mg/kg
From the results it cannot be excluded that residues above the LOQ (0.05 mg/kg) occur in succeeding crops. This is confirmed by the results of a field test with residues of nicobifen in wheat plant at 0.10 mg/kg and in wheat straw at 0.75 mg/kg.

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

Food of animal origin: (milk, muscle, liver) Nicobifen and Metabolite M510F01 stable for 5 months
Food of plant origin (Wheat plant, wheat grain, wheat straw, oilrape seed, sugar beet, white cabbage, peach, pea): Nicobifen stable for 24 months

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)
Grapes	N	1 x 0.86, 1x 1.40, 1x 1.45, 1x 1.71, 1 x 1.76, 1 x 1.79, 2 x 2.01, 1 x 3.26, 1 x 3.40, 1 x 3.49, 1 x 3.69mg/kg	Fruit	5.0 mg/kg (proposed)	1.90 mg/kg
	S	1 x 0.52, 1 x 0.84, 1 x 1.07, 1 x 1.21, 1 x 1.39, 1 x 1.45, 1 x 1.54, 1 x 1.72, 1 x 1.74, 1 x 2.32, 1 x 2.33, 1 x 3.15, 1 x 3.22			1.54 mg/kg
Beans	N	1 x 0.13, 1 x 0.22, 1 x 0.26, 1 x 0.29, 1 x 0.47, 1 x 0.50, 1 x 0.53, 1 x 0.67, 1 x 0.83 mg/kg	Pods with seed	2.0 mg/kg (proposed)	0.47 mg/kg
	S	1x 0.62, 1 x 0.95			0.79 mg/kg
	S Glasshouse	1 x 0.06, 2 x 0.28, 1 x 0.29, 1 x 0.61, 1 x 0.69, (1 x 1.65, 1 x 1.67) mg/kg			0.45 mg7kg
Peas	N	5 x < 0.05, 1 x 0.05, 2 x 0.07, 1 x 0.23 mg/kg	Seeds	0.3 mg/kg (proposed)	0.05 mg/kg
	S	1 x 0.06, 1 x 0.07 mg/kg			
Rape	N	10 x < 0.05 mg/kg	Seeds	0.05 mg/kg (proposed)	0
	S	2 x < 0.05 mg/kg			0

(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

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	No hen feeding study conducted	No pig feeding study conducted. Metabolism in rat and ruminant similar
Liver	< 0.10		
Kidney	< 0.10		
Fat	< 0.10		
Milk	< 0.02		
Eggs	not applicable		

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

ADI	0.04 mg/kg bw/d
TMDI (European Diet) (% ADI)	0.011 mg/kg bw (28.1 %)
NEDI (% ADI)	not calculated
Factors included in NEDI	-
ARfD	Not assigned
Acute exposure (% ARfD)	Not applicable

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

Crop/processed crop	Number of studies	Transfer factor	% Transference *
Grapes:			
must, cold	1 (4 trials)	0.32 – 0.52	
must, after short time heating	1 (4 trials)	0.45 – 0.48	
must, after mash heating	1 (4 trials)	0.09 – 0.18	
wine, from must, cold	1 (4 trials)	0.26 – 0.47	
wine from must, after short time heating	1 (4 trials)	0.36 – 0.46	
Wine from must, after mash heating	1 (4 trials)	0.08 – 0.12	
wet pomace	1 (4 trials)	1.95 – 3.41	
Peas:			
Peas/Washed peas	1 (4 trials, only one trial with residues > 0.05 in RAC))	0.50	
Peas/Wash water	“	0.43	
Cooked peas, canned peas, boiled water, vegetable stock	“	< 0.36 (residues in processed product < 0.05 mg/kg)	

* 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)

Food of plant origin	
Grapes	5 mg/kg
Beans	2 mg/kg
Peas	0.3 mg/kg
Rape	0.05 mg/kg
Food of animal origin	
Milk	0.02 mg/kg*
Fat	0.1 mg/kg*
Liver	0.1 mg/kg*
Kidney	0.1 mg/kg*

* sum of nicobifen and metabolite M510F01 (and its conjugates)

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)

Mineralization after 100 days	8 %/15 % (after 119 d) diphenyl-^{14}C/pyridine-^{14}C
Non-extractable residues after 100 days	49 %/33 % (after 119 d) diphenyl-^{14}C/pyridine-^{14}C
Relevant metabolites - name and/or code, % of applied (range and maximum)	None

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

Anaerobic degradation	74 - 77 % parent after 120 d, 15 % BR no major metabolites
Soil photolysis	After 15 d: 91 % parent, 6 % BR, 0.2 % CO_2 , no major metabolites, DT_{50}: 135 d

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

Method of calculation	Model Maker 3.0.3 or Timme, Frehse and Laska
Laboratory studies (range or median, with n value, with r^2 value)	DT_{50lab} (20 °C, aerobic): 108 - 384 d (5 soils)
	DT_{90lab} (20 °C, aerobic): 442 d
	DT_{50lab} (10 °C, aerobic): 583 d
	DT_{50lab} (20 °C, anaerobic): 261/345 d
	degradation in the saturated zone: not relevant
Field studies (state location, range or median with n value)	DT_{50f}: 28 - 208 d, 5 locations (3 in Germany, 2 in Spain). DT_{50} Values standardised to 20 °C: 139 d.
	DT_{90f}: > 1 year
Soil accumulation and plateau concentration	Minimum and maximum plateau concentration predicted with the simulation model FOCUS PEARL for three vines scenarios (Hamburg, Piacenza - central Europe and Sevilla - southern Europe) with application of 1*0.6 kg as/ha of nicobifen per year, and for three beans scenarios (Jokioinen - northern Europe, Hamburg – central Europe and Sevilla - southern Europe) with application of 2*0.5 kg as/ha each year. FOCUS interception and average standardised field half-life (DT_{50} of 139 d) are considered for calculation.

Scenario	Interception (%)	Crop type	Minimum Plateau [kg/ha]	Maximum Plateau [kg/ha]
Jokioinen	80	Beans	0.31	0.50
Hamburg	80	Beans	0.18	0.37
Sevilla	80	Beans	0.05	0.25
Hamburg	85	Vines	0.08	0.17
Piacenza	85	Vines	0.03	0.12
Sevilla	85	Vines	0.02	0.11

Soil adsorption/desorption (Annex IIA, point 7.1.2)

K_f /K_{oc}	Koc: 507 – 1110 (n = 6) Average: 771 1/n = 0.839 – 0.887
K_d	Kf: 3.3 - 27.8
pH dependence (yes / no) (if yes type of dependence)	no

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

Column leaching	Not required
Aged residues leaching	0 % radioactivity in leachate
Lysimeter/ field leaching studies	Not required, no leaching expected. See also PECgw

PEC (soil) (Annex IIIA, point 9.1.3)

Method of calculation	Degradation according to 1st order kinetics DT50: 314 d (standardised $DT_{50\ field}$ to 15 °C)
Application rate	Maximum accumulated application per year 1.0 kg as/ha (bean scenario) with no interception

$PEC_{(s)}$	Multiple application Actual concentration	Multiple application Time weighted average
Initial	1.333	1.333
Short term		
24 h	1.330	1.332
2 d	1.327	1.330
4 d	1.322	1.327
Long term		
7 d	1.313	1.323
28 d	1.254	1.293
50 d	1.194	1.262
100 d	1.070	1.197

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

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)		pH 4: stable
		pH 7: stable
		pH 9: stable
Photolytic degradation of active substance and relevant metabolites		DT_{50} not reported (stable)
Readily biodegradable (yes/no)		No
Degradation in water/sediment (**laboratory study**)	- DT_{50} water	Pond system: 9 d; river system: 3
	- DT_{90} water	Pond system: 133 d; river system: 43 d
	- DT_{50} whole system	Values far exceeding the duration of the experiment, for both systems and both labelling positions
	- DT_{90} whole system	Reliable extrapolation not possible.
Mineralization		0.5 % after 100 d
Non-extractable residues		Pond system: 13 %; river system: 10 % after 100 d
Distribution in water / sediment systems (active substance)		(diphenyl -^{14}C) Pond system: 17.4 % in water / 68.6 % in sediment (after 100 d) River system: 6.1 % in water / 80.2 % in sediment (after 100 d)
Distribution in water / sediment systems (metabolites)		No metabolites observed in water and sediment
Degradation in water/sediment (**outdoor study**)	- DT_{50} water	21 d (half-life)
	- DT_{50} water	16 d (best fit)

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

Method of calculation	Degradation in the water phase according to first order kinetics (half -life 21 d from the outdoor study), 30 cm water body
Application rate	Grape vine scenario, 600 g as/ha Beans scenario 2 x 500 g as/ha
Main routes of entry	Drift, runoff and drainage

PECinitial (Grapevine, Beans scenario)

DRIFT (30 cm depth of water body)	Grapevine (µg/L)	Beans (µg/L)
1 m buffer	-	12.0
3 m buffer	15.0	-
5 m buffer	10.0	-
10 m buffer	3.0	-
PEC_{ini}, runoff (no buffer)	1.71	3.72
PEC_{ini}, runoff (5 m buffer)	1.39	3.03
PEC_{ini}, drainage	1.49	2.80

PEC Grapevine

Time/integ-ration period	3 m buffer		5 m buffer		10 m buffer	
	PEC_{act}	PEC_{twa}	PEC_{act}	PEC_{twa}	PEC_{act}	PEC_{twa}
d	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
0	15.0	15.0	10.0	10.0	3.0	3.0
1	14.5	14.8	9.7	9.8	2.9	3.0
2	14.0	14.5	9.4	9.7	2.8	2.9
3	13.6	14.3	9.1	9.5	2.7	2.9
4	13.1	14.1	8.8	9.4	2.6	2.8
7	11.9	13.4	7.9	8.9	2.4	2.7
14	9.5	12.0	6.3	8.0	1.9	2.4
21	7.5	10.8	5.0	7.2	1.5	2.2
28	6.0	9.8	4.0	6.5	1.2	2.0
42	3.8	8.1	2.5	5.4	0.8	1.6
100	0.6	4.4	0.4	2.9	0.1	0.9

PEC Beans

The PEC_{ini} for the bean scenario by runoff and drainage are higher than the PEC_{ini} resulting from drift for the 5 m buffer zone (3.72 and 2.80 µg/L). The actual and twa PEC values for the beans scenario are therefore calculated after the runoff event, assuming 5 m buffer.

Time/Integration-Period	PEC_{act} 1 m buffer Entry by spray drift	PEC_{twa} 1 m buffer Entry by spray drift	PEC_{act} 5 m buffer Entry by runoff	PEC_{twa} 5 m buffer Entry by runoff
d	µg/L	µg/L	µg/L	µg/L
0	12.0		3.0	
1	11.6	12.0	2.9	3.0
2	11.2	11.8	2.8	2.9
3	10.8	11.6	2.7	2.9
4	10.5	11.4	2.7	2.8
7	9.5	10.9	2.4	2.7
14	7.5	9.7	1.9	2.4
21	6.0	8.8	1.5	2.2
28	4.7	8.1	1.2	2.0
42	3.0	7.0	0.8	1.6
100	0.4	3.9	0.1	0.9

PEC (sediment)

Method of calculation	Maximum concentration in sediment as observed in the outdoor water/sediment study (28.2 %)
Application rate	Grapevine: 1 x 600 g as/ha Beans: 2 x 500 g as/ha Entry by spray drift

Drift	Grapevine mg/kg	Beans mg/kg
1 m buffer	-	0.078
3 m buffer	0.098	-
5 m buffer	0.065	0.012
10 m buffer	0.020	-

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

Method of calculation and type of study (*e.g.* modelling, monitoring, lysimeter)	FOCUS PEARL 1.1.1 DT_{50}: 139 d (standardised $DT_{50\ field}$ to 20 °C) Koc: 771 (average value)
Application rate	Grapevine, 1 x 600 g as/ha Beans, 2 x 500 g as/ha

$PEC_{(gw)}$

Maximum concentration Average annual concentration	PEC_{gw} (80^{th} percentile), calculated for several scenarios did not exceed 0.1 µg/L (year average)

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

Direct photolysis in air	Photolytically stable in water. Photolysis in air not expected. Not stable under influence of radicals, (see DT_{50} photochemical oxidative degradation).
Quantum yield of direct phototransformation	$< 2.45 \times 10^{-4}$
Photochemical oxidative degradation in air	$DT_{50:} < 1.1$ d
Volatilization	from plant surfaces:about 1 % in 24 hours
	from soil: about 0.5 % in 24 hours

PEC (air)

Method of calculation	Due to low volatility and fast photochemical oxidative degradation, contamination of the compartment air not expected. PEC not calculated.

$PEC_{(a)}$

Maximum concentration	Not calculated

Definition of the Residue (Annex IIA, point 7.3)

Relevant to the environment	Active substance only.

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 (rat)
Long-term toxicity mammals	NOAEL 100 ppm (rat multi-generation study)
Acute toxicity to birds	LD50 > 2000 mg/kg (bobwhite quail)
Dietary toxicity to birds	LC50 > 5000 ppm (bobwhite quail and mallard duck)
Reproductive toxicity to birds	NOEC 300 ppm (bobwhite quail), 1000 ppm (mallard duck)

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

Residue estimates:

Insects in grapes: 12.6 mg/kg (based on RUD 21 mg/kg from Fischer and Bowers); fruit: 5.8 mg/kg (measured); vegetation in field crops: 15.5 mg/kg initially (based on RUD 31 mg/kg from Hoerger and Kenaga) and 8.2 mg/kg twa (averaging period = 3 w, DT50=10 days)

Food consumption related to body weight:

Insectivorous bird 94 %, insectivorous mammal 100 %, frugivorous bird 87 %, herbivorous bird 44 %, herbivorous mammal 28 %

Application rate (kg as/ha)	Crop	Category (*e.g.* insectivorous bird)	Time-scale	TER	Annex VI Trigger
0.6	Grapes	Insectivorous bird	acute	> 170	10
0.6	Grapes	Insectivorous bird	short-term	> 390	10
0.6	Grapes	Insectivorous bird	long-term	23	5
0.6	Grapes	Insectivorous mammal	acute	> 390	10
0.6	Grapes	Insectivorous mammal	long-term	8	5
0.6	Grapes	Frugivorous bird	acute	> 400	10
0.6	Grapes	Frugivorous bird	short-term	> 860	10
0.6	Grapes	Frugivorous bird	long-term	52	5
0.5	Field crops	Herbivorous bird	acute	> 294	10
0.5	Field crops	Herbivorous bird	short-term	> 322	10
0.5	Field crops	Herbivorous bird	long-term	37	5
0.5	Field crops	Herbivorous mammal	acute	> 1160	10
0.5	Field crops	Herbivorous mammal	long-term	12	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/L)
Laboratory tests				
L. macrochirus	Nicobifen	static – 96 h	LC_{50}	> 4.0
O. mykiss		static – 96 h	LC_{50}	2.7
O. mykiss		flow-through – 97 d (ELS)	NOEC	0.125
D. magna		static – 48 h	EC_{50}	5.33
D. magna		semistatic – 21 d	NOEC	1.31
P. subcapitata		static – 96 h	E_rC_{50}	3.75
			E_bC_{50}	1.34
C. riparius		static – 28 d	NOEC	2.0
Activated slugde		static – 0.5 h	Respiration rate	> 1000
O. mykiss	BAS 501 01 F	static – 96 h	LC_{50}	100
D. magna		static – 48 h	EC_{50}	50
P. subcapitata		static – 72 h	E_rC_{50}	4.50
			E_bC_{50}	3.37
Microcosm or mesocosm tests				
Not required.				

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
1 x 0.600	grapevines	*O. mykiss*	acute	3	169	100
		O. mykiss	long-term	3	14	10
		D. magna	acute	3	333	100
		D. magna	long-term	3	113	10
		P. subcapitata	short-term	3	84	10
		C. riparius	long-term	3	125	10
2 x 0.500	beans	*O. mykiss*	acute	1	380	100
		O. mykiss	long-term	1	30	10
		D. magna	acute	1	751	100
		D. magna	long-term	1	252	10
		P. subcapitata	short-term	1	189	10
		C. riparius	long-term	1	282	10

Bioconcentration

Bioconcentration factor (BCF)	89 – 125
Annex VI Trigger for the bioconcentration factor	> 100 for non readily biodegradeble substances
Clearance time (CT_{50})	1.0 d
(CT_{90})	3.3 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 (active substance)	LD_{50} = 100 µg as/bee
Acute contact toxicity (active substance)	LD_{50} = 100 µg as/bee
Acute oral toxicity (formulation)	LD_{50} = 166 µg/bee
Acute contact toxicity (formulation)	LD_{50} = 200 µg/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				
0.6	grape	oral	3.61	50
0.6	grape	contact	3	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 (g as/ha)	Endpoint	Effect	Annex VI Trigger
Laboratory tests						
Typhlodromus pyri	Protonymphs	BAS 510 01 F	46	Mortality Fecundity	0	30
Typhlodromus pyri	Protonymphs	BAS 510 01 F	115	Mortality Fecundity	0	30
Typhlodromus pyri	Protonymphs	BAS 510 01 F	288	Mortality Fecundity	0 +14	30
Typhlodromus pyri	Protonymphs	BAS 510 01 F	700	Mortality Fecundity	0 3	30
Typhlodromus pyri	Protonymphs	BAS 510 01 F	1800	Mortality Fecundity	0 +3	30
Aphidius rhopalosiphi	Imagines	BAS 510 01 F	355.5	Mortality Fecundity	0	30
Aphidius rhopalosiphi	Imagines	BAS 510 01 F	533.5	Mortality Fecundity	0	30
Aphidius rhopalosiphi	Imagines	BAS 510 01 F	800	Mortality Fecundity	0	30
Aphidius rhopalosiphi	Imagines	BAS 510 01 F	1200	Mortality Fecundity	11 25	30
Aphidius rhopalosiphi	Imagines	BAS 510 01 F	1800	Mortality Fecundity	11 34	30
Chrysopa carnea	Larvae	BAS 510 01 F	1200	Mortality Fecundity	2 11	30
Pardosa spp.	Adults	BAS 510 01 F	1200	Mortality Food uptake	0 5	30
Poecilus cupreus	Imagines	BAS 510 01 F	1200	Mortality Food uptake	0 5	30

Field tests

Test material	Species	Test	No. of appl.	Dosage per appl. (g as/ha)	Effect (%) Final bonitur *
	Predatory mites				
BAS 510 01 F	*T. pyri*	Field	3	600	0 / 0
BAS 510 01 F	*T. pyri*	Field	3	30	0 / 6
BAS 510 01 F	*T. pyri*	Field	3	600	39.7 / 2.8
BAS 510 01 F	*T. pyri*	Field	3	30	33.6 / 5.0
BAS 510 01 F	*T. pyri*	Field	3	600	21 / 9
BAS 510 01 F	*T. pyri*	Field	3	30	12 / 9

* 7 / 27 days resp. 29 days after last application

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

Acute toxicity	LC50 > 1000 mg BAS 510 01 F / kg (corrected > 500 mg form./kg) > 1000 mg nicobifen / kg (corrected > 500 mg as/kg)
Reproductive toxicity	NOEC 3.6 kg BAS 510 01 F /ha (corrected 1.8 kg form./ha; equivalent to 1.197 mg nicobifen/kg)

Field tests

Two field tests on the formulation BAS 510 01 F were conducted with 3 x 0.6 kg/ha and 3 x 1.2 kg/ha. One year after the last application there was a not significant reduction in abundance and biomass of earthworms of about 30 % in case of the higher application rate. No long-lasting effects on overall abundance and biomass of earthworms were observed for the lower test concentration. However for both test concentrations effects on single species of about 30 % in comparison to control still exist one year after the last application.

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

Application rate (kg as/ha)	Crop	Time-scale	TER	Annex VI Trigger
1 x 0.600	grape	acute	625	10
		longterm	1.5	5
2 x 0.500	beans	acute	375	10
		longterm	0.9	5

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

Nitrogen mineralization	No effects up to 12 kg BAS 510 01 F/ha (equivalent to 6 kg as/ha)
Carbon mineralization	No effects up to 12 kg BAS 510 01 F/ha (equivalent to 6 kg as/ha)

Level 3

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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

Nicobifen is the proposed ISO common name for 2-Chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide.

Residues of nicobifen in food of plant and animal origin, soil, drinking water, surface water, air and tissues can be determined using GC-MS, GC-ECD and/or LC-MS-MS. Analytical methods for metabolites are only needed for food of animal origin.
Analytical methods for body fluids are not submitted. Because of the classification of the active substance, such method is not necessary according to Directive 96/46/EC

The available data on mammalian toxicology, mutagenicity and animal metabolism are considered to adequately support the risk evaluation of **nicobifen** in humans.
Concerning toxicology and metabolism all studies required by Directive 91/414/EEC are available and were conducted according to Guideline requirements under Good Laboratory Practice regulations.

Concerning toxicology and metabolism (rats/mice/dogs) all studies required by Directive 91/414/EEC are available and were conducted according to Guideline requirements under Good Laboratory Practice regulations.

The metabolism of nicobifen in plants was investigated in grapes, lettuce and beans. 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 nicobifen was investigated in lactating goats and laying hens.
For monitoring purposes the residue definition for food of animal origin is proposed as nicobifen and metabolite M510F01 (including its conjugate).
For risk assessment bound residues in liver and minor metabolites in milk (M510F53) should be considered too.

The residue situation for the intended uses of nicobifen in grapes, beans, peas and rape seed is covered by a sufficient number of residue trials. On basis of these data the possible intake of residues by consumers was calculated. In a chronic risk assessment no unacceptable risk for consumers could be identified. An acute risk is not to be expected since there was no necessity to set an Acute Reference Dose (ARfD).

Due to its persistent nature in soil and its ability to be transported systemically in plants the parent compound nicobifen may occur in crops grown in rotation. A confined rotational crop study as well as field trials indicate that residue levels above 0.05 mg nicobifen/kg are possible in crops grown in rotation.

The behavior of nicobifen in soil is characterised by slow degradation. Half lifes of nicobifen in aerobic soils range from 108 to 384 days under laboratory conditions. No metabolites in

significant amounts were formed beside low levels of intermediary products. Bound residues were formed in amounts up to 49 % TAR after 119 days. Mineralisation up to 15 % within 119 days was observed. Certain metabolites were detectable under anaerobic conditions, in which degradation was slowed.
In field dissipation studies DT_{50} were shorter and ranged from 27 days in Spain to 208 days in Germany. However, DT_{90} was not reached within one year after application and accumulation studies were initiated. The accumulation issue was also approached by modelling. Accumulation plateau is expected to be reached within the first years, depending on the actual scenario.
Adsorption/desorption and aged residue column leaching studies clearly show that there is no risk of contamination of ground water with nicobifen. This was also supported by the calculation of the predicted environmental concentration in the ground water using current models.

Nicobifen is hydrolytically stable between pH 4 and pH 9. In the water/sediment study performed in the dark, the major elimination pathway for nicobifen in the water is binding to the sediment.
Under irradiated conditions a metabolite M510F64 was formed in sediment, which was not detected in other studies. This metabolite remained below 10 % of total applied radioactivity and was of transient nature.

Volatilisation studies from plant and soil surfaces showed that nicobifen has a very low volatilisation potential. Even if small amounts of nicobifen reach the troposphere, they will be degraded by photochemical processes.

According to the recommended pattern of use the risk to terrestrial vertebrates (birds and mammals), aquatic organisms, bees, other non-target arthropods, earthworms, soil micro-organisms, soil non-target macro-organisms and non-target terrestrial plants is considered to be low.

3.2 Proposed decision concerning inclusion in Annex I

Concerning the submitted data a postponement of the inclusion of the active substance nicobifen in Annex I of Directive 91/414/EEC is recommended pending submission and evaluation of further information on residues in succeding crops.

A potential risk has been identified with respect to the long term effects on earthworms. The available data so far cover application rates up to 1 kg as/ha and year. Member States may pay particular attention to this when authorising uses other than those described in this monograph.

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

Further trials are required regarding the residue situation in succeeding crops since the results of the rotational crop study and of a field test indicate that residues of nicobifen above the LOQ could occur in succeeding crops.

Level 4

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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.

Physical and chemical properties of the plant protection product
None.

Data on application and further information
Data on application
None.

Further information
None.

Toxicology and metabolism
None.

Methods of analysis
Analytical methods for residue analysis
None.

Residue data
Further trials are required regarding the residue situation in succeeding crops since the results of the rotational crop study and of a field test indicate that residues of nicobifen above the LOQ could occur in succeeding crops.(AIIA 6.6 / AIIIA 8.5)

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.

Physical and chemical properties of the plant protection product

Shelf life of the plant protection product (Section B.2.2.7.3). (AIIIA, 2.7)
Justification: The announced study has to be provided.

Methods of analysis

Analytical methods for residue analysis

Additional validation data for the analytical methods to determine the active substance in soil and water. (AIIA 4.2.2.-4.2.3 / AIIIA 5.2))
Justification: Uncorrected recoveries were not calculated in the report.

Toxicology and metabolism

None.

Residue data

If authorisations will be sought for beans or peas in southern Europe, then further residue trials for southern Europe are required. (AIIA 6.3 / AIIIA 8.2)
Justification:
For beans as a major crop a full data set (8 trials) is necessary for both European regions.
For peas a data set of four trials is required for South Europe.

If authorisation will be sought for indoor application on beans further trials are required. (AIIA 6.3 / AIIIA 8.2)
Justification:
Beans from two indoor trials showed significantly higher residues. It is not clear whether this is due to application at later growth stages or to temperature influence (low temperatures in November and December).

Ecotoxicology

None.

Monograph

08 November 2002

Nicobifen

Alternative common name proposed to ISO in August 2002:
Boscalid

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)

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]
Anonymous	AIIIA-1.4	1999	Safety data sheet: Wettol D 3. BASF DocID 1999/1004035 not GLP, unpublished BEI2001-193	N	BAS
Anonymous	AIIIA-1.4	1996	Safety data sheet: Ufoxane 3A. Reg. Doc.#BASF 96/11290 not GLP, unpublished BEI2001-192	N	BAS
Anonymous	AIIIA-1.4	2000	Safety data sheet: Wacker Antifoam Emulsion SRE. BASF DocID 2000/1018666 not GLP, unpublished BEI2001-191	N	BAS
Anonymous	AIIIA-1.4	2001	Safety data sheet: Ammonium Sulfate Industrial Grade. BASF DocID 2001/1001807 not GLP, unpublished BEI2001-190	N	BAS
Heinz, W.	AIIA-1.8	2001	BAS 510 F Description of the Manufacturing Process. 2001/1000043 not GLP, unpublished CHE2001-407	Y	BAS
Heinz, W.	AIIA-1.9; AIIA-1.10	2001	BAS 510 F (proposed common name: Nicobifen) TGAI Composition of the Technical Grade Active Ingredient. 2001/1000044 not GLP, unpublished CHE2001-408	Y	BAS
Heinz, W.	AIIA-1.11	2001	Characterization of the BAS 510 F Toxicology Batches (N 26, N37, N 46) Determination of the Active Ingredient and Impurities. 2001/1000841 GLP, unpublished CHE2001-410	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]
Heinz, W.	AIIA-1.11	2001	Characterization of five Batches of BAS 510 F Technical Grade Active Ingredient Determination of Active Ingredient and Process-related Impurities. 2001/1000045 GLP, unpublished CHE2001-409	Y	BAS
Schmider, F. and Becker-Arnold, R.	AIIA-1.8	2001	Confidential information from Document M-II. not GLP, unpublished CHE2001-559	N	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]
anonym	AIIA-2.1	2002	DSC-Plot to BASF DocID 1999/10991. not GLP, unpublished CHE2002-385	Y	BAS
Daum, A.	AIIA-2.1.1; AIIA-2.1.3; AIIA-2.4	1999	Determination of the melting point and the appearance of Reg. No. 300355 (BAS 510F). 1999/10991 GLP, unpublished CHE2001-397	Y	BAS
Daum, A.	AIIA-2.5	1999	UV-, NMR-, IR-, MS-Spectra of Reg. No. 300355 (BAS 510F). 1999/10832 GLP, unpublished CHE2001-400	Y	BAS
Daum, A.	AIIA-2.6	1998	Determination of the solubility of BAS 510 .. F (Reg.-No. 300355) in water at 20°C by column elution method and by HPLC. 1998/10961 GLP, unpublished CHE2001-401	Y	BAS
Daum, A.	AIIA-2.7	1998	Determination of the solubility of BAS 510.. F (Reg.-No. 300355 pure active ingredient (PAI) in organic solvents at 20°C. 1998/10953 GLP, unpublished CHE2001-402	Y	BAS
Daum, A.	AIIA-2.8	1998	Determination of the Octanol/Water-partition Coefficient of Reg.-No. 300355 (BAS 510 .. F) by HPLC. 1998/11082 GLP, unpublished CHE2001-403	Y	BAS
Daum, Ansgar	AIIA-2.9.4	1998	Determination of the pKa of Reg.No. 300355 (BAS 510 .. F) in water at 20 °C. BASF Reg.Doc.# 98/10967 GLP, unpublished WAS2001-155	Y	BAS
Gödde, M.	AIIIA-2.2; AIIIA-2.3	2000	Safety characteristics of the crop protection product BAS 510 01 F. BASF DocID 2000/1018467 GLP, unpublished PHY2001-335	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]
Gödde, M.	AIIIA-2.3	2001	Auto-flammability classification of the crop protection product BAS 510 01 F according to the UN-Recommendation on the Transport of Dangerous Goods (UN-Bowes-Cameron-Cage-Test). BASF DocID 2001/1009132 GLP, unpublished PHY2001-330	Y	BAS
Kästel, R.	AIIA-2.1.1; AIIA-2.2; AIIA-2.4; AIIA-2.14	1998	Physical and Chemical Properties of PS 300 355. 1998/10774 GLP, unpublished CHE2001-398	Y	BAS
Kästel, R.	AIIA-2.2; AIIA-2.14	1999	Physical Properties of 300 355 (PAI). 1999/10203 GLP, unpublished CHE2001-399	Y	BAS
Kästel, R.	AIIA-2.3.1	1999	Physical Properties of 300 355 (PAI). Reg.Doc.# BASF 99/10203 GLP, unpublished LUF2001-146	Y	BAS
Kästel, R.	AIIIA-2.1; AIIIA-2.4; AIIIA-2.6; AIIIA-2.7; AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.5; AIIIA-2.8.6; AIIIA-2.8.8; AIIIA-4.1	2001	Shelf life in original container of the formulation BAS 510 01 F - 12 Month storage - Physical and chemical properties INTERIM REPORT. BASF DocID 2001/1014636 GLP, unpublished PHY2002-84	Y	BAS
Kästel, R.	AIIIA-2.1; AIIIA-2.4; AIIIA-2.5; AIIIA-2.6; AIIIA-2.7; AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.5; AIIIA-2.8.6; AIIIA-2.8.8	2000	Physical and chemical properties of BAS 510 01 F. BASF DocID 2000/1017017 GLP, unpublished PHY2001-336	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]
König, W.	AIIA-2.11	2002	Statement of Analysis. 2002/1003388 not GLP, unpublished CHE2002-386	Y	BAS
Löffler, U.	AIIA-2.11.1; AIIA-2.11.2; AIIA-2.12; AIIA-2.13; AIIA-2.15	1998	Evaluation of safety characteristics according to 92/32/EEC. 1998/11078 GLP, unpublished CHE2001-404	Y	BAS
Ohnesorge, U.	AIIA-2.3.2	2000	Henry`s Law Constant for 300355. BASF DocID 2000/1001009 not GLP, unpublished LUF2001-147	Y	BAS
Schneider, K.-H.	AIIIA-2.9	2000	Physical and Chemical Compatibility in Aqueous Tank Mixtures of BAS 510 01 F with other products. BASF DocID 2000/1018466 not GLP, unpublished PHY2001-334	Y	BAS
von Götz, N.	AIIA-2.9.1	1999	Hydrolysis of BAS 510 F. BASF Reg.Doc.# 19 99/11285 GLP, unpublished WAS2001-153	Y	BAS
von Götz, N.	AIIA-2.9.2; AIIA-2.9.3	1999	Aqueous Photolysis of BASF 510 F. BASF #1999/11804 GLP, unpublished LUF2001-269	Y	BAS
von Götz, N.	AIIA-2.10	1994	Photochemical oxidative degradation of BAS 510 F (QSAR Estimates). BASF DocID 1999/11874 not GLP, unpublished LUF2001-149	Y	BAS

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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 protec-tion claimed Y/N	Owner[3]
Gerlach, H.	AIIA-3.7	2000	Safety data sheet. BASF DocID 2000/1013142 not GLP, unpublished CHE2001-405	Y	BAS
Kaltz, G.	AIIIA-4.1	2000	# 63 - 20 Corrosiveness of BAS 510 01 F. BASF DocID 2000/1018457 not GLP, unpublished PHY2001-332	Y	BAS
Kästel, R.	AIIIA-2.1; AIIIA-2.4; AIIIA-2.6; AIIIA-2.7; AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.5; AIIIA-2.8.6; AIIIA-2.8.8; AIIIA-4.1	2001	Shelf life in original container of the formulati-on BAS 510 01 F - 12 Month storage - Physi-cal and chemical properties INTERIM REPORT. BASF DocID 2001/1014636 GLP, unpublished PHY2002-84	Y	BAS
Ohnsorge, U.	AIIIA-4.2	2000	BAS 510 01 F; Effectiveness of Procedures for Cleaning Application Equipment and Protecti-ve Clothing. BASF DocID 2000/1017238 not GLP, unpublished PHY2001-331	Y	BAS
Schenk, W.	AIIA-3.9	2000	Possible procedures for the decontamination of water from BAS 510 F (proposed). 2000/1012375 not GLP, unpublished CHE2001-406	Y	BAS
Schreiner	AIIIA-4.1	2000	EU Performance Tests. BASF DocID 2000/1012266 not GLP, unpublished PHY2001-333	Y	BAS

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[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]
Bross, M.	AIIA-4.2.1	2001	Investigations on the extractability of 14C-BAS 510 F residues from plant matrices; study code 73479. 2001/1001739 GLP, unpublished MET2001-268	Y	BAS
Class, T.	AIIA-4.2.1	2001	Assessment and validation of the adapted multi-residue method DFG S19 for the determination of BAS 510 F and its metabolite M510F01 in animal matrices; report no. P/B 453 G. 2000/1017227 GLP, unpublished MET2001-261	Y	BAS
Eisert, R.	AIIA-4.1	1999	Validation of analytical method CP 290/1, determination of Reg.-No. 300355 in Reg.-No. 300355 (TGAI) using HPLC. 1999/10906 GLP, unpublished CHE2001-412	Y	BAS
Eisert, R.	AIIA-4.1	1999	Determination of Reg. No. 300355 in techn. active ingredient by HPLC. 1999/1003614 not GLP, unpublished CHE2001-395	Y	BAS
Fabian, E.	AIIA-4.2.1	2001	The validation of BASF method 476/0: the determination of BAS 510 F residues (as M510F53) in liver and milk by microwave treatment; study code 96997. 2000/1017224 GLP, unpublished MET2001-270	Y	BAS
Funk, H. und Mackenroth, Ch.	AIIA-4.2.1	2001	Validation of BASF method no. 445/0: determination of BAS 510 F in plant matrices; study code 41840. 2000/1012404 GLP, unpublished MET2001-266	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]
Funk, H.and Mackenroth, Ch.	AIIA-4.2.1	2001	Determination of the stability of 205259 (BAS 480 F), 242009 (BAS 49 F), 285028 (BAS 505 F) and 300355 (BAS 510 F) in different solvents; study code 41841. 2000/1014856 GLP, unpublished MET2001-258	Y	BAS
Grosshans, F.	AIIA-4.2.1	2001	The validation of BASF method 471/0: The determination of BAS 510F and the metabolite M510F01 in animal matrices; study code 42392. 2000/1017223 GLP, unpublished MET2001-269	Y	BAS
Grosshans, F.	AIIA-4.2.1	2001	The stability of BAS 510F and the metabolites M510F01, M510F49, M510F51 and M510F53 in Acetonitrile; study code 42393. 2000/1017225 GLP, unpublished MET2001-259	Y	BAS
Grote, Ch.	AIIA-4.2.3	2001	Validation of analytical method no. 411/0, GC/MS determination of BAS 510 F ai residues in surface water; study code 110241. 2001/1008955 GLP, unpublished MET2001-265	Y	BAS
Heinz, W.	AIIA-4.1	2001	Validation of Analytical Method CP 368: Determination of Impurities in Technical BAS 510 F by GC/MS. 2001/1000047 GLP, unpublished CHE2001-392	Y	BAS
Heinz, W.	AIIA-4.1	2001	Validation of Analytical Method CP 367: Determination of Impurities in Technical BAS 510 F by HPLC. 2001/1000046 GLP, unpublished CHE2001-391	Y	BAS
Heinz, W.	AIIA-4.1	2001	Method CP 368: Determination of Impurities in Technical BAS 510 F by GC/MS. 2000/1017127 not GLP, unpublished CHE2001-390	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]
Heinz, W.	AIIA-4.1	2001	Method CP 367: Determination of Impurities in Technical BAS 510 F by HPLC. 2000/1017126 not GLP, unpublished CHE2001-389	Y	BAS
Kampke-Thiel, K.	AIIA-4.2.1	2001	Independent laboratory validation of the adapted multi-residue method DFG S19 for the determination of BAS 510 F and its metabolite M510F01 in animal matrices; PTRL Europe Study No. P453G. 2000/1017226 GLP, unpublished MET2001-262	Y	BAS
Keller, W.	AIIA-4.2.2	1998	Validation of analytical method no. 408/1, GC-MS determination of BAS 510 F active ingredient residues in soil and sediment after methanol extraction; study code 48541. 1998/11314 GLP, unpublished MET2001-263	Y	BAS
Keller, W.	AIIA-4.2.3	1998	Validation of analytical method no. 411, determination of BAS 510 F ai residues in water; study no. 41877. 1998/10922 GLP, unpublished MET2001-264	Y	BAS
Reichert, N.	AIIA-4.2.1	2001	Independent laboratory validation of a method of analysis for the determination of BAS 510 F in white cabbage, rape (seed), hop, and lettuce; IF-100/35725-00. 2000/1014886 GLP, unpublished MET2001-267	Y	BAS
Türk, W.	AIIA-4.1	1998	Validation of HPLC-Method CP 290: Determination of Reg.-No. 300355 in Reg.-No. 300355 technical active ingredient (TGAI). 1998/10027 GLP, unpublished CHE2001-396	Y	BAS
Weeren, R.D. and Pelz, S.	AIIA-4.2.1	1999	Validation of DFG method S19 for the determination of BAS 510 F in various plant materials; Az. M8020/99. 1999/11461 GLP, unpublished MET2001-260	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	2000	Validation of analytical method 460, determination of BAS 510 F (Reg.no. 300355) in air by GC-MS; study code 41886. 2000/1014992 GLP, unpublished MET2001-271	Y	BAS
Ziegler, H.	AIIIA-5.1	1999	Validation of the analytical method CF-A 571: Determination of Reg.-No. 300355 in water dispersible granules (BAS 510 01 F). 1999/10419 GLP, unpublished CHE2001-414	Y	BAS
Ziegler, H.	AIIIA-5.1	1999	Determination of the content of the active ingredient Reg. No. 300355 in BAS 510 01 F using GC. 1999/1003818 not GLP, unpublished CHE2001-413	Y	BAS

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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]
Anonym	AIIA-5.8.1	1985	Chlorbenzoesäure und ihre Isomeren. German MAK Dokumentation, 1985, 1-4 1985/1000225 not GLP, published TOX2001-737	N	-
Anonym	AIIA-5.10	1999	Draft summary record: Commission group of specialised experts in the fields of carcinogenicity, mutagenicity and reprotoxicity. Meeting at Arona, 1-2 September 1999. European Chemicals Bureau., 1999, 1-12 1999/1004397 not GLP, published TOX2001-743	N	-
Anonym	AIIA-5.10	1979	Carbimazole and other antithyroid agents. Martindale The Extra Pharmacopoeia. Edited by N. W. Blacow and A. Wade, London The Pharmaceutical Press., 26, 1979, 379-385 1979/1000181 not GLP, published TOX2001-741	N	-
Anonym	AIIIA-7.2.3.1	1998	Policy science advisory council for exposure. From US EPA. #BASF 98/11675 not GLP, published TOX2001-702	N	-
Costigan, M.	AIIA-5.10	1998	The relevance of rat thyroid gland tumours to humans. HSE Toxicology Unit Bootle., 1998, 1-14 1998/1001262 not GLP, published TOX2001-742	N	-
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.1	1999	In vitro chromosome aberration assay with BAS 510 F in V79 cells. 32M0179/974076 ! 1999/10978 GLP, unpublished TOX2001-724	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.1	2000	In vitro unscheduled DNA synthesis (UDS) assay with BAS 510 F in primary rat hepatocytes. 81M0179/974096 ! 2000/1011413 GLP, unpublished TOX2001-725	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-5.4.1	2000	In vitro gene mutation test with BAS 510 F in CHO cells (HPRT locus assay). 50M0179/974097 ! 2000/1000180 GLP, unpublished TOX2001-723	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.1	1998	Salmonella typhimurium/escherichia coli reverse mutation assay (standard plate test and preincubation test) with BAS 510 F (reg. no. 300 355). 40M0179/974089 ! 98/11440 GLP, unpublished TOX2001-722	Y	BAS
Engelhardt, G. and Hoffmann, H.D.	AIIA-5.4.2	1999	Cytogenetic study in vivo with BAS 510 F in the mouse micronucleus test after two intraperitoneal administrations. 26M0179/974095 ! 1999/11048 GLP, unpublished TOX2001-726	Y	BAS
Gamer, A.O. and Hoffmann, H.D.	AIIA-5.2.3	1998	BAS 510 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure. 13I0179/977011 ! #BASF 98/10803 GLP, unpublished TOX2001-711	Y	BAS
Gamer, A.O. and Hoffmann, H.D.	AIIIA-7.1.1	2001	BAS 510 01 F - Acute oral toxicity study in Wistar rats. 10A0295/001046 ! 2001/1000119 GLP, unpublished TOX2001-696	Y	BAS
Gamer, A.O. and Hoffmann, H.D.	AIIIA-7.1.2	2001	BAS 510 01 F - Acute dermal toxicity study in rats. 11A0295/001047 ! 2001/1000120 GLP, unpublished TOX2001-697	Y	BAS
Gamer, A.O. and Hoffmann, H.D.	AIIIA-7.1.3	2000	BAS 510 01 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure. 13A0295/007008 ! 2001/1000121 GLP, unpublished TOX2001-698	Y	BAS
Grosshans, F. and Knoell, H.-E.	AIIA-5.1	2001	The metabolism of 14C-BAS 510F (reg. no. 300355) in rats. 41855 ! 2000/1017220 GLP, unpublished TOX2001-706	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]
Kaufmann, W., Schilling, K., Mellert, W. and Van Ravenzwaay, B.	AIIA-5.7	2001	BAS 510 F - Developmental neurotoxicity study in Wistar rats administration in the diet. 67R0179/97167 ! 2001/1000118 GLP, unpublished TOX2001-736	Y	BAS
Leibold, E. and Hoffmann, H.D.	AIIA-5.1	2001	14C-BAS 510 F - Study of the dermal absorption in rats. 01B0426/976047 ! 2001/1000111 GLP, unpublished TOX2001-704	Y	BAS
Leibold, E., Hoffmann, H.D. and Hildebrand, B.	AIIA-5.1	2000	14C-BAS 510 F - Study of the biokinetics in rats. 02B0426/976030 ! 2000/1014183 GLP, unpublished TOX2001-703	Y	BAS
Mellert, W., Deckardt, K., Kaufmann, W. and Hildebrand, B.	AIIA-5.3.1	2000	BAS 510 F - Repeated dose dermal toxicity study in Wistar rats administration for 4 weeks. 33C0179/97151 ! 2000/1013240 GLP, unpublished TOX2001-718	Y	BAS
Mellert, W., Deckardt, K., Kaufmann, W. and Hildebrand, B.	AIIA-5.3.2	2000	BAS 510 F - Subchronic oral toxicity study in C57BL mice administration in the diet for 3 months. 60C0179/97060 ! 2000/1000188 GLP, unpublished TOX2001-720	Y	BAS
Mellert, W., Deckardt, K., Kaufmann, W. and Hildebrand, B.	AIIA-5.3.2	2000	BAS 510 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months. 50S0179/97058 ! 2000/1012190 GLP, unpublished TOX2001-719	Y	BAS
Mellert, W., Deckardt, K., Kaufmann, W., Heider, K. and Van Ravenzwaay, B.	AIIA-5.5	2001	BAS 510 F - Carcinogenicity study in Wistar rats administration in the diet for 24 months. 82C0179/97090 ! 2001/1000115 GLP, unpublished TOX2001-729	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]
Mellert, W., Deckardt, K., Kaufmann, W., Heider, K. and Van Ravenzwaay, B.	AIIA-5.5	2001	BAS 510 F - Chronic toxicity study in Wistar rats administration in the diet for 24 months. 82C0179/97091 ! 2001/1000114 GLP, unpublished TOX2001-728	Y	BAS
Mellert, W., Deckardt, K., Küttler, K. and Van Ravenzwaay, B.	AIIA-5.5	2001	BAS 510 F - Carcinogenicity study in C57BL mice administration in the diet for 18 months. 76C0179/97103 ! 2001/1000116 GLP, unpublished TOX2001-730	Y	BAS
Mellert, W., Deckardt, K., Leibold, E. and Van Ravenzwaay, B.	AIIA-5.8.2	2001	BAS 510 F - Hormone and enzyme induction study in Wistar rats administration in the diet for 4 weeks. 99C0179/97174 ! 2001/1000141 GLP, unpublished TOX2001-739	Y	BAS
Mellert, W., Kaufmann, W. and Hildebrand, B.	AIIA-5.7	2000	BAS 510 F - Acute oral neurotoxicity study in Wistar rats. 20C0179/97144 ! 2000/1018638 GLP, unpublished TOX2001-734	Y	BAS
Mellert, W., Kaufmann, W. and Van Ravenzwaay, B.	AIIA-5.7	2001	BAS 510 F - Subchronic oral neurotoxicity study in Wistar rats administration in the diet for 3 months. 50C0179/97148 ! 2001/1000113 GLP, unpublished TOX2001-735	Y	BAS
Mellert, W., Kaufmann, W., Leibold, E., Deckardt, K. and Hildebrand, B.	AIIA-5.8.2	1999	BAS 510 F - Hepatic enzyme induction study in Wistar rats administration in the diet for 2 weeks. 99C0179/97063 ! 1999/10522 GLP, unpublished TOX2001-738	Y	BAS
Odell, W.D., Wilber, J.F. and Utiger, R.D.	AIIA-5.10	1967	Studies of thyrotropin physiology by means of radioimmunoassay. Recent Progress in Hormone Research. Edited by Gregory Pincus, Academic Press, New York and London., 23, 1967, 47-85 #BASF 67/10025 not GLP, published TOX2001-740	N	-

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. and Hellwig, J.	AIIA-5.6.2	2000	BAS 510 F - Prenatal developmental toxicity study in Himalayan rabbits oral administration (gavage). 40R0179/97127 ! 2000/1013425 GLP, unpublished TOX2001-733	Y	BAS
Schilling, K. and Hellwig, J.	AIIA-5.6.2	2000	BAS 510 F - Prenatal developmental toxicity study in Wistar rats oral administration (gavage). 30R0179/97140 ! 2000/1015001 GLP, unpublished TOX2001-732	Y	BAS
Schilling, K., Deckardt, K., Kaufmann, B. and Hildebrand, B.	AIIA-5.3.2	2000	BAS 510 F - Subchronic oral toxicity study in beagle dogs administration in the diet for 3 months. 31D0179/97101 ! 2000/1012306 GLP, unpublished TOX2001-721	Y	BAS
Schilling, K., Gembardt, C. and Van Ravenzwaay, B.	AIIA-5.6.1	2001	BAS 510 F - Two-generation reproduction toxicity study in Wistar rats continuous dietary administration. 70R0179/97136 ! 2001/1000117 GLP, unpublished TOX2001-731	Y	BAS
Thornley, K. and Bryson, S.	AIIA-5.1	2001	(14C)-BAS 510F: Rates of penetration through rat and human skin using an in vitro system. 729/204 ! 53H0179/979094 ! 2001/1000112 GLP, unpublished TOX2001-705	Y	BAS
Wiemann, C.	AIIA-5.2.1	2000	Amendment no. 1: BAS 510 F - Acute oral toxicity in rats. 10A0179/971052 ! 2000/1018715 GLP, unpublished TOX2001-708	Y	BAS
Wiemann, C.	AIIA-5.2.2	2000	Amendment no. 1: BAS 510 F - Acute dermal toxicity in rats. 11A0179/971053 ! 2000/1018711 GLP, unpublished TOX2001-710	Y	BAS
Wiemann, C.	AIIA-5.2.4	2000	Amendment no. 1: BAS 510 F - Acute dermal irritation / corrosion in the rabbit. 14H0179/972089 ! 2000/1018712 GLP, unpublished TOX2001-713	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.	AIIA-5.2.5	2000	Amendment no. 1: BAS 510 F - Acute eye irritation in the rabbit. 13H0179/972090 ! 2000/1018713 GLP, unpublished TOX2001-715	Y	BAS
Wiemann, C.	AIIA-5.2.6	2000	Amendment no. 1: BAS 510 F - Maximization test in guinea pigs. 30H0179/972091 ! 2000/1018714 GLP, unpublished TOX2001-717	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.1	1998	BAS 510 F - Acute oral toxicity in rats. 10A0179/971052 ! #BASF 98/10643 GLP, unpublished TOX2001-707	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.2	1998	BAS 510 F - Acute dermal toxicity in rats. 11A0179/971053 ! #BASF 98/10642 GLP, unpublished TOX2001-709	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.4	1998	Study on the acute dermal irritation/corrosion of BAS 510 F in the rabbit. 14H0179/972089 ! #BASF 98/10640 GLP, unpublished TOX2001-712	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.5	1998	Study on the acute eye irritation of BAS 510 F in the rabbit. 13H0179/972090 ! #BASF 98/10641 GLP, unpublished TOX2001-714	Y	BAS
Wiemann, C. and Hellwig, J.	AIIA-5.2.6	1998	BAS 510 F - Maximization test in guinea pigs. 30H0179/972091 ! #BASF 98/10638 GLP, unpublished TOX2001-716	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.4	2001	BAS 510 01 F - Acute dermal irritation / corrosion in rabbits. 18H0295/002086 ! 2001/1000122 GLP, unpublished TOX2001-699	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.5	2001	BAS 510 01 F - Acute eye irritation in rabbits. 11H0295/002087 ! 2001/1000123 GLP, unpublished TOX2001-700	Y	BAS
Wiemann, C. and Hellwig, J.	AIIIA-7.1.6	2001	BAS 510 01 F - Modified Buehler Test (9 inductions) in guinea pigs. 33H0295/002088 ! 2001/1000124 GLP, unpublished TOX2001-701	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., Deckardt, K., Kaufmann, W., Kolling, A. and Hildebrand, B.	AIIA-5.5	2000	BAS 510 F - Chronic oral toxicity study in beagle dogs administration in the diet for 12 months. 33D0179/97118 ! 2000/1016881 GLP, unpublished TOX2001-727	Y	BAS

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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]
Blaschke, U.	AIIA-6.3	2001	Determination of the Magnitude of the Residue of the Active ingredient of BAS 510 00 F and BAS 510 01 F in/on Grape Raw Agricultural Commodity Specimens from Supervised field Trials in Northern and Southern France in 1999. BASF DocID: 2000/1000251 GLP, unpublished RIP2001-336	Y	BAS
Blaschke, U.	AIIA-6.3	2001	Determination of the magnitude and decline of the residue of BAS 510 KA F in/on grape raw agricultural commodity specimens from supervised field trials in Northern and Southern France in 1998. BASF DocID: 2000/1000250 GLP, unpublished RIP2001-333	Y	BAS
Fabian, E.; Grosshans, F.	AIIA-6.2	2001	The Metabolism of 14C-BAS 510F in Lactaing Goat. BASF DocID: 2000/1017221 GLP, unpublished RIP2001-331	Y	BAS
Funk, H.; Mackenroth, C.	AIIA-6.3	2001	Investigation of the Stability of Residues of BAS 510 F in Plant Matrices under normal Storage Conditions. BASF DocID: 2000/1014855 GLP, unpublished RIP2001-351	Y	BAS
Funk, H.; Mackenroth, C.	AIIA-6.6	2001	Determination of the residues of BAS 510 F in wheat obtained from the trial year 2000. BASF DocID.: 2000/1000989 GLP, unpublished RIP2001-375	Y	BAS
Funk, H.; Mackenroth C.	AIIA-6.6	2000	Determination of the residues of BAS 510 F in wheat obtained from the trial year 2000. BASF DocID.: 2000/1014853 GLP, unpublished RIP2001-374	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]
Funk, Horst; Mackenroth, Christiane	AIIA-6.0	2001	Investigation of the Stability of Residues of BAS 510 F in Plant Matrices under Storge Conditions. 2001/1015028 GLP, unpublished RIP2002-192	Y	BAS
Grosshans, F.	AIIA-6.4	2001	Investigation of the Stability of Residues of BAS 510 F and M510F01 in Sample Material of Animal Origin under Usual Storage Conditi-ons. BASF DocID: 2000/1017229 GLP, unpublished RIP2001-354	Y	BAS
Hamm, R.T.	AIIA-6.1	1999	Metabolism of BAS 510 F in Lettuce. BASF DocID: 1999/11240 GLP, unpublished RIP2001-328	Y	BAS
Hamm, T.R.; Veit, P.	AIIA-6.6	2001	Confined Rotational Crop Study with 14C-BAS 510 F. BASF DocID.: 2000/1014862 GLP, unpublished RIP2001-373	Y	BAS
Heck, W.; Funk, H.; Mackenroth, C.	AIIA-6.3	2001	Study on the residue behavior of BAS 510 F in peas after treatment with BAS 510 01 F under field conditions in Germay, Denmark and Swe-den, 1999. BASF DocID: 2000/1014848 GLP, unpublished RIP2001-347	Y	BAS
Heck, W.; Mackenroth, C.	AIIA-6.3	2001	Study on the residue behavior of BAS 510 F in peas after treatment with BAS 510 01 F under field conditions in Germay, Denmark and Swe-den, 2000. BASF DocID: 2000/1014852 GLP, unpublished RIP2001-349	Y	BAS
Leibold, E.; Hoffmann, H.D.	AIIA-6.2	2000	14C-BAS 510 F- Absorption, Distribution and Excretion after Repeated Oral Administration in Lactating Goats. BASF DocID: 2000/1012353 GLP, unpublished RIP2001-330	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.; Funk, H.; Mackenroth, C.	AIIA-6.3	2000	Study on the residue behavior of BAS 510 F in grapes after treatment with BAS 510 00 F and BAS 510 01 F under field conditions in Germany and Spain, 1999. BASF DocID: 2000/1012411 GLP, unpublished RIP2001-337	Y	BAS
Meumann, H.; Funk, H.; Mackenroth, C.	AIIA-6.3	2000	Study on the residue behavior of BAS 510 F in grapes after treatment with BAS 510 KA F under field conditions in Germany and Spain, 1998. BASF DocID: 2000/1012410 GLP, unpublished RIP2001-334	Y	BAS
Meumann, H.; Funk, H.; Mackenroth, C.	AIIA-6.5.2	2000	Study on the residue behavior of BAS 510 F in grapes in grapes and grape process fractions after treatment with BAS 510 01 F under field conditions in Germany, 1999. BASF DocID: 2000/1012412 GLP, unpublished RIP2001-356	Y	BAS
Nietschmann, D.; Lam W.W.	AIIA-6.2	2000	Nature of Residues of 14C-BAS 510 F in Laying Hens. BASF Doc No.: 2000/5154 GLP, unpublished RIP2001-332	Y	BAS
Perny, A.	AIIA-6.3	2001	Residue study in Green Peas following treatment with the preparation BAS 510 01 F under Field conditions in France in 2000. BASF DocID: 2000/1014878 GLP, unpublished RIP2001-350	Y	BAS
Perny, A.	AIIA-6.3	2001	Residue study in Beans following treatment with the preparation BAS 510 01 F under Field conditions in France in 2000. BASF DocID: 2000/1014876 GLP, unpublished RIP2001-346	Y	BAS
Perny, A.	AIIA-6.3	2001	Residue study in Oil Seed Rape following treatment with the preparation BAS 510 01 F under Field conditions in France in 2000. BASF DocID: 2000/1014877 GLP, unpublished RIP2001-341	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]
Rabe, U.; Schlüter, H.	AIIA-6.1	2001	Metabolism of BAS 510 F in Grapevine. BASF DocID: 2000/1014860 GLP, unpublished RIP2001-327	Y	BAS
Raunft, E.	AIIA-6.3	2001	Study on the residue behavior of BAS 510 F in winter rape after treatment with BAS 510 01 F under field conditions in Germany, Sweden and Great Britain, 2000. BASF DocID: 2000/1014851 GLP, unpublished RIP2001-340	Y	BAS
Raunft, E.; Funk, H.; Mackenroth, C.	AIIA-6.3	2000	Determination of the residues of BAS 510 F in winter rape following treatment with BAS 510 01 F under field conditions in Denmark, France, Germany and Great Britain, 1999. BASF DocID: 2000/1012409 GLP, unpublished RIP2001-339	Y	BAS
Scharf, J.	AIIA-6.5.1	1998	Hydrolysis of BAS 510 F at 90°C, 100°C, and 120°C. BASF Doc.: 1998/10878 GLP, unpublished RIP2001-355	Y	BAS
Scharm, M.	AIIA-6.5.2	2001	Determination of the Residues of Reg. No. 300355 in Peas and Processed Products following treatment with BAS 510 01 F under field Conditions in Germany 2000. BASF DocID.: 2000/1014885 GLP, unpublished RIP2001-372	Y	BAS
Schulz, H.	AIIA-6.3	2001	Determination of the Residues of BAS 510 F in Peas following treatment with BAS 510 01 F under field Conditions in France 1999. BASF DocID: 2000/1014879 GLP, unpublished RIP2001-348	Y	BAS
Schulz, H.	AIIA-6.3	2000	Determination of the Residues of BAS 510 F in Grapes Following Treatment with BAS 510 00 F and BAS 510 01 F under field conditions in Italy and France 1999. BASF DocID: 2000/1014880 GLP, unpublished RIP2001-338	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]
Schulz, H.	AIIA-6.3	2000	Determination of the Residues of Reg. No. 300355 in Grapes following Treatment with bas 510 KA F under Field Conditions in Italy and Northern France 1998. BASF DocID: 2000/1014881 GLP, unpublished RIP2001-335	Y	BAS
Tilting, N.	AIIA-6.4	2001	Residues in Milk and Edible Tissues Following Oral Administration of BAS 510 F to Lactating Dairy Cattle. BASF DocID: 2000/1017228 GLP, unpublished RIP2001-352	Y	BAS
Treiber, S.	AIIA-6.3	2001	Study on the residue behavior of BAS 510 F in bush beans after treatment with BAS 510 01 F under field conditions in Denmark, France and Germany, 2000. BASF DocID: 2000/1014850 GLP, unpublished RIP2001-345	Y	BAS
Treiber, S.; Funk, H.; Mackenroth, C.	AIIA-6.3	2001	Study on the residue behavior of BAS 510 F in bush- and climbing beans after treatment with BAS 510 01 F under greenhouse conditions in Spain, 2000. BASF DocID: 2000/1014849 GLP, unpublished RIP2001-344	Y	BAS
Treiber, S.; Funk, H.; Mackenroth, C.	AIIA-6.3	2000	Study on the residue behavior of BAS 510 F in bush-and climbing beans after treatment with BAS 510 01 F under greenhouse condition in Spain, 1999. BASF DocID: 2000/1014847 GLP, unpublished RIP2001-343	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]
Treiber, S.; Funk, H.; Mackenroth, C.	AIIA-6.3	2000	Study on the residue behavior of BAS 510 F in bush beans after treatment with BAS 510 01 F under field conditions in Germany and Denmark, 1999. BASF DocID: 2000/1014846 GLP, unpublished RIP2001-342	Y	BAS
Veit, P.	AIIA-6.1	2001	Metabolism of 14C-BAS 510 F in Beans. BASF DocID: 2000/1014861 GLP, unpublished RIP2001-329	Y	BAS

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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]
anonym	AIIIA-9.1.3; AIIIA-11	2001	Fate and behaviour in the environment. Document M-III not GLP, unpublished BOD2001-329	Y	BAS
anonym	AIIIA-9.2.1; AIIIA-9.2.3; AIIIA-11	2001	Fate and behaviour in the environment. Document M-III not GLP, unpublished WAS2001-152	Y	BAS
Anonym	AIIIA-9.1.3; AIIIA-11	2001	Fate and behaviour in the environment. Document M-III not GLP, unpublished LUF2001-145	Y	BAS
Bayer, H. u. Grote, Ch.	AIIA-7.1.1.2.2	2001	Field soil dissipation of BAS 510 F (300355) in formulation BAS 510 KA F. BASF Reg.Doc.#2000/10 13295 GLP, unpublished BOD2001-292	Y	BAS
Ebert, D.	AIIA-7.2.1.3.2	2000	Degradation of BAS 510 F in Aerobic Aquatic Environment. BASF DocID 2000/1000135 GLP, unpublished WAS2001-148	Y	BAS
Ebert, D. u. Harder, U.	AIIA-7.1.1.2.1	2000	Degradation of 14C-chloronicotinic acid (Reg.No. 107371) in soil under aerobic conditions. BASF Doc ID 2000/1013280 GLP, unpublished BOD2001-286	Y	BAS
Ebert, D. u. Harder, U.	AIIA-7.1.1.2.1	2000	The Degradation Behaviour of 14C-BAS 510 F in Different Soils (DT50 / DT90). BASF Doc ID 2000/1013279 GLP, unpublished BOD2001-284	Y	BAS
Gottesbüren, B.	AIIIA-9.2.3	2001	Calculation of Predicted Environmental Concentrations (PECsed) for BAS 510 F in Sediment. BASF DocID 2000/1017053 not GLP, unpublished WAS2001-159	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]
Gottesbüren, B.	AIIIA-9.2.3	2001	Calculation of Predicted Environmental Concentrations (PECsw) for BAS 510 F in Static Surface Water. BASF DocID 2000/1017051 not GLP, unpublished WAS2001-158	Y	BAS
Götz, N.	AIIA-7.2.2	2000	Volatilization of BAS 510 F after Application of BAS 510 01 F on Soil and on Plant Surface. BASF DocID 2000/1014979 GLP, unpublished LUF2001-134	Y	BAS
Hauck, T.	AIIA-7.1.1.2.2; AIIIA-9.1.3	2001	Calculation of the Accumulation Potential and the Predicted Environmental Concentrations for BAS 510 F in Soil (PECsoil) after Repeated Application. BASF DocID 2000/1017050 not GLP, unpublished BOD2001-301	Y	BAS
Hauck, T.	AIIIA-9.1.3	2001	Calculation of Predicted Environmental Concentrations for BAS 510 F in Soil (PECsoil). BASF DocID 2000/1017049 not GLP, unpublished BOD2001-330	Y	BAS
Hein, W.	AIIA-7.1.1.2.1	1998	Influence of a Pretreatment with BAS 510 F on the Degradation of BAS 510 F in Soil (Einfluss der Vorbehandlung mit BAS 510 F auf das Abbauverhalten von BAS 510 F im Boden). BASF98/ 10607 GLP, unpublished BOD2001-290	Y	BAS
Kellner, O.	AIIA-7.1.1.2.1	1999	Degradation of 14C-Reg. No. 363 487 in Aerobic Soil. BASF Reg.Doc.#1999/11102 GLP, unpublished BOD2001-285	Y	BAS
Kellner, O.	AIIA-7.2.1.3.2	2001	Degradation and distribution of BAS 510 F in a water-sediment system under outdoor conditions. BASF DocID 2000/1017038 GLP, unpublished WAS2001-149	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. u. Grote, Ch.	AIIA-7.1.1.2.2	2001	Accumulation behaviour of BAS 510 F under field conditions over a 3-year-period (1998 - 2000) after application onto vegetables. BASF DocID 2000/1017040 GLP, unpublished BOD2001-296	Y	BAS
Kellner, O. u. Grote. Ch.	AIIA-7.1.1.2.2	2001	Accumulation behaviour of BAS 510 F under field conditions over a 3-year-period (1998-2000) after application onto grapes in a viney-ard. BASF DocID 2000/1017039 GLP, unpublished BOD2001-294	Y	BAS
Kellner, O. u. Keller, W.	AIIA-7.1.1.2.2	2000	Field soil dissipation of BAS 510 F (300 355) in formulation BAS 510 KB F (1997 - 1998). BASF DocID 2000/1000123 GLP, unpublished BOD2001-291	Y	BAS
Platz, K.	AIIA-7.1.1.2.2; AIIIA-9.1.3	2001	Comparison of actual residues in soil after repeated application of BAS 510 F (accumula-tion study) with expected residues for repeated applications onto vegetable crops. BASF DocID 2000/1017046 not GLP, unpublished BOD2001-298	Y	BAS
Platz, K.	AIIA-7.1.1.2.2; AIIIA-9.1.3	2001	Comparison of actual residues in soil after repeated application of BAS 510 (accumulati-on study) with expected residues for repeated applications onto a viney-ard . BASF DocID 2000/1017045 not GLP, unpublished BOD2001-295	Y	BAS
Platz, K.	AIIA-7.1.1.2.2; AIIIA-9.1.3	2001	Assessment whether field dissipation studies with BAS 510 F can be used to estimate trans-formation rates in soil and standardisation of half-lives to reference conditions. BASF Doc ID 2000/1017044 not GLP, unpublished BOD2001-293	Y	BAS
Platz, K.	AIIA-7.2.1.3.2	2001	Estimation of the kinetic parameters of the degradation of BAS 510 F in an aerobic water sediment system under outdoor conditions. BASF DocID 2000/1017047 not GLP, unpublished WAS2001-150	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.	AIIIA-9.2.1; AIIIA-9.2.3	2001	Assessment whether field dissipation studies with BAS 510 F can be used to estimate trans-formation rates in soil and standardisation of half-lives to reference conditions. BASF Doc ID 2000/1017044 not GLP, unpublished WAS2001-133	Y	BAS
Richter, T.	AIIA-7.1.3.1; AIIA-7.1.3.2	2001	Investigation of the leaching behaviour of aged and non-aged BAS 510 F residues in soil. BASF DocID 2000/1000965 not GLP, unpublished BOD2001-320	Y	BAS
Richter, T.	AIIA-7.1.3.1; AIIA-7.1.3.2	2001	Investigation of the leaching behaviour of aged and non-aged BAS 510 F residues in soil. BASF DocID 2000/1017037 GLP, unpublished BOD2001-305	Y	BAS
Seher, A	AIIA-7.1.2	1998	Soil Adsorption / Desorption Study of 300 355 (Bas 510 F). Reg. Doc.# BASF 98/10513 GLP, unpublished BOD2001-303	Y	BAS
Staudenmaier, H.	AIIA-7.1.1.1.2	2000	Anaerobic metabolism of BAS 510 F in soil (14C-pyridine-label). BASF DocID 2000/1014990 GLP, unpublished BOD2001-288	Y	BAS
Staudenmaier, H. u. Schäfer, C.	AIIA-7.1.1.1.2	2000	Anaerobic metabolism of BAS 510 F in soil (diphenyl-14C-label). BASF DocID 2000/1014986 GLP, unpublished BOD2001-287	Y	BAS
Stephan, A.	AIIA-7.1.1.1.1; AIIA-7.1.1.2.1	1999	Metabolism of BAS 510 F (14C-Diphenyl and 14C-Pyridin) in soil under aerobic conditions. BASF Reg.Doc.#1999/11807 GLP, unpublished BOD2001-283	Y	BAS
Stephan, A.	AIIA-7.1.1.2.1; AIIIA-9.1.3	2000	Storage stability of BAS 510 F in soil. BASF DocID 2000/1000136 GLP, unpublished BOD2001-302	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	2001	Calculation of Predicted Environmental Concentrations (PECgw) for BAS 510 F in Groundwater on an European Level. BASF DocID 2000/1017048 not GLP, unpublished WAS2001-157	Y	BAS
von Götz, N.	AIIA-7.1.1.1.2	2000	Soil Photolysis of BAS 510 F. BASF DocID 2000/1014989 GLP, unpublished BOD2001-289	Y	BAS
Werner, D.I.	AIIA-7.2.1.3.1	1999	Determination of the Biodegradabiltity of BAS 510 F in the Manometric Respirometry Test according to GLP, EN 45001 and ISO 9002. Reg.Doc.#BASF 99/10290 GLP, unpublished WAS2001-147	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]
Bühler, A.	AIIIA-10.5.1	2000	Effect of BAS 510 01 F on the ground dwelling predator Poecilus cupreus (Coleoptera, Carabidae) in a laboratory trial. 2000/1012479 GLP, unpublished ANA2001-427	Y	BAS
Chapleo, S., Caley, C.Y.	AIIA-8.2.3	2000	Bioaccumulation and metabolism of [14C]-BAS 510F in rainbow trout. 2000/1017222 ! 394178 ! 18219 GLP, unpublished WAT2001-367	Y	BAS
Dohmen, P.	AIIA-8.2.4	2001	Effect of BAS 510 F on the immobility of Daphnia magna Straus in a 48 hour static, acute toxicity test. 2000/1018537 ! 41898 GLP, unpublished WAT2001-378	Y	BAS
Dohmen, P.	AIIA-8.2.7	2001	Effects of BAS 510 F on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system. 2000/1018538 ! 44152 GLP, unpublished WAT2001-381	Y	BAS
Ehlers, E.	AIIIA-10.6.1	2002	Addendum No. 1 to Final Report: 7584023 Field Studie to Evaluate the Effects of BAS 510 01 F on Earthworms. 2002/1000252 GLP, unpublished ARW2002-14	Y	BAS
Ehlers, H.A.	AIIIA-10.6.1	2001	Interim Report: Field Study to Evaluate the Effects of BAS 510 01 F on Earthworms (Season 2000). 2001/1000102 GLP, unpublished ARW2001-79	Y	BAS
Ehlers,H.A.	AIIIA-10.6.1.3	2001	Final Report: Field Study to Evaluate the Effects of BAS 510 01 F on Earthworms. 2001/1014681 GLP, unpublished ARW2001-169	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]
Goßmann, A.	AIIIA-10.5.1	2000	Effects of BAS 510 01 F on the Predatory Mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the Laboratory - Dose Response Design -. 2000/1018520 GLP, unpublished ANA2001-425	Y	BAS
Hisgen	AIIA-8.2.5	2001	BAS 510 F - Determination of the chronic effect on the reproduction of the water flea Daphnia magna Straus. 2000/1018539 ! 00/0618/51/2 GLP, unpublished WAT2001-379	Y	BAS
Hommen, U.	AIIIA-10.6.1.3	2002	Final Report: Statistical evaluation of the earthworm field study with the test substance BAS 510 01 F (study code: 61000; BASF Doc ID 2001/1014661). BASF DocID 2002/1004385 not GLP, unpublished ARW2002-44	Y	BAS
Ipach, R.	AIIIA-10.5.1	2000	Effects of "BAS 510 01 F" on predatory mites (Typhlodromus pyri) under typical vine culture conditions on grape vines, Germany 2000. 2000/1014931 GLP, unpublished ANA2001-429	Y	BAS
Jatzek, H.-J.	AIIIA-10.2.1	2001	BAS 510 01 F - Determination of the acute effect on the swimming ability of the water flea Daphnia magna Straus. 2000/1018540 ! 00/0295/50/1 GLP, unpublished WAT2001-384	Y	BAS
Kling, A.	AIIIA-10.4	2000	Assessment of the Side Effects of BAS 510 01 F to the Honey Bee, Apis mellifera L. in the Laboratory. 20001059/01-BLEU GLP, unpublished BIE2001-25	Y	BAS
Krieg, W.	AIIIA-10.6.1	2001	Interim Report: Field study to evaluate the effects of BAS 510 01 F earthworms (season 2000). 2001/1000101 GLP, unpublished ARW2001-78	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	2000	Effects of BAS 510 01 F on Growth and Reproduction of the Earthworm Eisenia foetida. 2000/1011482 GLP, unpublished ARW2001-77	Y	BAS
Krieg, W.	AIIIA-10.6.1.3	2001	Final Report: Field study to evaluate the effects of BAS 510 01 F on earthworms. 2001/1014661 GLP, unpublished ARW2001-168	Y	BAS
Krieg, W.	AIIIA-10.6.2	2001	Effects of BAS 510 01 F on the organic matter degradation under field conditions (litter bag method) study 2. 2001/1000107 GLP, unpublished ARW2001-83	Y	BAS
Krieg, W.	AIIIA-10.6.2	2001	Effects of BAS 510 01 F on the organic matter degradation under field conditions (litter bag method) study 1. 2001/1000106 GLP, unpublished ARW2001-82	Y	BAS
Krieg, W. and Schick, H.	AIIIA-10.6.2	2001	Monitoring of Collembola populations following an exposure to BAS 510 01 F in the field (grassland). 2001/1000105 GLP, unpublished ARW2001-81	Y	BAS
Kubitza, J.	AIIA-8.2.6	2001	Effect of BAS 510 F on the growth of the green alga Pseudokirchneriella subcapitata. 2000/1018524 ! 41893 GLP, unpublished WAT2001-380	Y	BAS
Kubitza, J.	AIIIA-10.2.1	2001	Effect of BAS 510 01 F on the growth of the green alga Pseudokirchneriella subcapitata. 2000/1018525 ! 59064 GLP, unpublished WAT2001-385	Y	BAS
Lührs, U.	AIIA-8.4.1	1999	Acute Toxicity (14 Days) of BAS 510 to the Earthworm Eisenia fetida (Savigny 1826) in Artificial Soil. 1999/10816 GLP, unpublished ARW2001-84	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]
Lührs, U.	AIIIA-10.6.1	2000	Acute Toxicity (14 Days) of BAS 510 01 F to the Earthworm Eisenia fetida (Savigny 1826) in Artificial Soil. 2000/1014952 GLP, unpublished ARW2001-76	Y	BAS
Meister, A.	AIIIA-10.6.2	2001	Effects of BAS 510 01 F on Reproduction of the Collembola Folsomia candida in Artificial soil. 2001/1015075 GLP, unpublished ARW2002-15	Y	BAS
Meister, A.	AIIIA-10.6.2	2001	Effects of BAS 510 01 F on Reproduction of the Collembola Folsomia candida in Artificial Soil. 2001/1000104 GLP, unpublished ARW2001-80	Y	BAS
Moll, M. and Groer, M.	AIIIA-10.5.1	2000	Effects of BAS 510 01 F on the Parasitoid Aphidius rhopalosiphi (Hymenoptera, Braconidae) in the Laboratory - Dose Response Test -. 2000/1018519 GLP, unpublished ANA2001-424	Y	BAS
Müther, J.	AIIIA-10.5.1	2001	A Field Study to Evaluate the Effects of BAS 510 01 F Against the Predatory Mite Typhlodromus pyri Scheuten in Vines. 2000/1014938 GLP, unpublished ANA2001-430	Y	BAS
Nienstedt, K.	AIIIA-10.5.1	2001	BAS 510 01 F: Acute Toxicity Test With Spiders, Pardosa Sp: (Araneae: Lycosidae). 2000/1018518 GLP, unpublished ANA2001-428	Y	BAS
Oberwalder, Ch. and Schmidt, O.	AIIA-8.6; AIIIA-10.8	2000	BAS 510 01 F: Effects on non-target plants in the greenhouse - A limit test. 2000/1018515 GLP, unpublished PFL2001-63	Y	BAS
Sack, D.	AIIA-8.3	1999	Effect of Reg. No. 300 355 on the Honeybee (Apis mellifera L.) in Laboratory Trials. BASF 1999/10823 GLP, unpublished BIE2001-27	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]
Ufer, A.	AIIIA-10.5.1	2001	Effect of BAS 510 01 F on Populations of the Predatory Mite Typhlodromus Pyri, Scheuten in a Field Study (Vineyard). 2000/1014939 GLP, unpublished ANA2001-431	Y	BAS
Ufer, A.	AIIIA-10.5.1	2000	Effect of BAS 510 01 F on the Green Lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in a Laboratory Trial. 2000/1014932 GLP, unpublished ANA2001-426	Y	BAS
Wachter, S.	AIIIA-10.7.1	2001	Assessment of the Side Effects of BAS 510 01 F on the Activity of the Soil Microflora, Nitrogen Turnover. 2000/1018517 GLP, unpublished BMF2001-66	Y	BAS
Wachter, S.	AIIIA-10.7.1	2001	Assessment of the Side Effects of BAS 510 01 F on the Activity of the Soil Microflora, Short-Term Respiration. 2000/1018516 GLP, unpublished BMF2001-65	Y	BAS
Werner, D.I.	AIIA-8.7	1999	Determination of the inhibition of oxygen consumption by activated sludge by BAS 510 F in the activated sludge respiration inhibition test according to GLP, EN 45001 and ISO 9002. 99/10289 ! 98/0715/08/1 GLP, unpublished WAT2001-382	Y	BAS
Zok, S.	AIIA-8.1.1	1999	Report BAS 510 F - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus). 11W0179/97043 GLP, unpublished AVS2001-119	Y	BAS
Zok, S.	AIIA-8.1.2	1999	Test Report: BAS 510 F - Avian dietary LC50 test in chicks of the mallard duck (Anas platyrhynchos). 32W0179/97045 GLP, unpublished AVS2001-121	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-8.1.2	1999	Test Report: BAS 510 F - Avian dietary LC50 test in chicks of the bobwhite quail (Colinus virginianus). 31W0179/97042 GLP, unpublished AVS2001-120	Y	BAS
Zok, S.	AIIA-8.1.3	2000	Report: BAS 510 F - 1-generation reproduction study on the mallard duck (Anas platyrhynchos) by administration in the diet. 72W0179/97122 GLP, unpublished AVS2001-123	Y	BAS
Zok, S.	AIIA-8.1.3	2000	Report: BAS 510 F - 1-generation reproduction study on the bobwhite quail (Colinus virginianus) by administration in the diet + Amendment No. 1 to the report. 71W0179/97044 GLP, unpublished AVS2001-122	Y	BAS
Zok, S.	AIIA-8.2.1	2001	BAS 510 F Acute toxicity study on the bluegill (Lepomis macrochirus Raf.) in a static system (96 hours). 2001/1001727 ! 14F0179/975132 GLP, unpublished WAT2001-364	Y	BAS
Zok, S.	AIIA-8.2.1	2001	BAS 510 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 2001/1001726 ! 12F0179/975131 GLP, unpublished WAT2001-363	Y	BAS
Zok, S.	AIIA-8.2.2	1999	BAS 510 F - Sublethal toxic effects on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a flow-through system (28 days). 1999/10927 ! 42F0179/975054 GLP, unpublished WAT2001-365	Y	BAS
Zok, S.	AIIA-8.2.2.1	1999	BAS 510 F - Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss Walbaum 1792). 1999/11847 ! 52F0179/975051 GLP, unpublished WAT2001-366	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	2000	BAS 510 01 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 2000/1018528 ! 12F0295/005010 GLP, unpublished WAT2001-383	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Monograph

08 November 2002

Nicobifen

Alternative common name proposed to ISO in August 2002:
Boscalid

Volume 3

Annex B

Summary, Scientific Evaluation and Assessment

Rapporteur Member State: Germany

Contents

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

B-1: Identity

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)

Applicant:

BASF Aktiengesellschaft
Agricultural Center
Product Registration Management
P.O. Box 120
D-67114 Limburgerhof

Contact:

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Telephone: +49 (0) 621 60-27343
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Telephone: +49 (0) 621 60-27300
Telefax: +49 (0) 621 60-28135

BASF Agro S.A.S
Departement Homologation
49, Avenue Georges Pompidou
F-92300 Levallois-Perret

Christine Tourateir
Telephone: +33 (1) 49 64 54 43
Telefax: +33 (1) 49 64 57 29

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

Nicobifen (ISO, proposed)

B.1.1.3 Chemical name (Annex IIA 1.4)

IUPAC: 2-Chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide

CAS: 2-Chloro-*N*-(4'-chloro[1,1'-biphenyl]-2-yl)-3-pyridinecarboxamide

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

BAS 510 F, Reg. No. 300355, PS 300355

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

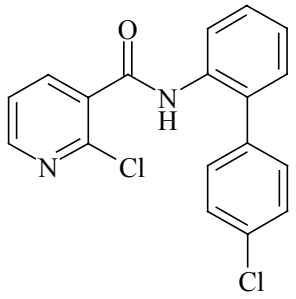
CAS: 188425-85-6
CIPAC: 673
EEC: not assigned
EINECS: not assigned

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

Molecular formular: $C_{18}H_{12}Cl_2N_2O$

Molecular mass: 343.21 g/mol

Structural formula:



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

Manufacturer:
BASF Aktiengesellschaft
Crop Protection Division
P.O. Box 120
D-67114 Limburgerhof

Person to contact: Dr. Wolfgang Türk
Production Crop Protection
Telephone: +49 (0) 621 60-79145
Telefax: +49 (0) 621 60-79519

Manufacturing site:
Pilot plant at BASF AG, Ludwigshafen

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)

≥ 960 g/kg (minimum purity)

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 510 01 F" (preliminary designator)
(country specific alternatives are under consideration)

Code Number:	Plant Protection Product:	BAS 510 01 F
	Active Substance:	BAS 510 F (proposed common name: nicobifen)
	BASF internal No.	Reg. No. 300355

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)

Water dispersible granule (WG)

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	Heinz, W.	2001	BAS 510 F Description of the Manufacturing Process. 2001/1000043 not GLP, unpublished CHE2001-407	Y	BAS
AIIA-1.8	Schmider, F. and Becker-Arnold, R.	2001	Confidential information from Document M-II. not GLP, unpublished CHE2001-559	N	BAS
AIIA-1.9; AIIA-1.10	Heinz, W.	2001	BAS 510 F (proposed common name: Nicobi-fen) TGAI Composition of the Technical Grade Active Ingredient. 2001/1000044 not GLP, unpublished CHE2001-408	Y	BAS
AIIA-1.11	Heinz, W.	2001	Characterization of the BAS 510 F Toxicology Batches (N 26, N37, N 46) Determination of the Active Ingredient and Impurities. 2001/1000841 GLP, unpublished CHE2001-410	Y	BAS
AIIA-1.11	Heinz, W.	2001	Characterization of five Batches of BAS 510 F Technical Grade Active Ingredient Determination of Active Ingredient and Pro-cess-related Impurities. 2001/1000045 GLP, unpublished CHE2001-409	Y	BAS
AIIIA-1.4	Anonymous	1999	Safety data sheet: Wettol D 3. BASF DocID 1999/1004035 not GLP, unpublished BEI2001-193	N	BAS
AIIIA-1.4	Anonymous	1996	Safety data sheet: Ufoxane 3A. Reg. Doc.#BASF 96/11290 not GLP, unpublished BEI2001-192	N	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	Anonymous	2000	Safety data sheet: Wacker Antifoam Emulsion SRE. BASF DocID 2000/1018666 not GLP, unpublished BEI2001-191	N	BAS
AIIIA-1.4	Anonymous	2001	Safety data sheet: Ammonium Sulfate Industrial Grade. BASF DocID 2001/1001807 not GLP, unpublished BEI2001-190	N	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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 nicobifen

PAS: Pure active substance (min purity: 99.4 %)
TAS: Technical active substance (purity: 98.18 %)

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	Y	142.8-143.8 °C (capillary method) 145.0 °C (DSC method)	Acceptable	Daum, 1999 (CHE2001-397)
B.2.1.1.2 (IIA 2.1)	Boiling point of purified active substance	PAS	EEC A 2 (DSC method)	Y	The as decomposed at ca 300 °C.	Acceptable	Daum, 1999 (CHE2001-397)
B.2.1.1.3 (IIA 2.1)	Temperature of decomposition or sublimation	PAS	EEC A 2 (DSC method)	Y	According to the author the DSC plot show at ca 300 °C an exothermic effect which can be interpreted as decomposition. No further endothermic effect was observed. Therefore, sublimation or boiling of the as can be excluded.	Acceptable	Daum, 1999 (CHE2001-397)

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 method)	Y Y	d_4^{20} = 1.381 d_4^{20} = 1.394	Acceptable not required additional info	Kästel, 1999 (CHE2001-399) Kästel, 1998 (CHE2001-398)
B.2.1.3.1 (IIA 2.3)	Vapour pressure of purified active substance	PAS	Weight loss per area and time (internal method used)	Y	7.2×10^{-9} hPa at 20°C 1.5×10^{-8} hPa at 25°C	Acceptable	Kästel R, 1999 (LUF2001-146)
B.2.1.3.2 (IIA 2.3)	Volatility, Henry's law constant of purified active substance	PAS	Calculation	N (not requir)	5.178×10^{-8} (kPa m³/mol)	Acceptable	Ohnsorge U, 2000 (LUF2001-147)
B.2.1.4.1 (IIA 2.4)	Appearance: physical state	PAS TAS	Visual assessment	Y	PAS: crystalline solid TAS: powder, solid		Daum, 1999 (CHE2001-397) Kästel, 1998 (CHE2001-398)
B.2.1.4.2 (IIA 2.4)	Appearance: colour	PAS TAS	Visual assessment	Y	PAS: white TAS: white		Daum, 1999 (CHE2001-397) Kästel, 1998 (CHE2001-398)
B.2.1.4.3 (IIA 2.4)	Appearance: odour	PAS TAS	Olfactory assessment	Y	PAS: odourless TAS: faint smoky		Daum, 1999 (CHE2001-397) Kästel, 1998 (CHE2001-398)

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	UV/VIS	Y	λ_{max} [nm] ε 207 31534 228 19834 290 1529 300 531	Acceptable	Daum, 1999 (CHE2001-400)
			IR NMR MS	Y	Spectra are consistent with given structure of nicobifen.	Acceptable	
B.2.1.5.2 (IIA 2.5)	Spectra for impurities of toxicological, ecotoxicological or environmental concern		UV/VIS IR NMR MS		No toxicologically, ecotoxicologically or environmentally significant components.		
B.2.1.6 (IIA 2.6)	Solubility in water of purified active substance	PAS	OECD 105 (EEC A 6) (column elution method)	Y	4.64 mg/L (20 °C)	Acceptable	Daum, 1998 (CHE2001-401)

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		N	**Solvent — Solubility (20 °C)** *n*-Heptane <10 g/L Toluene 20-25 g/L Dichloromethane 200-250 g/L Methanol 40-50 g/L Acetone 160-200 g/L Ethyl acetate 67-80 g/L N,N-Dimethylformamide >250 g/L Acetonitrile 40-50 g/L 1-Octanol <10 g/L 2-Propanol <10 g/L olive oil <10 g/L	Acceptable	Daum, 1998 (CHE2001-402)
B.2.1.8 (IIA 2.8)	Partition coefficient of purified active substance	PAS	OECD 117 (HPLC-method)	Y	log $P_{o/w}$ = 2.96 at 21 °C (pH 7.1). Investigation into the effect of pH was not applicable, the compound is not ionised in the range of pH 4 to 10.	Acceptable	Daum, 1998 (CHE2001-403)
B.2.1.9.1 (IIA 2.9)	Hydrolysis rate of purified active substance	PAS	EPA 161-1, EC Method C7	Y	Stable at pH 4, 7 and 9 DT50 not calculated because exceeding twice the exper. period.	Acceptable	Goetz von N, 1999 (WAS2001-153)
B.2.1.9.2 (IIA 2.9)	Direct phototransformation in purified water of purified active substance	PAS	FAO revised Guideline on Environ. Criteria rev.3, EPA 161-2	Y	Stable. No degradation observed.	Acceptable	Goetz von N, 1999 (LUF2001-269)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.9.3 (IIA 2.9)	Quantum yield of direct photodegradation	PAS	FAO revised Guideline on Environ. Criteria rev.3, EPA 161-2	Y	Smaller than 2.45×10^{-4}	Acceptable	Goetz von N, 1999 (LUF2001-269)
B.2.1.9.4 (IIA 2.9)	Dissociation constant (pK_a) of purified active substance	PAS	OECD 112	Y	No dissociation observed.	Aceptable	Daum A, 1998 (WAS2001-155)
B.2.1.10 (IIA 2.10)	Stability in air, indirect phototransformation	(PAS)	EC 94/37	N	$t_{1/2} = 8.8 \times 10^{-12}$ cm³ molecule^{-1}s^{-1}	Acceptable	Goetz von N, 2000 (LUF2001-149)
B.2.1.11.1 (IIA 2.11)	Flammability of active substance as manufactured	TAS	EEC A10	Y	The as does not burn under test conditions. Therefore, the as is not considered highly flammable.	Acceptable	Löffler, 1998 (CHE2001-404)
B.2.1.11.2 (IIA 2.11)	Auto-flammability of active substance as manufactured	TAS	EEC A 16	Y	No autoflammability was observed up to 400 °C.	Acceptable	Löffler, 1998 (CHE2001-404)
B.2.1.12 (IIA 2.12)	Flash point of the active substance as manufactured		EEC A 9	Y	Test was not conducted, because the melting point of the as is higher than 40 °C.	Acceptable	Löffler, 1998 (CHE2001-404)
B.2.1.13 (IIA 2.13)	Explosive properties of active substance as manufactured		EEC A 14	Y	Test was not conducted, because the chemical structure of the as gives no evidences of explosive properties.	Acceptable	Löffler, 1998 (CHE2001-404)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.14 (IIA 2.14)	Surface tension	TAS PAS	EEC A 5	Y	66.0 mN/m 0.5% (w/w) and 61.7 mN/m 1.0% (w/w) both at 20 °C 72.1 mN/m 0.5% (w/w) and 72.4 mN/m 1.0% (w/w) both at 20 °C	Acceptable not required additional info	Kästel, 1998 (CHE2001-398) Kästel, 1998 (CHE2001-399)
B.2.1.15 (IIA 2.15)	Oxidising properties of active substance as manufactured		EEC A17	Y	Test was not conducted, because of the chemical structure of the as gives no evidences of oxidising properties.	Acceptable	Löffler, 1998 (CHE2001-404)

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

Nicobifen (pure and technical active substance) is a white solid. A melting point of 143 – 145 °C was determined for PAS. The as decompose from 300 °C. The relative density determined at 20 °C is 1.38. The water solubility is 4.64 mg/L (20 °C) and the log $P_{o/w}$ is 2.96. The test substance is soluble in acetone, acetonitrile, dichloromethane, ethyl acetate and methanol (40 – > 250 g/L). Lowest solubility are observed in *n*-heptane (< 10 g/L). The substance is not highly flammable or autoflammable, not explosive and without oxidising properties.

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

Product name: BAS 510 01 F (containing 505 g/kg nicobifen, WG)

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	Grey brown	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.1.2 (IIIA 2.1)	Appearance: odour	Olfactory assess-ment	Faint aromatic	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.1.3 (IIIA 2.1)	Appearance: physical state	Visual assessment	Solid	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.2.1 (IIIA 2.2)	Explosive properties		The as and the formulants have no potential for explosivity due to their chemical structure. The substances has no chemical groups indicating ex-plosive properties. Examples are given in table A6.1 of appendix 6 in the Manual of Tests and Criteria of the United Nations. The decomposition energy of the substance is less than 500 J/g.	Acceptable	Goedde, M. (2000) PHY2001-335

Section (Annex point)	Study	Method	Results	Comment / Conclusion	Reference
B.2.2.2.2 (IIIA 2.2)	Oxidising properties		The test has not been carried out because neither the active ingredient nor the formulants have oxidising properties. The substance contains oxygen and chlorine but this elements are not bonded to other electronegative elements (see appendix 6 in the Manual of Tests and Criteria of the United Nations).		Goedde, M. (2000) PHY2001-335
B.2.2.3.1 (IIIA 2.3)	Flash point		Not relevant.		
B.2.2.3.2 (IIIA 2.3)	Flammability	EEC A 10	Not highly flammable.	Acceptable	Goedde, M. (2000) PHY2001-335
B.2.2.3.3 (IIIA 2.3)	Auto-flammability	EEC A 16 UN-Bowes-Cameron-Cage-Test	Result: The relative self-ignition temperature is 348 °C Result: Almost no temperature rise in a 100 mm test cube has been observed. Therefore BAS 510 01 F is not considered to be a self-heating substance according to the UN recommendations on the Transport of Dangerous Goods (Division 4.2).	Acceptable	Goedde, M. (2000) PHY2001-335 Goedde, M. (2001) PHY2001-330
B.2.2.4.1 (IIIA 2.4)	Acidity/alkalinity		Not necessary.	Acceptable	
B.2.2.4.2 (IIIA 2.4)	pH	CIPAC MT 75.2	5.8 at 1 % concentration in CIPAC water D; After accelerated storage for 2 weeks at 54 °C: 5.5 at 1 % concentration in CIPAC water D	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.5.1 (IIIA 2.5)	Kinematic viscosity		Not applicable.		
B.2.2.5.2 (IIIA 2.5)	Dynamic viscosity		Not applicable.		

Section (Annex point)	Study	Method	Results	Comment / Conclusion	Reference
B.2.2.5.3 (IIIA 2.5)	Surface tension	EEC 5 1.6.1 (Ring Method)	69.9 mN/m (at 0.01 % concentration) 65.8 mN/m (at 0.6 % concentration)	Acceptable	
B.2.2.6.1 (IIIA 2.6)	Relative density		Not applicable.		
B.2.2.6.2 (IIIA 2.6)	Bulk (tap) density	CIPAC MT 169	Pour: 548 g/L Tap: 620 g/L	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.7.1 (IIIA 2.7)	Storage stability	CIPAC MT 46	Physical and chemical stable after storage for 2 weeks at 54 °C. There is less than 2 % decrease in the active substance content. The alteration of the observed physical properties (pH-range, suspensibility, dispersibility, wet sieving, dust content) are negligible.	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.7.2 (IIIA 2.7)	Low temperature stability		No liquid preparation.		
B.2.2.7.3 (IIIA 2.7)	Shelf-life	GIFAP Monograph 17	Report not available.	Study still in progress.	
B.2.2.8.1 (IIIA 2.8.1)	Wettability	CIPAC MT 53.3	21 s (without swirling in CIPAC water D)	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.8.2 (IIIA 2.8.2)	Persistant foaming	CIPAC MT 47.1	Foam after 1 min: 0 ml (at 0.6 % concentration in CIPAC water D)	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.8.3.1 (IIIA 2.8.3)	Suspensibility	CIPAC MT 168	105 % (at 0.6 % concentration in CIPAC water D) After accelerated storage for 2 weeks at 54 °C: 105 % (at 0.6 % concentration in CIPAC water D)	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.8.3.2 (IIIA 2.8.3)	Spontaneity of dispersion	CIPAC MT 174	100 % After accelerated storage for 2 weeks at 54 °C: 101 %	Acceptable	Kaestel, R. (2000) PHY2001-336

Section (Annex point)	Study	Method	Results	Comment / Conclusion	Reference
B.2.2.8.4 (IIIA 2.8.4)	Dilution stability		Not applicable.	Acceptable	
B.2.2.8.5 (IIIA 2.8.5)	Wet sieve test	CIPAC MT 167	Residue on a 75 µm sieve: 0 % After accelerated storage for 2 weeks at 54 °C: Residue on a 75 µm sieve: 0 %	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.8.6.1 (IIIA 2.8.6)	Particle size distribution	Laser light diffraction spectrometry, MALVERN Mastersizer S CIPAC MT 170 Air jet sieving	Suspension medium: Water 10 % < 0.6 µm 90 % < 4.6 µm R </= 10 %: 75 µm R </= 90 %: 500 µm R >/= 50 µm: < 0.1 %	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.8.6.2 (IIIA 2.8.6)	Dust content	CIPAC MT 171	1.6 mg, After accelerated storage for 2 weeks at 54 °C: 1.1 mg.	Acceptable	Kaestel, R. (2000) PHY2001-336
B.2.2.8.6.3 (IIIA 2.8.6)	Friability and attrition		Not necessary.		
B.2.2.8.7.1 (IIIA 2.8.7)	Emulsifiability, emulsion stability and re-emulsifiability		Not applicable.		
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	CIPAC MT 172	100 % (through a 5 mm sieve spontaneously)	Acceptable	Kaestel, R. (2000) PHY2001-336

Section (Annex point)	Study	Method	Results	Comment / Conclusion	Reference
B.2.2.8.8.2 (IIIA 2.8.8)	Pourability (rinsa-bility)		Not applicable.		
B.2.2.8.8.3 (IIIA 2.8.8)	Dustability		Not applicable.		

Section (Annex point)	Study	Method	Results	Comment / Conclusion	Reference
B.2.2.9.1 (IIIA 2.9)	Physical compatibility with other products	ASTM method E 1518-93	41 different mixtures of BAS 510 01 F with other plant protection products were tested. All of them were determined to be compatible in aqueous tank mixtures. Test substances: BAS 510 01 F and BAS 9095 3 l (Karate WG) or BAS 9095 1 l (Karate EC) or BAS 9038 1 l (Mavrik Flo) or BAS 9005 0 l (Pirimor) or BAS 00 F or BAS 518 01 F (Cabrio Top) or BAS 525 00 F or BAS 490 02 F (Discus) or BAS 9210 0 F (Vento) or BAS 9106 0 F (Topas) or BAS 9233 1 F (Prosper) or BAS 175 01 F (Kumulus DF) or BAS 9125 0 F (Cuproxat flüssig) or BAS 266 04 F (Dithane Ultra) or BAS 499 00 F (Kauritil DF) or BAS 430 06 F (Aviso DF) or BAS 440 03 F (Aviso Cup DF) or BAS 9062 0 F (Mikal flash) or BAS 9277 1 F (Valiant flash) or BAS 9278 0 F (Equation Pro) or BAS 9223 0 F (Forum FP) or BAS 9178 0 F (Forum) or BAS 9139 0 F (Aktuan) or BAS 9279 1 F (Ridomil Gold Combi) or BAS 9104 1 l (Cascade) or Lannate Liquido or BAS 9075 1 l (Ordoval) or BAS 318 01 l (Masai) or BAS 9063 0 l (Insegar) or BAS 9137 0 l (Mimic) or Xen Tari or BAS 9007 0 l Torque or BAS 9108 0 l (Kiron) or Gusathion 20 SC or BAS 9053 0 l (Nomolt) or BAS 9069 1 l (Apollo) or BAS 222 28 F (Polyram WG) or BAS 537 00 F or Ultrazid 40 or BAS 152 11 l (Perfekthion) or BAS 9277 0 F (R6 Triplo F)	Acceptable	Schneider, K.-H. (2000) PHY2001-334

Section (Annex point)	Study	Method	Results	Comment / Conclusion	Reference
B.2.2.9.2 (IIIA 2.9)	Chemical compa-bility with other products	ASTM method E 1518-93	There were no indications of chemical reactions between the mixed products.	Acceptable	Schneider, K.-H. (2000) PHY2001-334
B.2.2.10 (IIIA 2.10)	Adherence and dis-tribution to seeds		No seed dressing formulation.		

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 510 01 F is a grey brown, free flowing water dispersible granule with a faint aromatic odour. It has neither explosive nor oxidising properties and it is not highly flammable. Its pH-value of 5.65 ± 0.15 lies within the naturally occurring acidic range. Although the shelf life test has not finished yet due to the accelerated storage stability test one can expect that the results will confirm its stability allowing storage 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-2.1	anonym	2002	DSC-Plot to BASF DocID 1999/10991. not GLP, unpublished CHE2002-385	Y	BAS
AIIA-2.1.1; AIIA-2.1.3; AIIA-2.4	Daum, A.	1999	Determination of the melting point and the appearance of Reg. No. 300355 (BAS 510F). 1999/10991 GLP, unpublished CHE2001-397	Y	BAS
AIIA-2.1.1; AIIA-2.2; AIIA-2.4; AIIA-2.14	Kästel, R.	1998	Physical and Chemical Properties of PS 300 355. 1998/10774 GLP, unpublished CHE2001-398	Y	BAS
AIIA-2.2; AIIA-2.14	Kästel, R.	1999	Physical Properties of 300 355 (PAI). 1999/10203 GLP, unpublished CHE2001-399	Y	BAS
AIIA-2.3.1	Kästel, R.	1999	Physical Properties of 300 355 (PAI). Reg.Doc.# BASF 99/10203 GLP, unpublished LUF2001-146	Y	BAS
AIIA-2.3.2	Ohnesorge, U.	2000	Henry`s Law Constant for 300355. BASF DocID 2000/1001009 not GLP, unpublished LUF2001-147	Y	BAS
AIIA-2.5	Daum, A.	1999	UV-, NMR-, IR-, MS-Spectra of Reg. No. 300355 (BAS 510F). 1999/10832 GLP, unpublished CHE2001-400	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	Daum, A.	1998	Determination of the solubility of BAS 510 .. F (Reg.-No. 300355) in water at 20°C by column elution method and by HPLC. 1998/10961 GLP, unpublished CHE2001-401	Y	BAS
AIIA-2.7	Daum, A.	1998	Determination of the solubility of BAS 510.. F (Reg.-No. 300355 pure active ingredient (PAI) in organic solvents at 20°C. 1998/10953 GLP, unpublished CHE2001-402	Y	BAS
AIIA-2.8	Daum, A.	1998	Determination of the Octanol/Water-partition Coefficient of Reg.-No. 300355 (BAS 510 .. F) by HPLC. 1998/11082 GLP, unpublished CHE2001-403	Y	BAS
AIIA-2.9.1	von Götz, N.	1999	Hydrolysis of BAS 510 F. BASF Reg.Doc.# 19 99/11285 GLP, unpublished WAS2001-153	Y	BAS
AIIA-2.9.2; AIIA-2.9.3	von Götz, N.	1999	Aqueous Photolysis of BASF 510 F. BASF #1999/11804 GLP, unpublished LUF2001-269	Y	BAS
AIIA-2.9.4	Daum, Ansgar	1998	Determination of the pKa of Reg.No. 300355 (BAS 510 .. F) in water at 20 °C. BASF Reg.Doc.# 98/10967 GLP, unpublished WAS2001-155	Y	BAS
AIIA-2.10	von Götz, N.	1994	Photochemical oxidative degradation of BAS 510 F (QSAR Estimates). BASF DocID 1999/11874 not GLP, unpublished LUF2001-149	Y	BAS
AIIA-2.11	König, W.	2002	Statement of Analysis. 2002/1003388 not GLP, unpublished CHE2002-386	Y	BAS
AIIA-2.11.1; AIIA-2.11.2; AIIA-2.12; AIIA-2.13; AIIA-2.15	Löffler, U.	1998	Evaluation of safety characteristics according to 92/32/EEC. 1998/11078 GLP, unpublished CHE2001-404	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-2.1; AIIIA-2.4; AIIIA-2.6; AIIIA-2.7; AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.5; AIIIA-2.8.6; AIIIA-2.8.8; AIIIA-4.1	Kästel, R.	2001	Shelf life in original container of the formulation BAS 510 01 F - 12 Month storage - Physical and chemical properties INTERIM REPORT. BASF DocID 2001/1014636 GLP, unpublished PHY2002-84	Y	BAS
AIIIA-2.1; AIIIA-2.4; AIIIA-2.5; AIIIA-2.6; AIIIA-2.7; AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.5; AIIIA-2.8.6; AIIIA-2.8.8	Kästel, R.	2000	Physical and chemical properties of BAS 510 01 F. BASF DocID 2000/1017017 GLP, unpublished PHY2001-336	Y	BAS
AIIIA-2.2; AIIIA-2.3	Gödde, M.	2000	Safety characteristics of the crop protection product BAS 510 01 F. BASF DocID 2000/1018467 GLP, unpublished PHY2001-335	Y	BAS
AIIIA-2.3	Gödde, M.	2001	Auto-flammability classification of the crop protection product BAS 510 01 F according to the UN-Recommendation on the Transport of Dangerous Goods (UN-Bowes-Cameron-Cage-Test). BASF DocID 2001/1009132 GLP, unpublished PHY2001-330	Y	BAS
AIIIA-2.9	Schneider, K.-H.	2000	Physical and Chemical Compatibility in Aqueous Tank Mixtures of BAS 510 01 F with other products. BASF DocID 2000/1018466 not GLP, unpublished PHY2001-334	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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 Primary action

Nicobifen is envisaged to be used as a fungicide.

B.3.1.2 Function

Nicobifen controls several fungal pathogens belonging to the four major classes of plant pathogenic fungi.
Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound. Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.

B.3.1.3 Field of use envisaged

Nicobifen will be used under field conditions in several agricultural and horticultural , ornamentals and viticulture.

B.3.1.4 Harmful organisms controlled and crops protected

Uses supported by available data are on grapes: *Botrytis cinerea* and *Uncinula necator*; on oilseed rape: *Sclerotinia sclerotiorum*, *Alternaria brassiceae*, *Phoma lingam* (*Leptoshaeria maculans*); on peas: *Sclerotinia sclerotiorum*, *Botrytis cinerea*; on beans (*Vicia*): *Botrytis cinerea*, *Sclerotinia sclerotiorum*.

Further uses envisaged are on oilseed rape: *Botrytis cinerea*; on beans (French): *Botrytis cinerea*, *Sclerotinia sclerotiorum*; on lettuce: *Botrytis cinerea*, *Sclerotinia sclerotiorum*; on potato: *Alternaria* spp.; on cabbages: *Alternaria* spp., *Botrytis cinerea*, *Sclerotinia sclerotiorum*; on tomato: *Botrytis cinerea*, *Leveillula taurica*; on peppers: *Botrytis cinerea*, *Leveillula taurica*; on winter leeks: *Stemphylium botryosum*; on stonefruit: *Monilinia fructigena*, *Monilinia laxa*; on pomefruit: *Podosphaera leucotricha*, *Venturia inaequalis*; on strawberry: *Botrytis cinerea*; on hops: *Sphaerotheca humuli*; on cucumber: *Erysiphe cichoracearum*, *Sphaerotheca fuliginea*; on melon: *Erysiphe cichoracearum*, *Sphaerotheca fuliginea*; on roses: *Sphaerotheca pannosa*; on lilies: *Botrytis* spp.; on bulb ornamentals: *Botrytis* spp.

B.3.1.5 Mode of action

Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound. Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.

B.3.1.5.1 Active substance

Nicobifen is a member of the class of carboxin fungicides. Nicobifen effectively controls several fungal pathogens belonging to the four major classes of plant pathogenic fungi.
The mode of action of nicobifen at the molecular level is the inhibition of the mitochondrial succinate dehydrogenase (SDH, complex II). This enzyme is part of tricarboxylic acid cycle (citrate cycle, Krebs cycle). It belongs also to a class of flavoproteins, which enter electrons into the mitochondrial respiration chain. Therefore, inhibition of succinate dehydrogenase by nicobifen affects both the carbon flow into crucial metabolites and the yield of ATP. The shortage of building blocks for amino acids and sugars together with the reduced energy yield severely interferes with basic principles of growth and maintenance of a living cell.
The efficacy of inhibition of SDH strongly depends on the species. In phytopathogenic fungi as *Botrytis cinerea*, SDH is strongly inhibited by a low concentration of nicobifen. In the yeast *Saccharomyces cerevisiae*, inhibition is still observed but a higher concentration is necessary and the mammalian enzyme from pig liver is almost resistant to nicobifen.
Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal groth and reproduction necessary for disease development).

B.3.1.5.2 Active metabolites

Not relevant here, since the fungicidal effect is directly caused by the parent active substance.

B.3.1.5.3 Formation of active metabolites

Not relevant here, since the fungicidal effect is directly caused by the parent active substance.

B.3.1.6 Information on the occurrence or possible occurrence of the development of resistance of the target organisms

B.3.1.6.1 Mechanism of resistance

No resistant field isolates of the intended target pathogens for nicobifen have so far been found. There is, however, published information on the putative mechanism of resistance in some fungal pathogens to carboxin which is also a carboxanilide product. It is currently thought that mutations which lead to amino acid substitutions in the iron-sulphur protein sub-unit of succinate dehydrogenase confer resistance to carboxin in *Ustilago maydis* (Keon et al., 1991) and *Mycosphaerella graminicola (Septoria tritici)* (Skinner et al., 1998).

B.3.1.6.2 Evidence of resistance

There is no evidence of field resistance to nicobifen in any of the target pathogens. Evidence of resistance comes mainly from resistance to other carboxanilides shown by mutant fungal strains. Carboxin-resistant mutant stains or laboratory strains have been studied in *Ustilago maydis* (Keon et al., 1991), *Ustilago hordei* (Ben-Yephet et al., 1975), *Aspergillus nidulans* (Gunatilleke et al., 1976), *Sclerotinia sclerotiorum*, *Fusarium solani pisi* (Bochow et al., 1971), and *Mycosphaerella graminicola* (Skinner et al., 1998).
Reports of naturally occurring field resistance to carboxanilides are restricted to *Ustilago nuda* (Leroux and Berthier, 1988), (Newcombe and Thomas, 1991) and *Puccinia horiana* (Grouet et al., 1981).

B.3.1.6.3 Cross resistance

There is no indication yet of cross-resistance between nicobifen and the other carboxanilide fungicides mentioned in the above section on evidence of resistance. Whether such cross-resistance exists has not yet been tested - because there is no activity on the pathogens in question.

B.3.1.6.4 Baseline Sensitivity

The baseline sensitivities to nicobifen have been determined *for Botrytis cinerea* (using both in vitro and in vivo methods), *Uncinula necator* and *Sphaerotheca fuliginea*.
The results are summarised in the following table:

Pathogen	Mean ED50	Range ED50	Mean ED98	Range ED98
Botrytis cinerea (in vitro) n=129	0.07	0.01-0.21	0.70	0.1-2.21
Botrytis cinerea (In vitro) n=116	2.1	0.5-5.3	6.5	1.1-16.0
Sphaerotheca fuliginea n=48	1.1	0.5-2.7	6.6	2.6-14.3
Uncinula necator n=45	0.6	0.2-1.2	1.9	0.5-5.5

B.3.1.6.5 Resistance risk assessment of unrestricted use pattern

The following table shows a risk assessment based on some of the most important risk factors (inherent to the pathogen, fungicide and agronomic practices) specified in the EPPO Guidelines. The table includes all the pathogens presently envisaged as targets for nicobifen. It is imprtant to note here that most of the Target pathogens mentioned in the table are intended for a range of ready-mix products containing nicobifen and other products that have a different mode of action to the carboxanilides.
The table gives a transparent overview on how the resistance risk has been assessed for each target pathogen.
Explanatory notes outlining how the risk classes are allocated are given after the table.
For the pathogen codes (Bayer codes) shown in the table the following distinctions are necessary:

SPHRMA*	-	*Sphaerotheca alchemillae* on strawberries
SPHRMA	-	*Sphaerotheca humili* on hops
COLLSP*	-	*Colletotrichum acutatum* on strawberries
GNOMSP*	-	*Gnomonia fragariae* on strawberries
ERYSSP*	-	*Erysiphe heraclei* on carrots
STEMP*	-	*Stemphylium botryosum* on leeks

Resistance risk assessment for carboxanilide compounds on different diseases in Europe.

risk scoring by classes		
0 – 30	very low risk	I
31 – 60	low risk	II
61 – 80	tolerable risk	III
81 – 100	increased risk	IV
101 – 120	high risk	V
>120	very high risk	VI

Disease	Crop	# of generations (weight) *4	spore dispersal *3	Spore production *2	history of resistance *2	occur-rence *3	Resistance to carboxinilides *5	cropping type *3	Number of applications *4	% of applications *10	score	Risk class
maximum	-	6	6	6	6	6	6	6	15	100%	202	VI
BOTRCI	grapevine	3	6	6	5	3	1	6	3	75%	104	V
BOTRCI	beans	3	6	6	5	3	1	1	3	75%	89	IV
BOTRCI	peas	3	6	6	5	3	1	1	2	50%	82	IV
BOTRCI	Strawberry	3	6	6	5	3	1	2	4	80%	96	IV
BOTRCI	Cabbage	3	6	6	5	3	1	1	4	80%	93	IV
BOTRCI	Lettuce	3	6	6	5	3	1	1	2	40%	81	IV
SCLESC	oilseed rape	1	1	1	1	4	1	1	2	100%	49	II
SCLESC	Beans	1	1	1	1	4	1	1	3	75%	51	II
SCLESC	Peas	1	1	1	1	4	1	1	2	50%	44	II
SCLESC	Cabbage	1	1	1	1	4	1	1	4	80%	55	II
SCLESC	Lettuce	1	1	1	1	4	1	1	2	40%	43	II
ALTESP	oilseed rape	4	4	5	1	2	1	1	2	100%	72	III
ALTESP	Cabbage	4	4	5	1	2	1	1	4	80%	78	III
ALTEDA	Carrot	4	4	5	1	2	1	1	3	100%	76	III
MONISP	Stonefruit	3	6	4	4	4	1	6	5	80%	109	V
SPHRMA*	Strawberry	4	6	3	5	4	1	2	4	80%	97	IV
SPHRMA	Hops	4	6	4	3	4	1	6	3	40%%	99	IV
COLLSP*	Strawberry	3	3	2	3	4	1	2	4	80%	78	III
GNOMSP*	Strawberry	1	3	2	1	2	1	2	4	80%	60	II
MYCOBR	Cabbage	3	5	3	3	3	1	1	4	80%	80	III
ERYSSP*	Carrot	3	5	4	2	2	1	1	3	100%	75	III
STEMSP*	Leek	3	6	3	2	3	1	1	3	60%	75	III
CLADAP	Leek	3	2	2	1	3	1	1	3	60%	59	II
PHYTPO	Leek	3	6	2	1	3	1	1	3	60%	71	III

PUCCAL	Leek	4	6	4	1	3	1	1	3	60%	79	III
RHIZSO	Lettuce	1	2	1	1	3	1	1	2	40%	43	II
LEVETA	pepper/tomato	4	5	3	1	3	1	2	3	40%	75	III
VENTIN	apple	3	4	4	5	4	1	6	4	33%	96	IV
PODOLE	apple	4	6	4	4	4	1	6	4	33%	104	V
UNCINE	grapevine	4	6	4	4	4	1	6	3	30%	100	IV
SPHRFU	curcurbitaceae	5	6	6	5	5	1	2	4	80%	110	V
SPHRPA	rose	4	6	6	5	5	1	6	15	30%	157	VI
BOTRTU	Tulip /bulbs	3	6	6	5	5	1	1	8	80%	115	V
BOTREL	lillies	3	6	6	5	5	1	1	12	66%	130	VI
BOTRTU	Tulip /bulbs	3	6	6	5	5	1	1	8	80%	115	V
BOTREL	lillies	3	6	6	5	5	1	1	12	66%	130	VI

Explanatory notes for the resistance risk scoring method

These notes explain the method used to obtain the resistance score and corresponding risk classes in the section "Resistance risk assessment of unrestricted use pattern".

The resistance risk score for each target disease is obtained into consideration a range of risk factors. These risk factors have been described in the EPPO "Guideline for the efficacy evaluation of plant protection products". The risk factors include those that can be considered inherent to both the target pathogen and the fungicide product, and also those that are related to certain conditions of use which can be defined as the agronomic risk.

The resistance risk factors used in the scoring method are shown in the table below. Each risk factor is assigned a fixed numerical „weight" (Shown with an asterisk) and also a variable assessment index from 1 – 6 based on the characteristics which are shown in the table.

Type of risk	Risk factor	Weighted value	Assessment index
inherent to target disease	Number of Generations	4	1 = only one generation / year 3 = 2 - 5 generations / year 6 = >10 generations / year
	spore dispersal	3	1 = only once per year 3 = mainly by rain splash 6 = via wind
	spore production	2	1 = very sparse spore production 3 = abundant spore production under favourable conditions 6 = abundant spore production under all conditions
	History of resistance to fungicides	2	1 = resistance unknown for the pathogen 3 = resistance known but not of practical importance 6 = resistance common to various fungicide groups
	Occurrence	3	1 = seldom a problem 3 = disease always present but easy to control 6 = disease difficult to control every year
inherent to fungicide product	Resistance known to this mode of action	5	1 = no resistance known 3 = shifting type resistance known 6 = disruptive type resistance known
agronomic risk	Cropping type	3	1 = full crop rotation 3 = max. 3 years mono-cropping 6 = full mono-cropping
	Number of Applications	4	intended (unrestricted) number of applications
	% of total applications	10	% of all fungicide applications against target pathogen

All values between 1 – 6 are used in the assessment index according to how well they describe the resistance factor.

The risk scores are obtained by multiplying the assessment indices with the fixed weighted value for each risk factor and the adding the results.

The maximum score possible from the scoring method is 202. This is obtained by awarding the maximum assessment index (6) for each of the risk factors and assuming that all (100 %) of a total of 15 applications are made with the same fungicide.

The scores obtained for each target pathogen are divided into the 6 categories of resistance risk which are shown at the top of the table.

Score	Risk class
0 – 30	I = very low risk
31 – 60	II = low risk
61 – 80	III = tolerable risk
81 – 100	IV = increased risk
101 – 120	V = high risk
> 120	VI = very high risk

Use pattern
The number of applications is an unrestricted use pattern is shown in the table for the resistance risk assessment. Further details of intended use pattern will be presented in the Resistance Risk Analysis to support each individual product containing nicobifen as an active ingredient.

Acceptability of the resistance risk
An overview of the resistence risk of an unrestricted use is shown in the table of risk assessments. Many of the target pathogens represent a low or tolerable (acceptable) risk. Others are assessed to represent an increased or high (unacceptable) risk where consideration will be given to possible modifiers. The notifier plans to deal with this on a product by product basis according to the target pathogens and whether it is a nicobifen solo product or a ready-mix product containing nicobifen as one of the active ingredients in the Annex III documentation.

B.3.1.6.6 Management strategy

The management strategies will depend on the products containing nicobifen as an active ingredient, the target pathogens for those products and the resistance risk assessments for the pathogens which are outlined in the above table.
In very general terms it can be stated here that a maximum of two applications of nicobifen are to be recommended for the solo product. The notifier recommends for read-mix products containing nicobifen as one of the active ingredients the maximum number of three applications with the exception of pomefruits (Max. four applications) and various ornamentals where the total number of fungicide applications per year is relatively high. For the target pathogens where an increased or high risk of resistance is assessed (see above table), the notifier plans to include further modifiers and to outline these in the dossiers submitted for the individual products.

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

B.3.2.1 Field of use envisaged

Nicobifen is envisaged to be used as a fungicide under field conditions in several agricultural and horticultural , ornamentals and viticulture.

B.3.2.2 Mode of action

Nicobifen controls several fungal pathogens belonging to the four major classes of plant pathogenic fungi.
Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound. Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.

B.3.2.3 Details of existing and intended uses

Uses supported by available data are on grapes: *Botrytis cinerea* and *Uncinula necator*; on oilseed rape: *Sclerotinia sclerotiorum*, *Alternaria brassiceae*, *Phoma lingam (Leptoshaeria maculans*); on peas: *Sclerotinia sclerotiorum*, *Botrytis cinerea*; on beans (Vicia): *Botrytis cinerea, Sclerotinia sclerotiorum.*

Further uses envisaged are on oilseed rape: *Botrytis cinerea*; on beans (French): *Botrytis cinerea, Sclerotinia sclerotiorum*; on lettuce: *Botrytis cinerea, Sclerotinia sclerotiorum*; on potato: *Alternaria spp*.; on cabbages: *Alternaria spp., Botrytis cinerea, Sclerotinia sclrotiorum*; on tomato: *Botrytis cinerea*, *Leveillula taurica*; on peppers: *Botrytis cinerea*, *Leveillula taurica*; on winter leeks: *Stemphylium botryosum*; on stonefruit*: Monilinia fructigena*, *Monilinia laxa*; on pomefruit: *Podosphaera leucotricha*, *Venturia inaequalis*; on strawberry: *Botrytis cinerea*; on hops: *Sphaerotheca humuli*; on cucumber*: Erysiphe cichoracearum*, *Sphaerotheca fuliginea*; on melon: *Erysiphe cichoracearum*, *Sphaerotheca fuliginea*; on roses: *Sphaerotheca pannosa;* on lilies: *Botrytis spp*.; on bulb ornamentals: *Botrytis spp*.

B.3.2.4 Application rate

The envisaged application rates in grapes against *Botrytis cinerea* are 0.15-0.6 kg/ha active substance (as). one time per year depending on the developmental stage of the plant; in oilseed rape against *Sclerotinia sclerotiorum*, *Alternaria brassicae* and *Phoma lingam* 0.25 kg/ha as one time per year; in bean against *Botrytis cinerea* and *Sclerotinia sclerotiorum* 0.5 kg/ha as in two applications per year.
The application rate of the existing uses of BAS 510 01 F in grape vines, oilseed rape, peas, and beans are:

Crop	Rate of BAS 510 01 F	Spray (water) volume (max)
Grape vines	1.2 kg/ha	1600 L/ha
Oilseed rape	0.5 kg/ha	300 L/ha
Peas	1.0 kg/ha	400 L/ha
Beans	1.0 kg/ha	300 L/ha

Data about other intended uses are not yet available.

B.3.2.5 Concentration of the active substance in material used (diluted spray)

The envisaged concentrations in grapes against *Botrytis cinerea* are 0.15-0.6 kg/ha active substance (as) in 400-1600 L depending on the developmental stage of the plant; in oilseed

rape against *Sclerotinia sclerotiorum, Alternaria brassicae* and *Phoma lingam* 0.25kg/ha as in 200-400 L; in bean against *Botrytis cinerea* and *Sclerotinia sclerotiorum* 0.5kg/ha as in 300 L. Data about other intended uses are not yet available.

Depending on the figures on application rate and spray volume (see above) the concentrations in ready-to use spray of existing uses are as follows:

Crop	Concentration of formulation (BAS 510 01 F, 50% WG in the spray	Concentration of a.s. Nicobifen in the spray
Grape vines	0.075 %	0.38 g/L
Oilseed rape	0.17 %	0.83 g/L
Peas	0.25 %	1.25 g/L
Beans	0.33 %	1.67 g/L

B.3.2.6 Method of application

In all cases the application method is spraying by means of each type of spraying equipment which is normally used for applying fungicides in practical viticulture/agriculture. The diluent is water. The water volumes required are outlined above.

B.3.2.7 Number and timing of applications

The products are intended to be used one to two times per year depending on the crop and the developmental stage of the plant. For the existing uses the data are the following:

Crop	Max number of application	Timing of the first application	Timing of the second application
Grape vines	1	4 weeks before harvest	-
Oilseed rape	2	GS 51-55	GS 65
Peas	2	2 weeks before harvest	1 week before harvest
Beans	2	2 weeks before harvest	1 week before harvest

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

It is to assume that there are no phytotoxic effects on succeeding crops by the use of the product.

B.3.3 Summary of data on application

Nicobifen is envisaged to be used as a fungicide under field conditions in several agricultural and horticultural, ornamentals and viticulture and controls several fungal pathogens belonging to the four major classes of plant pathogenic fungi.
Uses exist in grape vines, oilseed rape, peas, and beans
Nicobifen has preventative and curative properties. It inhibits spore germination, germ tube elongation, mycelial growth, and sporulation (all major stages of fungal growth and reproduction necessary for disease development). Nicobifen is a systemic compound.

Depending on the type of formulation, it penetrates into the plant when applied to leaves (or roots), and it is then translocated acropetally.
The envisaged application rates in grapes against *Botrytis cinerea* are 0.15-0.6 kg/ha active substance (as) one time per year depending on the developmental stage of the plant; in oilseed rape against *Sclerotinia sclerotiorum*, *Alternaria brassicae* and *Phoma lingam* 0.25 kg/ha as one time per year; in bean against *Botrytis cinerea* and *Sclerotinia sclerotiorum* 0.5 kg/ha as in two applications per year. Data about other intended uses are not yet available.
In all cited cases the application method is spraying.
The products are intended to be used one to two times per year depending on the crop and the developmental stage of the plant (exceptions see below).
There is no evidence of field resistance to nicobifen in any of the target pathogens. Evidence of resistance comes mainly from resistance to other carboxanilides shown by mutant fungal strains.
There is no indication yet of cross-resistance between nicobifen and the other carboxanilide fungicides mentioned in the above section on evidence of resistance. Whether such cross-resistance exists has not yet been tested - because there is no activity on the pathogens in question.
Many of the target pathogens represent a low or tolerable (acceptable) risk. Others are assessed to represent an increased or high (unacceptable) risk where consideration will be given to possible modifiers. The notifier plans to dealt with this on a product by product basis according to the target pathogens and whether it is a nicobifen solo product or a ready-mix product containing nicobifen as one of the active ingredients.
The notifier points out that the management strategies will depend on the products containing nicobifen as an active ingredient, the target pathogens for those products and the resistance risk assessments for the pathogens.
A maximum of two applications of nicobifen are to be recommended by the notifier for the solo product. Following the notifier, for ready-mix products containing nicobifen as one of the active ingredients the maximum number of applications will be three with the exception of pomefruits (Max. four applications) and various ornamentals where the total number of fungicide applications per year is relatively high. For the target pathogens where an increased or high risk of resistance is assessed (see above table), the notifier plans to include further modifiers and will outline these in the dossiers submitted for the individual products.

Summary of uses supported by available data Nicobifen (BAS 510 01 F)

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grape	**EU (North & South)**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 - 81	1	-	0.038 - 0.060	1000 - 1600	0.600	28	
Oil seed rape	**EU (North)**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63 - 65	2	4-6 weeks	0.062 - 0.125	200 - 400	0.250	-	
Oil seed rape	**EU (South)**	BAS 510 01 F	F	*Alternaria Sclerotinia, Phoma*	WG	500	spraying	30, 63 - 65	2	4-6 weeks	0.100 - 0.050	200 - 400	0.200	-	
Peas	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 - 10	0.125	400	0.500	7	
Beans	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 – 10	0.166	300	0.500	7	

(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)

Ref.: Anonymous, 2000 (CHE2001-405, Material safety data sheet)

B.3.4.1.1 Handling

Information on safe handling:
Avoid dust formation.
Protection against fire and explosion:
prevent formation of dust / air mixtures
prevent electrostatic charge – sources of ignition should be kept well clear – fire extinguishers should be kept handy

Personal Precautions:

Wear protective equipment, removal of ignition sources, provision for sufficient ventilation/respiratory protection, control of dust, prevention of skin and eye contact.

Respiratory protection:	if breathable dust is formed wear suitable breathing apparatus
Hand protection	chemical resistant gloves
Eye Protection:	tightly fitting safety goggles
Body Protection:	safety shoes, disposable protective clothing

B.3.4.1.2 Storage

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

B.3.4.1.3 Transport

Land transport ADR/RID

ADR/RID GGVS/E class	9
Number/letter	12c
UN-No.	3077

Maritime transport IMDG/GGVSee

IMDG/GGV See class	9
Marine pollutant	yes
UN-No.	3077
Proper technical name	environmentally hazardous substance, solid, n. o. s.

Air transport ICAO TI and IATA-DGR

ICAO/IATA class	9
UN-No.	3077
Proper technical name	environmentally hazardous substance, solid, n. o. s.

B.3.4.1.4 Fire fighting measures

Suitable extinguishing media:
water spray, dry extinguishing media, foam

The following can be given off in fire:
carbon monoxide, nitrogen oxides, hydrogen chloride

Fire fighting protective equipment:
Wear breathing equipment operated independently of ambient air, and protective firefighting suit.

Fire fighting instructions:
If 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 extinguishing water, do not allow to reach sewerage or effluent systems.

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

Unwanted amounts of nicobifen can be destroyed best by combustion in a licensed incinerator (see also B.3.4.3).

Decontamination of equipment, packing a.s.o. is achieved by washing with water.

Controlled incineration
The halogen content of nicobifen 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.

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

Incineration of the contaminated solid. In the case of contamination of water the undissolved amount of the product is to be separated by appropriate measures (e. g. filtration). The aqueous phase is to be treated with approximately 200 mg/L of activated powdered carbon for at least 12 hrs. 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).

Reference: Schenk, 2000 (CHE2001-406)

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

B.3.5.1 Packaging (type, materials, size, etc.), compatibility of the preparation with proposed packaging materials (Annex IIIA 4.1)

B.3.5.1.1 Description of packaging (Annex IIIA 4.1.1)

BAS 510 01 F is to be marketed in a square block bottom paper bag, laminated with polyethylene on the inner side. The bag is heat sealed.

1 kg bag:	material:	Laminated paper with polyethylene
	size:	153 mm (L) x 72 mm (W) x 381 mm (H)
	seal:	heat -sealing
5 kg bag:	material:	Laminated paper with polyethylene
	size:	240 mm (L) x 120 mm (W) x 560 mm (H)
	seal:	heat -sealing

B.3.5.1.2 Suitability of packaging (Annex IIIA 4.1.2)

Reference number:	PHY2001-333
Report:	Schreiner (2000) EU Performance Tests BASF AG, Ludwigshafen, Germany unpublished
Guidelines:	None
GLP:	No

The packaging is suitable according to ADR Method 3552 (drop test) for transporting solids

B.3.5.1.3 Resistance of packaging material to its contents (Annex IIIA 4.1.3)

Reference number:	PHY2001-332
Report:	Kaltz G. (2000) Corrosiveness of BAS 510 01 F BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany unpublished
Guidelines:	None
GLP:	No

During the handling or storage of BAS 510 01 F, corrosiveness of the formulation towards containers or the packaging material (Lupolen) was not observed.

A 2-year shelf-life study including evaluation of any interaction of BAS 510 01 F with the original container material was started in 07/2000. An interim report can be submitted after

one year, and a final report will be available approximately two years after the start of the study.

B.3.5.2 Procedures for cleaning application equipment and protective clothing (Annex IIIA 4.2)

Reference number:	PHY2001-331
Report:	Ohnsorge U. (2000) BAS 510 01 F (preliminary designator): Effectiveness of procedures for cleaning application equipment and protective clothing BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany unpublished
Guidelines:	None
GLP:	No

When the field sprayer is cleaned with water according to the use instructions after application of BAS 510 01 F, a contamination equal or less than 0.06 % of the original a.i. concentration is to be expected in the subsequent spray mix.
Therefore, cleaning the sprayer solely with water is considered completely adequate in the case of BAS 510 01 F.

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

B.3.5.3 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.

B.3.5.4 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIIA 4.4)

Handling:

Open containers should only be handled in well-ventilated areas. Make provisions for product and fire-fighting water to be retained.

Storage (Warehouse / User level):

No chemical hazards are inherent to BAS 510 01 F. Store out of reach of unauthorized persons. Keep away from food, feed and consumable items. 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 510 01 F as if it were a water pollutant. Make sure that the product does not enter any drains, water courses or the ground. In addition for warehouse storage: provide retention facilities.

For more detailed information see:

- Guidelines for the safe handling of pesticides during their formulation, packing, storage and transport (GIFAP)
- Guidelines for the safe warehousing of crop protection products (GCPF)
- Sichere Lagerung von Pflanzenschutz- und Schädlingsbekämpfungsmitteln (IVA).

Transport:

As of today BAS 510 01 F is class 9, No. 12c, UN No. 3077 (RID/ADR, IMDG, IATA) for transport.
Follow the general rules and good practices for transport.
Do not stow BAS 510 01 F together with food, feed and consumable items.

Fire:

BAS 510 01 F is a water dispersible granule (WG) formulation. 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 and HCl.

In case of fire, water, foam-water mixtures, dry powder or carbon dioxide can be used as extinguishing media for BAS 510 01 F. Fight fire if safe to do so.

Wear respiratory equipment:

in well ventilated areas:	full-face mask with combination filter, e.g ABEK-P2 (offers no protection against carbon monoxide !)
in enclosed premises:	respirator with independent air supply

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 in accordance with local regulations.

Protective clothing and equipment proposed:

If BAS 510 01 F is handled while not enclosed:

Avoid direct contact with the product.
Wear goggles to protect eyes.
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 soaked clothing.
Wash hands before break and at the end of work.

B.3.5.5 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. If necessary, use personal protective equipment.
Spillages of BAS 510 01 F may be taken up using an industrial vacuum cleaner. Place collected material in closeable containers.
Use a damp cloth to clean floors and other objects after removal of the collected material. Adding a detergent will enhance the cleaning process. Place used cleaning materials in closeable receptacles.

Disposal of damaged packaging, adsorbents, and other materials

BAS 510 01 F as well as its 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 Measures:

<u>General Advice</u>:

Remove person from danger zone.
Remove contaminated clothing.

<u>Upon Inhalation</u>:

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:

Immediately rinse mouth and then drink plenty of water.
Call medical help immediately.

B.3.5.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA4.6)

BAS 510 01 F as well as its 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.

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

A neutralization procedure for BAS 510 01 F is not applicable since the product shows no acidic or alkaline behaviour (see 2.4.2).

A detailed procedure for the decontamination of water from the active ingredient BAS 510 F by adsorption onto activated carbon is described in Annex II, 3.9.

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

Due to a halogen content of less than 60 % in the active ingredient, combustion in a waste incinerator plant does not raise concern about the formation of halogenated dibenzodioxins/-furans.

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

For purposes of disposal, combustion of BAS 510 01 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. by 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 exhaust gases.

Empty primary packages of BAS 510 01 F shall be triple rinsed as described in the ECPA "Guidelines for rinsing agrochemical containers", 1993. 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.
In order to minimize waste of packages, it is recommended that empty and rinsed containers are delivered to local container collection stations. If these are not existing, empty and rinsed containers must be rendered unusable, and then they may be disposed off according to local regulations.

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

No other methods for disposal of BAS 510 01 F than those described under 4.6.2 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	Gerlach, H.	2000	Safety data sheet. BASF DocID 2000/1013142 not GLP, unpublished CHE2001-405	Y	BAS
AIIA-3.9	Schenk, W.	2000	Possible procedures for the decontamination of water from BAS 510 F (proposed). 2000/1012375 not GLP, unpublished CHE2001-406	Y	BAS
AIIIA-4.1	Kaltz, G.	2000	# 63 - 20 Corrosiveness of BAS 510 01 F. BASF DocID 2000/1018457 not GLP, unpublished PHY2001-332	Y	BAS
AIIIA-2.1; AIIIA-2.4; AIIIA-2.6; AIIIA-2.7; AIIIA-2.8.1; AIIIA-2.8.2; AIIIA-2.8.3; AIIIA-2.8.5; AIIIA-2.8.6; AIIIA-2.8.8; AIIIA-4.1	Kästel, R.	2001	Shelf life in original container of the formulation BAS 510 01 F - 12 Month storage - Physical and chemical properties INTERIM REPORT. BASF DocID 2001/1014636 GLP, unpublished PHY2002-84	Y	BAS
AIIIA-4.1	Schreiner	2000	EU Performance Tests. BASF DocID 2000/1012266 not GLP, unpublished PHY2001-333	Y	BAS

[3] 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[3]
AIIIA-4.2	Ohnsorge, U.	2000	BAS 510 01 F; Effectiveness of Procedures for Cleaning Application Equipment and Protecti-ve Clothing. BASF DocID 2000/1017238 not GLP, unpublished PHY2001-331	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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):

Nicobifen

Hazard symbol:	N
Indication of danger:	Dangerous to the (aquatic) environment
Risk phrases:	R51/53 Toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment

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 510 01 F

Hazard symbol:	N
Indication of danger:	Dangerous to the (aquatic) environment
Risk phrases:	R51/53 Toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment

B.4.3 References relied on

No references submitted

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

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 (Annex IIA 4.1)

The method uses reversed-phase HPLC with a Nucleosil 100-5 C18 column and UV-detection at 205 nm and external calibration to determine nicobifen in the active substance as manufactured.The product is dissolved in acetonitrile/water. The solutions are directly injected into the HPLC system for separation and detection.

Ref.: Eisert, 1999 (CHE2001-395)

Specificity, linearity, accuracy and repeatability

Specificity

Identification of the active ingredient is based on comparison of the HPLC retention time of the active ingredient in the technical active substance to the retention time of the reference substance.

No analytical signal interferences are observed.

Linearity

The test substance (five samples with different analyte concentrations varying between 50 % and 150 % of the test concentration usually applied in the method) shows a linear analyte response in the investigated concentration range. A coefficient of correlation (r) of > 0.9999 was observed, and typical results for the linear regression graph were a slope of 71393 and an intercept of 14288.

Accuracy

The analyses of five samples of technical grade active substance fortified with defined amounts of pure active substance yielded mean recoveries between 99.6 % and 99.9 % (mean: 99.8 %) .

Repeatability

Based on the analysis of five samples of the pure active substance the relative standard deviation (% RSD) was 0.49 %. The analysis of five samples of technical material yielded a relative standard deviation of 0.46 %.
The acceptance of the % RSD values was confirmed by a comparison of the results with the % RSD reference values calculated according to the modified Horwitz equation.

Conclusions

The validation data of method with respect to precision, accuracy, linearity and specificity prove that the method is suitable for the determination of nicobifen in the active substance as manufactured.

Ref.: Eisert, 1999 (CHE2001-412)
Tuerk, 1998 (CHE2001-396)

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

Confidential information, see Annex C.

CIPAC Methods

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

B.5.1.2 Analytical method for formulation analysis (plant protection product) (Annex IIIA 5.1)

A GC method with a DB-1 capillary column is used for the quantitation of the active substance in the water dispersible granule (WG) formulation BAS 510 01 F. Separation and quantification is achieved with FID detection and calibration using an internal standard (benzyl butyl phthalate).
The active ingredient is dissolved in acetone, and the solutions are directly injected into the GC-FID system for separation and detection.

Ref.: Ziegler, CHE2001-413)

Specificity,linearity,accuracy and repeatability

Specificity

The identification of the active ingredient is based on a comparison of the retention time of the respective gas chromatographic peak of the test substance with that of the reference substance.
No analytical interferences of the a.i. with components of the formulation matrix are observed.

Linearity

The test substance (five samples with different analyte concentrations varying between 50 % and 150 % of the test concentration usually applied in the method) shows a linear analyte response in the investigated concentration range. A coefficient of correlation (r) of > 0.9999 was observed, and typical results for the linear regression graph were a slope of 0.0815 and an intercept of 0.0021.

Accuracy

The analyses of six samples of a BAS 510 01 F blank formulation fortified with defined amounts of pure active substance yielded a mean recovery of 100.28 %.

Repeatability

The analysis of six samples of a typical BAS 510 01 F formulation yielded a repeatability of 0.303 % relative standard deviation (RSD).
No outliers were observed.

Conclusions
The validation data of method with respect to precision, accuracy, linearity and specificity prove that the method is suitable for the determination of nicobifen in water dispersible granule formulations (BAS 510 01 F).

Ref.: Ziegler, 1999 (CHE2001-414)

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

BAS 510 01 F 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 accelerated storage at 54° C for 14 days. Therefore, no respective method is required.

CIPAC Methods

To date, no CIPAC methods exist for the analysis of nicobifen (the active substance of BAS 510 01 F) in either technical or formulated material.

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

According to the new version of the standard multi-method S 19 the sample material is extracted with acetone/water 2+1 (v/v). In case of lemons, the pH value has to be adjusted to 7-8 by means of $NaHCO_3$. Rape seed is extracted with acetone/acetonitril using Calflo E. For liquid/liquid partition, ethyl acetate/cyclohexane (1+1) and sodium chloride is added and after repeated mixing, excess water is separated.

The evaporated residue of an aliquot of the organic phase is cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1+1) as eluant and an automated gel permeation chromatograph. The residue containing fraction is concentrated, followed by an additionally clean-up by mini silica gel chromatography and analysed by gas chromatography using a fused silica capillary column (DB-5) and a mass selective detector (MSD). The monitoring ions were m/z = 140, 112, 342 (Weeren and Pelz, 1999). The method allows the determination of nicobifen by GC/MSD which is a highly selective detection technique. Therefore, no additional confirmation technique is regarded as necessary.

The independent laboratory validation of this method elaborated by the Institut Specht und Partner was performed by Reichert (2001) in Fresenius laboratories.

For validation data see Table B.5.2-1.

In BASF method 445/0 (Funk and Mackenroth, 2001) nicobifen is extracted with a methanol/water/hydrochloric acid mixture and after subsequent centrifugation an aliquot of the supernatant is purified using liquid/liquid partitioning with cyclohexane. After evaporation of cyclohexane to dryness the residue is dissolved in methanol/water for HPLC-MS/MS quantitation. The transition ions m/z = 343 -> 271 and m/z = 343 -> 307 can be used for quantification. Due to the high specifity of HPLC-MS/MS and the different transitions proposed a confirmatory technique is not regarded as necessary.

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

Reference	Sample Matrix	Test Substance	Fortific. level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Weeren and Pelz (1999)	Tomato	Nicobifen	0.01 0.1	94 93	8.9 4.2	5 5
	Lemon		0.01 0.1	100 / 94 [1)] 101 / 98 [1)]	8.9 / 8.2 [1)] 6.2 / 1.4 [1)]	5 / 5 [1)] 5 / 5 [1)]
	Wheat, grain		0.01 0.1	93 83	5.8 4.5	5 5
	Oilrape, seed		0.02 0.2	86 82	14 10	5 5
Reichert, (2001)	White cabbage	Nicobifen	0.01 0.1	70 77	6 9	5 5
	Rape, seed		0.02 0.2	71 76	10 9	5 5
	Hop		0.05 0.5	63 56	9 16	5 5
	Lettuce		0.01 0.1	71 78	9 10	5 5
Funk and Mackenroth (2000)	Apple	Nicobifen	0.05 0.5	95 88	1.8 13	5 5
	Sour cherry		0.05 0.5	91 86	4.9 3.5	5 5
	Grapes		0.05 1.0	97 103	2.3 11	5 5
	Strawberry		0.05 0.5	102 104	2.5 8.2	5 5
	Carrot		0.05 0.5	87 85	2.9 4.6	5 5
	Onion		0.05 0.5	101 98	5.7 5.9	5 5
	Tomato		0.05 0.5	98 90	11 0.9	5 5
	Broccoli		0.05 0.5	109 94	19 2.2	5 5
	White cabbage		0.05 0.5	90 93	9.3 3.8	5 5
	Leek		0.05 1.0	85 80	15 14	5 5
	Dwarf bean		0.05 0.5	96 93	6.5 3.8	5 5
	Oilrape, seed		0.05 0.5	91 95	11 1.8	5 5

[1)] extraction in presence of $NaHCO_3$

Additional information:

The stability of nicobifen in methanol, methanol/water, iso-octane and acetonitrile was determined under two different conditions: at 4°C in the dark and at room temperature with daylight. In methanol, methanol/water and acetonitrile a solution of BAS 510 F was stable under all chosen conditions. In iso-octane nicobifen was only stable at 4°C in the dark. At room temperature and with daylight nicobifen is degrading rapidly. It is recommended not to use iso-octane for preparation of the standards. The standards should not be stored longer than 30 days (Funk and Mackenroth, 2001).

As the extraction procedure used in the residue analytical method 445/0 (see II A 4.2.1/1) and the multi residue method S19 slightly deviates from those used in the metabolism studies, ^{14}C-nicobifen treated plant material was extracted according to these methods and the results thereof were compared. Purpose of this study is to verify the extraction efficiency of the "cold methods" in extracting the total radioactive residue (TRR).

The results show that comparable or slightly higher amounts of radioactivity were released by extraction with methanol/ water/hydrochloric acid (70/25/5)and acetone/ water (70/30).

The extractability with methanol/water/HCl ranged from 62.5% TRR (wheat straw) to 99.0% TRR (green bean). For acetone/water, the extractability ranged from 60.9% TRR (wheat grain) to 98.8% TRR (green bean). The data are presented in Table B.5.2-2

The HPLC metabolite profiles were comparable with the profiles obtained in the course of the metabolism studies (Bross, 2001).

Table B.5.2-2: Comparison of extractability of nicobifen obtained with different extraction solvents

		Extraction results					
Plant material		**metabolism study**		**Method 445/0**		**Multi method S 19**	
		mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Green beans	**TRR**	1.027	100	0.901	100	0.934	100
	ERR	1.010	98	0.892	99	0.923	99
	RRR	0.017	2	0.009	1	0.011	1
Dry beans	**TRR**	0.205	100	0.151	100	0.162	100
	ERR	0.165	81	0.129	85	0.141	87
	RRR	0.040	20	0.022	15	0.021	13
Grapes	**TRR**	1.181	100	1.291	100	1.185	100
	ERR	1.100	93	1.126	87	1.115	94
	RRR	0.081	7	0.165	13	0.070	6
Lettuce	**TRR**	0.067	100	0.069	100	0.071	100
	ERR	0.063	94	0.065	94	0.067	95
	RRR	0.004	6	0.004	6	0.004	6
Wheat grain	**TRR**	0.023	100	0.024	100	0.023	100
	ERR	0.008	35	0.015	63	0.014	61
	RRR	0.015	65	0.009	38	0.009	39
Wheat straw	**TRR**	3.226	100	3.177	100	3.176	100
	ERR	2.487	77	2.630	83	2.659	84
	RRR	0.739	23	0.546	17	0.516	16
Radish roots	**TRR**	0.098	100	0.095	100	0.101	100
	ERR	0.091	93	0.088	93	0.092	91
	RRR	0.007	7	0.007	7	0.008	8

RRR = residual radioactive residue
ERR = extractable radioactive residue
TRR = total radioactive residue

B.5.2.2 Foodstuff of animal origin

In study No P453G (Class, 2000) residues of nicobifen and its metabolite M510F01 which is also present as conjugate are extracted from animal matrices by methanol. An aliquot of the filtered extract is concentrated and treated with enzymes (ß-glucuronidase/arylsulfatase) for deconjugation of the metabolite. Water, acetone, sodium chloride and ethyl ace-

tate/cyclohexane (1/1) are added to achieve a homogeneous partition of the analytes into the organic phase. An aliquot of the organic extract is cleaned-up by gel permeation chromatography followed by acetylation of the phenolic metabolite M510F01 with acetic anhydride and further fractionation on silica gel. The determination of nicobifen and the acetylated metabolite M510F01 is achieved by gas chromatography with electron capture detection (GC/ECD). As confirmatory method a mass selective determination (GC/MS) is described.

The independent laboratory validation of previous method of PTRL Europe was performed by Kampke-Thiel (2001) in a BASF-laboratory.

For validation data see Table B.5.2-3.

Table B.5.2-3: Validation data for analytical methods for the determination of nicobifen residues in food of animal origin

Reference	Sample Matrix	Test Substance	Fortific. level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Class (2000)	Cow Milk	Nicobifen	0.01 0.1	82 88	12 6	5 5
		M510F01	0.01 0.1	93 101	16 8	5 5
	Cow Muscle	Nicobifen	0.025 0.25	95 84	6 6	5 5
		M510F01	0.025 0.25	93 92	14 15	5 5
	Cow Fat	Nicobifen	0.025 0.25	105 91	15 10	5 5
		M510F01	0.025 0.25	85 86	9 17	5 5
	Cow Kidney	Nicobifen	0.025 0.25	93 89	13 9	5 5
		M510F01	0.025 0.25	87 99	9 4	5 5
	Cow Liver	Nicobifen	0.025 0.25	91 83	15 7	5 5
		M510F01	0.025 0.25	89 86	15 19	5 5
	Hen Egg	Nicobifen	0.025 0.25	97 89	17 4	5 5
		M510F01	0.025 0.25	80 78	14 12	5 5
Kampke-Thiel (2001)	Cow Milk	Nicobifen	0.01 0.1	84 95	4 4	5 5
		M510F01	0.01 0.1	97 107	11 5	5 5
	Cow Liver	Nicobifen	0.025 0.25	74 74	3 1	5 5
		M510F01	0.025 0.25	92 93	3 5	5 5

Reference	Sample Matrix	Test Substance	Fortific. level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Grosshans (2000)	Cow Milk	Nicobifen	0.01	86.0	3.6	5
			0.1	88.7	7.7	5
		M510F01	0.01	88.4	5.8	5
			0.1	84.9	8.6	5
	Cow Cream	Nicobifen	0.01	72	1.5	5
			0.1	90	4.7	5
		M510F01	0.01	90	1.7	5
			0.1	94	2.3	5
	Cow Muscle	Nicobifen	0.025	86	4.0	5
			0.25	95	1.5	5
		M510F01	0.025	89	2.1	5
			0.25	86	1.4	5
	Cow Fat	Nicobifen	0.025	80	5.4	5
			0.25	81	8.5	5
		M510F01	0.025	81	4.0	5
			0.25	83	7.4	5
	Cow Kidney	Nicobifen	0.025	83	1.9	5
			0.25	91	3.9	5
		M510F01	0.025	82	2.5	5
			0.25	82	4.6	5
	Cow Liver	Nicobifen	0.025	87	6.3	5
			0.25	96	8.7	5
		M510F01	0.025	91	10	5
			0.25	92	6.2	5
	Hen Egg	Nicobifen	0.01	83	3.8	5
			0.1	93	3.1	5
		M510F01	0.01	83	6.1	5
			0.1	89	8.2	5
Fabian (2000)	Cow Milk	M510F53	0.01	95	4.5	5
			0.1	100	4.2	5
	Cow Liver	M510F53	0.05	91	2.7	5
			0.5	98	3.3	5

Additional information:

In BASF-method 471/0 (Grosshans, 2000 a) nicobifen is extracted with methanol. An aliquot corresponding to 5 g sample is taken for further work-up. The methanol extract is evaporated to dryness, redissolved in buffer solution and incubated with ß-glucuronidase / arylsulfatase to cleave the glucuronide. Then a liquid / liquid partition with ethyl acetate is carried out and the organic phase is purified on SPE C18 and if necessary on SPE silica gel columns. The final determination of the analytes nicobifen and M510F01 is performed by LC-MS-MS. The method which is classified as data generation method allows the determination of nicobifen and M510F01 by LC-MS-MS which is a highly selective detection technique. Therefore, no additional confirmation technique is regarded as necessary.

The BASF-method 476 was developed to determine bound residues of nicobifen as metabolite M510 F53 in liver. It was also possible to apply the method on milk extracts to determine minor milk metabolites. Acetonitrile and concentrated acetic acid are added to a sample of homogenised liver material. In the case of milk, an acetonitrile extract is taken and concentrated acetic acid is added. The mixture is treated for 0.5 h at 170 °C in the microwave oven. The mixture is evaporated to a crude solution and saturated sodiumchloride solution is added. The pH is adjusted to ~pH 12 with potassiumhydroxide. A liquid / liquid partition with iso-octane is carried out. The organic phase is purified on SPE Silica (liver and milk) and C18 (liver), respectively. The final determination is performed by GC/MS (Fabian, 2000 b). The method

which is classified as data generation method by the applicant allows the determination of M510F53 by GC / MS which is a highly selective detection technique.

The stability of nicobifen and the metabolites M510F01, M510F49, M510F51 and M510F53 in acetonitrile was investigated using HPLC / UV determination. All solutions showed no decrease of concentration. However, it is recommended that the solutions not be used as standards if they have been stored for more than 60 days (Grosshans, 2001 b).

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

According to BASF-method 408/1 (Keller, 1998 a) nicobifen is extracted from soil by shaking twice with methanol. The methanol extracts are separated by centrifugation. An aliquot of the combined methanol extracts is evaporated to dryness. The residue is dissolved in n-hexane and cleaned up with a silica gel SPE column using n-hexane/ethyl acetate (1 + 1) as eluent. The eluate is evaporated to dryness and the residue is redissolved in acetone containing an internal standard 2-chloro-N-(4'-methyl-biphenyl-2yl)-nicotinamide (Reg. No. 304813) for GC/MS determination. The final quantification is done by GC/MS. No significant interferences were observed. The monitoring ions are m/z = 140, 142, 342, 344

Uncorrected recoveries were not calculated in the report.

For validation data see Table B.5.3-1.

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

Reference	Sample matrix	Test substance	Fortific. level [ppm]	Mean recovery* [%]	RSD [%]	No. of replicates
Keller (1998 a)	Standard soil 2.2	Nicobifen	0.01 0.1 1.0	79 88 84	5.9 3.5 3.3	5 5 5
	US soil	Nicobifen	0.01 0.1 1.0	82 89 91	3.2 1.6 5.3	5 5 5
	Sediment	Nicobifen	0.01 0.1 1.0	77 86 98	6.3 4.2 3.6	5 5 5

* corrected recoveries

B.5.3.2 Water

According to the BASF-method 411 (Keller, 1998 b) enrichment of the analyte nicobifen from water is achieved by adsorption on a C_{18} SPE column. The column is dried and the analyte is eluted with n-hexane/ethyl acetate (1 + 1, v : v). The eluate is evaporated to dryness and the residue is redissolved in acetone containing an internal standard for GC/MS determination.

The final quantification is done by GC/MS. The monitoring ions are m/z = 140, 142, 342, 344. The validation for surface water was performed by Grothe (2001).

Uncorrected recoveries were not calculated in the reports.

For validation data see Table B.5.3-2.

Table B.5.3-2: Validation data for analytical methods for the determination of nicobifen residues in water

Reference	Sample matrix	Test substance	Fortific. level [µg/kg]	Mean recovery* [%]	RSD [%]	No. of replicates
Keller (1998 b)	Tap water	Nicobifen	0.05** 0.5 5.0	77 84 88	2.2 3.9 2.4	5 5 5
	Leachate water	Nicobifen	0.05** 0.5 5.0	97 96 102	7.6 6.5 2.1	5 5 5
Grothe (2001)	Surface water	Nicobifen	0.05 0.5** 5.0	114 105 99	0.7 4.4 4.6	5 5 5

* corrected recoveries
** LOQ

B.5.3.3 Air

For determination of nicobifen in air the BASF-method 460 (Zangmeister, 2000) was developed. After sampling of approximately 540 l air by sucking air (1.5 l/min) for approximately 6 hours through a Tenax absorber tube, the absorber tube is closed with 2 plastic caps and transported to the laboratory for analysis or stored at + 6°C (storage stability for 3 days proven).
For analysis, the Tenax adsorbent is extracted with acetone. The solvent is evaporated to dryness and the residue is redissolved in acetone. The final determination is done by GC-MS on a DB-XLB column using the ions m/z = 140, 142, 342. An additional confirmation technique is not regarded as necessary. No significant interferences were observed.

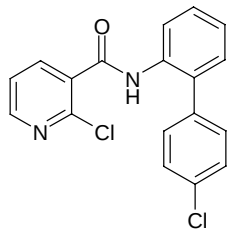
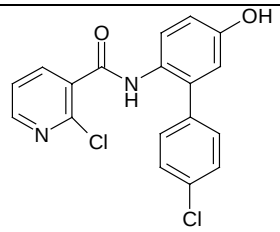
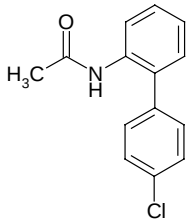
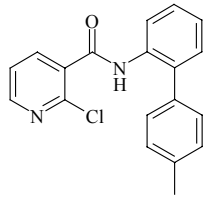
For validation data see Table B.5.3-3.

Table B.5.3-3: Validation data for the analytical method for the determination of nicobifen in air

Reference	Sample matrix	Test substance	Fortific. level [µg/m³]	Average recovery [%]	RSD [%]	No. of analyses
Zangmeister (2000)	Air	Nicobifen	1.5 133	100 92	2.7 4.7	5 6

B.5.3.4 Structural formulae and designation of compounds

Table B.5.3-4: Summery of compounds used as calibration standards or for fortification

Structural formulae	Designation of compounds
	Nicobifen Reg. No. 300355
	M510F01 Reg. No. 398794
	M510F53 Reg. No. 4035210
	Internal Standard Reg. No. 304813

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

B.5.4.1 Body fluids and tissues

Because nicobifen is not classified as toxic or as highly toxic no residue method is required for these matrices.

B.5.5 Evaluation and assessment

B.5.5.1 Formulation analysis

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

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

Impurities in the technical active substance are determined by a HPLC method on a reversed phase column with UV detection. Trace amounts of some other organic impurities are quantified by capillary gas chromatography/mass spectrometry (GC/MS). Quantification is achieved by external calibration.

Nicobifen in the formulation is determined by a GC internal standard method on a DB-1 capillary column with flame ionisation detection an calibration using an internal standard.

The methods are fully validated.

B.5.5.2 Residue analysis

For the assessment of the analytical methods for the determination of nicobifen 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	0.02	mg/kg	proposed MRL (nicobifen and M510F01 incl. its conjugates)
meat, fat, liver kidney, eggs	0.05	mg/kg	proposed MRL (nicobifen and M510F01 incl. its conjugates)
soil	0.05	mg/kg	general limit according to Directive 96/46/EC
drinking water	0.1	µg/L	EU drinking water limit
surface water	125	µg/L	NOEC of Oncorhynchus mykiss as most sensitive species
air	30	µg/m³	based on a proposed systemic AOEL of 0.1 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 only needed for food of animal origin. The metabolite M510F01 can be analysed according to Class (2001) and Kampke-Thiel (2001). For all other matrices nicobifen is the only relevant analyte.

According to these criteria adequate analytical methods are available for the determination of nicobifen in plant material, food of animal origin, soil, drinking water, surface water and air and for the determination of M510F01 and its conjugates in food of animal origin. Additional validation data are required for the methods to determine the active substance in water and soil.

Analytical methods for body fluids are not submitted. Because of the classification of the active substance a method is not necessary according to Directive 96/46/EC.

Table B.5.5-1: Analytical methods for the determination of residues

Matrix		Method	Limit of quantification		Reference
crops	tomato, lemon, grain	GC-MS	0.01	mg/kg	Weeren and Pelz, 1999
	oilrape seed		0.02	mg/kg	
	apple, sour cherry, grapes, strawberry, carrot, onion, tomato, broccoli, cabbage, leek, dwarf beans, oilrape seed	LC-MS-MS	0.05	mg/kg	Funk and Mackenroth, 2001
	white cabbage, lettuce	GC-MS	0.01	mg/kg	Reichert, 2001
	oilrape seed		0.02	mg/kg	
	hops		0.05	mg/kg***	
	milk	GC-ECD	0.01	mg/kg*	Class, 2001
	muscle, liver, kiney, fat, eggs		0.025	mg/kg*	
	milk	GC-ECD	0.01	mg/kg*	Kampke-Thiel, 2001
	liver		0.025	mg/kg*	
	milk, cream, eggs	LC-MS-MS	0.01	mg/kg*	Grosshans, 2001
	muscle, liver, kidney, fat		0.025	mg/kg*	
	milk	GC-MS	0.01	mg/kg**	Fabian, 2001
	liver		0.05	mg/kg**	

Matrix		Method	Limit of quantification		Reference
soil		GC-MS	0.01	mg/kg	Keller, 1998
water	drinking, leaching	GC-MS	0.05	µg/L	Keller, 1998
	surface	GC-MS	0.5	µg/L	Grote, 2001
air		GC-MS	1.5	µg/m^3	Zangmeister, 2000

* identical for M510F01 including conjugates
** only M510F53 (due to bound residues in liver and minor metabolites in milk)
*** average recovery only 63 %

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 290/1, determination of Reg.-No. 300355 in Reg.-No. 300355 (TGAI) using HPLC. 1999/10906 GLP, unpublished CHE2001-412	Y	BAS
AIIA-4.1	Eisert, R.	1999	Determination of Reg. No. 300355 in techn. active ingredient by HPLC. 1999/1003614 not GLP, unpublished CHE2001-395	Y	BAS
AIIA-4.1	Heinz, W.	2001	Validation of Analytical Method CP 368: Determination of Impurities in Technical BAS 510 F by GC/MS. 2001/1000047 GLP, unpublished CHE2001-392	Y	BAS
AIIA-4.1	Heinz, W.	2001	Validation of Analytical Method CP 367: Determination of Impurities in Technical BAS 510 F by HPLC. 2001/1000046 GLP, unpublished CHE2001-391	Y	BAS
AIIA-4.1	Heinz, W.	2001	Method CP 368: Determination of Impurities in Technical BAS 510 F by GC/MS. 2000/1017127 not GLP, unpublished CHE2001-390	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	Heinz, W.	2001	Method CP 367: Determination of Impurities in Technical BAS 510 F by HPLC. 2000/1017126 not GLP, unpublished CHE2001-389	Y	BAS
AIIA-4.1	Türk, W.	1998	Validation of HPLC-Method CP 290: Determination of Reg.-No. 300355 in Reg.-No. 300355 technical active ingredient (TGAI). 1998/10027 GLP, unpublished CHE2001-396	Y	BAS
AIIA-4.2.1	Bross, M.	2001	Investigations on the extractability of 14C-BAS 510 F residues from plant matrices; study code 73479. 2001/1001739 GLP, unpublished MET2001-268	Y	BAS
AIIA-4.2.1	Class, T.	2001	Assessment and validation of the adapted multi-residue method DFG S19 for the determination of BAS 510 F and its metabolite M510F01 in animal matrices; report no. P/B 453 G. 2000/1017227 GLP, unpublished MET2001-261	Y	BAS
AIIA-4.2.1	Fabian, E.	2001	The validation of BASF method 476/0: the determination of BAS 510 F residues (as M510F53) in liver and milk by microwave treatment; study code 96997. 2000/1017224 GLP, unpublished MET2001-270	Y	BAS
AIIA-4.2.1	Funk, H. und Mackenroth, Ch.	2001	Validation of BASF method no. 445/0: determination of BAS 510 F in plant matrices; study code 41840. 2000/1012404 GLP, unpublished MET2001-266	Y	BAS
AIIA-4.2.1	Funk, H.and Mackenroth, Ch.	2001	Determination of the stability of 205259 (BAS 480 F), 242009 (BAS 49 F), 285028 (BAS 505 F) and 300355 (BAS 510 F) in different solvents; study code 41841. 2000/1014856 GLP, unpublished MET2001-258	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	Grosshans, F.	2001	The validation of BASF method 471/0: The determination of BAS 510F and the metabolite M510F01 in animal matrices; study code 42392. 2000/1017223 GLP, unpublished MET2001-269	Y	BAS
AIIA-4.2.1	Grosshans, F.	2001	The stability of BAS 510F and the metabolites M510F01, M510F49, M510F51 and M510F53 in Acetonitrile; study code 42393. 2000/1017225 GLP, unpublished MET2001-259	Y	BAS
AIIA-4.2.1	Kampke-Thiel, K.	2001	Independent laboratory validation of the adapted multi-residue method DFG S19 for the determination of BAS 510 F and its metabolite M510F01 in animal matrices; PTRL Europe Study No. P453G. 2000/1017226 GLP, unpublished MET2001-262	Y	BAS
AIIA-4.2.1	Reichert, N.	2001	Independent laboratory validation of a method of analysis for the determination of BAS 510 F in white cabbage, rape (seed), hop, and lettuce; IF-100/35725-00. 2000/1014886 GLP, unpublished MET2001-267	Y	BAS
AIIA-4.2.1	Weeren, R.D. and Pelz, S.	1999	Validation of DFG method S19 for the determination of BAS 510 F in various plant materials; Az. M8020/99. 1999/11461 GLP, unpublished MET2001-260	Y	BAS
AIIA-4.2.2	Keller, W.	1998	Validation of analytical method no. 408/1, GC-MS determination of BAS 510 F active ingredient residues in soil and sediment after methanol extraction; study code 48541. 1998/11314 GLP, unpublished MET2001-263	Y	BAS
AIIA-4.2.3	Grote, Ch.	2001	Validation of analytical method no. 411/0, GC/MS determination of BAS 510 F ai residues in surface water; study code 110241. 2001/1008955 GLP, unpublished MET2001-265	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	Keller, W.	1998	Validation of analytical method no. 411, determination of BAS 510 F ai residues in water; study no. 41877. 1998/10922 GLP, unpublished MET2001-264	Y	BAS
AIIA-4.2.4	Zangmeister, W.	2000	Validation of analytical method 460, determination of BAS 510 F (Reg.no. 300355) in air by GC-MS; study code 41886. 2000/1014992 GLP, unpublished MET2001-271	Y	BAS
AIIIA-5.1	Ziegler, H.	1999	Validation of the analytical method CF-A 571: Determination of Reg.-No. 300355 in water dispersible granules (BAS 510 01 F). 1999/10419 GLP, unpublished CHE2001-414	Y	BAS
AIIIA-5.1	Ziegler, H.	1999	Determination of the content of the active ingredient Reg. No. 300355 in BAS 510 01 F using GC. 1999/1003818 not GLP, unpublished CHE2001-413	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

B-6: Toxicology and metabolism

B.6 Toxicology and metabolism

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

Following oral administration to rats, radiolabelled nicobifen was rapidly but incompletely absorbed from the gastrointestinal tract, widely distributed and rapidly eliminated from the body. Based on recovery of the radiolabel in bile from bile-duct cannulated rats within 48 h and in urine from non-cannulated rats within 6 h of application, gastrointestinal absorption of an administered low and high dose was estimated to be approx. 44 % and 12 %, respectively. Blood/plasma kinetics revealed initial half-lives of approx. 8 h and terminal half-lives ranging between 20 and 40 h. AUC values of both dose levels indicated sublinear kinetics. Tissue distribution determined 8 h after administration revealed highest amounts of radioactivity in the GI tract, liver and adipose tissue in low-dose rats. In the high-dose group, a similar distribution was observed in males, while in females, highest concentrations were found in the GI-tract, liver, thyroid and kidney. There was no evidence of a cumulative potential of nicobifen. The administered low dose was completely recovered in excreta within 2 days (approx. 20 % via urine and 80 % via faeces). At the high dose level of 500 mg/kg bw, total excretion within 7 days was in the range of 93–106 % AD), while only 3–5 % AD was eliminated via the urine. There were no significant differences in the excretory pattern with regard to sex, radiolabel used or frequency of application.

The systemically available portion of nicobifen was rapidly and intensively metabolised to a large number of biotransformation products. The hydroxylation of the diphenyl moiety was the quantitatively most important pathway. Second important was the substitution of the Cl in the 2-chloropyridine moiety against SH by conjugation with glutathione. Partial cleavage of the glutathione moiety afforded the cysteine conjugate and finally the SH-compound, which was subsequently methylated or oxidised. In addition, the introduction of glutathione and a second hydroxy group into the diphenyl part of the molecule was observed. Combinations of these reactions and the conjugation of the OH-groups with glucuronic acid or sulphate and the conjugation of the SH-group with glucuronic acid led to the large number of metabolites. The cleavage of the amide bond is negligible because the 2-chloronicotinic acid was detected only in trace amounts. No major differences were observed with regard to label, sex and dose level.

B.6.1.1 Absorption, distribution and excretion in rats after single or repeated oral intake

Report: Leibold E. et al., 2000 (TOX2001-703)
^{14}C-BAS 510 F - Study of the biokinetics in rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1014183, unpublished
(Experimental work from 21 November 1997 – 8 October 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 87/302, OECD 417, EPA 870.7485, JMAFF

Deviations: None

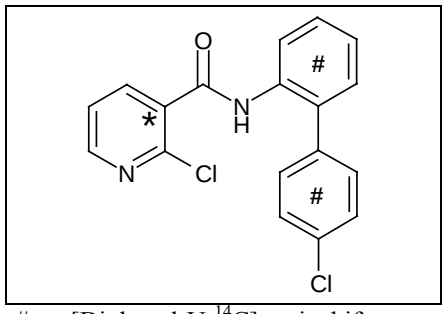
Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Radiolabelled nicobifen (diphenyl-ring–U-^{14}C); batch 641-1018 and 641-1101; chemical purity > 98 %, radiochemical purity: > 95 %.
Radiolabelled nicobifen (pyridin-ring-3-^{14}C); batch 640-1026; chemical purity > 97 %, radiochemical purity: > 95 %.
Non radiolabelled nicobifen; batch 01174-236; purity: > 99 %.

Test animals: Male and female Chbb-THOM Wistar rats (SPF), age (Day 1): at least 7 wk: bw males (Day 1, main tests): 221–304 g, bw females (Day 1): 163–206 g
Source: Boehringer Ingelheim Pharma KG, Biberach a.d. Riss, Germany

Figure B.6.1-1: Structure and position of the ^{14}C-label for both diphenyl and pyridine- labelled nicobifen



= [Diphenyl-U-^{14}C] – nicobifen
* = [Pyridine-3-^{14}C] – nicobifen

The key data of the toxicokinetic experiments performed with nicobifen are summarised in Table B.6.1-1 .

Table B.6.1-1: Rat ADME studies: Experimental design

Experiment No.	Type	Label	Dose [mg/kg bw]	Investigation period post application	Radiolabel recovery in
1	Excretion balance	Diphenyl	1 x 50	0–168 h	Urine, faeces, cage wash, blood cells, plasma, organs/tissues, contents of stomach and gut
2	Excretion balance	Diphenyl	1 x 500	0–168 h	Urine, faeces, cage wash, blood cells, plasma, organs/tissues, contents of stomach and gut
3	Excretion balance	Pyridine	1 x 500	0–168 h	Urine, faeces, cage wash, blood cells, plasma, organs/tissues, contents of stomach and gut
4	Excretion balance	Diphenyl	(14+1) x 500	0–120 h beginning after last dose	Urine, faeces, cage wash, blood cells, plasma, organs/tissues, contents of stomach and gut
5	Blood/plasma kinetics	Diphenyl	1 x 50	0.5–120 h	Plasma
6	Blood/plasma kinetics	Diphenyl	1 x 500	0.5–120 h	Plasma
7	Tissue distribution	Diphenyl	1 x 50	8, 17, 21 and 24	Blood cells, plasma, organs/tissues, contents of stomach and gut
8	Tissue distribution	Diphenyl	1 x 500	8, 18, 24 and 35 h	Blood cells, plasma, organs/tissues, contents of stomach and gut
9	Bile excretion	Diphenyl	1 x 50	48 h	Bile
10	Bile excretion	Diphenyl	1 x 500	48 h	Bile

The absorption, distribution, elimination and biokinetics of ^{14}C-nicobifen in male and female Wistar rats were investigated at dose levels of 50 and 500 mg/kg bw. The experiments were performed with ^{14}C-nicobifen labelled in the diphenyl-ring. An additional experiment investigating the balance and excretion pattern was performed with ^{14}C-nicobifen labelled in the pyridine-ring.

For the excretion balance studies four male and four female rats were used for each experiment. Excretion via the bile was determined separately for each dose level using four male and four female rats subjected to bile-duct cannulation surgery. For the pharmacokinetics studies four male and four female rats for each dose level were used. Tissue distribution was determined using 12 male and 12 female rats for each dose level.

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

Elimination
No radioactivity was detectable in expired air after administration of 500 mg/kg bw of the diphenyl- or pyridine-ring label. The overall recovery of administered radioactivity (AD) was in the range of 93.1–106.3 % in all experiments. The investigation of excretion in female rats administered diphenyl-radiolabelled nicobifen by single oral gavage had to be repeated, because in both low and high dose groups, unexpectedly high recoveries were obtained in feces at the 120-144-h sampling interval, which were not reproducible when repeating the experiment. Therefore, the excretion data for females listed in the Table B.6.1-2 and Table B.6.1-3 are derived from the second experiment; the metabolic profile obtained for females are also based on samples from the second experiment (see section B.6.1.2).

Table B.6.1-2: Rat ADME study: Overview of radiolabel recovery in excreta, carcass and tissues within 168 h following oral dosing

	Mean percentage of administered dose (%) recovered within 168 h							
	1x 50 mg/kg bw diphenyl-label		1x 500 mg/kg bw diphenyl-label		1x 500 mg/kg bw pyridine-label		(14+1)x 500 mg/kg bw diphenyl-label	
	male	female	male	female	male	female	male	female
Urine	16.38	25.74	2.73	5.67	5.21	3.80	2.64	3.99
Cage wash	0.26	0.03	0.41	0.12	0.10	0.30	0.11	0.18
Faeces	84.86	80.50	90.69	87.26	89.61	92.15	94.88	98.47
Carcass and tissues	0.04	0.07	0.05	0.05	0.04	0.02	0.11	1.1
Total recovery	**101.54**	**106.34**	**93.87**	**93.10**	**94.96**	**96.27**	**97.74**	**102.75**

As can be seen in Table B.6.1-3, elimination of radioactivity was rapid, with most of the radiolabel recovered within the first 24 hours following administration of the radiolabelled nicobifen. After single oral dosing of diphenyl-radiolabelled nicobifen at 50 mg/kg bw, the administered radiolabel was completely recovered in the excreta of males and females within 48 hours (approx. 20 % via urine and 80 % via the faeces).

Table B.6.1-3: Rat ADME-Study: Elimination of radiolabelled nicobifen

	Mean percentage of administered dose (%)								
	Urine			Faeces			Total eliminated		
	male	female	combined	male	female	combined	male	female	combined
1x 50 mg/kg bw diphenyl label									
0–6 h	2.68	5.35	4.02	0.01	0.01	0.01	2.69	5.36	4.03
0–12 h	7.72	11.49	9.61	41.77	0.51	21.14	49.49	12.00	30.75
0–24 h	13.43	21.74	17.59	71.93	68.15	70.04	85.36	89.89	87.63
0–48 h	15.88	25.03	20.46	83.44	79.41	81.43	99.32	104.44	101.88
0–72 h	16.14	25.41	20.78	84.64	80.07	82.36	100.78	105.48	103.13
0–168 h*	16.64	26.76	21.70	84.78	80.53	82.66	101.42	107.29	104.36
1x 500 mg/kg bw diphenyl label									
0–6 h	0.29	0.35	0.32	0.00	0.00	0.00	0.29	0.35	0.32
0–12 h	0.87	1.12	1.00	30.30	9.39	19.85	31.17	10.51	20.84
0–24 h	1.81	3.01	2.41	85.96	70.34	78.15	87.77	73.35	80.56
0–48 h	2.59	5.24	3.92	90.12	84.24	87.18	92.72	89.48	91.10
0–72 h	2.66	5.48	4.07	90.47	86.94	88.71	93.13	92.42	92.78
0–168 h*	3.14	5.81	4.48	90.72	87.27	89.00	93.86	93.08	93.47
1x 500 mg/kg bw pyridine label									
0–24 h	2.89	2.59	2.74	72.34	87.66	80.00	75.23	90.25	82.74
0–48 h	4.72	3.45	4.09	87.34	91.63	89.49	92.05	95.08	93.57
0–72 h	5.08	3.71	4.35	88.80	91.94	90.37	93.88	95.56	94.72
0–168 h*	5.32	4.12	4.72	89.63	92.15	90.89	94.95	96.27	95.61
(14 + 1)x 500 mg/kg bw diphenyl label									
0–24 h	1.60	2.56	2.08	77.96	88.49	83.23	79.56	91.05	85.31
0–48 h	2.46	3.77	3.12	94.05	98.01	96.03	96.51	101.78	99.15
0–72 h	2.61	3.92	3.27	94.75	98.37	96.56	97.36	102.29	99.83
0–120 h*	2.77	4.18	3.48	94.90	98.48	96.69	97.67	102.66	100.17

* urine values at last time-point of investigation include recoveries in cage wash

Radioactivity remaining in tissues and organs 168 hours post dosing was less than 0.3 µg eq./g at a dose level of 50 mg/kg bw except for the GI tract. Total radiolabel recovery of the administered low dose in tissues and organs amounted to approx. 0.05 % AD. Similar recovery values in carcass and tissues ranging between 0.02–0.05 % AD were obtained after

single high-dose treatment. After repeated high-dose treatment, recovery in tissues/carcasses of high-dose males and females was 0.11 and 1.1 % AD, respectively. Radioactivity remaining in tissues and organs 168 hours post dosing was less than 1 µg eq./g at a dose level of 500 mg/kg bw except for the GI tract.

Elimination appeared to be independent of sex or of the kind of radiolabel administered. However, a dose-dependency was observed, since increasing the dose from 50 to 500 mg/kg bw resulted in a reduction of urinary excretion (from 20 % to approx. 3–5 % AD) and in a concomitant increase in excretion via the faeces.

Biliary excretion

The biliary excretion of radiolabelled nicobifen was determined for a 48-h period in bile-duct cannulated rats administered either 50 or 500 mg nicobifen/kg bw. Results are summarised in Table B.6.1-4. Radiolabel recovery in bile was 39–40 % AD at the low-dose level and 11–12 % in high-dose group animals.

Table B.6.1-4: Rat ADME study: Recovery in bile-duct cannulated rats within 48 h post application

	Mean percentage of administered dose (%)			
	1x 50 mg/kg bw		**1x 500 mg/kg bw**	
	diphenyl-label		**diphenyl-label**	
Recovery in	**male**	**female**	**male**	**female**
Bile	39.29	39.92	10.69	11.93
Faeces	no data	no data	no data	no data
Urine	no data	no data	no data	no data
Organs/tissues	no data	no data	no data	no data

Absorption

Due to the lack of urinary excretion data from bile-duct cannulated rats, only a rough estimate of gastrointestinal absorption is possible. Based on the assumption that enterohepatic circulation is expected not to occur to a significant degree during the first 6 hours after treatment, it is considered to be justified to add the amount of radiolabel recovered in the 0–6-h urine from non-cannulated rats (approx. 4 % and 0.3 % of the administered low and high dose, respectively) to the biliary radiolable recovery obtained within 48 h post application in bile-duct cannulated rats (approx. 40 % and 12 % of the administered low and high dose, respectively). Thus, by this approach, the absorption estimates of 44 % and 12 % of the administered low and high dose are derived.

Note: By comparison of bile/urine recovery ratios for different sampling periods, the notifier considered that the amount of radiolabel recovered in the 0–12-h urine from non-cannulated rats was excreted via the kidney under total exclusion of enterohepatic circulation. Thus, it would be justified to add the amount recovered in 0–12-h urine from non-cannulated rats to the radiolabel recovery in bile from bile-duct cannulated rats for estimation of gastrointestinal absorption. The RMS does not agree with this approach for the following reasons:

1) Since different experimental conditions of non-cannulated and bile-duct cannulated rats result in different toxicokinetics, such mixed bile/urine ratios are difficult to interpret and are most probably not meaningful.

2) The kinetic data (see Table B.6.1-5) shows two plasma concentration maxima, the first occuring at approx. 0.5–1 h and the second occuring at approx. 8 h post application. In the opinion of the RMS, the second Tmax at 8-h is most probably due to enterohepatic circulation of radiolabelled nicobifen metabolites coming into play. Therefore, it cannot be excluded that a considerable amount of radiolabel recovered in the 6–12-h urine sample was already subject to prior enterohepatic circulation, i.e. would have been recovered in the bile of bile-duct cannulated rats.

Blood/plasma kinetics

The results of biokinetic investigations in plasma and blood are summarised in Table B.6.1-5. In rats exposed to a single oral dose of 50 mg/kg bw of ^{14}C-nicobifen, the plasma concentration/time curve showed 2 peaks. The first plasma peak was reached after 0.5 hour with peak levels of 0.99 µg eq./g in males and 1.40 µg eq./g in females. At the second plasma peak occurring after 8 hours, plasma levels were 1.54 and 1.58 µg eq./g in males and females, respectively. After the second peak, plasma concentrations declined biphasically to levels of 0.01 µg eq./g in males and females at 120 hours post dosing. The initial half life was found to be 7.2 hours in males and 8.2 hours in females. Terminal half lives in male and female rats were 41.7 and 30.1 hours, respectively. The AUC was 21.2 µg eq. x h/g in males and 24.4 µg eq. x h/g in females. In rats exposed to a single oral dose of 500 mg/kg bw of ^{14}C-nicobifen, the plasma concentration/time curve showed 2 peaks. The first plasma peak was reached 0.5–1 hour post dosing with peak levels of 2.61 µg eq./g in males and 3.52 µg eq./g in females. At the second plasma peak occurring after 8 hours, plasma levels were 4.46 and 3.77 µg eq./g in males and females, respectively. After the second peak, plasma concentrations declined to levels of 0.01 µg eq./g in males and 0.03 µg eq./g in females at 120 hours post dosing. The initial half life was calculated to be 8.0 h in males and 9.1 hours in females. Terminal half life was 20.2 h in males and 27.4 hours in females. The AUC was 68.4 µg eq. x h/g in males and 75.5 µg eq. x h/g in females.

Table B.6.1-5: Rat ADME study: Results of kinetic investigation in blood and plasma

	50 mg/kg bw		500 mg/kg bw	
	males	**females**	**males**	**females**
1st Cmax (µg eq./g)	0.99	1.40	2.61	3.52
1st Tmax (h)	1.00	0.5	0.5–1	0.5–1
2nd Cmax (µg eq./g)	1.54	1.58	4.46	3.77
2nd Tmax (h)	8	8	8	8
Initial T1/2 (h)	7.2	8.2	8.0	9.1
Terminal T1/2 (h)	41.7	30.1	20.2	27.4
AUC (µg eq. x h / g)	21.2	24.4	68.4	75.5

Thus, increasing the dose level by a factor of about 10 resulted in an increase of the AUC-values by a factor of approx. 3 in males and 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.

Table B.6.1-6: Rat ADME study: tissue distribution at 8 and 24 h time points

Tissue	Concentration of radioactive residues (as µg eq./g tissue)							
	Dose of 50 mg/kg bw				Dose of 500 mg/kg bw			
	Males		Females		Males		Females	
	8 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h
Blood cells	0.64	0.12	0.78	0.13	1.74	0.31	1.35	0.43
Plasma	1.14	0.14	1.52	0.17	3.52	0.40	2.84	0.51
Lung	2.15	0.30	1.82	0.31	4.31	0.68	3.76	0.88
Heart	1.35	0.08	1.25	0.09	3.14	2.50	2.74	0.38
Spleen	1.13	0.19	1.04	0.31	2.01	0.29	1.86	0.44
Kidney	5.96	0.58	7.87	0.67	11.96	1.53	16.11	2.07
Adrenal	3.10	0.45	3.05	0.28	8.47	2.31	8.79	3.18
Testes/ovaries	0.89	0.05	4.38	1.75	0.31	0.18	10.94	2.25
Uterus	-	-	3.28	1.41	–	-	5.64	3.03
Muscle	1.15	0.06	0.85	0.06	2.46	0.74	2.82	0.85
Brain	0.79	0.03	0.75	0.04	1.16	0.18	0.48	0.26
Adipose tissue	16.91	1.05	15.67	1.46	32.80	3.05	6.01	3.88
Bone	0.37	0.05	0.35	0.08	1.26	0.32	1.91	0.49
Bone marrow	1.57	0.20	1.66	0.66	15.78	1.21	5.06	3.49
Thyroid	3.82	0.78	2.80	1.01	15.02	1.70	17.11	1.32
Pancreas	5.32	0.99	4.62	1.63	7.24	1.71	5.47	1.15
Stomach contents	194.79	4.21	176.31	0.54	411.40	19.90	433.92	28.54
Stomach	78.89	0.94	40.56	0.79	103.82	3.06	77.11	6.60
Gut contents	852.09	21.09	717.02	30.90	6373.97	64.95	9216.82	138.38
Gut	74.87	8.68	87.13	10.10	202.97	18.59	256.61	25.56
Liver	16.47	1.16	13.62	1.13	39.12	2.86	22.65	3.17
Skin	3.10	0.22	2.97	0.36	8.06	1.08	8.51	0.88
Carcass	14.13	0.63	4.17	0.82	101.82	1.96	39.18	2.70

Tissue distribution

Mean tissue concentrations of radioactivity after single oral administration of ^{14}C-nicobifen at 50 and 500 mg/kg bw are summarised in Table B.6.1-6. 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. Tissue radioactivity concentrations declined with time and at a similar rate to the plasma concentrations. Throughout the time course of the experiments, highest radioactivity concentrations were found in the GI tract, liver and adipose tissue. In high-dose group females highest radiolabel concentrations were found in GI-tract, liver, thyroid and kidney.

Potential for accumulation

Based on generally low tissue levels and nearly complete elimination of the radiolabel regardless of single low or repeated high dose treatment (see Table B.6.1-2 and Table B.6.1-6), no evidence for an accumulation potential of nicobifen could be deduced from the available data.

Conclusion:

Following oral administration of nicobifen to rats, nicobifen was rapidly but incompletely absorbed from the gastrointestinal tract, widely distributed and rapidly eliminated from the body. Based on recovery of the radiolabel in bile, gastrointestinal absorption of a low and high dose within 48 h of application was estimated to be approx. 40 % and 12 % AD, respectively. Blood/plasma kinetics revealed initial half-lives of approx. 8 h and terminal half-lives ranging between 20 and 40 h. AUC values of both dose levels indicated a less than linear kinetics.

Tissue distribution determined 8 h after administration revealed highest amounts of radioactivity in the GI tract, liver and adipose tissue in low-dose rats. In the high-dose group, a similar distribution was observed in males, while in females, highest concentrations were found in the GI-tract, liver, thyroid and kidney. There was no evidence of a cumulative potential of nicobifen. Approx. 99 % of the administered low dose was recovered in excreta within 7 days (17 % via urine and cage wash, and 82 % via faeces). At the high dose level of 500 mg/kg bw, total excretion was similar (96–100 %), while only 3–5 % AD was eliminated via the urine. There were no significant differences in the excretory pattern with regard to sex, radiolabel used or frequency of application.

B.6.1.2 Metabolism

Report: Grosshans F. and Knoell H.E., 2001 (TOX2001-706)
The metabolism of ^{14}C-BAS 510 F (Reg.No. 300 355) in rats
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany
BASF RegDoc# 2000/1017220, unpublished
(Experimental work from September 1997 – October 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EPA 870.7485, EEC 87/302, JMAFF

Deviations: None that compromised the validity of the study results

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen;
Batch: 641-1018 (diphenyl-U-^{14}C), purity: > 99 %, specific activity: 5.23 MBq/mg; Batch: 640-1026 (pyridine-3-^{14}C), purity: > 97 %, specific activity 5.81 MBq/mg; Batch: 01174-236 (unlabelled), purity: > 99.4 %

Primarily for the purpose of metabolite identification, groups of 10 male and 10 female Wistar rats were orally dosed with 500 mg nicobifen/kg bw radiolabelled either at the diphenyl-U-^{14}C or at the pyridine-3-^{14}C moiety. Two further groups each consisting of four rats per sex were administered (pyridine-3-^{14}C)-labelled nicobifen either at 50 or 500 mg/kg bw for the analysis of metabolite patterns in plasma, liver and kidneys. Plasma was sampled and organs were removed at or near the presumed peak plasma level (t_{max}, 8 h post dose).

Patterns of radioactive metabolites in excreta (urine, faeces, bile), plasma, and tissues (liver and kidney) were analysed chromatographically. Relevant metabolites were identified by MS analysis and in some cases NMR analysis of isolated fractions. Metabolite patterns of samples generated in this study were compared with those obtained in a biokinetic study (see B.6.1.1) in which groups of rats were administered single oral high (500 mg/kg bw) and low (50 mg/kg bw) doses and repeated high doses (14 x non-radio-labelled, 1 x radio-labelled; 500 mg/kg bw) For an overview of the treatment groups and analysed samples refer to Table B.6.1-7.

Table B.6.1-7: Rat metabolism study: Summary of dose groups and analysed samples

Metabolism study						
Dose group	DX		DX	V		W
Nominal dose level [mg/kg bw]	1x 500		1x 500	1x 50		1x 500
^{14}C – label	Diphenyl		Pyridine	Pyridine		Pyridine
Samples analysed	urine, faeces		urine, faeces	plasma, tissues		plasma, tissues
Biokinetic study						
Dose group	1	2	3	4	9	10
Nominal dose level [mg/kg bw]	1x 50	1x 500	1x 500	(14+1)x 500	1x 50	1x 500
^{14}C – label	Diphenyl	Diphenyl	Pyridine	Diphenyl	Diphenyl	Diphenyl
Samples analysed	urine, faeces	urine, faeces	urine, faeces	urine, faeces	bile	bile

Findings:

Excretion of radioactivity

Within 96 h post application of 500 mg/kg bw nicobifen labelled either at the pyridine or the diphenyl moiety, the amounts of radioactivity excreted in urine and faeces were 3–5 % and 86–102 % AD, respectively, and thus comparable to the findings of the biokinetic study (see Table B.6.1-2).

Urine metabolites

After oral dose of [^{14}C]-nicobifen to male and female rats, a large number of metabolites was detected in urine (see Table B.6.1-8). Predominant metabolites were M510F01 (hydroxylated at the 4- position of the phenyl ring) and its glucuronic acid conjugate M510F02. For M510F01, the proportion excreted via urine ranged from 0.5 % to 3 % in the high dose groups (dose level: 500 mg/kg bw) and from 10 % to 16 % in the low dose group (dose level: 50 mg/kg bw). Metabolite M510F02 was detected in a range from 0.1 % to 4 % of the dose. M510F48 (exchange of Cl against S-glucuronide in the pyridine moiety) and M510F05 (Cl in the pyridine moiety substituted by cysteine) were found as additional metabolites (up to 2 % of the dose). Minor metabolites were M510F03, M510F04, M510F12, M510F20, and M510F42. Traces of parent could be detected. In the pyridine labelled groups, traces of M510F47 (chloronicotinic acid) were identified, whereas chloro-aminobiphenyl was not detected in the diphenyl labelled dose groups.

Table B.6.1-8: Rat metabolism study: Identified urinary metabolites

Radiolabel:		DiPh	DiPh	DiPh	Pyr	Pyr	DiPh
Experimental Group:		1	2	DX	DX	3	4
Dose [mg/kg bw]:		1x 50	1x 500	1x 500	1x 500	1x 500	15x 500
Metabolites identified	Sex	Total excretion in 0–48 h urine [% of administered dose]					
Nicobifen	m	–	0.16	–	–	0.07	0.11
	f	0.06	0.04	–	–	0.02	0.05
M510F01	m	9.58	1.04	0.57	0.51	2.93	1.34
	f	15.79	1.52	2.23	1.02	0.94	1.94
M510F02	m	2.95	0.69	1.73	2.74	0.08	0.22
	f	4.33	2.41	1.60	2.14	1.64	1.03
M510F03	m	–	–	–	–	–	–
	f	–	–	0.08	0.13	–	0.07
M510F04	m	0.08	–	–	–	0.01	–
	f	0.22	0.04	0.13	0.10	0.01	0.02
M510F05	m	0.48	0.09	0.34	0.40	0.17	0.04
	f	0.59	0.07	0.34	0.37	0.03	0.08
M510F06	m	faecal metabolites, not detected in urine extracts					
	f						
M510F11	m						
	f						
M510F12	m	–	–	–	–	0.34	0.06
	f	–	–	–	–	0.04	–
M510F20	m	0.57	0.05	0.02	–	0.26	0.14
	f	0.46	0.10	0.02	0.01	0.06	0.04
M510F42	m	0.18	0.22	0.10	0.13	0.48	0.26
	f	0.25	0.08	0.03	0.04	0.05	–
M510F47	m	–	–	–	0.07	0.10	–
	f	–	–	–	0.06	0.07	–
M510F48	m	1.10	0.03	0.37	0.44	0.04	0.02
	f	2.28	0.47	0.39	0.33	0.26	0.26
M510F63	m	faecal metabolite, not detected in urine extracts					
	f						
Total	m	14.94	2.28	3.13	4.29	4.48	2.19
	f	23.98	4.73	4.82	4.20	3.12	3.49

Faeces metabolites

Throughout all dose groups and independent of sex and label, the parent substance nicobifen was the major component and ranged from 57 % to 85 % of the dose in the high dose groups and from 30 % to 41 % in the low dose groups. M510F01 and M510F06 (exchange of Cl against SH in the pyridine moiety) were identified to be predominant metabolites in all dose groups. The metabolites M510F20 and M510F63 were mainly found in the low dose groups. As minor metabolites M510F05, M510F11 and M510F48 were found. For individual values see Table B.6.1-9.

Table B.6.1-9: Rat metabolism study: Identified faecal metabolites

Radiolabel:		DiPh	DiPh	DiPh	Pyr	Pyr	DiPh
Experimental Group:		1	2	DX	DX	3	4
Dose [mg/kg bw]:		1x 50	1x 500	1x 500	1x 500	1x 500	15x 500
Metabolites identified	Sex	Total excretion in 0–48 h faeces [% of administered dose]					
Nicobifen	m	41.00	80.37	80.46	75.63	72.91	85.15
	f	30.45	68.26	64.96	56.96	70.16	75.82
M510F01	m	21.81	4.10	4.32	8.18	4.84	2.46
	f	18.99	5.50	9.06	10.54	4.35	12.60
M510F02	m	urinary metabolites, not detected in faecal extracts					
	f						
M510F03	m						
	f						
M510F04	m						
	f						
M510F05	m	–	–	0.64	4.87	–	–
	f	1.85	–	0.70	1.52	–	–
M510F06	m	4.88	7.00	6.10	1.04	7.59	2.60
	f	7.57	3.00	10.29	12.24	3.81	1.41
M510F11	m	2.31	1.33	–	–	–	0.14
	f	0.53	0.58	–	–	–	–
M510F12	m	urinary metabolite, not detected in faecal extracts					
	f						
M510F20	m	6.21	–	0.80	1.05	–	–
	f	3.79	–	0.57	0.73	–	0.51
M510F42	m	–	–	–	–	–	–
	f	–	0.20	–	–	–	–
M510F47	m	urinary metabolite, not detected in faecal extracts					
	f						
M510F48	m	–	0.42	1.26	6.50	–	–
	f	2.84	0.63	1.05	0.38	–	–
M510F63	m	0.60	0.32	–	–	0.46	–
	f	4.01	1.35	–	–	0.25	–
Total	m	76.81	93.54	93.58	97.27	85.80	90.35
	f	70.03	79.52	86.63	82.37	78.57	90.34

Bile metabolites

Major metabolites in bile were M510F02 and M510F05. As minor components, the metabolite M510F01, and the metabolites M510F57 and M510F58 (introduction of a hydroxy group and cysteine into the diphenyl moiety) were identified. In addition traces of M510F50 (OH group at the pyridine part of the molecule) were found. For individual values see Table B.6.1-10.

Table B.6.1-10: Rat metabolism study: Identified metabolites in bile after single low and high dose administration of [diphenyl U-^{14}C]–radiolabelled nicobifen

Metabolite identity	Total excretion in % of dose	
	50 mg/kg bw female	500 mg/kg bw female
M510F01	1.71	0.28
M510F02	19.27	4.78
M510F03	1.48	0.21
M510F05	14.24	3.59
M510F22/F23	–	0.10
M510F57	1.32	0.41
M510F58	0.27	0.09

Liver metabolites

All metabolites were present in the liver with less than 0.3 % of the dose (see Table B.6.1-11). The metabolites M510F01, M510F02, M510F05, and M510F06 already known from urine and faeces were found in liver. In addition, the metabolite M510F46 (introduction of an OH-group and a glutathione group in the diphenyl ring moiety), the metabolite M510F45 (introduction of a glutathione group in the diphenyl ring moiety), and the metabolite M510F43 (exchange of Cl versus glutathione in the pyridine moiety) were identified.

Table B.6.1-11: Rat metabolism study: Identified metabolites in liver after single low and high dose administration of [pyridine 3-^{14}C]–radiolabelled nicobifen

Metabolite identity	Metabolite concentration in liver (µg equivalents per g)			
	50 mg/kg bw		500 mg/kg bw	
	male	female	male	female
Nicobifen	0.12	0.21	1.01	1.10
M510F01	0.84	0.73	2.19	2.50
M510F02	1.88	3.08	19.43	16.42
M510F05	0.13	0.29	2.08	1.13
M510F06	0.33	0.43	2.92	3.66
M510F42	0.17	-	-	-
M510F43	0.92	2.16	13.54	12.34
M510F45	0.66	0.41	4.04	8.25
M510F46	1.63	1.11	2.63	4.22
M510F47	-	-	0.21	-

Kidney metabolites

Kidney showed the metabolites M510F01, M510F02, M510F03, M510F05, M510F06, M510F48 and parent already known from excreta (Table B.6.1-12). These metabolites were detected in a range from <0.01 % to 0.06 % of the dose.

Table B.6.1-12: Rat metabolism study: Identified metabolites in kidney after single low and high dose administration of [pyridine 3-^{14}C]-radiolabelled nicobifen

Metabolite identity	Metabolite concentration in kidney (µg equivalents per g)			
	50 mg/kg bw		500 mg/kg bw	
	male	female	male	female
Nicobifen	0.68	1.69	6.92	10.48
M510F01	0.48	0.58	2.37	3.98
M510F02	1.72	1.18	5.60	5.95
M510F03	0.17	-	2.83	-
M510F05	0.27	3.04	2.61	28.85
M510F06	0.10	0.14	2.68	1.62
M510F42	0.20	-	-	-
M510F48	0.30	0.80	3.00	3.23

Plasma metabolites

M510F01, M510F02, M510F06, M510F48 and parent were detected in plasma at or below 0.01 % of the dose (see Table B.6.1-13):

Table B.6.1-13: Rat metabolism study: Identified metabolites in plasma after single low and high dose administration of [pyridine 3-^{14}C]-radiolabelled nicobifen

Metabolite identity	Metabolite concentration in plasma (µg equivalents per g)			
	50 mg/kg bw		500 mg/kg bw	
	male	female	male	female
Nicobifen	0.26	0.29	1.74	1.85
M510F01	0.07	0.09	0.22	0.26
M510F02	0.39	0.54	1.62	2.86
M510F06	0.10	0.19	1.38	1.86
M510F48	0.12	0.07	0.91	0.52

Metabolic pathway

After oral administration of [^{14}C]-nicobifen the unchanged parent compound was predominantly found in faeces and in trace amounts in urine, liver, and in plasma. Overall, the comparison of the sexes, the different labels, and the different dose levels resulted in no remarkable differences of the metabolite patterns. The absorbed parent compound was intensively metabolised following two main routes and three side routes.

At first, the diphenyl ring system was oxygenated at the 4-position of the phenyl ring followed mainly by conjugation with glucuronic acid and to a smaller extent by sulphate.

Second, the parent compound was transformed via substitution of the Cl of the 2-chloropyridine moiety against SH by conjugation with glutathione. The glutathione moiety was then cleaved to the cysteine conjugate followed by further cleavage to the SH-compound and subsequent S-methylation, S-glucuronidation or oxidation to a sulphate.

To a smaller extent the introduction of glutathione could occur at the diphenyl ring system followed by cleavage steps down to the SH-compound and subsequent S-methylation. Also to a smaller extend a second hydroxylation at the diphenyl ring moiety was observed. However, the cleavage of the parent compound at the amide bond showed to be negligible. The 2-

chloronicotinic acid was detected in trace amounts using the pyridine label whereas the other cleavage product chloro-aminobyphenyl could not be detected using the diphenyl label.

Combinations of these reactions led to a large number of observed metabolites, which resulted in a fairly complex metabolic pathway scheme (see Figure B.6.1-2). The structures of the identified metabolites can be found in Table B.6.1-14.

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

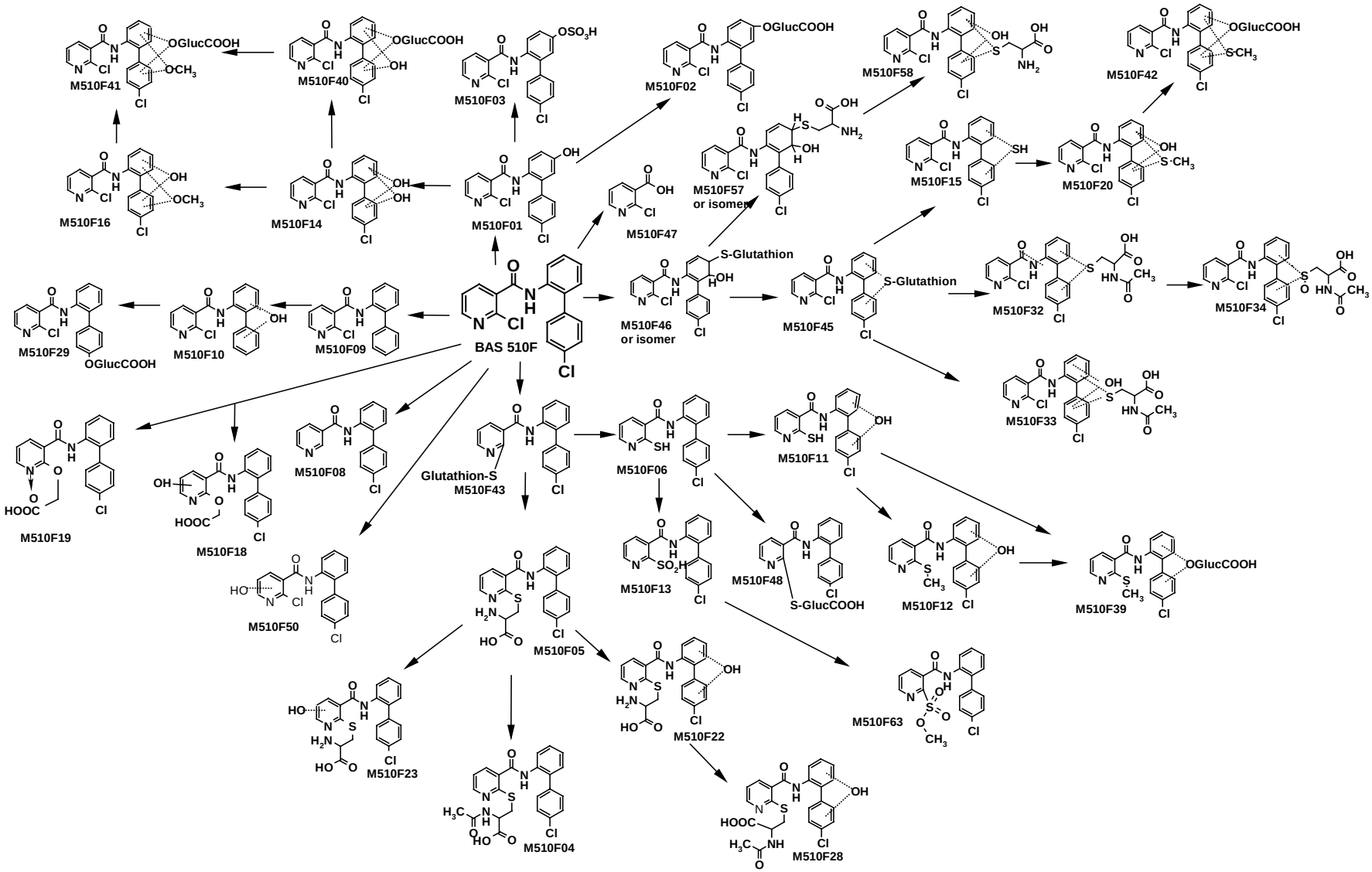
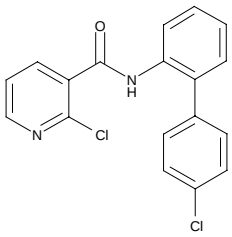
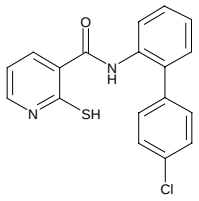
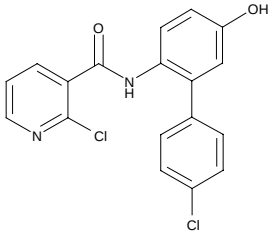
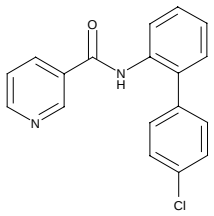
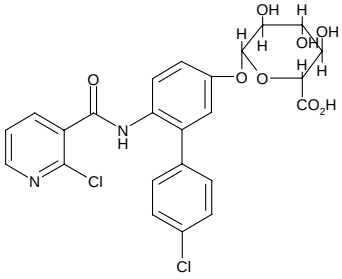
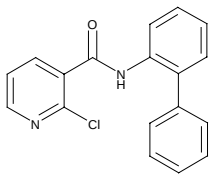
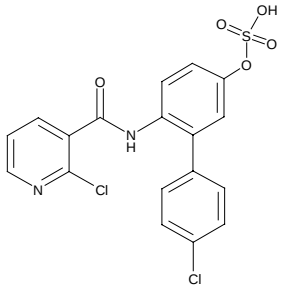
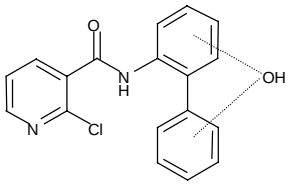
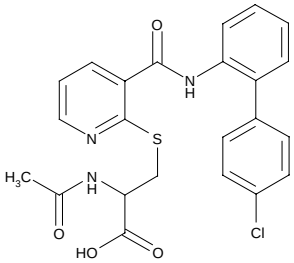
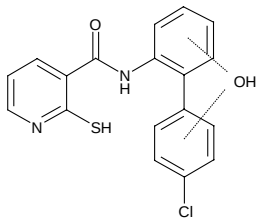
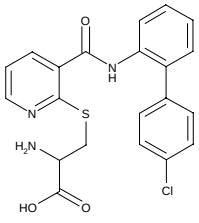
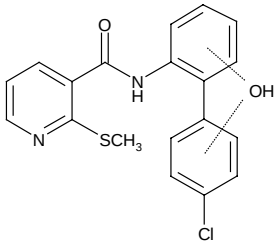
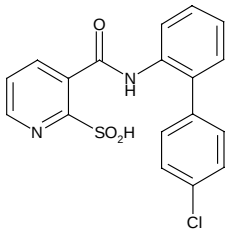
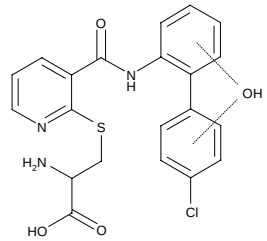
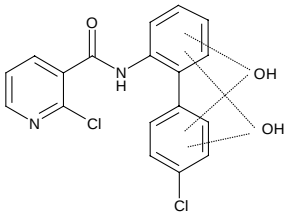
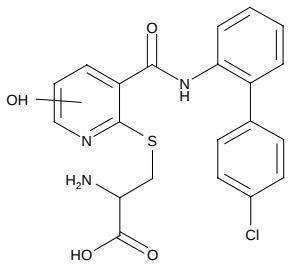
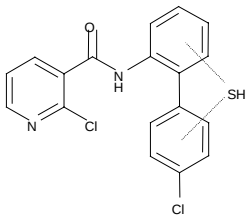
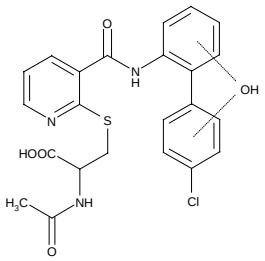
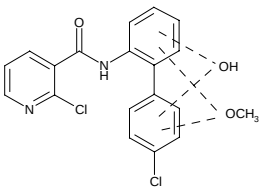
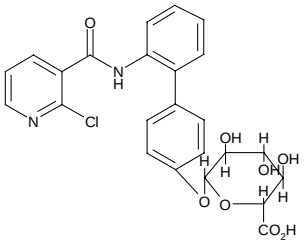
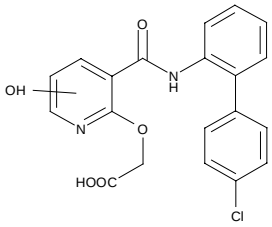
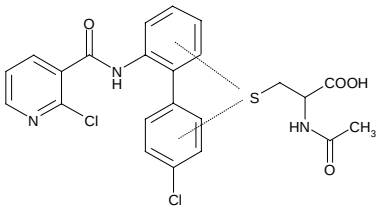
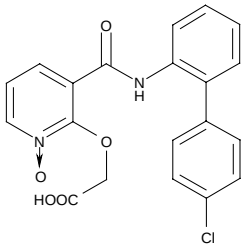
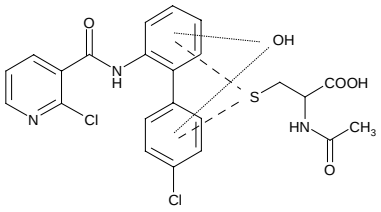
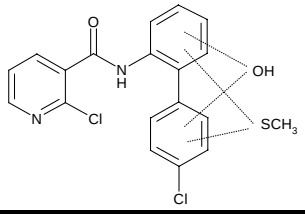
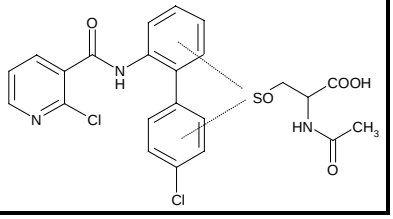
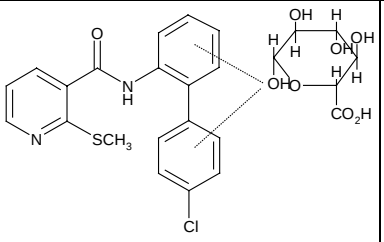
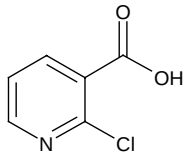
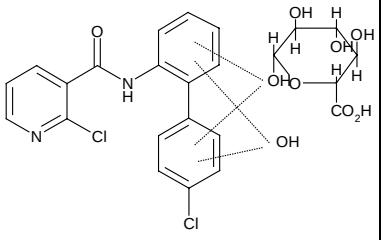
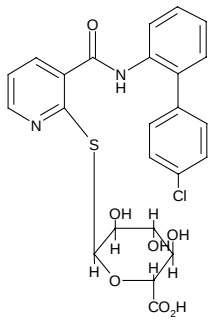
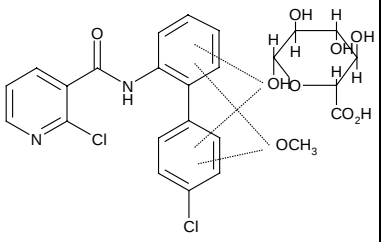
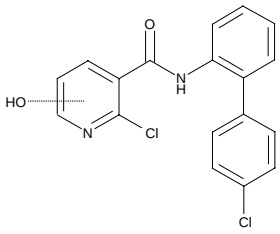
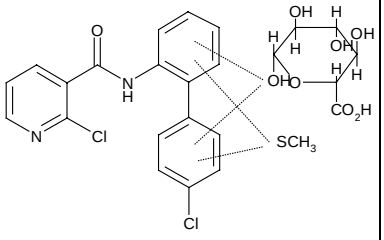
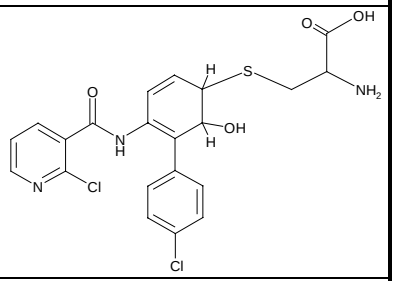
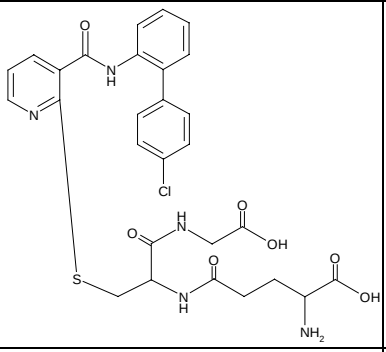
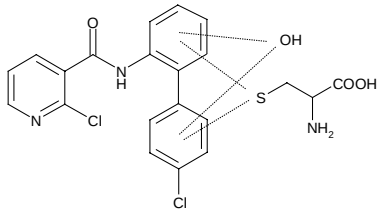
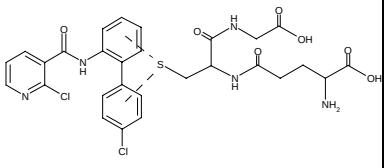
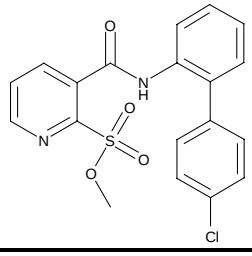
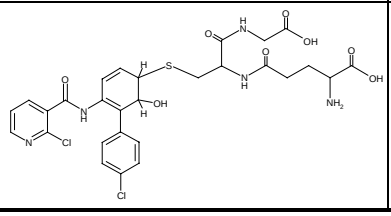


Table B.6.1-14: Rat metabolism study: Structures of identified metabolites in rat excreta, plasma, and tissues

Metabolite Code	Structure	Metabolite Code	Structure
Nicobifen		M510F06	
M510F01		M510F08	
M510F02		M519F09	
M510F03		M510F10	
M510F04		M510F11	
M510F05		M510F12	

Metabolite Code	Structure	Metabolite Code	Structure
M510F13		M510F22	
M510F14		M510F23	
M510F15		M510F28	
M510F16		M510F29	
M510F18		M510F32	
M510F19		M510F33	
M510F20		M510F34	

Metabolite Code	Structure	Metabolite Code	Structure
M510F39		M510F47	
M510F40		M510F48	
M510F41		M510F50	
M510Fy42		M510F57 or isomer	
M510F43		M510F58	
M510F45		M510F63	

Metabolite Code	Structure	Metabolite Code	Structure
M510F46 or isomer			

Conclusion:
After oral administration to male and female rats, the systematically available portion of nicobifen was rapidly and intensively metabolised to a large number of biotransformation products. The hydroxylation of the diphenyl moiety was the quantitatively most important pathway. Second important was the substitution of the Cl of the 2-chloropyridine part against SH by conjugation with glutathione. Partial cleavage of the glutathione moiety afforded the cysteine conjugate and finally the SH-compound which was subsequently methylated or oxidised. In addition, the introduction of glutathione and a second hydroxy group into the diphenyl part of the molecule was observed. Combinations of these reactions and the conjugation of the OH-groups with glucuronic acid or sulphate and the conjugation of the SH-group with glucuronic acid led to the large number of metabolites. The cleavage of the amide bond is negligible because the 2-chloronicotinic acid was detected only in trace amounts. No major differences were observed with regard to label, sex, and dose level.

Note: A discrepancy was noted regarding the reporting of the radiolabel recovery in two female dose groups (Metabolism report BASF Reg.Doc.No. 2000/1017220, page 44, table 4 versus Biokinetic report BASF Reg.Doc.No. 2000/1014183, page 35, table 2). Note that the figures of the male animals are the same in both reports, while the values for the female rats differ. A comment by the notifier is requested.

	% of the radioactivity administered							
	1x 50 mg/kg bw diphenyl-label (Gr. B)				1x500 mg/kg bw diphenyl label (Gr. D)			
	Biokinetic study 2000/1014183		Metabolism study 2000/1017220		Biokinetic study 2000/1014183		Metabolism study 2000/1017220	
	male	female	male	female	male	female	male	female
Urine	16.38	15.72	16.38	25.74	2.73	2.88	2.73	5.67
Cage wash	0.26	1.03	0.26	0.03	0.41	0.04	0.41	0.12
Faeces	84.86	79.27	84.86	80.50	90.69	97.35	90.69	87.26
Carcass and tissues	0.04	0.04	N.R.	N.R.	0.05	0.02	N.R.	N.R.
Total recovery	**101.54**	**96.06**	**101.50**	**106.27**	**93.87**	**100.29**	**93.83**	**93.05**

N.R. not reported

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

Nicobifen is characterised by a very low acute oral, dermal and inhalation toxicity. The substance is neither irritating to the skin nor to the eyes. It is not a skin sensitiser in the Maximisation Test. The results of the acute toxicity studies with nicobifen are summarised in Table B.6.2-1 below.

Table B.6.2-1: Summary of acute toxicity data for nicobifen

Type of study	Nicobifen purity	Results	Comments
Rat acute oral toxicity	95.3%	LD_{50} >5000 mg/kg bw	No mortality
Rat acute dermal toxicity	95.3%	LD_{50} >2000 mg/kg bw	No mortality, no signs of toxicity
Rat acute inhalation toxicity	95.3%	LC_{50} >6.7 mg/l air	4-h dust exposure; no mortality
Rabbit skin irritation	95.3%	Not irritating	very slight erythema only at 24 h
Rabbit eye irritation	95.3%	Not irritating	Slight and reversible conjunctival irritation.
Guinea pig skin sensitisation (M&K Test)	95.3%	Not a skin sensitiser	A few (4/19) animals displayed skin irritation in the challenge

Classification:
With respect to the prevailing EU classification schemes, a classification of nicobifen is not required.

B.6.2.1 Oral

Report: Wiemann C. and Hellwig J., 1998 (TOX2001-707)
BAS 510 F: Acute oral toxicity in rats,
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1998/10643, unpublished
(Experimental work from 12 August – 3 September 1997)

Amendment (Re-analysis of the test substance's stability):
Wiemann C., 2000 (TOX2001-708)
Amendment No. 1: BAS 510 F - Acute oral toxicity in rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1018715, unpublished

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 401, EEC 92/69, EPA 81-1

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 26, purity: 95.3%
Test animals: Male + female Wistar rats (Chbb: thom, SPF), bw 150–300 g (Day 1)
Source: Dr. K. Thomae GmbH, Biberach a.d. Riss, Germany

10 fasted Wistar rats (5/sex) were given a single oral dose of nicobifen at a dose level of either 2000 or 5000 mg/kg bw. The test substance was administered as preparation in 0.5% aqueous Tylose CB 30.000 (cleaned sodium carboxymethylcellulose from Hoechst AG) using an application volume of 10 and 20 ml/kg bw for the low and high dose level, respectively. All animals were observed for clinical signs of toxicity and mortality for up to 14 days post-dosing.

Findings:
The stability of the test substance in the vehicle was demonstrated and confirmed by re-analysis. The correctness of the concentration and its homogeneity were analytically confirmed.

There was no mortality in either males or females. Signs of toxicity noted at 5000 mg/kg bw included impaired general state, dyspnoea, staggering, excitation, erythema and piloerection in males and females. They occurred on day 1 in two males and in one female. All animals appeared normal within two days after application. No signs of toxicity were noted in the 2000 mg/kg bw dose group

Body weight development appeared to be normal. 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 >5000 mg/kg bw for male and female rats.

B.6.2.2 Dermal

Report: Wiemann C. and Hellwig J., 1998 (TOX2001-709)
BAS 510 F: Acute dermal toxicity in rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1998/10642, unpublished
(Experimental work from 11–25 September 1997)

Amendment (Re-analysis of the test substance's stability):
Wiemann C., 2000 (TOX2001-710)
Amendment No. 1: BAS 510 F - Acute dermal toxicity in rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1018711unpublished

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 402, EEC 92/69, EPA 81-2
Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 26, purity: 95.3%
Test animals: Male + female Wistar rats (Chbb: thom, SPF), bw 200–300 g (Day 1)
Source: Dr. K. Thomae GmbH, Biberach a.d. Riss, Germany

10 fasted Wistar rats (5/sex) were dermally exposed to nicobifen at 2000 mg/kg bw (limit dose) for 24 hours under semi-occlusive dressing. The test substance was administered as 0.5% aqueous Tylose CB 30.000 suspension (cleaned sodium carboxymethylcellulose from Hoechst AG) to an application area of approx. 50 cm² (corresponding to at least 10% of the body surface area). Animals were observed for mortality, clinical signs of toxicity, body weight development and skin changes for up to 14 days post-dosing. A gross pathological examination was performed on all animals at the end of the observation period.

Findings:
The stability of the test substance over the study period could be demonstrated. The stability of the test substance in the preparation, its concentration and its homogeneity was confirmed by analysis.

No mortality occurred during the 14-day post-application observation period. There were no clinical symptoms observed. Body weight development appeared to be normal. One day after application a well defined erythema was observed in a single female rat. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion:
The dermal LD_{50} was found to be >2000 mg/kg bw for male and female animals.

B.6.2.3 Inhalation

Report: Gamer A.O. and Hoffmann H.D., 1998 (TOX2001-711)
BAS 510 F - Acute inhalation toxicity study in Wistar rats - 4-hour dust exposure
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1998/10803, unpublished
(Experimental work from 6–20 October 1997)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 403, EPA 81-3, EPA 798.1150, EEC 92/69, EEC 93/21

Deviations: A low mean relative humidity of 20.3% resulted from the need to use compressed air for dust generation. Because of the relatively short exposure period, this deviation from guideline recommendations (30–70%) is not considered to have compromised the validity of the test results.

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen; batch: N 26, purity: 95.3%
Test animals: Male + female Wistar rats (Chbb: thom, SPF), age (Day 1): 8-9 wk
Source: Dr. K. Thomae GmbH, Biberach a.d. Riss, Germany

10 young adult Wistar rats (5/sex) were exposed by nose-only inhalation to nicobifen dust at an analytically-determined concentration of 6.7 mg/l air for 4 hours. Animals were observed for clinical signs of toxicity for up to 14 days post-exposure and all animals were subject to gross necropsy.

Findings:

The test substance was demonstrated to be stable and homogeneous. The homogenous distribution of atmospheres in this inhalation system had been proven in technical tests with model aerosols. The particle size distribution revealed a mass median aerodynamic diameter (MMAD) of 3.4 µm, which is within the respirable range. The geometrical standard deviation was 3.4.

No mortalities occurred in the test group. Clinical observations revealed attempts to escape, irregular and dragging respiration, respiratory sounds as well as urine-smeared fur, piloerection and squatting posture. All clinical signs had subsided by Day 3 post application. Body weight development was not adversely affected by the test substance exposure.

No macroscopic pathologic findings were noted in exposed animals at the end of the study.

Conclusion:

The inhalation LC_{50} was found to be > 6.7 mg/l (4 h) for males and females.

B.6.2.4 Skin irritation

Report: Wiemann C. and Hellwig J., 1998 (TOX2001-712)
Study on the acute dermal irritation/corrosion of BAS 510 F in the rabbit.
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1998/10640, unpublished
(Experimental work from 1–3 September 1997)

Amendment (Re-analysis of the test substance's stability):
Wiemann C., 2000 (TOX2001-713)
Amendment no. 1: BAS 510 F - Acute dermal irritation / corrosion in the rabbit.
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1018712, unpublished

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 404, EEC 92/69, B 4, EPA 81-5

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 26, purity: 95.3%
Test animals: Male and female White New Zealand rabbits, bw (Day 1): 3.9–4.0 kg
Source: Source: Dr. K. Thomae GmbH, Biberach a.d. Riss, Germany

Nicobifen (0.5 g) was applied dermally to the intact skin of 2 male and 4 female White New Zealand rabbits for 4 h on a 2.5 cm x 2.5 cm test patch under a semi-occlusive 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 72 hours after test material application. Skin readings were performed at 1 h, 24 h, 48 h and 72 h after removal of the patch.

Findings:
The stability of the test substance over the study period was confirmed. Skin findings are summarised in the following table:

Table B.6.2-2: Rabbit skin irritation study: Skin irritation grading values

Skin effect	Erythema				Oedema			
Reading (h)	**1**	**24**	**48**	**72**	**1**	**24**	**48**	**72**
Rabbit No.								
1	1*	0	0	0	0	0	0	0
2	1	0	0	0	0	0	0	0
3	1	1	0	0	0	0	0	0
4	2	1	0	0	0	0	0	0
5	1	1	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
Mean Score		**0.2**				**0.0**		

* Erythema extending beyond the area of exposure

The average score (24 to 72 hours) for dermal irritation was calculated to be 0.2 for erythema and 0.0 for oedema. Skin findings were reversible within 48 h in all animals.

Conclusion:
Nicobifen is not irritant to the skin according to EU classification and labelling criteria.

B.6.2.5 Eye irritation

Report: Wiemann C. and Hellwig J. 1998 (TOX2001-714)
Study on the acute eye irritation of BAS 510 F in the rabbit.
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1998/10641, unpublished
(Experimental work from 1–11 September 1997)

Amendment (Re-analysis of the test substance's stability):
Wiemann C., 2000 (TOX2001-715)
Amendment no. 1: BAS 510 F - Acute eye irritation in the rabbit.
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1018713, unpublished

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 405, EEC 92/69, B 5, EPA 81-4

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen; batch: N 26, purity: 95.3%

Test animals: Male and female White New Zealand rabbits, bw (Day 1): 2.7–3.0 kg
Source: Source: Dr. K. Thomae GmbH, Biberach a.d. Riss, Germany

The 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 h, 24 h, 48 h and 72 h after the application of the test material.

Findings:

The stability of the test substance over the study period was confirmed. The homogeneity of the test substance was confirmed.

Table B.6.2-3: Rabbit eye irritation study: mean readings and symptoms

Animal No.	Opacity	Iris	Conjunctiva						Symptoms
			Redness					Swelling	
	Mean	Mean	1-h	24-h	48-h	72-h	Mean	Mean	
1	0.0	0.0	1	1	0	0	0.33	0.0	None
2	0.0	0.0	1	1	1	0	0.67	0.0	None
3	0.0	0.0	1	1	0	0	0.33	0.0	None
4	0.0	0.0	1	1	0	0	0.33	0.0	None
5	0.0	0.0	1	1	0	0	0.33	0.0	None
6	0.0	0.0	1	1	0	0	0.33	0.0	None
Mean	**0.0**	**0.0**		**0.4**				**0.0**	

Signs of eye irritation were confined to transient, grade 1 conjunctival redness, which in the absence of conjunctival discharge or swelling was observed in all rabbits at 1 and 24 h, and in a single rabbit at 48 h post application. The mean grade for conjunctival redness amounted to 0.4. The findings were reversible within 72 h after application.

Conclusion:

Nicobifen is not irritant to the eye according to EU classification and labelling criteria.

B.6.2.6 Skin sensitisation

Report: Wiemann C. and Hellwig J., 1998 (TOX2001-716)
BAS 510 F - Maximization test in guinea pigs
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1998/10638, unpublished,
(Experimental work from 12 August–5 September 1997)

Amendment (Re-analysis of the test substance's stability):
Wiemann C., 2000 (TOX2001-717)
Amendment No. 1: BAS 510 F - Maximization test in guinea pigs
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1018714, unpublished

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, B 6, OECD 406

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; Batch/purity: N 26: 95.3%
Test animals: Pirbright White (Dunkin-Hartley) guinea pigs; bw (Day 1): 329–393 g
Source: Charles River GmbH - Wiga, Kisslegg, Germany

Nicobifen 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 for the test group and ten female animals in each of two control groups.

The concentrations of the test substance suitable for use in the main experiment were determined in a pre-test involving two 24-h percutaneous occlusive applications within 96 h. The maximum non-irritant concentration was 5% nicobifen in 1% aqueous Tylose CB 30.00, and the minimum irritant concentration (based on slight to well-defined erythema in a single animal after 2nd treatment) was 10% nicobifen in 1% aqueous Tylose CB 30.000 suspension.

The intradermal application of a 5% test substance preparation in 1% aqueous Tylose CB 30.000 resulted in a well defined erythema and a moderate oedema in all test animals. Two 24-hour percutaneous occlusive applications within 96 hours were performed. The minimum irritant concentration was found to be a 10–25% test substance preparation in 1% Tylose CB 30.000 in aqua bidest. The maximum non-irritant concentration was found to be a 5% test substance preparation in 1% Tylose CB 30.000 in aqua bidest.

The following concentrations for induction and challenge were selected on the basis of the pre-tests performed with 4 guinea-pigs per test concentration (Table B.6.2-4):

Table B.6.2-4: Guinea pig skin sensitisation: Test substance preparations for Maximisation test

Intradermal induction	test substance 5% in 1% Tylose CB 30.000 in aqua bidest. or in Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)
Percutaneous induction	test substance 25% in 1% Tylose CB 30.000 in aqua bidest.
1st challenge	test substance 5% in 1% Tylose CB 30.000 in aqua bidest.
2nd challenge	test substance 5% in 1% Tylose CB 30.000 in aqua bidest.

The induction phase consisted of both intradermal and percutaneous exposures. First, six intradermal injections in groups of two were given to each animal. One week later a percutaneous induction exposure was conducted. 2 x 4 cm filter paper strips containing the test substance formulation were applied to the skin of the shoulder under an occlusive

dressing for 48 h. The test animals were treated with the preparations as indicated in the table above. Control animals received the same treatment but without test material.

The first challenge was conducted 14 days after the percutaneous induction with a test substance preparation as indicated in the above table. Control group two received the same treatment but without test substance. 2 x 2 cm filter paper strips containing the test substance formulation were applied to the skin of the flank under an occlusive dressing for 24 h.

Skin irritation readings were made at 24 and 48 hours after removal of the patch.

Separate tests using alpha-hexylcinnamaldehyde as a positive control are conducted twice a year in the laboratory to determine the ability of the test procedures to detect sensitising compounds.

Findings:
The stability of the test substance over the study period was proven. The stability of the test substance in the vehicle was confirmed by analysis. Homogeneity of the preparation was ensured by stirring.

One animals of the test group and one animals of the vehicle control group died from pneumonia. This was assessed to be unrelated to treatment.

Upon percutaneous induction all of the test group animals (25% nicobifen in 1% Tylose CB 30.000 in aqua bidest.) and the vehicle control animals (1% Tylose 30.000 in aqua bidest.) exhibited erythema and oedema involving incrusted and partially open skin lesions (see Table B.6.2-5).

Table B.6.2-5: Guinea pig skin sensitisation: Results of M&K induction phase

	Number of animals showing skin reactions*	
Intradermal induction	Control**	Nicobifen
1:1 Freund's adjuvant : 0.9% aqueous saline	10/10	20/20
5% nicobifen in 1:1 Freund's adjuvant : 0.9% aqueous saline	–	20/20
1% Tylose CB 30.000 in aqua bidest.	0/10	–
5% nicobifen in 1% aqueous Tylose CB 30.000	–	20/20
Percutaneous induction		
1% Tylose CB 30.000 in aqua bidest.	10/10	–
25% nicobifen in 1% aqueous Tylose CB 30.000	–	20/20

* Intradermal induction: well-defined erythema and slight oedema (both grade 2);
Percutaneous induction: incrustation, partially open

** Results of 1st control group; data from 2nd control group not shown.

The number of animals with skin findings after the first challenge and second challenge is summarised in Table B.6.2-6 below.

Table B.6.2-6: Guinea pig skin sensitisation: Results of M&K challenge

Group	24 h	48 h	Total
Control group	0/10	0/10	0/10
Test group	3/19	4/19	4/19

Number of positive reactions/number of animals tested

Treatment with vehicle alone caused no dermal irritation. In the treatment group, very slight oedema (grade 1) was detected in a total of 3/19 guinea pigs after 24 h and in a total of 4/19 guinea pigs after 48 hours.

Discussion:

According to the classification criteria of the EEC Directive 93/21, a test substance is to be considered as a skin sensitiser if at least 30% of the test animals exhibit skin reactions upon challenge with the test substance. Since only 4/19 guinea pigs (21%) showed very slight skin reactions upon nicobifen challenge, a negative test result was concluded.

Conclusion:

It is concluded, that nicobifen has no sensitising potential to the skin of the guinea pig in the Magnusson & Kligman Maximisation Test according to EU classification criteria.

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

The short-term toxicity of nicobifen was studied in dietary 90-day studies in rats and mice, and in 90-day and 1-year studies in dogs. In addition, the short-term toxicity following dermal exposure was determined in a 28-day study in rats. The short-term toxicity studies with nicobifen are summarised in Table B.6.3-1.

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

Study **Dose levels** **Nicobifen purity**	**NOAEL** **males/females** **mg/kg bw/d**	**LOAEL** **males/females** **mg/kg bw/d**	**Effects**
Rat 90-day oral 0–100–500–2000–5000–15000 ppm purity: 95.3 %	34 / 40 (500 ppm)	137 / 159 (2000 ppm)	≥ 2000 ppm: altered clin.-chem. & haematological parameters; ↑ thyroid wt., follicular cell hypertrophy & hyperplasia (males) ≥ 5000 ppm: ↑ liver wt & centrilob. hypertrophy; ↑ thyroid wt. (both sexes)
Mouse 90-day oral 0–150–1000–4000–8000 ppm purity: 95.3 %	29 / 42 (150 ppm)	197 / 277 (1000 ppm)	≥ 1000 ppm: ↑ cholesterol; ↑ liver wt (both sexes), 4000 ppm: altered clin.-chem. parameters, fatty liver change
Dog 90-day oral 0–250–2500–25000 ppm purity: 94.4 %	7.6 / 8.1 (250 ppm)	78 / 82 (2500 ppm)	≥ 2500 ppm: ↑ liver wt (both sexes) ↑ AP, ↓ ALAT, ASAT (females) ↑ triglycerides (both sexes) 25000 ppm: initial ↓ bw, subsequently retarded bw gain (females) ↓ red blood cell parameters (females) ↑ AP, ↓ ALAT, ASAT (both sexes) ↑ thyroid wt (females)
Dog 1-year oral 0–200–800–2000–20000 ppm purity: 94.4 %	22 / 22 (800 ppm)	57 / 58 (2000 ppm)	≥ 2000 ppm: vomitus, ↓ bw & food efficiency, altered clinical chemical parameters ↑ thyroid wt & liver wt.
Rat 28-day dermal 0–10–250–1000 mg/kg bw/d purity: 96.3 %	1000	–	No systemic adverse effects; no signs of local irritation

Nicobifen has a very low toxic potential as demonstrated by the high dose levels which were administered. For all studies the high dose level was in the range of 1000 mg/kg bw/d. Even at this dose level, clinical signs of toxicity or adverse effects on food consumption or body weight gain were very rarely seen. The signs of toxicity observed in the three species tested were overall similar and consisted mainly of altered clinical-chemical changes.

Main target organ was the liver. Weight increases of the liver were observed in all three species. Histopathological changes, however, were minor (hypertrophy and fatty change) and suggest an adaptation of this organ to increased functional demand.

In rats and dogs, the thyroids were identified as a second target organ, as evidenced by weight increases (rats and dogs) and histopathologically by follicular cell hyperplasia (only rats).

In a 28-day dermal toxicity study in rats no substance-related systemic adverse effects were detected up to the highest dose level tested of 1000 mg/kg bw/d. There were no signs of local irritation in this study.

A particular species sensitivity was not clearly evident from comparison of LOAELs and NOAELs obtained in oral short-term toxicity studies with different species. The fact that the studies conducted with dogs yielded the lowest NOAEL and LOAEL values might just have well resulted from the choice of different dose levels tested in rats, mice and dogs, respectively. The 12-month oral dog study was identified as the most relevant short-term toxicity study for risk assessment purposes.

B.6.3.1 Oral studies

B.6.3.1.1 Rat, 90 Days

Report: Mellert W. et al., 2000 (TOX2001-719)
BAS 510 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/101219, unpublished
(Experimental work from October 1997 - January 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Test Method: EEC 87/302, OECD 408, EPA 82-1, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test Material: Nicobifen; batch: N 26, purity: 95.3 %

Test Animals: Male and female Wistar rats (Chbb:THOM (SPF)), supplied by Boehringer Ingelheim Pharma KG, Biberach, Germany

Nicobifen was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0–100–500–2000–5000 and 15000 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, clinico-chemical and haematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Findings:

The stability of the test substance during the study period was analytically proven. The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was analytically confirmed. The test substance intake is shown in Table B.6.3-2.

Table B.6.3-2: Rat 90-d study: test substance intake

Dietary dose level (ppm)		**100**	**500**	**2000**	**5000**	**15000**
Test substance intake (mg/kg bw/d)	males	7	34	137	347	1055
	females	8	40	159	395	1225

There were no mortalities or clinical signs of toxicity in any of the dose groups, and no effects on body weight gain or food consumption. The clinical-chemical and haematological parameters which were considered to have shown test substance related effects are listed in Table B.6.3-3.

Table B.6.3-3: Rat 90-d study: clinical chemical and haematological parameters

Parameter	Sex	Dose level (ppm)					
		0	100	500	2000	5000	15000
Prothrombin time (sec)	F	30.2	30.5	29.0	28.8	29.0	27.4**
Red blood cells (tera/l)	M	8.10	8.39	8.47	8.52**	8.87***	8.77**
Haemoglobin (mmol/l)	M	9.2	9.3	9.4	9.5	9.9**	9.7*
Haematocrit (l/l)	M	0.412	0.421	0.422	0.430*	0.450***	0.441**
AP (µkat/l)	F	4.05	4.70*	3.53**	3.06**	3.19**	2.99**
γ-GT (nkat/l)	M	12	10	11	30***	28**	47***
	F	14	14	18	21	37***	42***
Total protein (g/l)	M	62.85	61.92	62.14	62.05	67.13**	67.51**
	F	65.45	64.52	64.07	66.73	65.69	70.29*
Albumin (g/l)	M	33.02	33.30	33.75	33.24	35.37***	35.39**
	F	36.96	37.22	37.37	37.94	36.31	39.02
Globuline (g/l)	F	28.49	27.30	26.70*	28.79	29.37	31.27**
Cholesterol (mmol/l)	F	2.22	2.31	2.09	2.09	2.46	2.88**
Triglycerides (mmol/l)	M	3.09	2.62	3.34	2.96	2.93	2.21*
Bilirubin (µmol/l)	M	2.48	2.73	2.15	1.74**	1.61**	1.66**
Calcium (mmol/l)	M	2.70	2.79*	2.74	2.76	2.86***	2.86***

Statistical significance: * =p < 0.05; ** = p < 0.01; *** = p < 0.001 (Kruskal-Wallis + Mann-Whitney u-test, two-sided)

The reduction of alkaline phosphatase (APh) in females at doses ≥ 500 ppm was not considered to be an adverse effect as this parameter is determined to assess liver toxicity in case of an increase. There is no adverse effect associated with a reduction in APh.

There were no test substance related effects seen in urinalysis and ophthalmoscopy.

Organ weight determination revealed increased weight of the liver and the thyroids (see Table B.6.3-4)

Table B.6.3-4: Rat 90-d study: organ weights

Parameter	Sex	Dose level (ppm)					
		0	100	500	2000	5000	15000
Abs. spleen wt (g)	M	0.961	0.976	0.796	0.787	0.749	0.731*
Rel. spleen wt (%)	M	0.214	0.217	0.184	0.182	0.176	0.165**
Abs. liver wt (g)	M	13.945	14.655	14.027	14.333	15.027	16.639**
	F	7.505	7.590	7.842	7.664	8.190*	9.240**
Rel. liver wt (%)	M	3.116	3.251	3.234	3.315	3.539**	3.760**
	F	2.994	2.990	3.150	3.183	3.348**	3.727**
Abs. thyroid wt (g)	M	23.4	23.2	23.7	28.3**	24.2	31.4**
	F	17.3	17.1	17.2	18.5	20.3*	22.6**
Rel. thyroid wt (%)	M	0.005	0.005	0.005	0.007**	0.006	0.007**
	F	0.007	0.007	0.007	0.008	0.008**	0.009**

Statistical significance: * = p < 0.05; ** = p < 0.01 (Kruskal-Wallis + Wilcoxon-test, two sided)

Spleen weights were significantly decreased in males at 15000 ppm. Liver weights were considered test-substance related increased at doses of 5000 and 15000 ppm. The slight and statistically non-significant increase of absolute and relative thyroid weights in males of the 5000 ppm group is considered to be treatment-related because at this dose level

histopathological changes in the thyroid were observed (see Table B.6.3-5). Therefore an effect of the test substance on thyroid weight was concluded at doses ≥ 2000 ppm.

The histopathological investigations revealed changes in the liver and the thyroid, as shown in Table B.6.3-5.

Table B.6.3-5: Rat 90-d study: incidence of pathological changes

Parameter	Sex	Dose level (ppm)					
		0	100	500	2000	5000	15000
Liver							
Hepatocyte centrilobular hypertrophy	M	0 / 10	0 / 10	0 / 10	0 / 10	8 / 10	10 / 10
	F	0 / 10	0 / 10	0 / 10	0 / 10	2 / 10	7 / 10
Thyroid							
Follicular cell hypertrophy	M	1 / 10	2 / 10	3 / 10	7 / 10	7 / 10	8 / 10
	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Follicular cell hyperplasia	M	1 / 10	2 / 10	3 / 10	7 / 10	7 / 10	8 / 10
	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10

Thus the weight increases in the liver at 5000 and 15000 ppm could be correlated with histopathological changes. These changes are indicative of an adaptive response to increased functional (metabolic) demand. In the thyroid, the weight increase in males ≥ 2000 ppm were also associated with histopathological changes. In the females, however, despite the observed weight increase at 5000 and 15000 ppm there was no histopathological correlate.

Conclusion:
The NOAEL (no observed adverse effect level) in this 90-d dietary rat study was 500 ppm (34 mg/kg bw/d in males and 40 mg/kg bw/d in females). A LOAEL was established at 2000 ppm based on haematological and clinical-chemical effects and on pathological findings in the thyroid of males.

B.6.3.1.2 Mouse, 90 Days

Report: Mellert W. et al., 2000 (TOX2001-720)
BAS 510 F - Subchronic oral toxicity study in C57BL mice.
Administration in the diet for 3 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1000188, unpublished
(Experimental work from October 1997-January 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 408, EEC 87/302, EPA 82-1, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test Material: Nicobifen; batch: N 26, purity: 95.3 %

Test Animals: Male and female mice (C57BL/6JRj), supplied by Centre d´Elevage R. Janvier, France

Nicobifen was administered to groups of 10 male and 10 female C57BL mice at concentrations of 0; 150; 1000, 4000 and 8000 ppm in the diet over a period of 3 months.

Food consumption and body weight were determined once a 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. Clinico-chemical and haematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examination.

Findings:

The stability of the test substance over the study period was confirmed analytically. The stability of the test substance in the diet was verified. The homogeneity of the mixtures was verified prior to the study. The correctness of the concentrations was demonstrated. The test substance intake is shown in Table B.6.3-6.

Table B.6.3-6: Mouse 90-d oral study: test substance intake

Dietary dose level (ppm)		**150**	**1000**	**4000**	**8000**
Test substance intake (mg/kg bw/d)	males	29	197	788	1518
	females	42	277	1184	2209

Nicobifen did not induce clinical signs of toxicity or mortality in any of the dose groups. There were no test substance related adverse effects on body weight, food consumption and haematology at any dose level.

The clinical-chemical findings, which are considered to be test substance related adverse effects are listed in Table B.6.3-7:

Table B.6.3-7: Mouse 90-d oral study: clinical chemical changes

Parameter	**Sex**	**Dose level (ppm)**				
		0	**150**	**1000**	**4000**	**8000**
Total protein (g/l)	M	62.49	62.46	61.13	59.90**	59.21**
	F	60.33	58.17	58.75	58.54	57.30
Albumin (g/l)	M	38.77	38.81	37.92	37.53**	36.82**
Globulins (g/l)	M	23.72	23.65	22.76	22.39**	22.06**
	F	21.18	21.05	20.51	19.92	19.67
Cholesterol (mmol/l)	M	2.57	2.53	2.26**	1.91***	1.84***
	F	1.76	1.96	1.66	1.59	1.57
ALAT (µkat/l)	F	1.03	0.98	1.13	1.23*	1.21*

Statistical significance: * = $p < 0.05$; ** = $p < 0.02$; *** = $p < 0.002$
(Kruskal-Wallis + Mann-Whitney u-test / two sided)

Thus, test substance related adverse effects were noted at 4000 and 8000 ppm. The only observation at 1000 ppm in clinical chemistry was a decrease of cholesterol in males.

Organ weight determination and histopathological investigations revealed the following test substance related changes:

Table B.6.3-8: Mouse 90-d oral study: Test substance related organ weight and histopathological changes

Parameter	Sex	Dose level (ppm)				
		0	150	1000	4000	8000
Abs. liver wt (mg)	M	1096.8	1113.5	1228.2*	1220.0**	1396.5**
	F	886.9	955.6*	946.6	995.2*	1085.0
Rel. liver wt (%)	M	4.392	4.509	5.005**	4.969**	5.658**
	F	4.723	4.872	5.091*	5.227*	5.700**
Fatty liver change, diffuse, grade 4 (incidence)	M	0 / 10	0 / 10	0 / 10	3 / 10	5 / 10
	F	0/10	0/10	1/10	0/10	1/10

Statistical significance: * = $p < 0.05$; ** = $p < 0.01$; (Kruskal-Wallis- + Wilcoxon-test / two sided)

Table B.6.3-9: Mouse 90-d oral study: Historical control values of C57Bl mice

	Male				Female			
	Absolute liver wt (mg)		Relative liver wt (%)		Absolute liver wt (mg)		Relative liver wt (%)	
Study period	individual	Mean	individual	Mean	individual	Mean	individual	Mean
10.88–01.89	1182	1208	4.321	4.352	916	970	4.962	4.951
12.88-03.89	1215		4.282		**1053**		**5.071**	
10.90-01.91	1218		**4.184**		965		4.944	
11.90-02.91	1141		4.396		953		5.026	
12.91-03.92	1172		**4.581**		1026		5.100	
03.92-06.92	1198		4.375		957		5.064	
09.93-12.93	**1443**		4.283		1001		4.719	
11.97-01.98*	**1097**		4.392		**887**		**4.723**	

* Data from current study

By comparison with historical control data of the test facility (see Table B.6.3-9), the significantly increased absolute liver weights at 1000 ppm in both species were well within historical control ranges, suggesting a substance-independent effect. However, statistically significantly increased relative liver weights at 1000 ppm were outside historical ranges in both sexes. In view of the liver being the target organ with further liver changes observable at higher dose levels, at least the significant increase in relative liver weights of males at ≥ 1000 ppm (by 14 %) is considered to be an adverse substance-related effect. The reduced protein values in males and the increased values of alanine aminotransferase (ALAT) in females indicate that liver function is impaired at higher dose levels.

Conclusion:
The NOAEL of this study was 150 ppm (29 mg/kg bw/d in males, 42 mg/kg bw/d in females). The LOAEL was 1000 ppm (197 mg/kg bw/d in males, 277 mg/kg bw/d in females) based on effects on cholesterol levels and on absolute and relative liver weight.

B.6.3.1.3 Dog oral toxicity studies

B.6.3.1.3.1 Dog, 90 Days

Report: Schilling K. et al. 2000 (TOX2001-721)
BAS 510 F - Subchronic oral toxicity study in Beagle dogs.
Administration in the diet for 3 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1012306, unpublished
(Experimental work from January 1999 - April 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 87/302, EPA 82-1, OECD 409, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test Material: Nicobifen; batch: N 37, purity: 94.4 %
Test Animals: Male and female pure-bred Beagle dogs (supplied by BASF's own Beagle breed)

Nicobifen was administered to groups of five male and five female pure-bred Beagle dogs at dietary concentrations of 0, 250, 2500 and 25000 ppm for 3 months.

Food consumption of the animals was determined daily and their body weight once a week. The animals were examined at least once each working day for any signs of toxicity and a check for any 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 haematological examinations as well as urinalyses were carried out once before and two times during the administration period.

Ophthalmological examinations were carried out 12 days before the beginning of the administration period and on study day 91.

All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Findings:
The stability of the test substance over the study period was proven analytically. The stability of the test substance in the diet and dietary preparation was verified. The homogeneity of the mixtures was verified. The correctness of the concentrations was demonstrated. The test substance intake is shown in Table B.6.3-10.

Table B.6.3-10: Dog 90-d study: test substance intake

Dietary dose level (ppm)		250	2500	25000
Test substance intake (mg/kg bw/d)	males	7.6	78	729
	females	8.1	82	825

There was no test substance related mortality or clinical signs of toxicity at any dose level. The faeces in all 25000 ppm males and females and transiently in 3 males and 3 females from the 2500 ppm group were light brown discoloured, partly with soft consistency.

At the high dose level (25000 ppm) a slight body weight loss and retarded body weight gain was observed during the initial phases of treatment in both sexes. Thereafter, body weight gain was reduced in females only. At the end of the study control females had gained 1 kg of body weight whereas the 25000 ppm females had gained only 0.2 kg. At the end of the study in high dose males there was no difference in body weight gain compared to controls.

Food consumption in high dose males was 96 % of the total food provided vs. 100 % in the controls. Food efficiency in high dose animals of both sexes (particularly females) was reduced.

There were no findings in ophthalmology in any dose group.

Clinical-chemical and haematological examinations revealed some test substance related changes, as shown in Table B.6.3-11.

Table B.6.3-11: Dog 90-d study: haematological and clinical-chemical changes

Parameter	Sex	Day	Dose level (ppm)			
			0	250	2500	25000
Red blood cells (terra/l)	F	90	7.35	7.27	7.59	6.56*
Haemoglobin (mmol/l)	F	90	10.6	10.2	10.8	9.5*
APh (µkat/l)	M	43	4.57	5.56	5.50	12.95**
		87	3.60	4.97	5.17	9.99**
	F	44	3.85	4.39	6.44	8.13
		90	3.05	4.24	5.70**	8.80**
ALAT (µkat/l)	M	43	0.59	0.53	0.47	0.33
		87	0.69	0.57	0.58	0.35**
	F	44	0.74	0.63	0.42**	0.40
		90	0.98	0.66	0.43**	0.47*
ASAT (µkat/l)	M	43	0.49	0.46	0.48	0.39
		87	0.52	0.48	0.44	0.38**
	F	44	0.49	0.51	0.57	0.41
		90	0.69	0.47	0.38**	0.37**
Triglycerides (mmol/l)	M	43	0.38	0.41	0.52	0.81**
		87	0.30	0.40*	0.44*	0.60**
	F	44	0.39	0.43	0.51	0.71**
		90	0.33	0.44	0.49*	0.66**

Statistical significance: * = $p < 0.05$; ** = $p < 0.01$ (Kruskal-Wallis + Mann-Whitney u-test)

The values of triglycerides in the dose groups 250 and 2500 ppm were within the historical range (male: mean: 0.38 mmol/l, minimum: 0.24 mmol/l, maximum: 0.45 mmol/l, female:

mean: 0.45 mmol/l, minimum: 0.38 mmol/l, maximum: 0.51 mmol/l) the numerical differences, which attained sometimes statistical significance were due to unusually low control values. Therefore theses differences to the control value are not considered to be adverse effects.

Organ weight determinations showed an increased weight of the liver in 25000 ppm and 2500 ppm males and females. Moreover, an increase in thyroid weight was noted for 25000 ppm females, as shown in Table B.6.3-12.

Table B.6.3-12: Dog 90-d study: liver and thyroid weight

Parameter	Sex	Dose level (ppm)			
		0	250	2500	25000
Abs. liver wt (g)	M	357.234	401.440	420.836*	506.576**
	F	321.882	332.170	381.932*	479.736**
Rel. liver wt (%)	M	3.257	3.301	3.653	4.151*
	F	2.854	3.013	3.454*	4.651**
Abs. thyroid wt (g)	M	0.830	0.964	0.924	0.954
	F	0.794	0.798	0.862	1.084
Rel. thyroid wt (%)	M	0.008	0.008	0.008	0.008
	F	0.007	0.007	0.008	0.010**

Statistical significance: * = $p < 0.05$; ** = $p < 0.01$ (Kruskal-Wallis-H- + Wilcoxon-test)

There were no test substance related gross- or histopathological changes observed at any dose level.

Conclusion:
The no observed adverse effect level (NOAEL) for Beagle dogs under the conditions of the study was 250 ppm (males: 7.6 mg/kg bw/day; females: 8.1 mg/kg bw/day), based on clinical-chemical findings and effects on organ weight of liver and thyroid observed at the LOAEL of 2500 ppm. The increased levels of triglycerides in dose groups 250 and 2500 ppm are due to unusually low control values and were within historical range.

B.6.3.1.3.2 Dog, 1 year

Report: Wiemann C. et al., 2000 (TOX2001-727)
BAS 510 F - Chronic oral toxicity study in Beagle dogs - Administration in the diet for 12 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1016881, unpublished
(Experimental work from October 1998 – October 1999)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz. Rheinland-Pfalz, Mainz)

Guideline: EEC 87/302, OECD 452, EPA 870.4100, JMAFF

Deviations: No deviations.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test Material: Nicobifen; batch: N 37, purity: 94.4 %
Test Animals: Male and female pure-bred Beagle dogs (supplied by BASF's own Beagle breed)

Nicobifen was administered to groups of 5 male and 5 female pure-bred Beagle dogs at dietary concentrations of 0, 200, 800, 2000 and 20000 ppm for about 12 months.

Food consumption of the animals was determined daily and their body weight once a week. The animals were examined at least once each working day for any signs of toxicity and a check for any 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 haematological examinations as well as urinalyses were carried out once before and after approximately 3, 6 and 12 months of test substance administration.

Ophthalmological examinations were carried out 7 days before the beginning of the administration period and on study day 359.

All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

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. The test substance intake is shown in Table B.6.3-13.

Table B.6.3-13: Dog 1-year study: test substance intake

Dietary dose level (ppm)		200	800	2000	20000
Test substance intake (mg/kg bw/d)	males	5.5	22	57	544
	females	5.8	22	58	593

No mortality occurred during the study.

At the high dose level (20000 ppm) the following clinical signs of toxicity were observed:
Vomitus during the later part of the study period occurred in a single female. The faeces of all high dose animals appeared light brown discoloured and were of soft consistency.

Body weight in high dose females was initially decreased, and body weight gain was reduced for the rest of the study period. Body weight gain was also reduced in 2000 ppm females.

Alanine aminotransferase (ALAT) was decreased in males (2000 and 20000 ppm) and in females (20000 ppm). Aspartate aminotransferase (ASAT) was decreased in females (20000 ppm). The decrease of both enzymes in blood is not considered to be a toxicologically adverse effect. Relevant findings are listed in Table B.6.3-14.

Table B.6.3-14: Dog 1-year study: findings

Parameter	Month	Sex	Dose level (ppm)				
			0	200	800	2000	20000
Body weight (kg)	12	F	13.5	12.6	13.3	12.0	12.1
APh (µkat/l)	3	M	3.63	3.33	4.74	6.67**	8.75**
		F	3.78	4.76	4.89	5.92	10.45**
Triglycerides (µmol/l)	3	M	0.36	0.39	0.45	0.45	0.65**
		F	0.39	0.48	0.52*	0.53	0.69**
Cholesterol (µmol/l)	3	F	4.12	4.49	5.23**	4.84	6.59**
Total protein (g/l)	3	F	60.63	60.05	63.44	62.82	65.70**
Globulins (g/l)	3	F	28.65	28.92	30.93	30.43	33.30**
Chloride (mmol/l)	3	M	114.2	114.4	112.4	114.1	112.1**
		F	114.3	114.1	112.6	113.5	110.3**
Abs. liver wt (g)	12	F	384.7	366.6	409.8	421.0	544.6*
Rel. liver wt (%)	12	F	2.849	2.918	3.087	3.507*	4.426**
Abs. thyroid wt (g)	12	M	0.954	1.226	0.898	1.322*	1.468*
		F	1.160	1.140	1.030	1.160	1.650
Rel. thyroid wt (%)	12	M	0.007	0.009	0.007	0.010*	0.011*
		F	0.009	0.009	0.008	0.010	0.013*

Statistical significance * = $P < 0.05$, ** = $P < 0.02$, (Kruskal-Wallis + Mann-Whitney u-test)

The strongest effects on alkaline phosphatase (APh), triglycerides, cholesterol, total protein, globulins and chloride were seen at 3 months. Most of these effects were either no longer observable or not statistically significant at 12 months.

There were no gross or microscopical changes observed in any of the organs.

There were no test substance related effect at 800 and 200 ppm.

Conclusion:
The no observed adverse effect level (NOAEL) in this 12 month dog study was 800 ppm (males: 21.8 mg/kg bw/day; females: 22.1 mg/kg bw/day), based on significant effects on alkaline phosphatase and organ weights of liver and thyroid observed at the LOAEL of 2000 ppm.

B.6.3.2 Dermal studies

B.6.3.2.1 Rat, 28 Days

Report: Mellert W. et al., 2000 (TOX2001-718)
BAS 510 F - Repeated dose dermal toxicity study in Wistar rats - Administration for 4 weeks
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1013240, unpublished
(Experimental work from April 1999 – May 1999)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, OECD 410, EPA 870.3200

Deviations: No deviations.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test Material: Nicobifen; batch: N 46, purity: 96.3 %
Test Animals: Male and female Wistar rats ((Chbb:THOM (SPF), supplied by Boehringer Ingelheim Pharma KG)

Nicobifen was administered to groups of 10 male and 10 female Wistar rats by dermal route (6 hours/day; 5 days/week, seem-occlusive dressing) for 4 weeks at doses of 0 (vehicle control; 0.5 % aqueous CMC and 0.5 % Cremophor EL solution); 100; 250 and 1000 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, clinico-chemical and haematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Findings:
The stability of the test substance in the vehicle was verified. The homogeneity of the mixtures was verified. The correctness of the concentrations was demonstrated.

One high dose female died on day 13 of the administration. The cause of death was considered to be related to septicaemia. The findings causative for the mortality were localised in the uterus (pyometra) and urinary tract (papillary necrosis and severe suppurative pyelonephritis

of the kidney, dilation and inflammation of the urinary bladder and ureter). These changes were not test substance related.

There were no signs of skin irritation at any dose level.

The only finding in this study was a slight, but statistically significant, reduction in total bilirubin in the serum of high dose males (3.02 µmol/l in controls vs. 2.64 µmol/l at 1000 mg/kg bw/d, $p < 0.05$).

The marginal reduction of bilirubin may be related to the induction of hepatic metabolic enzymes. Nicobifen has been shown to increase the activity of phase I and II liver enzymes. Increased activity of phase II enzymes would enhance the elimination of serum bilirubin and thus explain the slight reduction of total bilirubin in the serum. These changes (in absence of any other pathologically relevant observations) are signs of an adaptation rather than toxicologically relevant adverse effects. Therefore, the reduction in total bilirubin in high dose males is not considered to be an adverse effect.

There were no other test substance related changes in any dose group.

Conclusion:
The no observed adverse effect level for systemic toxicity after a 28–d dermal administration in rats was 1000 mg/kg bw/d in both sexes. There were no signs of dermal irritation.

B.6.3.2.2 Rat, 90 days

A 90 day dermal study was not considered necessary because the NOAEL for systemic toxicity in the 28-day dermal toxicity study in rats was 1000 mg/kg bw/d.

B.6.4 Genotoxicity (Annex IIA 5.4)

The potential genotoxicity of nicobifen was investigated in a series of both in vitro and in vivo studies. All regular end points for genetic damage (point mutations, chromosome damage and DNA-damage and repair) were assessed: In *in vitro* investigations, nicobifen was evaluated for its potential genotoxicity using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis 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 negative test result of this study corroborated the evidence obtained *in vitro* that nicobifen has no chromosome-damaging potential. In conclusion, there is no evidence from the available *in vitro* and *in vivo* data to assume mutagenic or genotoxic properties of nicobifen. The results of these studies are summarised in Table B.6.4-1.

Table B.6.4-1: Summary of mutagenicity studies

Study/strains/species	Test conditions	Test substance purity	Results
***In vitro* studies**			
Bacterial reverse mutation (Ames test) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100; *E. coli* WP2 uvrA	with and without S9-mix	95.3%	Negative
Mammalian forward mutation (HPRT test); Chinese hamster ovary (CHO) cells	with and without S9-mix	94.4%	Negative
Mammalian chromosome aberration test Chinese hamster V79 cells	with and without S9-mix	94.4%	Negative
Unscheduled DNA synthesis Rat primary hepatocytes	–	94.4%	Negative
***In vivo* studies**			
Mouse micronucleus test	0–500–1000–2000 mg/kg bw/d (1 i.p. injection/day, 2 days)	94.4%	Negative

B.6.4.1 *In vitro* testing

B.6.4.1.1 *In vitro* gene mutation in bacterial cells

Report: Engelhardt G. and Hoffmann H.D., 1998 (TOX2001-722)
Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with BAS 510 F (Reg.No. 300 355)
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1998/11440, unpublished
(Experimental work in September 1997)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 471 (adopted 21 July 1997), EEC 92/69 B 14, EEC 92/69 B 13

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 26, purity: 95.3%
Test strains: *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537 and TA 98 and *Escherichia coli* strain WP2 uvrA

Nicobifen was tested for its mutagenic potential based on the 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. The study consisted

of a standard plate test (with the dose range of 0–22–110–550–2750–5500 µg/plate) and a preincubation test (with doses ranging from 0–20–100–500–2500–5000 µg/plate) both with and without metabolic activation (Aroclor induced Sprague-Dawley rat liver S-9 mix). 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 (with and without S9 mix) and positive controls (see Table B.6.4-2) were tested.

Table B.6.4-2: Bacterial mutagenicity test: Positive controls used

Test strains	Positive test compounds (concentration) used for assays ...	
	... without S9-mix	... with S9 mix
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (5 µg/plate in DMSO)	2-aminoanthracene (2-AA) (2.5 µg/plate in DMSO)
TA 100		
TA 98	4-nitro-*o*-phenylenediamine (NOPD) (10 µg/plate in DMSO)	
TA 1537	9-aminoacridine (AAC) (100 µg/plate in DMSO)	
E.coli WP2 uvrA	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) (10 µg/plate in DMSO)	2-aminoanthracene (2-AA) (60 µg/plate in DMSO)

Nicobifen was considered positive in this test if the following criteria were met:
- at least a doubling of the negative control mutation rate,
- a dose-response relationship, and
- reproducibility of the test results

Findings:
The stability of the test substance throughout the study period was guaranteed. The stability of nicobifen in the vehicle DMSO and in water was determined analytically.

Nicobifen precipitated at concentrations of 500 µg/plate and higher. Weak bacteriotoxicity (reduced background growth, slight decrease in the number of revertants, reduction in the titre) was observed starting from 500 – 2750 µg/plate onwards, depending on the strain and the test conditions.

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 controls.

Conclusion:
According to the results of the study, nicobifen is not mutagenic in the Ames reverse mutation assay.

B.6.4.1.2 *In vitro* gene mutation in mammalian cells

Report: Engelhardt G. and Hoffmann H.D., 2000 (TOX2001-723)
In vitro gene mutation test with BAS 510 F in CHO cells (HPRT locus assay)
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1000180, unpublished
(Experimental work from 11 May 1998 – 9 September 1999)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 476 (adopted 21 July 1997), EEC 87/302

Deviations: None that compromised the validity of the study

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen; batch: N 37, purity 94.4%
Test system: Chinese hamster ovary (CHO) cells

Nicobifen 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, with or without the addition of Aroclor 1254-induced Sprague-Dawley rat liver S-9 mix (exogenous metabolic activation).

In an initial range-finding cytotoxicity test, the cloning efficiency was reduced at doses > 500 µg/ml (with and without S-9 mix). Test substance precipitation was observed at doses > 50 µg/ml. According to these results, two separate experiments were designed: after an attachment period of 20–24 h, the cells were exposed for 4 h to a range of concentrations listed in Table B.6.4-3 both with and without metabolic activation, followed by an expression phase of 7–9 days.

Table B.6.4-3: Gene mutation in mammalian cells: Concentrations tested

Experiment	S9-mix	Test concentrations [µg/ml]						
1	without and with	0	15.625	31.25	62.5	125	250	500
2	without	0	3.125	6.25	12.5	25	50	100
	with	0	10.24	25.6	64	160	400	1000

The following positive control substances were used to demonstrate the sensitivity of the test method and the activity of the S-9 mix:

Without metabolic activation: Ethyl methane sulfonate (300 µg/ml)
With metabolic activation: Methylcholanthrene (10 µg/ml)

For the selection of mutants, 6 x 300000 cells from each treatment group were seeded in six 75-cm² flasks with selection medium (TG medium) at the end of the expression period and the

flasks then returned to the incubator for about 1 week. Subsequently, the colonies of each test group were fixed with methanol, stained with Giemsa and counted.

The criteria for a positive response were:

- Increases of the corrected mutation frequencies above the concurrent negative control values and above 15 mutants per 106 clonable cells and/or the evidence of a dose-response relationship in the increase in mutant frequencies,
- evidence of reproducibility of any increase in mutant frequencies, and
- a statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Findings:
The stability of the test substance throughout the study period was determined by re-analysis. The stability of nicobifen in the vehicle DMSO and in water has been determined analytically.

Test substance precipitation was observed at concentrations of approximately 31.25 µg/ml and higher. On the basis of the findings from the 1st experiment, 1000 µg/ml was selected as top dose for the 2nd experiment both with and without S-9 mix.

In the 2nd experiment, without S-9 mix, the selected doses exhibited a higher toxic effect than had been expected. Therefore, the experimental part without metabolising system was discontinued due to the extreme cytotoxicity observed at 64 µg/ml and onward (extremely low cell density). The two lower doses of 10.24 µg/ml and 25.6 µg/ml were not considered for further mutagenicity testing as only two doses would not have been in accordance with the requirements of the OECD-Guideline where at least four analysable ones are required. Therefore, the doses for the 2nd repeat experiment without S-9 mix were selected taking into consideration the results of the 1st experiment and the 2nd discontinued experiment.

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. ethyl methane sulfonate and methylcholanthrene, led to the expected increase in the frequencies of forward mutations.

Based on the results of the present study, the test substance did not cause an increase in the mutant frequencies either without S-9 mix or after adding a metabolising system in three experiments performed independently of each other. The mutant frequencies at any dose were comparable to the concurrent vehicle control values and were within the range of historical control data.

Conclusion:
Thus, under the experimental conditions of this assay, nicobifen does not induce forward mutations *in vitro* in the CHO/HPRT mutation assay.

B.6.4.1.3 *In vitro* chromosome aberration in mammalian cells

Report: Engelhardt G. and Hoffmann H.D., 1999 (TOX2001-724)
In vitro chromosome aberration assay with BAS 510 F in V79 cells
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1999/10978, unpublished
(Experimental work from 3 February – 19 May 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 473 (adopted 21 July 1997), EEC 92/69 B 10

Deviations: None that compromised the validity of the study

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 37, purity 94.4%
Test system: Chinese hamster lung V79 fibroblast cells

Nicobifen was assessed for its potential to induce structural chromosomal aberrations in V79 cells in vitro both in the presence and in the absence of an exogenous metabolising system, for which an S-9 mix prepared from Aroclor 1254-induced Sprague-Dawley rat liver was used.

Based on the results of an initial range-finding cytotoxicity test, two separate experiments were conducted according to the design summarised in the table below:

Table B.6.4-4: Mammalian gene mutation in V79 cells: experimental design

Experiment	Exposure duration (h)	Harvest time (h)	S9-mix	Test concentrations [µg/ml]			
1	4	18	with & without	0	20	100	500
2	4	28	with	0	125	250	500
	18		without	0	31.25	62.5	125
	28			0	125	–	–

The cell cycle of the untreated V79 cells is about 13–14 hours under the selected culture conditions. Thus, the selected first sampling time of 18 hours was within the 1–1.5fold range of the normal cell cycle duration, 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-metaphases). 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 or 100 cells for each culture in the case of the concurrent positive controls, were analysed for chromosomal aberrations. For each experiment two cultures were used.

The criteria for a positive response were:

- A dose-related and reproducible significant increase in the number of structural chromosomal aberrations.
- The proportion of aberrations exceeded both the concurrent negative control range and the negative historical control range.

A test substance is generally considered non clastogenic in this test system if:

- there was no significant increase in the number of chromosomally damaged cells at any dose above concurrent control frequencies.
- the aberration frequencies were within the historical control range.

Findings:
The stability of the test substance throughout the study period was verified by re-analysis. The stability of nicobifen in the vehicle DMSO and in water was determined analytically. Homogeneity was achieved by mixing.

Test substance precipitation occurred at concentrations of 62.5 µg/ml and higher. Based on the results obtained from determination of the mitotic index, no suppression of mitotic activity was observed under any of the experimental conditions. Cell counts indicated that growth inhibition did not occur. However, cell attachment was slightly reduced (i.e. few cells rounded) at 500 µg/ml. Osmolality and pH values were not influenced by test substance treatment.

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. ethyl methanesulfonate and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations.

On the basis from the results of the present study, the test substance did not cause any biologically relevant and dose-dependent increase in the number of structurally aberrant metaphases incl. and excl. gaps at both sampling times either without S-9 mix or after adding a metabolising system in two experiments performed independently of each other. No increase in the frequency of cells containing numerical aberrations was demonstrated either.

Conclusion:
Nicobifen is not a chromosome-damaging (clastogenic) agent under in vitro conditions in V79 cells.

B.6.4.1.4 *In vitro* DNA damage and repair in mammalian cells

Report: Engelhardt G. and Hoffmann H.D., 2000 (TOX2001-725)
In vitro unscheduled DNA synthesis (UDS) assay with BAS 510 F in primary rat hepatocytes
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1011413, unpublished
(Experimental work from 14 May – 30 November 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 482, EEC 87/302

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 37, purity 94.4%
Test system: Primary hepatocytes isolated from Wistar rats

Nicobifen was tested for its ability to induce DNA repair synthesis (unscheduled DNA synthesis; UDS) in primary Wistar rat hepatocytes in vitro. Two independent experiments were carried out. Based on results of a preliminary cytotoxicity test, cells were exposed to either 0–1–5–10–50 µg/ml in the first experiment. Subsequently, a second experiment was conducted in which a slightly different spacing of concentrations was chosen (0–6.25–12.5–25–50 µg/ml) in consideration of cytotoxicity information obtained in the first experiment.

The quantification of UDS was performed microscopically using 2 or 3 slides per test group; slides were coded before microscopic evaluation. 25–50 cells in good morphological conditions 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 nuclear grain (NG) count (= number of silver grains overlying the nucleus) and the cytoplasmic grain (CG) count (= number of grains in two or three nucleus-equivalent areas adjacent to the nucleus) were performed with an automatic image analyser (ARTEK). Based on NG and CG counts the following parameters were calculated:

- Net Nuclear Grain (NNG) count of each cell (= nuclear grain count minus cytoplasmic grain count; NG - CG)
- the mean nuclear grain (NG) count
- the mean cytoplasmic grain (CG) count
- the mean net nuclear grain (NNG) count
- % cells in repair (% cells showing NNG ≥ 0)
- % cells in repair (% cells showing NNG ≥ 5)

A positive test result was concluded if the following criteria were met:

- A dose-related increase in the mean NNG count
- Mean NNG ≥ 0 at one of the test points.
- ≥ 20% cells in repair (≥ 20% cells showing NNG ≥ 5) in combination with a further dose-related increase

A marginally positive response (requiring confirmation/clarification in an independent experiment) was concluded in case of

- a dose-related increase in the percentage of cells in repair ≥ 5 outside the values of both the concurrent negative control and the historical control data base (≥ 3 < 20), and
- a dose-related increase in the mean NNG count near to but without exceeding zero.

The test result of the in vitro UDS assay is considered to be negative if the test article produces both values for NNG counts and % cells in repair which are in the range of the negative control data.

Findings:
The stability of the test substance throughout the study period was verified by re-analysis. The stability of nicobifen in the vehicle DMSO and in water over a period of 4 hours was determined analytically. The homogeneity was ensured by mixing.

Test substance precipitation was observed at concentrations ≥ 50 µg/ml. Concerning cytotoxicity the following observations were made:

- LDH activity was slightly increased in the 1st experiment at doses ≥ 50 µg/ml and in the 2nd experiment at doses ≥ 25 µg/ml.
- Lactate concentration was reduced at doses ≥ 100 µg/ml (1st experiment) and ≥ 25 µg/ml (2nd experiment).
- Cell morphology was affected from about 10 µg/ml (1st experiment) or 12.5 µg/ml (2nd experiment) onward.

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.

On the basis of the results from the present study, the test substance did not lead to 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 nicobifen is negative in the in vitro UDS assay using primary rat hepatocytes.

B.6.4.2 *In vivo* testing

B.6.4.2.1 *In vivo* mouse micronucleus test

Report: Engelhardt G. and Hoffmann H.D., 1999 (TOX2001-726)
Cytogenetic study in vivo with BAS 510 F in the mouse micronucleus test after two intraperitoneal administrations
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1999/11048, unpublished
(Experimental work from 11–15 May 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 474 (adopted 21 July 1997), EEC 92/69, method B.12

Deviations: None that compromised the validity of the study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 37, purity 94.4%
Test animals: Male NMRI mice (Charles River Deutschland GmbH)

Nicobifen was tested for clastogenicity and for the ability to have spindle poison effects in male NMRI mice using the micronucleus test method. For this purpose, the test substance, suspended in an aqueous 0.5% CMC (carboxymethylcellulose) formulation, was administered twice (at a 24-h interval) by intraperitoneal injection at dose levels of 0, 500, 1000 and 2000 mg/kg body weight in a volume of 20 ml/kg body weight.

The animals were sacrificed and the bone marrow of the two femora was prepared 24 hours after the second administration. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also registered.

The test chemical is to be considered positive in this assay if the following criteria are met:

- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent negative control range and the negative historical control range.

Findings:
The stability of the test substance throughout the study period was verified by re-analysis. The stability of nicobifen in aqueous media and the correctness of the concentrations in the vehicle was analytically confirmed. Homogeneity was achieved by constant mixing.

Animals which were administered the vehicle or the positive control substances cyclophosphamide or vincristine did not show any clinical signs of toxicity. However, the administration of the test substance led to piloerection and squatting posture in all dose groups. These clinical signs of toxicity lasted for 24 to 48 hours.

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 nicobifen was detected: the ratio of polychromatic to normochromatic erythrocytes was always in the same range as that of the control values in all dose groups.

According to the results of the present study, the intraperitoneal administration of nicobifen did not lead to any increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was always in the same range as that of the negative control in all dose groups and at all sacrifice intervals.

Conclusion:
Nicobifen gave a negative test result in the *in-vivo* mouse micronucleus test. There was no evidence of any chromosome-damaging (clastogenic) effects. In addition, there were no indications of any impairment of chromosome distribution in the course of mitosis.

B.6.4.2.2 *In vivo* DNA damage and repair

Because of the unambiguous negative result of the in vitro UDS assay with nicobifen, no *in vivo* study was performed.

B.6.4.2.3 *In vivo* testing of germ cells

The results of the *in vitro* as well as the *in vivo* studies demonstrated that nicobifen has no mutagenic or genotoxic potential. Therefore, there was 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)

The chronic toxicity and carcinogenicity studies are summarised in Table B.6.5-1:

Table B.6.5-1: Summary of long-term toxicity studies

Study Dose levels Nicobifen purity	NOAEL males/females mg/kg bw/d	LOAEL males/females mg/kg bw/d	Effects
Rat 24-mo oral diet (combined oral chronic and carcinogenicity) 0–100–500–2500–15000 ppm purity: 94.4%	4.4 / 5.9 (100 ppm)	22 / 30 (500 ppm)	≥ 500 ppm: ↑ γ-GT, ↓ total bilirubin (males), ↑ hepatocellular hypertrophy ≥ 2500 ppm: ↓ bw (females, slight) signs of anaemia (females) clin-chem. changes indicative of liver toxicity, ↑ path. changes in thyroid and liver (both sexes) ↑ thyroid follicular cell adenomas (slight) 15000 ppm: general toxicity, treatment discontinued
Mouse 18-mo oral diet (carcinogenicity) 0–80–400–2000–8000 ppm purity: 94.4%	13 / 90 (80 / 400 ppm)	65 / 443 (400 / 2000 ppm)	≥ 400 ppm: ↓ bw (males) ↑ relative liver weight (males) ≥ 2000 ppm: ↓ bw (both sexes) ↑ abs. liver wt, hepatocellular hypertrophy (females) 8000 ppm: ↑ liver wt, hepatocellular hypertrophy (both sexes)

In a combined chronic toxicity/carcinogenicity study in rats, dietary exposure to a concentration of 15000 ppm resulted in overt toxicity indicating the maximum tolerated dose had been exceeded. Accordingly, treatment at the top dose level was discontinued. At the next lower concentration (2500 ppm), however, there were still sufficient signs of toxicity (reduced body weight in females, indications of an anaemic effect in females, clinical chemical changes indicating liver toxicity in both sexes as well as pathological changes to the liver and the thyroid).

In the carcinogenicity study there was a slight increase in the incidence of thyroid follicular cell adenomas at the high dose. A similar increase was not observed in the chronic toxicity study, possibly because the effect is so weak that the number of animals were not high enough to detect it. The concomitant changes to the thyroid (hypertrophy and hyperplasia), however, suggest that the slight increase in the carcinogenicity study is likely to be treatment related.

From a mechanistic point of view the thyroid changes can be linked to an increased metabolism of thyroid hormones (T3 and T4) due to increased phase II (conjugation) hepatic

activity. The reduced thyroid hormone levels trigger, by means of a feedback mechanism, the release of increased amounts of TSH in an attempt to restore homeostatic conditions. Due to continued treatment with nicobifen, the metabolic activity of the liver, however, remains elevated resulting in a continuously increased breakdown of thyroid hormone and continuously increased TSH levels. Chronic stimulation of the thyroid due to increased TSH levels is well known to results in follicular cell hypertrophy, hyperplasia and ultimately in benign thyroid tumours in rats. According to various publications the rat is particularly sensitive to this secondary mechanism.

Nicobifen does not need to be classified with respect to its tumourigenic potential in rats of the following reasons:

1. Nicobifen is clearly non genotoxic.
2. For non genotoxic agents, the mechanism of action must be determined. Nicobifen was shown to enhance the metabolism of thyroid hormones.
3. Nicobifen has a very low potency of the tumourigenic effect, as indicated by the marginal increase of (thyroid follicular cell) adenomas in rats. There was no increase in the incidence of carcinomas. There was no tumourigenic response in the thyroid in mice.

In conclusion, the marginal increase of thyroid follicular cell adenomas in rats is not considered to be relevant to man.

A carcinogenicity study in mice was conducted up to a maximum tolerated dose as evidenced by significant body weight depression (8 - 10% in both sexes) at 8000 ppm. Liver weights were increased at the high dose level, histopathology revealed hypertrophy. Moderate effects on body weights were seen at 2000 ppm (both sexes) and in 400 ppm males. Liver weights were also increased in 2000 ppm females, histopathology revealed hypertrophy. There was no evidence of a carcinogenic effect of nicobifen in mice at any dose level.

B.6.5.1 Rat oral chronic toxicity study

Report: Mellert W. et al. 2001, (TOX2001-728)
BAS 510 F - Chronic toxicity study in Wistar rats - Administration in the diet for 24 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000114, unpublished
(Experimental work from January 1998 - February 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 87/302, OECD 452, EPA/FIFRA, Subdivison F; para. 83-1, JMAFF

Deviations: No deviations.

Acceptability: The study is considered to be acceptable.

Material and Methods

Test material: Nicobifen; batch: N 37, purity: 94.4%
Test animals: Male and female Wistar rats (Chbb:THOM (SPF), supplied by Boehringer Ingelheim Pharma KG)

Nicobifen was administered to groups of 20 male and 20 female Wistar rats at dietary concentrations of 0 ppm; 100 ppm, 500 ppm; 2500 ppm and 15000 ppm for 24 months in this chronic toxicity study. The 15000 ppm dose level was discontinued after approximately 17 months of treatment.

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.

Ophthalmologic examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinico-chemical and haematological examinations were carried out at approximately 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.

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. The test substance intake is shown in Table B.6.5-2.

Table B.6.5-2: Rat chronic toxicity: test substance intake

Dietary dose level (ppm)		**100**	**500**	**2500**	**15000***
Test substance intake (mg/kg bw/d)	males	4.4	22	110	739
	females	5.9	30	150	1000

* treated for 17 months

The 15000 ppm treatment group was discontinued after approximately 17 months of administration due to reduction in body weight gain of male and female animals which deteriorated over time. (11.2% and 21.3% reduction, respectively, by Day 511).

Body weights in the chronic toxicity study were not affected for males dosed up to 2500 ppm. For females the body weights of the 2500 ppm group were reduced by 7 – 8% from Day 539 until 630. Mortality in females of the 2500 ppm group was lower (5 %) than the control value (35 %) but it is doubtful if this is a true substance related effect.

Food consumption was not affected.

Haematological and clinical chemical examinations after 12 months revealed the following changes (see Table B.6.5-3):

Table B.6.5-3: Rat chronic toxicity: haematological and clinical-chemical changes after 12 months of administration

Parameters	Sex	Dose level (ppm)				
		0	100	500	2500	15000
White blood cells [giga/l]	M	7.01	6.54	6.94	6.17	5.35***
	F	3.78	3.56	3.60	3.43	4.15
Haemoglobin [mmol/l]	M	8.8	8.9	8.8	8.7	8.9
	F	8.8	8.7	8.7	8.7	8.3***
Haematocrit [l/l]	M	0.391	0.396	0.389	0.387	0.400
	F	0.384	0.385	0.381	0.382*	0.364***
MCV [fl]	M	50.6	50.2	49.0***	49.9	49.2**
	F	52.1	51.6	52.0	50.9*	50.8**
MCH [fmol]	M	1.13	1.13	1.11	1.12	1.10**
	F	1.16	1.15	1.16	1.13	1.13**
Prothrombin time [sec]	M	34.0	32.2	32.0	32.6	33.5
	F	26.3	26.7	26.9	25.9	24.7**
γ-GT [nkat/l]	M	14	15	20**	34***	109***
	F	12	12	13	20**	45***
Total protein [g/l]	M	64.90	66.25	65.39	65.40	68.66***
	F	73.65	73.88	73.29	77.48*	79.04***
Albumin [g/l]	M	28.45	29.28	28.97	28.51	30.05***
	F	34.23	34.32	33.60	35.52	35.35
Globulins [g/l]	M	36.45	36.97	36.41	36.89	38.61**
	F	39.42	39.56	39.69	41.96**	43.69***
Cholesterol [mmol/l]	M	2.29	2.32	2.45	2.49	2.41
	F	2.41	2.55	2.53	2.87***	3.47***
Total bilirubin [µmol/l]	M	2.19	2.11	1.44**	1.21***	1.52***
	F	2.01	1.93	1.81	1.69	1.70
Triglycerides [mmol/l]	M	5.64	6.14	6.38	4.99	3.39***
	F	4.41	4.62	3.86	4.34	2.92**

Statistical significance: * = $p < 0.05$; ** = $p < 0.02$; *** = $p = 0.002$ (Kruskal-Wallis + Mann-Whitney u-test / two sided)

Note:
Alanine aminotransferase (ALAT) activities were decreased in the males of group 3 on Day 176 and 547. In the females significantly reduced serum ALAT activities were found in group 3 on Day 87, 177, 366 and 400 and in groups 1 and 2 on Day 177 and 366. Aspartate aminotransferase (ASAT) activities were decreased in the females of group 3 on Day 177. Decreased alkaline phosphatase (APh) activities were observed in the males of group 3 at each time interval and in group 2 on Day 86 and 176. In the females APh activities were reduced in the animals of group 3 on Day 87, 177, 366, 400 and 548 and in group 2 on Day 87. Gamma-Glutamyltransferase activities (GGT)were significantly increased in the males of groups 3 at each time interval and in group 2 on Day 86, 176 and 365 and in group 1 on Day 86. In group 3 females GGT activities were elevated on Day 87 and 366.
In the animals of group 3 bilirubin levels were decreased almost throughout the study and in males of group 2 lower bilirubin levels were detected on Day 365 and 547. Reduced total bilirubin concentrations were found in the sera of all treated females on Day 87 and 400 and in the females of group 3 on Day 721. Increased total protein and globulin levels were measured in males and females of group 3 almost at each time point. Serum albumin concentrations were higher in the males of group 3 on Day 720. Cholesterol levels were increased in the males of group 3 on Day 395. In the females of group 3 serum cholesterol levels were higher than controls on Day 87, 366, 400 and 548 and in group 2 on Day 400.

The organ weight determinations revealed the following test substance related changes:
Increased mean absolute weight of thyroid glands in males (31%) and increased mean relative weight of liver in females (11%) at 2500 ppm.

The histopathological investigations also showed changes in liver and thyroid:
In the thyroid at 2500 ppm there was an increase in diffuse thyroid follicular cell hypertrophy in males and females. Moreover, an increase in focal thyroid follicular cell hyperplasia in males and females was observed at this dose level.

In the liver, centrilobular liver cell hypertrophy was seen in males and females of the 2500 ppm group as well as in one male of the 500 ppm group.
A slightly increased number of eosinophilic foci of cellular alteration in liver of males was noted at 500 and 2500 ppm.

There were no test substance related effects at 100 ppm.

In the carcinogenicity study with nicobifen in rats, a slight increase in the number of thyroid gland follicular cell adenomas was observed at 2500 ppm (see B.6.5.2). In this chronic toxicity study such an effect was not noted as demonstrated by the incidences shown in Table B.6.5-4:

Table B.6.5-4: Rat chronic toxicity: incidence of thyroid follicular cell adenomas

	Incidence of thyroid gland follicular cell adenomas (No. adenomas / No. Thyroids examined)			
	0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**
Males	0 / 20	0 / 20	2 / 20	1 / 20
Females	0 / 20	0 / 20	1 / 20	0 / 20

Conclusion:
The no observed adverse effect level (NOAEL) in this 24 month chronic toxicity study in rats was 100 ppm (males: 4.4 mg/kg bw/d; females: 5.9 mg/kg bw/d), based on haematological and clinical-chemical effects and on pathological findings in the liver at the LOAEL of 500 ppm. In this chronic toxicity study nicobifen did not demonstrate a carcinogenic effect.

B.6.5.2 Rat carcinogenicity study

Report: Mellert W. et al., 2001 (TOX2001-729)
BAS 510 F - Carcinogenicity study in Wistar rats - Administration in the diet for 24 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000115, unpublished
(Experimental work from February 1998 - February 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 87/302, OECD 451, EPA/FIFRA, Subdivision F; para 83-2, JMAFF

Deviations: No deviations.

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen; batch: N 37, purity: 94.4%

Test animals: Male and female Wistar rats (Chbb:THOM (SPF) supplied by Boehringer Ingelheim Pharma KG

Nicobifen was administered to groups of 50 male and 50 female Wistar rats at dietary concentrations of 0 ppm; 100 ppm, 500 ppm; 2500 ppm and 15000 ppm for 24 months in this carcinogenicity study. The 15000 ppm dose level was discontinued after approximately 17 months of treatment.

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, organ weight determination, followed by histopathological examinations.

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. The test substance intake is shown in Table B.6.5-5.

Table B.6.5-5: Rat carcinogenicity: test substance intake

Dietary dose level (ppm)		**100**	**500**	**2500**	**15000**
Test substance intake (mg/kg bw/d)	males	4.6	23	116	769
	females	6.0	30	156	1024

The 15000 ppm treatment group was discontinued after approximately 17 months of administration for the following reasons:

Body weight in the high dose females started to decrease after about 6 months of treatment. This decline became more rapid after about 14 months. By this time the body weights in the females of the 2500 ppm group were also statistically significantly reduced. At the time that the treatment of the 15000 ppm females was discontinued, their body weights were 86.8% of the controls. As expected, the decline in body weight also became progressively worse in the 2500 ppm females. At the end of the study their body weight was 84.1% of the controls (i.e. lower than the high dose terminated after about 17 months). In males, at that time, although there was no apparent effect on body weight, there was an increase in mortality in the high dose group. Whereas in the controls the first animal died on Day 420, the first animal in the 15000 ppm group had died already on Day 196. On study Day 532 there had been a

progressive increase in mortality in the 15000 ppm males. As it was expected that this trend would continue too, this group was also discontinued.

Simultaneously, in the chronic toxicity study with nicobifen in rats adverse effects on haematological and clinical chemical parameters were observed at a dose of 15000 ppm.

In this carcinogenicity study, body weight at the end of the study was reduced in the 2500 ppm females (15.9%) as shown in table B.6.5-6. Body weight gain in these animals was 24.2% lower than in the controls.

Table B.6.5-6: Rat carcinogenicity body weight after 24 months

Parameter	Sex	Dietary dose level (ppm)			
		0	100	500	2500
Body weight (g)	M	699.3	695.9	711.2	700.0
	F	425.8	410.0	397.0	358.0**

Statistical significance: ** = $p < 0.01$ (Anova + Dunnett's test / two sided

Food consumption was not affected.

In the thyroid the following changes were noted (see Table B.6.5-7 and Table B.6.5-8):

Table B.6.5-7: Rat carcinogenicity: absolute and relative thyroid weight

Dietary dose level (ppm)	Absolute thyroid weight (mg)		Relative thyroid weight (%)	
	Males	Females	Males	Females
0	38.872	30.583	0.006	0.008
100	37.583	29.075	0.006	0.008
500	41.703	30.184	0.006	0.008
2500	45.818**	30.600	0.007**	0.009

Statistical significance: ** = $p < 0.01$ (Kruskal-Wallis-H- + Wilcoxon-test; two-sided)

Table B.6.5-8: Rat carcinogenicity: incidence of non-neoplastic lesions in the thyroid gland

Dietary dose level (ppm)	Diffuse follicular cell hypertrophy		Focal follicular cell hyperplasia	
	Males	Females	Males	Females
0	2	2	1	2
100	5	0	1	2
500	6	0	1	1
2500	22	4	9	7

n = 50 animals per sex and dose group

In the liver the following changes were noted (see Table B.6.5-9):

Table B.6.5-9: Rat carcinogenicity incidence of histopathological findings in the liver

Dietary dose level (ppm)	Centrilobular hypertrophy		Eosinophilic foci	
	Males	Females	Males	Females
0	0	0	3	0
100	0	0	4	0
500	2	0	8	0
2500	27	11	9	0

n = 50 animals per sex and dose group

The incidence of eosinophilic liver cell foci is numerically increased in males of the 2500 and 500 ppm groups. As the size of the foci was only increased in the 2500 ppm males (showing eosinophilic cytoplasmic inclusions) it is assessed that a test substance related effect is only indicated at this dose level.

The absolute organ weight of kidneys was decreased in females at 500 ppm (-4.8%) and at 2500 ppm (-7.5%). But the relative organ weight of kidneys in females was not lower than the control values.

In males the number of animals with diffuse papillary transitional cell hyperplasia in the urinary bladder was slightly increased in the high dose group (7 animals in the control group, 13 animals in the high dose group). The hyperplasia was accompanied by an inflammation in the urinary bladder.

There was a slight, numerical increase in the number of thyroid follicular cell adenomas in high dose males and females as shown in Table B.6.5-10.

Table B.6.5-10: Rat carcinogenicity: incidence of thyroid follicular cell adenomas

Dietary dose level (ppm)	Males	Females
0	0	0
100	0	1
500	1	0
2500	4	3

n = 50 animals per sex and dose group

There were no test substance related effects at 100 ppm.

Discussion:
The incidence of thyroid follicular cell adenomas was slightly increased at the high dose. A similar increase was not observed in the chronic toxicity study, possibly because the effect is so weak that the number of animals were not high enough to detect it. The concomitant changes to the thyroid (hypertrophy and hyperplasia), however, suggest that the slight increase in the carcinogenicity study is likely to be treatment related.

From a mechanistic point of view the thyroid changes can be linked to an increased metabolism of thyroid hormones (T3 and T4) due to increased phase II (conjugation) hepatic activity. The reduced thyroid hormone levels trigger, by means of a feedback mechanism, the release of increased amounts of TSH, in an attempt to restore homeostatic conditions. Due to continued treatment with nicobifen, the metabolic activity of the liver, however, remains elevated resulting in a continuously increased breakdown of thyroid hormone and continuously increased TSH levels. Chronic stimulation of the thyroid due to increased TSH levels is well known to result in follicular cell hypertrophy, hyperplasia and ultimately in benign thyroid tumours in rats. The rat, however, is particularly sensitive to this secondary mechanism for the following reasons:

The decrease of thyroid hormone is related to an induction of hepatic enzymes in rats. It is well known that hepatic metabolic capacity, as well as the possibility of induction is far more pronounced in rats than in humans.

Humans have a substantial reserve supply of thyroid hormone, much of which is carried in thyroxine-binding globulin, a serum protein that is missing in laboratory rodents [see Odell et al., 1967 (TOX2001-740)]. Therefore, release of stored thyroid hormones causes serum hormone levels to stay normal for weeks in euthyroid humans [see Martindale, 1979 (TOX2001-741)] and for weeks to several months in hyperthyroid individuals [see Odell et al., 1967 (TOX2001-740)] despite daily doses of anti-thyroid drugs, sufficient to completely block synthesis.

Thirdly, under conditions of prolonged thyroid insufficiency, caused for example by dietary iodine deficiency, the primary human response resulting from increased TSH levels, is goitre rather than neoplasia [see Costigan, 1998 (TOX2001-742)].

The evaluation of thyroid neoplasia in the rodent bioassay was also a major issue of a group of Specialised Experts by request of the European Chemicals Bureau Pesticide Working Group in 1999 [see TOX2001-743].

The following criteria were given for the determination of the relevance (and classification) of a thyroid tumour response in rodents:

- For chemicals causing thyroid tumours in rodents, it must first be determined if the compound is genotoxic or not. Nicobifen is clearly non genotoxic.
- For non genotoxic agents, the mechanism of action must be determined. Nicobifen was shown to enhance the metabolism of thyroid hormones.
- Finally, the potency of the tumourigenic effect should be assessed. Nicobifen has a very low potency, as indicated by the marginal increase of (thyroid follicular cell) adenomas in rats. There was no increase in the incidence of carcinomas. There was no tumourigenic response in the thyroid in mice.

Thus, in accordance with the criteria put forward [see TOX2001-743], nicobifen does not need to be classified with respect to its tumourigenic potential in rats.

Conclusion:
The no observed adverse effect level (NOAEL) in this 24 month carcinogenicity study in rats was 100 ppm (males: 4.6 mg/kg bw/d; females: 6.0 mg/kg bw/d), based on histopathological findings (increased incidence of eosinophilic foci) in the liver of male rats at the NOAEL of 500 ppm.

The marginal increase of thyroid follicular cell adenomas observed in rats is not considered to be relevant to man. The absence of such a finding in mice is further evidence for the unusual sensitivity of the rat for the described mode of action.

B.6.5.3 Carcinogenicity mouse

Report: Mellert W. et al., 2001 (TOX2001-730)
BAS 510 F - Carcinogenicity study in C57BL mice - Administration in the diet for 18 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000116, unpublished
(Experimental work from February 1998 - August 1999)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 87/302, OECD 451, EPA/FIFRA, Subdivision F; para 83-2; JMAFF

Deviations: No deviations.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 37, purity: 94.4%
Test animals: Male and female C57BL/6J Rj mice, supplied by Centre d`Elevage R. Janvier, France

Nicobifen was administered to groups of 50 male and 50 female C57BL mice at dietary concentrations of 0 ppm, 80 ppm, 400 ppm, 2000 ppm and 8000 ppm 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, organ weight determination followed by histopathological examinations.

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. The test substance intake is shown in Table B.6.5-11.

Table B.6.5-11: Mouse carcinogenicity: test substance intake

Dietary dose level (ppm)		80	400	2000	8000
Test substance intake (mg/kg bw/d)	males	13	65	331	1345
	females	18	90	443	1804

There was no test substance related increase in mortality or clinical signs of toxicity in this study.

Food consumption was not adversely affected. Body weight was reduced in the 8000 ppm group in both sexes and in the 2000 ppm and 400 ppm groups in males (see Table B.6.5-12):

Table B.6.5-12: Mouse carcinogenicity: body weight at the end of the study

Dietary dose (ppm)	Body weight / g (%)			
	Males		Females	
0	34.4	(100%)	28.0	(100%)
80	33.8	(98.1%)	27.6	(98.6%)
400	32.6*	(94.6%)	27.1	(96.9%)
2000	32.1**	(93.2%)	28.0	(99.9%)
8000	31.3**	(90.8%)	25.9**	(92.6%)

Statistical significance: * = $p < 0.05$; ** = $P < 0.01$ (Anova + Dunnett`s test, two sided)

Body weight gain was reduced in 8000 ppm males (23.8%) and females (20.5%), 2000 ppm males (18.1%) and 400 ppm males (13.6%).

Blood smears did not indicate any test substance related effects.

Organ weight determination revealed an increase of absolute and relative liver weight in the 8000 ppm males and females as well as in the 2000 ppm females. The relative liver weight was also increased in the 2000 ppm and 400 ppm males (see Table B.6.5-13):

Table B.6.5-13: Mouse carcinogenicity: absolute and relative liver weight

Dose level (ppm)	Males		Females	
	Abs. liver wt (mg)	Rel. liver wt (%)	Abs. liver wt (mg)	Rel. liver wt (%)
0	1260	3.992	1167	4.677
80	1235	4.000	1251	5.109
400	1246	4.204**	1150	4.773
2000	1329	4.568**	1261**	5.059**
8000	1465**	5.118**	1289**	5.518**

Statistical significance * = $p < 0.05$; ** = $p < 0.01$ (Kruskal-Wallis-H- + Wilcoxon-test, two sided)

In males, the absolute and relative adrenal weights were increased in all treatment groups compared to the controls. This, however, is related to the fact that the absolute and relative control adrenal weights were the lowest in historical control data. The adrenal weights of all the treatment groups are within the historical range and the increase did not show a clear dose-response relationship.

Relative testes weight was increased in all treatment groups. However, this increase did not show a clear dose-response relationship and there was no effect on absolute testes weight. Therefore, this is related to the slightly lower body weights rather than to a test substance related effect on the testes. This conclusion is confirmed by the absence of histopathological changes in this organ.

The absolute kidney weights were significantly decreased in males of group 3 (-7.3%) and 4 (-12.4%) and in females of group 2 (-4.4%) and 4 (-4.5%). The relative kidney weight did not show a significant weight change. Furthermore there were no histopathological findings for the decreased kidney weights. Therefore the reduction of kidney weights is considered incidental.

Histopathological investigations demonstrated an increased incidence of periportal hypertrophy of hepatocytes in 8000 ppm males and females as well as in 2000 ppm females (see Table B.6.5-14). There was no evidence of a carcinogenic response.

Table B.6.5-14: Mouse carcinogenicity: incidence of periportal hepatocellular hypertrophy

Dietary dose level (ppm)	Incidence of periportal hepatocellular hypertrophy	
	Males	Females
0	0 / 50	0 / 50
80	0 / 50	0 / 50
400	0 / 50	0 / 50
2000	0 / 50	10 / 50
8000	29 / 50	45 / 50

Conclusion:
The no observed adverse effect level (NOAEL) in this 18 month carcinogenicity toxicity study in mice was 80 ppm for males (13 mg/kg bw/d) and 400 ppm for females (90 mg/kg bw/d) based on effects on body weight and body weight gain and on relative liver weight at the LOAEL of 400 ppm. Nicobifen is not carcinogenic in mice.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

The reproduction toxicity of nicobifen was investigated in a two-generation reproduction study as well as in developmental toxicity studies in rats and rabbits. Results of all reproduction toxicity studies are summarised in Table B.6.6-1:

Table B.6.6-1: Summary of reproduction toxicity studies

Study dose levels purity	Target	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Effects
Rat 2-generation study 0–100–1000–10000 ppm purity: 94.4 %	Parental toxicity	6.7 (100 ppm)	67 (1000 ppm)	≥ 1000 ppm: ↑ hepatocell. hypertrophy 10000 ppm: ↓ bw gain & feed intake ↑ liver wt & hepatocyte degeneration
	Fertility	667 (10000 ppm)	–	No effects observed
	Offspring toxicity	6.7 (100 ppm)	67 (1000 ppm)	≥ 1000 ppm: ↓ bw gain 10000 ppm: ↑ Male F2 pup mortality during days 0–4 p.p.
Rat teratogenicity 0–100–300–1000 mg/kg bw/d purity: 94.4 %	Maternal toxicity	1000	–	No effects observed
	Developmental toxicity	300	1000	1000 mg/kg bw/d: ↑ Incomplete ossification of the thoracic centrum
Rabbit teratogenicity 0–100–300–1000 mg/kg bw/d purity: 94.4 %	Maternal toxicity	100	300	300 mg/kg bw/d: 1 doe with abortion and reduced / discoloured faeces 1000 mg/kg bw/d: 4 does with abortion ↓ feed intake, bw & bw gain
	Developmental toxicity	300	1000	1000 mg/kg bw/d: ↑ Incomplete ossification of the thoracic centrum

Nicobifen had no adverse effects on reproductive performance or fertility of the F0 or F1 parental animals of all substance-treated groups up to a dose of 10000 ppm (667 mg/kg bw/d). Signs of general toxicity/systemic effects occurred in both parental generations at 1000 and 10000 ppm. The effects at 10000 ppm were characterised by decreased food consumption and reduced body weights during parts of the administration period. Pathology showed statistically significantly increased liver weights, centrilobular hypertrophy of liver cells and centrilobular liver cell degeneration in single or all male and/or female animals. Systemic effects at 1000 ppm were confined to an increased incidence of centrilobular hepatocellular hypertrophy, which occurred in few F0 and F1 parental animals. No substance-related effects were noted at 100 ppm. Substance-induced signs of developmental toxicity were observed in progeny of the F0 and F1 parents at 1000 and 10000 ppm. At 10000 ppm a slightly increased pup mortality of the F2 litters was noted between days 0 and 4 post partum only. Pup body weight development was impaired in both F1 and F2 litters. At 1000 ppm, slightly decreased body weight gains were recorded for the male F2 pups only. 100 ppm did not induce any indication of developmental toxicity. The NOAEL for parental toxicity of the test substance was established at 100 ppm (6.7 mg/kg bw/d) for the F0 and F1 parental males and females. The NOAEL for developmental toxicity was 1000 ppm (67 mg/kg bw/d) for the male and female F1 and female F2 progeny and 100 ppm (6.7 mg/kg bw/d) for the male F2 progeny.

In the developmental toxicity study in rats, incomplete ossification of the thoracic centrum was observed at the highest dose tested (1000 mg/kg bw/d) in the absence of overt maternal toxicity. At this limit dose level there were also no signs of maternal toxicity. However, results from the 90-day oral feed study in rats indicate that liver toxicity would have been detected in dams at 1000 mg/kg bw/d.

In the rabbit developmental toxicity study, incomplete ossification of the thoracic centrum was also observed at significantly increased incidences at the highest dose level (1000 mg/kg bw/d). At this dose level there was overt maternal toxicity (clinical signs of toxicity, reduced body weight and body weight gain). At 300 mg/kg bw/d clinical signs (abortion and discoloured/reduced faeces) were observed in a single animal only. Thus, the NOAELs for maternal and for developmental toxicity were 100 mg/kg bw/d and 300 mg/kg bw/d, respectively.

B.6.6.1 Multigeneration studies in mammals

Report: Schilling K. et al., 2001 (TOX2001-731)
BAS 510 F - Two-generation reproduction toxicity study in Wistar rats - Continuous dietary administration
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000117, unpublished
(Experimental work from 8 September 1998 – 7 June 1999)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 416 (compliant with Draft June 2000), EEC 87/302, EPA/OPPTS 870.3800, JMAFF

Deviations: None

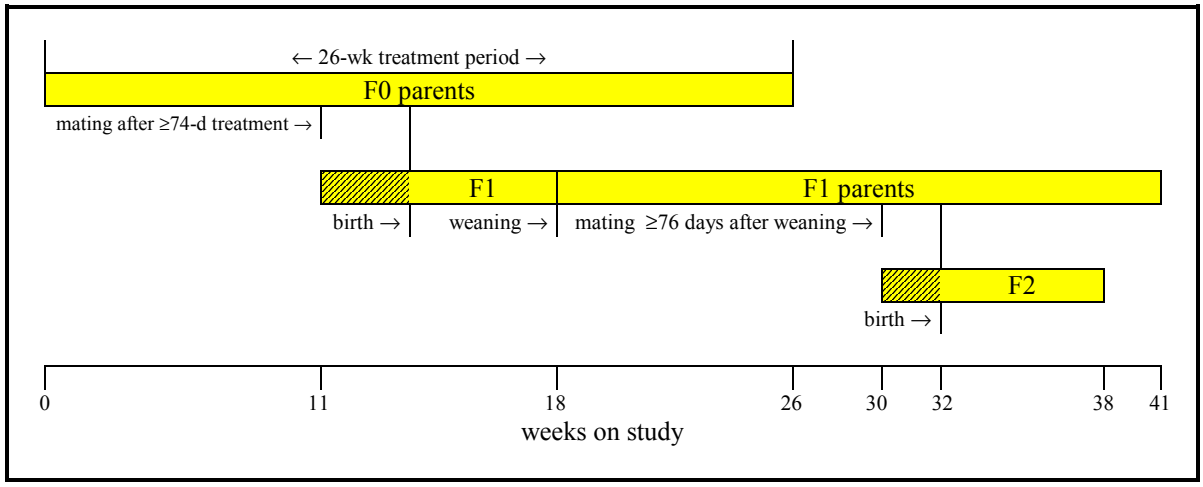
Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen; batch: N 37; purity: 94.4 %
Test animals: Male and female Wistar rats (Chbb THOM (SPF), age (Day 1): 35 (±2) days; bw range, males: 100.1–145.7 g, females: 93.3–132.1 g
Source: Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany

Nicobifen was administered to groups of 25 male and 25 female sexually immature Wistar rats (F0 parental generation) as a constant homogeneous addition to the food at different concentrations (0; 100; 1000 or 10000 ppm). The experimental design is outlined in Figure B.6.6-1.

Figure B.6.6-1: Rat 2-gen. study: Experimental design



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; 100; 1000 or 10000 ppm of the test substance post weaning, and the breeding program was repeated to produce F2 litter. For standardisation, each individual litter with litter size > 8 pups was adjusted on day 4 p.p. to contain 8 pups (ideally 4 pups/sex).

The study was terminated with the terminal sacrifice of the F2 weanlings and F1 adult animals. Test diets containing nicobifen 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 pre-mating (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.

Oestrus 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 oestrus stage of each female was determined on the day of scheduled sacrifice. Different sperm parameters (motility, sperm head count, morphology) were assessed in all F0 and F1 generation males at scheduled sacrifice or shortly thereafter.

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 pre-putial 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) and subjected to an extensive histopathological examination, special attention being paid to the organs of the reproductive system. A

quantitative assessment of primordial follicles, growing follicle and antral follicles in the ovaries was performed for all control and high dose F0 and F1 parental females.

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. The estimates for the mean test substance intake are given in Table B.6.6-2. The intake estimates obtained using the standard conversion factor was used for calculating the NOAEL and LOAEL, 15 (as recommended by JMPR).

Table B.6.6-2: Rat 2-gen. study: Test substance intake

	Test substance intake (mg/kg bw/d)					
Feed concentration:	**100 ppm**		**1000 ppm**		**10000 ppm**	
Generation:	**F0**	**F1**	**F0**	**F1**	**F0**	**F1**
Males	10.1	12.3	101.2	123.9	1034.5	1295.4
Mean intake, males	*11*		*113*		*1165*	
Females (pre-mating)	10.7	12.5	106.8	124.7	1062.0	1299.6
Females gestation	8.7	9.2	88.7	94.2	907.4	952.9
Females lactation*	14.8	14.8	149.4	155.2	1456.7	1456.1
Mean intake, females	*12*		*120*		*1189*	
Default calculation Males & females**	**6.7**		**67**		**667**	

* days 1–14 p.p. only
** based on default conversion factor 15 proposed by JMPR (WHO) to be used for rat multi-generation studies

There were no mortalities or test substance related clinical signs of toxicity in any of the dose groups. The following test substance related effects were noted:

Parental toxicity
No mortalities were observed in any of the F0 and F1 parental animals under study. No clinical observations considered to be treatment-related were made in any of the F0 and F1 treatment groups.

In males, treatment-related effects on body weight were confined to the high-dose group of the F1 generation, in which statistically significant body weight decreases compared to controls were encountered during almost the entire 18-wk treatment period; at the end of treatment, body weight and body weight gains were reduced by about 9 %. No corresponding effects were observed in males of the F0 generation. Assessment of food consumption by F0 and F1 males did not reveal any treatment-related changes.

In females at 10000 ppm, body weight loss was observed in F0 rats during pre-mating (up to 3.2 % for wk 9) and lactation (up to 5.1 % on day 7). Body weight gain was statistically significantly reduced in F0 rats during pre-mating (wk 6–7) and during gestation, especially during days 7–14 (approx. 19 % reduction). No corresponding body weight reductions were seen in F1 females, although reduction of food consumption of high-dose group F1 females reached statistical significance during lactation days 4–14 (reduction up to 8 %).

The weights of liver, kidney and spleen showed both dose-dependent and statistically significant changes in the F0 and F1 generations (see Table B.6.6-3):

Table B.6.6-3: Rat 2-gen. study: organ weight changes in parental animals

Parameter	Sex	F0 generation, dose level (ppm)				F1 generation, dose level (ppm)			
		0	100	1000	10000	0	100	1000	10000
Terminal bw [g]	M	459	463	452	454	503	484	483	456** (-9 %)
	F	273	267	267	264	283	279	275	282
Abs. spleen wt. [g]	M	0.872	0.856	0.774* (-11 %)	0.714** (-18 %)	0.901	0.866	0.757** (-16 %)	0.708** (-21 %)
	F	0.608	0.585	0.563 (-7 %)	0.546 (-10 %)	0.633	0.610	0.578* (-9 %)	0.570** (-10 %)
Rel. spleen wt. [%]	M	0.190	0.185	0.172** (-10 %)	0.157** (-17 %)	0.180	0.179	0.158** (-12 %)	0.155** (-14 %)
	F	0.222	0.219	0.212 (-4.5 %)	0.209 (-6 %)	0.224	0.220	0.211	0.202** (-10 %)
Abs. liver wt. [g]	M	16.6	16.2	16.6	17.1 (+3 %)	17.8	18.3	17.7	18.09
	F	9.86	9.75	10.08	11.46** (+16 %)	10.4	10.5	10.5	12.6** (+21 %)
Rel. liver wt. [%]	M	3.61	3.50	3.66	3.76 (+4 %)	3.53	3.79	3.68	3.97** (+13 %)
	F	3.60	3.65	3.79	4.35** (+21 %)	3.68	3.77	3.83	4.47** (+21 %)
Abs. kidney wt. [g]	M	2.99	2.94	2.85	2.87	3.06	3.01	2.96	2.74** (-10 %)
	F	2.08	1.98* (-5 %)	2.01	1.93** (-7 %)	1.99	1.93	1.94	1.86** (-7 %)
Rel. kidney wt. [%]	M	0.651	0.637	0.632	0.632	0.610	0.624	0.613	0.602
	F	0.762	0.742	0.755	0.732 (-4 %)	0.705	0.693	0.707	0.657** (-7 %)

Statistical significance: * $p \leq 0.05$ ** $p \leq 0.01$ (Kruskal-Wallis-H and Wilcoxon-Test two-sided)

Treatment-related histopathological findings in parental animals were confined to the liver (see Table B.6.6-4).

Table B.6.6-4: Rat 2-gen. study: Histopathological findings in parental animals

LIVER	Sex	F0 generation, dose level (ppm)				F1 generation, dose level (ppm)			
		0	100	1000	10000	0	100	1000	10000
centrilobular hypertrophy of hepatocytes	M	0 / 25	0 / 25	9 / 25	25 / 25	1 / 25	0 / 25	10 / 25	25 / 25
	F	0 / 25	0 / 25	6 / 25	25 / 25	0 / 25	0 / 25	8 / 25	25 / 25
liver cell degeneration	M	0 / 25	1 / 25	0 / 25	3 / 25	0 / 25	0 / 25	0 / 25	8 / 25
	F	0 / 25	0 / 25	0 / 25	1 / 25	0 / 25	0 / 25	0 / 25	0 / 25

Centrilobular hypertrophy of hepatocytes was seen in 10000 and 1000 ppm males and females (F0 and F1 generations). In addition, an increased incidence of liver cell degeneration was noted in 10000 ppm males and one female (F0 generation) and in males of the F1 generation.

Fertility effects

In F0 animals, no substance-related adverse effects at any dose level on reproductive performance, on sperm and ovarian parameters or on the oestrus cycle were observed (see Table B.6.6-5).

In F1 animals, two of three high-dose group females with a slightly irregular oestrus cycle failed to mate, thus resulting in a slightly reduced mating index of 23/25 or 92 % (historical control range: 96–100 %). One of the two females showed fibroplasia of the cervix uteri and dilation of the uterus horns, which might have contributed to the failure of mating. All in all, the slightly reduced mating index is considered to be an incidental finding. The incidences of other parameters either lacked a dose-related response or were within historical control ranges and therefore not considered treatment related.

Table B.6.6-5: Rat 2-gen. study: Reproduction parameters

Parameter	Gen.	Dose level (ppm)				Historical control range
		0	100	1000	10000	
Mating index	F0	100 %	100 %	100 %	100 %	96–100 %
	F1	100 %	100 %	100 %	92 %	
Fertility index	F0	100 %	100 %	88 %	100 %	88–100 %
	F1	100 %	100 %	92 %	88 %	
Sperm motility	F0	89 %	86 %	83 %	89 %	65–99 %
	F1	89 %	88 %	87 %	83 %*	

Statistical significance: * = $p \leq 0.05$ (Wilcoxon-test with Bonferoni-Holm adjustment (one-sided))

Effects on F1 and F2 pups / litters

There were no substance-related differences between the control and the 100, 1000, and 10000 ppm F1 pup concerning mortality, sex ratio, clinical signs, and sexual maturation parameters, based on the lack of statistical significance or dose-relationship and on comparison with historical control data.

The viability index (indicator for increased perinatal pup mortality between Days 0–4 p.p.) was significantly reduced in the F2 high-dose group (86 % vs. 93 %, historical control range:: 83–99 %), but not in the corresponding F1 group (see Table B.6.6-6).

Table B.6.6-6: Rat 2-gen. study: Litter data and pup body weight (gain)

Parameter	Gen.	Dose level (ppm)				Historical control range
		0	100	1000	10000	
Delivered pups per litter (mean no. pups/dam)	**F1**	15.8 ^{D}	13.2*	14.2	13.0**	11.1–16.4 pups/litter
	F2	14.2 ^{D}	13.4	15.5	13.0	
Viability index (Days 1–4 p.p.)	**F1**	95 % ^{Fi}	93 %	92 %	94 %	83–99 %
	F2	93 % ^{Fi}	91 %	97 %	86 %**	
Lactation index (Days 4–21 p.p.)	**F1**	99 % ^{Fi}	100 %	99 %	99 %	93–100 %
	F2	100 % ^{Fi}	100 %	100 %	99 %	
Pup bw gain (g) (M+F, Days 4–21 p.p.)	**F1**	41.1 ^{D}	40.6	42.2	37.2*	No data
	F2	43.8 ^{D}	42.3	41.6	37.4**	
Pup bw (g) (M+F, Day 21 p.p.)	**F1**	49.5 ^{D}	49.4	51.0	45.9*	40.0–57.5 g
	F2	53.1 ^{D}	51.7	50.1	46.2**	
Pup bw (g) (M, Day 21 p.p.)	**F1**	50.3 ^{D}	50.6	51.8	46.7*	40.0–57.5 g
	F2	54.6 ^{D}	52.4	50.8*	47.0**	

Statistical significance: * = $p \leq 0.05$; ** $p \leq 0.01$ (^{D}: Dunnett test, two-sided; ^{Fi}: Fisher's Exact test, one sided)

Statistically significant differences in body weights or body weight gains were not evident between treatment-groups during the first four days after birth, but developed subsequently during the lactation period (Days 4–21) in both sexes at 10000 ppm and in males at 1000 ppm. At the end of lactation (Day 21 p.p.), the reduction of the mean body weights amounted to about 7 % for F1 pups and 12–14 % for F2 pups (both sexes combined). In addition, body weight gains during Days 4–21 were significantly reduced in the high-dose group pups (F1: -9.5 %, F2: -14.6 %, both sexes combined) and in F2 males at 1000 ppm (-6.6 %; -9.6 %). Impairment in pup body weights / body weight gains of the F1 and F2 high-dose groups and of the F2 mid-dose male group were considered to be treatment-related. The results of the organ weight determinations are summarised in Table B.6.6-7.

Table B.6.6-7: Rat 2-gen. study: Pup organ weight changes

Parameter (Day 21 p.p.)	Sex	F1 pups (N= 20–24 pups/group)				F2 pups (N= 22–25 pups/group)			
		Dose levels (ppm				Dose levels (ppm)			
		0	100	1000	10000	0	100	1000	10000
Abs. brain wt.		1.459 (–)	1.464 (+0.3 %)	1.461 (+0.1 %)	1.430 (-2.0 %)	1.489 (–)	1.461 (-1.9 %)	1.469 (-1.3 %)	1.462 (-1.8 %)
[g]		1.409 (–)	1.423 (+1.0 %)	1.433 (+1.7 %)	1.410 (+0.1 %)	1.435 (–)	1.426 (-0.6 %)	1.430 (-0.3 %)	1.413 (-1.5 %)
Rel. brain wt.	M	2.899 (–)	2.888 (-0.4 %)	2.784 (-4.0 %)	3.048* (+5.1 %)	2.710 (–)	2.837 (+4.7 %)	2.970** (+9.6 %)	3.123** (+15 %)
[% bw]	F	2.848 (–)	2.924 (+2.7 %)	2.956 (+3.8 %)	3.095** (+8.7 %)	2.838 (–)	2.772 (-2.3 %)	2.942 (+3.7 %)	3.107** (+9.5 %)
Abs. thymus wt.	M	0.178 (–)	0.175 (-1.7 %)	0.185 (+3.9 %)	0.163 (-8.4 %)	0.200 (–)	0.166** (-17 %)	0.176* (-12 %)	0.162** (-19 %)
[g]	F	0.180 (–)	0.174 (-3.3 %)	0.193 (+7.2 %)	0.162* (-10 %)	0.193 (–)	0.181 (-6.2 %)	0.195 (+1.0 %)	0.175 (-9.3 %)
Rel. thymus wt.	M	0.343 (–)	0.342 (-0.3 %)	0.343 (±0 %)	0.348 (+1.5 %)	0.363 (–)	0.316** (-13 %)	0.353 (-2.7 %)	0.345 (-5.0 %)
[% bw]	F	0.362 (–)	0.353 (-2.5 %)	0.394 (+8.8 %)	0.355 (-1.9 %)	0.382 (–)	0.349* (-8.6 %)	0.398 (+4.2 %)	0.380 (-0.5 %)
Abs. spleen wt	M	0.222 (–)	0.213 (-4.1 %)	0.222 (±0 %)	0.201 (-9.5 %)	0.260 (–)	0.242 (-6.9 %)	0.215** (-17 %)	0.189** (-27 %)
[g]	F	0.232 (–)	0.211 (-9.1 %)	0.220 (-5.2 %)	0.199 (-14 %)	0.238 (–)	0.237 (-0.5 %)	0.218 (-8.4 %)	0.196* (-18 %)
Rel. spleen wt	M	0.431 (–)	0.409 (-5.1 %)	0.424 (-1.6 %)	0.423 (-1.9 %)	0.467 (–)	0.459 (-1.7 %)	0.429 (-8.1 %)	0.397** (-15 %)
[% bw]	F	0.461 (–)	0.426 (-7.6 %)	0.448 (-2.8 %)	0.436 (-5.4 %)	0.468 (–)	0.456 (-2.6 %)	0.443 (-5.3 %)	0.422 (-9.8 %)

Statistical significance: * $p \leq 0.05$ ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two-sided)

Organ weight changes observed in F1 pups at 10000 ppm were considered to have resulted from body weight reductions and therefore were not indicative of a direct effect. By assessment of organ weight changes in F2 pups, the statistically significant reduction in absolute and relative spleen weight in 10000 ppm male pups was considered to be treatment-related. However, as there were no pathological changes in the spleen of parental animals, the weight reduction was considered to be related to the reduced body weight development in these pups, rather than to a specific effect of the test substance on the spleen. All other differences in absolute and/or relative F2 pup organ weights were assessed as incidental and as being without any toxicological significance. There were no test substance related effects at 100 ppm.

Conclusion:
Nicobifen had no adverse effects on reproductive performance or fertility of the F0 or F1 parental animals of all substance-treated groups (100, 1000 and 10000 ppm). Therefore, under the conditions of this study the NOAEL (no observed adverse effect level) for reproductive performance and fertility is 10000 ppm (1165 mg/kg bw/d) for the F0 and F1 parental rats.

The NOAEL for parental toxicity was 100 ppm (6.7 mg/kg bw/d), based on increased incidence of increased liver weights and centrilobular hepatocellular hypertrophy at 1000 ppm (67 mg/kg bw/d) and above. At 10000 ppm (667 mg/kg bw/d), the highest dose tested, additional effects observed were significantly reduced body weight / body weight gain and food consumption and degeneration of hepatocellular cells.

The NOAEL for reproductive toxicity was also 100 ppm based on impairment of pup body weight development at 1000 ppm in F2 male pups and slightly increased pup mortality of the F2 litters at 10000 ppm, noted between Days 0–4 p.p. only.

B.6.6.2 Developmental toxicity

B.6.6.2.1 Rat

Report: Schilling K. and Hellwig J., 2000 (TOX2001-732)
BAS 510 F - Prenatal developmental toxicity study in Wistar rats - Oral administration (gavage)
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1015001, unpublished
(Experimental work from 27 October– 25 November 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 414 (Draft Document, March 1998); EEC 87/302, EPA 870.3700, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch: N 37; purity: 94.4 %
Test animals: Female Wistar rats (Chbb THOM (SPF)), 10–11 wk old (Day 0 p.c.)
Source: Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany

Nicobifen was administered as an aqueous suspension to 25 mated female Wistar rats/group by stomach tube at doses of 0, 100; 300 and 1000 mg/kg bw on day 6 through day 19 post coitum (p.c.). A standard dose volume of 10 ml/kg body weight was used for each group. The

control group was dosed with the vehicle only (0.5 % Tylose CB 30.000 in double distilled water).

Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked each day.

On Day 20 p.c., all females were sacrificed and assessed by gross pathology (including weight determinations of the unopened uterus and the placentae). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead foetuses) were determined. The foetuses were removed from the uterus, sexed, weighed and further investigated for any external findings. Thereafter, nearly one half of the foetuses of each litter was examined for soft tissue findings and the remaining foetuses for skeletal (incl. cartilage) findings. Foetal morphology findings were described using the glossary of Wise et al. (1997), while classification of these findings as malformation or variation was based on the terms and definitions proposed by Chahoud et al. (1999).

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.

Maternal toxicity
No substance-related effects were observed up to the highest dose tested.
NOAEL (maternal toxicity): 1000 mg/kg bw/d

Developmental (embryo-foetal) toxicity
Sex distribution, weight of placentae, weight of foetuses, incidences of external, soft tissue and skeletal malformations, and of external variations were not influenced by nicobifen administration.

Soft tissue examination of the foetuses revealed increased incidences of the following variations: "dilated renal pelvis", "dilated ureter", "dilated cerebral ventricle" and "total foetal soft tissue variations" in one or several treatment groups when compared to control incidences (see Table B.6.6-8). However, none of these increases were statistically significant, the incidences failed to show a dose-related increase and/or were within historical control ranges. Therefore the observed increased incidences of soft tissue variations were not considered to be related to nicobifen treatment.

Table B.6.6-8: Rat developmental toxicity: Foetal soft tissue variations

FINDING	Dose level (mg/kg bw/d)				Historical control	
	0	100	300	1000	Mean (%)	Range (%)
DILATED RENAL PELVIS:						
Foetal incidence (%)	18/155 (12)	20/118 (17)	22/159 (14)	27/139 (19)	I (13.4) II (13.6)	I (5.4–20.5) II (7.6–21.6)
Litter incidence (%)[Fi]	12/22 (55)	10/18 (56)	12/22 (55)	15/21 (71)	I (55.3) II (50.4)	I (27.3–80.0) II (30.4–68.0)
Affected foetuses/litter (mean %) [Wi]	12.6±14.1	16.7±19.8	13.0±14.6	20.5±18.8	I (13.5) II (14.4)	I (5.0–21.0) II (7.5–21.9)
DILATED URETER / HYDROURETER:						
Foetal incidence (%)	2/155 (1.3)	1/118 (0.8)	6/159 (3.8)	3/139 (2.2)	I (0.6) II (1.4)	I (0–1.2) II (0.5–3.6)
Litter incidence (%)[Fi]	2/22 (9.1)	1/18 (5.6)	5/22 (23)	3/21 (14)	I (4.3) II (7.0)	I (0–8.0) II (3.2–14.3)
Affected foetuses/litter (mean %)[Wi]	1.4 ± 4.6	0.8 ± 3.4	3.4 ± 7.0	2.7 ± 7.2	I (0.7) II (1.6)	I (0–1.2) II (0.5–5.9)
DILATED CEREBRAL VENTRICLE: ***No dose-relation***						
Foetal incidence (%)	0/155 (0)	1/118 (0.8)	0/159 (0)	1/139 (0.7)	No data	No data
Litter incidence (%)[Fi]	0/22 (0)	1/18 (5.6)	0/22 (0)	1/21 (4.8)	No data	No data
Affected foetuses/litter (mean %)[Wi]	0±0	0.9±3.9	0±0	0.6±2.7	No data	No data
TOTAL FOETAL SOFT TISSUE VARIATIONS: ***Within historical control range***						
Foetal incidence (%)	18/155 (12)	21/118 (18)	22/159 (14)	28/139 (20)	I (13.4)	I (5.4–20.5)
Litter incidence (%)[Fi]	12/22 (55)	11/18 (61)	12/22 (55)	15/21 (71)	I (55.3)	I (27.3–80)
Affected foetuses/litter (mean %)[Wi]	12.6±14.1	17.6±19.4	13.0±14.6	21.1±18.7	I (13.5)	I (5.0–21.0)

Statistics: Fi = Fisher's Exact Test (one sided); Wi = Wilcoxon-test (one-sided)
[I] Recent historical control data (2 studies, 1997–1998) with updated classification of foetal findings as malformations and variations
[II] Old historical control data (10 studies, 1996–1997) with classification of foetal findings as malformations, variations and retardations

A statistically significant increased incidence of "incomplete ossification of thoracic centrum" was observed at the top dose level, and was the only skeletal variation to show a dose-dependent increase (see Table B.6.6-9).

Table B.6.6-9: Rat developmental toxicity: Foetal skeletal variations

FINDING	Dose level (mg/kg bw/d)				Historical control[a]	
	0	100	300	1000	Incidence (Mean %)	Range (%)
INCOMPLETE OSSIFICATION OF THE THORACIC CENTRUM: – cartilage unchanged						
Foetal incidence (%)	5/164 (3.0)	4/128 (3.1)	3/174 (1.7)	14/148 (9.5)	9/341 (2.6)	1.9–3.3
Litter incidence (%)[Fi]	3/22 (14)	3/18 (17)	2/22 (9.1)	10/21* (48)	9/47 (19.1)	13.6–24.0
Affected foetuses/litter (mean %) [Wi]	3.0±8.2	3.4±8.3	1.6±5.7	9.2±12.0*	2.7	1.8–3.5
INCOMPLETE OSSIFICATION OF THE THORACIC CENTRUM: – cartilage not stained due to a technical error						
Foetal incidence (%)	0/164 (0.0)	0/128 (0.0)	0/174 (0.0)	3/148 (2.0)	–	
Litter incidence (%)[Fi]	0/22 (0.0)	0/18 (0.0)	0/22 (0.0)	1/21 (4.8)		
Affected foetuses/litter (mean %) [Wi]	0.0±0.0	0.0±0.0	0.0±0.0	1.8±8.2		
TOTAL FOETAL SKELETAL VARIATIONS:						
Foetal incidence (%)	145/164 (88)	111/128 (87)	160/174 (92)	132/148 (89)	272/341 (79.8)	78.1–81.6
Litter incidence (%)[Fi]	22/22 (100)	18/18 (100)	22/22 (100)	21/21 (100)	47/47 (100)	100
Affected foetuses/litter (mean %) [Wi]	87.8±12.1	86.8±17.8	91.8±12.8	89.4±13.5	79.9	78.0–82.0

Statistics: Fi = Fisher's Exact Test (one sided); Wi = Wilcoxon-test (one-sided)
[a] Recent historical control data (2 studies, 1997–1998) with updated classification of foetal findings as malformations and variations

Discussion:
The increase in the incidence of "incomplete ossification of thoracic centrum" observed at 1000 mg/kg bw/d was statistically significant compared to control incidences and exceeded the historical control range. In addition, a similar increase was observed in the rabbit teratogenicity study. On this basis, it appears that this ossification disorder is substance-related, even though no associated findings were seen in other parts of the vertebral column.

Conclusion:
Under the conditions of this developmental toxicity study, the oral administration of nicobifen to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 19 p.c.) resulted in delayed ossification of the thoracic centrum in offspring of dams administered the limit dose of 1000 mg/kg bw/d. No developmental effects were observed at 300 mg/kg bw/d. Although overt maternal toxicity was not evident up to the limit dose of 1000 mg/kg bw/d, results from the 90-day oral feed study in rats indicate that liver toxicity would have been detected in dams at 1000 mg/kg bw/d. Thus, a higher sensitivity of the offspring cannot be concluded based on the overall weight of evidence.
NOAEL (developmental toxicity, rat): 300 mg/kg bw/d

B.6.6.2.2 Rabbit

Report: Schilling K. and Hellwig J., 2000 (TOX2001-733)
BAS 510 F - Prenatal developmental toxicity study in Himalayan rabbits. Oral administration (gavage)
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1013425, unpublished
(Experimental work from 13 September – 22 October 1998)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 414 (Draft Guideline August 1997); EEC 87/302, EPA 870.3700, JMAFF

Deviations: None that were considered to have compromised the validity of the study.

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen; batch: N 37; purity: 94.4 %
Test animals: Female Himalayan rabbits (Chbb : HM (outbred strain), 34–36 wk old (Day 0, post insemination), mean bw (Day 0 p.i.): 2713 g;
Source: Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany

Nicobifen was tested for its developmental toxicity in Himalayan rabbits. The test substance was administered as an aqueous suspension to 25 inseminated female Himalayan rabbits/group by stomach tube at doses of 0, 100, 300 and 1000 mg/kg bw on day 7 through day 28 post insemination (p.i.). A standard dose volume of 10 ml/kg bw was used. The control group was dosed with the vehicle only (0.5 % Tylose CB 30.000 in *aqua bidest.*).

Body weights were recorded at 2–3 day intervals, food consumption and state of health of the animals was determined daily throughout the study period.

On Day 29 p.i., all surviving females were sacrificed and assessed by gross pathology (including weight determination of the unopened uterus and the placentae). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead foetuses) were determined. The foetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal findings. In accordance to draft update of OECD guideline 414, the heads of one-half of the foetuses (and those foetuses with head malformations) were removed and processed for evaluation of soft tissue alterations. Foetal morphology findings were described using the glossary of Wise et al. (1997), while classification of these findings as malformation or variation was based on the terms and definitions proposed by Chahoud et al. (1999).

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.

Maternal toxicity
No substance-related effects on mortality were observed. At 1000 mg/kg bw, 4 does aborted between gestation Days 27–29, three of which showing no clinical symptoms while the fourth dam exhibited discoloured faeces and reduced defecation. At 300 mg/kg bw/d, substance-induced signs of maternal toxicity consisted only of one doe with abortion as well as reduced defecation in this female a few days prior to abortion. Mean food consumption and body weight gain was significantly reduced only at 1000 mg/kg bw during the period of nicobifen administration (Days 7–28 p.i.). The net maternal body weight change was statistically significantly lower at 1000 mg/kg bw/d. Moreover, the carcass weight (terminal bw minus weight of unopened uterus was statistically significantly reduced by about 6 % in the high-dose-group. No treatment-related adverse effects were observed at 100 mg/kg bw/d. 24 - 25 females/ group became pregnant. Test substance related findings in dams are summarised in Table B.6.6-10 below:

Table B.6.6-10: Rabbit developmental toxicity: Maternal toxicity

FINDING	Dose level (mg/kg bw/d)			
	0	100	300	1000
Found dead (indications of misgavaging)	1 / 25	0 / 25	1 / 25	0 / 25
Not pregnant	0 / 25	1 / 25	0 / 25	0 / 25
Abortion / early delivery (Days 26–29 p.i.)	0 / 25	0 / 25	1 / 25	4 / 25
No. pregnant dams with scheduled sacrifice on Day 29 p.i.	24 / 25	24 / 25	23 / 25	21 / 25
Discoloured / reduced faeces	0 / 25	0 / 25	1 / 25	1 / 25
Food consumption, Days 7–28 p.i. [g/d]	76.1 ± 7.3	69.0 ± 13.4	68.3 ± 10.4	56.2 ± 11.1 (-26 %)
Terminal body weight [g]	2889	2891	2895	2752* (-4.7 %)
Body weight gain, Days 7–28 p.i., [g]	146.8 ± 120.9 (± 0 %)	125.5 ± 96.3 (-15 %)	115.0 ± 114.6 (-22 %)	27.8 ± 182.3** (-81 %)
Gravid uterus [g]	288.1	359.7*	320.5	298.4
Mean carcass weight [g][a]	2601.1 (± 0 %)	2531.6 (-3 %)	2574.4 (-1 %)	2453.8* (-6 %)
Mean corrected weight gain [g][b]	-125.3 (± 0 %)	-220.6** (-76 %)	-190.3 (-52 %)	-233.3** (-86 %)

Statistics: Dunnett-test (two-sided); * : $p \leq 00.5$; ** : $p \leq 00.1$
[a] Carcass weight = terminal bw minus uterine weight (Day 29)
[b] Corrected bw gain = terminal bw minus weight of the unopened uterus on Day 29 p.i. minus bw on Day 7 p.i.

Thus, under the conditions of this developmental toxicity study, the oral administration of nicobifen to pregnant Himalayan rabbits from implantation to one day prior to the expected day of parturition (days 7 - 28 p.i.) elicited overt maternal toxicity at 1000 mg/kg bw/d

(increased incidence of abortions or early delivery in four does, reduced food consumption, impairments in body weight gain, reductions in mean carcass weight and corrected body weight gain) and possibly caused abortion in a single animal at 300 mg/kg bw/d. There were no adverse effects on the female rabbits at 100 mg/kg bw/d.

There were no substance-induced, dose-related influences on the gestational parameters (sex distribution, placentae weight, weight of foetuses) at any dose level.

There were no signs of substance-related developmental toxicity at any dose level upon external and soft tissue examination. A statistically significantly increased incidence of incomplete ossification of the thoracic centrum was found at the top dose level, with a dose-related increase seen at lower dose levels. No associated finding was noted in other parts of the vertebral column. All of the other noted skeletal variations appeared without relation to dose, were without biologically relevant differences between groups and/or could be found at comparable frequency in the historical control data that were included in original study report. The incidence of total skeletal variations was comparable to the concurrent control group on a litter basis, or even lower when based on mean affected foetuses per litter (see Table B.6.6-11).

Table B.6.6-11: Rabbit developmental toxicity: Foetal skeletal variations

FINDING	Dose level (mg/kg bw/d)				Historical control[a]	
	0	100	300	1000	Mean (%)	Range (%)
INCOMPLETE OSSIFICATION OF THE THORACIC CENTRUM:						
Foetal incidence (%)	1 / 135 (0.7)	4 / 183 (2.2)	6 / 145 (4.1)	16 / 136 (12)	0.1	0.0–0.6
Litter incidence (%)[Fi]	1 / 23 (4.3)	4 / 24 (17)	3 / 22 (14)	7 / 21[*] (33)	0.7	0.0–4.2
Affected foetuses/litter (mean %)[Wi]	0.7±3.5	1.8±4.2	2.9±9.0	8.3±17.2[**]	0.1	0.0–0.5
TOTAL FOETAL SKELETAL VARIATIONS:						
Foetal incidence (%)	82 / 135 (61)	127 / 183 (69)	86 / 145 (59)	84 / 136 (62)	No data	
Litter incidence (%)[Fi]	21 / 23 (91)	24 / 24 (100)	21 / 22 (95)	19 / 21 (90)		
Affected foetuses/litter (mean %)[Wi]	59.7±31.7	68.2±20.0	59.8±26.5	56.5±26.7		

Statistics: Fi = Fisher's Exact Test (one sided); Wi = Wilcoxon-test (one-sided)
[a] Historical control data (8 studies, 1995–1998; Himalayan rabbit Chbb:HM (outbred strain; supplier: Thomae/Boehringer Ingelheim Pharma KG, Biberach, Germany),

Discussion:
The notifier was of the opinion that the increased frequency of incompletely ossified thoracic centrum is incidental and not related to treatment, mainly because no associated finding was noted in other parts of the vertebral column and the total incidence of skeletal variations was similar to control levels. The RMS does not concur with this view for the following reasons:

4. Incomplete ossification of the thoracic centrum was observed at significantly increased incidences in rat foetuses as well,

5. at 1000 mg/kg bw/d, both the observed litter incidence and the number of affected foetuses per litter were statistically significantly increased over control levels, both incidences clearly exceeded the historical control range, and
6. a dose related increase in the foetal incidence was observed, with the historical control range exceeded in all test groups.

The slightly increased litter incidences observed at lower dose levels were not dose-related and statistically non-significant, and therefore not considered to be treatment-related.

Conclusion:
The NOAEL for maternal toxicity is 100 mg/kg bw/d, based on a single doe with abortion and reduced defecation at 300 mg/kg bw/d, and more pronounced effects at the top dose level of 1000 mg/kg bw/d.
NOAEL (developmental toxicity, rabbits): 300 mg/kg bw/d, based on statistically significantly increased delayed ossification of the thoracic centrum at 1000 mg/kg bw/d.

B.6.7 Delayed neurotoxicity (Annex IIA 5.7)

Three oral neurotoxicity studies with nicobifen were conducted in rats (see Table B.6.7-1). In the acute neurotoxicity study, piloerection observed on the day of treatment in 2 of 20 rats was the only clinical sign of toxicity to be seen at the top dose level (2000 mg/kg bw). This finding was considered to reflect an unspecific reaction of the animals to excessive dosing and was therefore not regarded as a substance-related effect. No adverse reaction to treatment was observed in any animals at 1000 mg/kg bw or lower dose levels. No signs of neurotoxicity were observed at any dose level.

In the 90 day oral neurotoxicity study in rats, there were no test substance related adverse effects at any dose level and there were no signs of neurotoxicity at any dose level. The no observed effect level was 15000 ppm, i.e. 1050 mg/kg bw/d in males and 1272 mg/kg bw/d in females.

In a developmental neurotoxicity study, the slight reduction on pup body weight (at 10000 and 1000 ppm) were in line with similar effects seen in the 2-generation study in rats, where at these dose levels, parental toxicity was noted in form of hepatotoxicity. No signs of developmental neurotoxicity were noted up to the highest concentration of 10000 ppm (1442 mg/kg bw/d), which was clearly above a recommended limit dose of 1000 mg/kg bw/d.

In conclusion, nicobifen is neither neurotoxic to adult rats nor to the developing rat. As there were no neurotoxic effects observed in any of the studies with nicobifen, studies on the delayed neurotoxicity in hens were not triggered.

Table B.6.7-1: Summary of neurotoxicity studies

Study dose levels purity	Target	NOAEL (males / females) mg/kg bw/d	LOAEL (males / females) mg/kg bw/d	Effects
Rat acute oral neurotoxicity 0–500–1000–2000 mg/kg bw purity: 96.3 %	Neurotox.	2000	–	No specific neurotoxic effects.
	General tox.	1000	2000	2000 mg/kg bw: Piloerection in 2/10 females on Day 0
Rat 90-day oral neurotoxicity 0–150–1500–15000 ppm purity: 96.3 %	Neurotox.	1050 / 1273 (15000 ppm)	–	No effects observed
Rat developmental neurotoxicity 0–100–1000–10000 ppm purity: 96.3 %	Maternal tox.	1442 (10000 ppm)	–	No effects observed
	General tox. in offspring	14 (100 ppm)	147 (1000 ppm)	1000 ppm: ↓ bw and bw gains (Days 1-4 p.p.) 10000 ppm: ↓ bw and bw gains until weaning ↓ abs. brain wt, brain length (Day 11 p.p. male pups only)
	Neurotox. in offspring	1442 (10000 ppm)	–	No effects observed

B.6.7.1 Rat acute neurotoxicity

Report: Mellert W. et al., 2000 (TOX2001-734)
BAS 510 F - Acute oral neurotoxicity study in Wistar rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2000/1018638, unpublished
(Experimental work from 21 December 1998 – 15 January 1999)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD Test Guideline No. 424 (adopted 21 July 1997); EEC 92/32; EPA Health Effects Test Guidelines, OPPTS 870.6200 (Neurotoxicity Screening Battery)

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material:	Nicobifen; batch No. N 46; purity: 96.3 %
Test animals:	Male and female Wistar rats [Chbb:THOM (SPF)], age (Day 0): 49 d; mean bw (Day 0): males 243 g (range 220–268 g), females 164 g (133–188 g); supplier: Boehringer Ingelheim Pharma KG, Biberach, Germany

Nicobifen was administered to groups of 10 male and 10 female Wistar rats as a single oral administration by gavage at dose levels of 0; 500; 1000 and 2000 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. Functional observational batteries (FOBs) and motor activity measurements were carried out in all animals before administration (Day –7), on Day 0 (within a few hours after dosing), as well as on Days 7 and 14. FOBs consisted of four parts, starting with passive observations, followed by removal from the home cage and open field observations in a standard arena. Thereafter sensorimotor tests and reflex tests were conducted. The measurement of motor activity was performed in the dark with 4 infrared beams per cage over a 60-min period. Five animals per sex and dose were anaesthetised and killed by perfusion fixation, visible organs assessed by gross necroscopy and sections from the brain, spinal chord and peripheral nervous system were prepared and examined by light microscopy. The remaining animals were killed under CO_2-anaesthesia without any further examinations.

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 (2000 mg/kg bw) piloerection was observed in two females during the FOB on Day 0. This finding was considered to reflect an unspecific reaction of the animals to excessive dosing and was therefore not regarded as a substance-related effect. Grip strength of forelimbs was statistically significantly decreased in high-dose males on Day 7. As no effect was seen on Days 0 and 14, it is rather unlikely that this was a true substance-related effect. Light microscopic investigation of the central and peripheral nervous system did not reveal any substance-dependent changes in the organ samples examined.

Conclusion:

No signs of neurotoxicity were observed. The no observed effect level for neurotoxicity was 2000 mg/kg bw in both sexes. The NOAEL for general toxicity was 1000 mg/kg bw/d, based on transient piloerection in 2 of 20 rats at 2000 mg/kg bw.

B.6.7.2 Rat 90-day oral neurotoxicity

Report: Mellert W. et al., 2001 (TOX2001-735)
BAS 510 F - Subchronic oral neurotoxicity study in Wistar rats - Administration in the diet for 3 months
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000113, unpublished
(Experimental work from 9 February –28 May 1999)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD Test Guideline No. 424 (adopted 21 July 1997); EEC 92/32; EPA Health Effects Test Guidelines, OPPTS 870.6200 (Neurotoxicity Screening Battery)

Deviations: None that compromised the validity of the study results

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Nicobifen; batch No. N 46; purity: 96.3 %

Test animals: Male and female Wistar rats [Chbb:THOM (SPF)], age (Day 0): 49 d; mean bw (Day 0): males 248 g (range 232–271 g), females 177 g (159–194 g); supplier: Boehringer Ingelheim Pharma KG, Biberach, Germany

Nicobifen was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 150, 1500 and 15000 ppm 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 prior to the start of the administration (Day –7) as well as on Days 22, 50 and 85. FOBs consisted of four parts, starting with passive observations, followed by removal from the home cage and open field observations in a standard arena. Thereafter sensorimotor tests and reflex tests were conducted. The measurement of motor activity was performed in the dark with 4 infrared beams per cage over a 60-min period.

Five animals per sex and dose were anaesthetised and killed by perfusion fixation, visible organs assessed by gross necroscopy and sections from the brain, spinal chord and peripheral nervous system were prepared and examined by light microscopy. The remaining animals were killed under CO_2-anaesthesia without any further examinations.

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.6.7-2: Rat 90-d neurotoxicity study: Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw)	
	Males	Females
150	10.5	12.7
1500	103.1	124.5
15000	1050.0	1272.5

There were no test substance related adverse effects at any dose level. There were no signs of neurotoxicity at any dose level.

Conclusion:
The no observed effect level for neurotoxicity was 15000 ppm (equivalent to 1050 mg/kg bw/d in males and 1272 mg/kg bw/d in females), the highest feed concentration tested. Nicobifen was not neurotoxic under the conditions of this study.

B.6.7.3 Delayed neurotoxicity in hens

As there were no neurotoxic effects observed in any of the studies with nicobifen, studies on the delayed neurotoxicity in hens were not triggered.

B.6.7.4 Rat developmental neurotoxicity

Report: Kaufmann W. et al., 2001 (TOX2001-736)
BAS 510 F - Developmental Neurotoxicity study in Wistar rats. Administration in the diet
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000118, unpublished
(Experimental work from 23 April – 18 July 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD Draft Guideline No. 426 (October 1999)
EPA/OPPTS 870.6300 ("Developmental neurotoxicity study", Aug 1998)

Deviations: Reflex ontogeny measurements were not conducted (at least two determinations before Day 21 p.p. required according to OECD Draft Guideline 426)
Dosing conditions on Day of parturition were not reported

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch No. N 46; purity: 96.3 %
Test animals: Male and female Wistar rats CRL:WI (GLX/BRL/HAN) IGS BR, male and female animals derived from different litters according to the breeder; supplier: Charles River, Sulzfeld, Germany

Nicobifen was tested for its effect on the embryonic, foetal and postnatal development of the nervous system in Wistar rats in this developmental neurotoxicity study. The test substance was administered continuously as a homogeneous addition to the food in different concentrations (0–100–1000–10000 ppm) to 35 mated female Wistar rats/group from Day 6 post coitum (p.c.) to Day 21 post partum (p.p.). The dams were allowed to litter and rear their offspring until Day 4 (standardisation of litters) or 21 after parturition.

Examination of dams
The state of health, as well as the nesting, littering and lactation behaviour of all dams (35/group) was checked each day. Food consumption was determined regularly during gestation (Days 0, 6, 13 and 20) and lactation periods (Days 1, 7 and 14). Body weights were determined regularly during gestation (Days 0, 6, 13 and 20) and lactation periods (Days 1, 7, 14 and 21 p.p.). The intake of test substance was calculated from the food consumption and body weight data. A detailed clinical examination outside the cage (open field observations) was performed in 10 dams/group on Days 7 and 14 p.c. and on Days 7 and 14 p.p. (see Table B.6.7-3).

Table B.6.7-3: Rat developmental neurotoxicity: Parameters assessed in open field examinations of dams and selected offspring

• Behaviour when handling	• Fur	• Skin
• Posture	• Salivation	• Respiration
• Activity/arousal level	• Tremors	• Convulsions
• Abnormal movements	• Gait abnormalities	• Lacrimation
• Palpebral closure	• Exophthalmos	• Faeces (appearance/consistency)
• Urine	• Pupil size	• Other abnormalities observed

Female fertility index, gestation index and live birth index were calculated for the litters. After the offspring were weaned (Day 21 p.p.), the dams were sacrificed and discarded without any further examinations. Dams without any litter were also discarded after the uterus had been stained for the evidence of early resorptions.

Examination of offspring
Offspring number and status (sex, liveborn or stillborn) of all delivered offspring were determined as soon as possible on the day of birth. At the same time, the offspring were also examined for macroscopically evident changes.

After parturition, only those litters were used for further examination which consisted of at least 8 offspring and whose date of littering (Day 0 p.p.) occurred within a timeframe of three consecutive days.

The state of health (and viability/mortality) of offspring were checked each day. Offspring were weighed on the day after birth and on Day 4, 11, 17 and 21 p.p., and after weaning once a week. The sex ratio was calculated at Day 0 and Day 21 p.p. Following standardisation of litter size on Day 4 p.p., selected pups from each standardised litter were allocated to five specific subsets (I–V), each consisting of 10 pups per sex and treatment group, as indicated in Table B.6.7-4 below:

Table B.6.7-4: Rat developmental neurotoxicity study: scope of investigations (subsets)

Subsets	Number of pups selected	Test procedure
I	10/sex/group	Day 11 p.p.: Perfusion fixation, brain weights and neuropathology of the brain
II	10/sex/group	Open field observation (OFO) and motor activity (MA)
III	10/sex/group	Auditory startle test and Day 60 (±2) p.p.: Perfusion fixation, brain weights and neuropathology)
IV	10/sex/group	Day 21 (±2) p.p.: Learning and memory test
V	10/sex/group	Day 60 (±2) p.p.: Learning and memory test

The sexual maturation of all offspring from subsets I–III and V were investigated daily beginning on Day 27 and 40 p.p. for females and males, respectively. The exact day of vaginal opening in males and the exact day of preputial separation in males was recorded and the body weight of the respective animals on this day was determined.

Sexual maturation (day of preputial separation/vaginal opening) of all selected offspring except from offspring of subset IV was evaluated daily with examinations initiating on Day 40 and Day 27 p.p. for male and female offspring, respectively. and the respective body weights were determined.

A detailed clinical examination outside the cage (open field observations, see Table B.6.7-3), measurements of motor activity, as well as auditory startle and learning and memory (water maze test) tests were performed in selected offspring.

Neuropathological examinations and determinations were carried out in pups selected for perfusion fixation (10 per sex, group and study section [one male and one female per litter]) on Day 11 p.p. (subset I) and Day 60 p.p. (subset III):

1. Brain weights (incl. olfactory bulb) and morphometric measurements of length and width of the whole brain were determined.
2. The following organs were carefully removed, processed histotechnically and examined by light microscopy (see Table B.6.7-5):

Table B.6.7-5: Rat developmental neurotoxicity: histopathological examinations

Tissue sections examined in pups fixed by perfusion on Day 11 p.p. and on Day 60 p.p.	
• Brain (cross sections of olfactory bulb, frontal lobe, parietal lobe with diencephalon, midbrain with occipital and temporal lobe, pons, cerebellum medulla oblongata)* • Eyes with retina and optical nerve • Olfactory epithelium (nose cavity, level III)	• Spinal chord (Cervical, thoracial, lumbar sections) • Pituitary gland • Gasserian ganglia with nerve • Gastrocnemius muscle • All gross lesions
Additional tissue sections examined in pups fixed by perfusion on Day 60 p.p.	
• Dorsal root ganglion • Dorsal root fibre • Ventral root fibre	• Proximal sciatic nerve • Proximal tibial nerve (at knee) • Distal tibial nerve (at lower leg)

* Tissues from control and high-dose group examined only

All offspring, which were not required for any examinations, all offspring standardised on Day 4 p.p., and all animals of subsets II, IV and V (on completion of the tests) were sacrificed and discarded without any further examinations.

Findings:
The stability of the test substance was analytically confirmed. The stability of the test substance in the diet was proven. The correctness of the concentrations and homogeneity were analytically confirmed.

Dams
The mean test substance intakes are summarised in Table B.6.7-6:

Table B.6.7-6: Rat developmental toxicity: Test substance intake

	Mean test substance intake (mg/kg bw/d)		
Dams	**100 ppm**	**1000 ppm**	**10000 ppm**
– Gestation period	9.6	108.6	1031.6
– Lactation period	18.3	186.0	1853.1
– Mean	**14**	**147**	**1442**

No substance-related effects were observed in dams up to the highest dose tested (10000 ppm or approx. 1442 mg/kg bw/d)

Offspring:
There were no signs of developmental neurotoxicity up to the highest dose tested (10000 ppm or approx. 1442 mg/kg bw/d). Substance-induced signs of transiently retarded physical development in form of impaired mean body weights/body weight gains were observed only during lactation in the offspring of the mid- and high-dose groups (see Table B.6.7-7).

Table B.6.7-7: Rat developmental neurotoxicity: Effects on pup body weight (gain)

Parameter	Sex	Body weight (gain) [g] at dose level (ppm)			
		0	100	1000	10000
Body weight (Day 1 p.p.)	M	6.4±0.5	6.2±0.6 (-3.1 %)	6.3±0.5 (-1.6 %)	6.1±0.6 (-4.7 %)
	F	6.2±0.5	5.9±0.5 (-4.8 %))	6.0±0.5 (-3.2 %)	5.8±0.6* (-6.5 %)
Body weight (Day 4 p.p. pre-/post-culling)	M	9.8±0.9	9.3±1.1 (-5.1 %)	9.0±0.9* (-8.2 %)	8.4±0.9** (-14 %)
	F	9.6±0.8	9.1±1.1 (-5.2 %)	8.7±0.8** (-9.4 %)	8.1±0.9** (-16 %)
Body weight (Day 21 p.p.)	M	47.1±4.3	49.3±3.9 (+4.7)	48.0±3.9 (+1.9 %)	43.6±3.2** (-7.4 %)
	F	45.6±4.1	47.6±2.7 (+4.4 %)	46.1±3.4 (+1.1 %)	42.5±2.9** (-6.8 %)
Body weight gain (Day 1–4 p.p.)	M	3.4±0.5	3.1±0.6 (-8.8 %)	2.7±0.5** (-21 %)	2.3±0.4** (-32 %)
	F	3.4±0.5	3.1±0.6 (-8.8 %)	2.7±0.5** (-21 %)	2.3±0.4** (-32 %)
Body weight gain (Day 4–21 p.p.)	M	37.3±3.7	40.0±3.5* (+7.2 %)	39.0±3.54 (+4.6 %)	35.3±2.9 (-5.4 %)
	F	36.0±3.4	38.6±2.4** (+7.2 %)	37.5±3.1 (+4.2 %)	34.4±2.4 (-4.4 %)

Statistical significance: * = $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett test, two-sided)

In pups of the mid- and high-dose group, treatment-related reductions in body weight development were noted, which were most pronounced during the period prior to standardisation of litter size (Day 1-4 p.p.). During the remaining period of lactation, mid-dose group pups gained weight to reach body weight values that were comparable to control levels on Day 21 p.p. High-dose group pups, however, were not able to compensate initial body weight losses during the lactation period: At the time point of weaning, mean body weights were statistically reduced by 7 % and body weight gains were also about 7 % lower when compared to control values. No statistical differences in body weight parameters were detectable between treatment groups after weaning.

Assessment of sexual maturity data revealed no treatment-related differences between groups. No substance-related findings were evident from open field observations and determination of motor activity. Results from the auditory startle test and from the water maze test (learning and memory assessment) gave no indication of a substance-related effect.

The neuropathological examination of a broad variety of different brain regions and associated tissues (Day 11 and 60(±2) p.p. groups subjected to perfusion fixation) and the peripheral nervous system (Day 60(±2) p.p. groups) did not reveal any substance-dependent changes in morphology. A few statistically significant differences concerning terminal body and brain weights are summarised in Table B.6.7-8 and Table B.6.7-9.

Table B.6.7-8: Rat developmental toxicity: Significant findings in pups killed on Day 11 and subjected to neuropathological examination

Parameter	Sex	Dose level (ppm)			
		0	100	1000	10000
Terminal body weight [g] Kw/Wi	M	21.8±2.4	22.7±1.9	21.4±1.5	19.8±1.6* (-9.2 %)
	F	20.9±1.7	22.7±1.8*	21.2±1.6	19.0±1.6* (-9.1 %)
Absolute brain weight [g] Kw/Wi	M	1.30±0.08	1.29±0.07 (-0.8 %)	1.24±0.07 (-4.6 %)	1.22±0.07* (-6.2 %)
	F	1.27±0.05	1.23±0.06 (-3.2 %)	1.21±0.09 (-4.7 %)	1.18±0.05** (-7.1 %)
Relative brain weight [%] Kw/Wi	M	6.00±0.44	5.71±0.59	5.81±0.25	6.15±0.47
	F	6.07±0.34	5.44±0.21* (-10.3 %)	5.69±0.26* (-6.3 %)	6.23±0.41 (+2.6 %)
Whole brain, length [cm] Wi/Bo	M	1.71±0.05	1.71±0.03	1.68±0.04	1.66±0.04* (-2.9 %)
	F	1.68±0.03	1.68±0.04	1.67±0.05	1.66±0.03 (-1.2 %)
Whole brain, width [cm] Wi/Bo	M	1.40±0.04	1.41±0.03	1.39±0.03 (-0.7 %)	1.38±0.03 (-1.4 %)
	F	1.40±0.03	1.38±0.02	1.37±0.03 (-2.1 %)	1.38±0.03 (-1.4 %)
Hippocampus right, length [µm] Wi	M	1201±104	–	–	1167±128 (-2.8 %)
	F	1207±103	–	–	1111±105* (-8.0 %)

Statistical significance: * = $p \leq 0.05$; ** $p \leq 0.01$
Kw/Wi = Kruskal-Wallis-H & Wilxoxon Test, two-sided;
Wi/Bo = Wilcoxon-Test (one-sided) with Bonferoni-Holm adjustment

The terminal body weights of male and female treated top-dose offspring of the Day 11 p.p. groups were significantly lower when compared with the control values. The same is true for the significantly lower absolute brain weights of the Day 11 p.p. offspring. In contrast to these results, the terminal body weights of 100 ppm females were increased. Whereas the relative brain weights were not changed in the top-dose groups, they were decreased in the low- and mid-dose groups. The linear measurements of the maximal length of the overall brain showed a weak significant decrease for male Day 11 p.p. offspring, but not for females. Only the right (but not the left) hippocampus of the females showed a slight but significant decrease, when compared with the controls. All other measurements for the Day 11 p.p. offspring revealed no significant changes.

The decreases of the terminal body weights and absolute brain weights observed in high-dose group pups killed during the lactation period (Day 11 p.p.) are considered to be treatment-related. The findings within this subgroup are in line with the observation of impaired body weight development established especially during the early lactation period. The reduced length of the whole brain in top-dose males is assessed as being related to the reduced body weights. The slightly reduced size of the right hippocampus observed in top-dose females (Day 11 p.p.) was not interpreted as a nicobifen-dependent selective effect on brain area growth and rather considered to be incidental, because only one side of the brain was involved, no corresponding neurohistological changes were observed, and no effect on the

hippocampus was observed in males. Observed reductions in relative brain weight were not dose-related and therefore not considered to be substance-related.

Table B.6.7-9: Rat developmental toxicity: Significant findings in pups killed on Day 60 p.p. and subjected to neuropathological examination

Parameter	Sex	Dose level (ppm)			
		0	100	1000	10000
Terminal body weight [g] Kw/Wi	M	265±26	260±21	265±21	258±23 (-2.6 %)
	F	172±13	170±15	173±10	168±11 (-2.3 %)
Absolute brain weight [g] Kw/Wi	M	2.02±0.08	2.04±0.04	2.06±0.07	1.98±0.06 (-2.0 %)
	F	1.91±0.06	1.90±0.04	1.93±0.04	1.87±0.07 (-2.1 %)
Relative brain weight[%] Kw/Wi	M	0.77±0.06	0.79±0.06	0.78±0.05	0.77±0.06
	F	1.11±0.06	1.12±0.09	1.12±0.06	1.11±0.06
Whole brain, length [cm] Wi/Bo	M	2.10±0.03	2.09±0.05	2.09±0.05	2.08±0.03 (-1.0 %)
	F	2.05±0.04	2.05±0.03	2.06±0.03	2.03±0.04 (-1.0 %)
Whole brain, width [cm] Wi/Bo	M	1.54±0.03	1.56±0.02	1.57±0.02	1.54±0.03
	F	1.52±0.02	1.51±0.02	1.52±0.02	1.50±0.02* (-1.3 %)
Hippocampus, right, length [µm] Wi	M	1830±76	–	–	1871±115
	F	1803±84	–	–	1788±74 (-0.8 %)

Statistical significance: * = $p \leq 0.05$; ** $p \leq 0.01$
Kw/Wi = Kruskal-Wallis-H & Wilcoxon Test, two-sided;
Wi/Bo = Wilcoxon-Test (one-sided) with Bonferoni-Holm adjustment

Neither terminal body weight nor brain weight parameters were significantly changed at the second time point of investigation on Day 60(±2) p.p. Only a slightly but significantly reduced width of the whole brain was found for the top-dose females, but not for the males. With reference to the linear measurements of major brain areas (including the hippocampus), no changes to control values were found for the top-dose animals in the treated day 60(±2) p.p. groups.

Discussion:
The observed slight reduction on pup body weight (at 10000 and 1000 ppm) is in line with similar effects seen in the 2-generation study in rats. At these dose levels parental toxicity was noted in form of hepatotoxicity.

Conclusion:
In this developmental neurotoxicity study, nicobifen had no adverse effects on the embryonic, foetal and postnatal development of the nervous system in Wistar rats at dose levels up to 10000 ppm, i.e. > 1000 mg/kg bw/d. Slight signs of impaired physical development in form of transiently reduced body weights and retarded body weight gains occurred in the offspring at

and above 1000 ppm (ca. 147 mg/kg bw/d). There were no apparent signs of general toxicity in the parental females at any dose levels.
NOAEL (maternal toxicity): 10000 ppm (1442 mg/kg bw/d)
NOAEL (developmental neurotoxicity): 10000 ppm (1442 mg/kg bw/d)
NOAEL (reproductive toxicity): 100 ppm (14 mg/kg bw/d)

B.6.8 Further toxicological studies (Annex IIA 5.8)

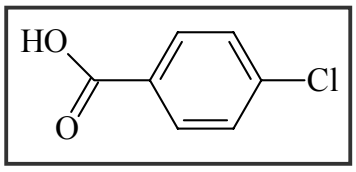
Para-chlorobenzoic acid was identified in the aquatic environment as degradation product of nicobifen. Results from a literature survey indicate that para-chlorobenzoic acid is more toxic than nicobifen after acute oral intake (LD_{50} 4170 mg/kg bw vs. > 5000 mg/kg bw). No adverse effects were reported in oral subchronic toxicity studies in rabbits (NOAEL: 1500 mg/rabbit/d) and rats (NOAELs: 26 mg/rat/d and 0.3 mg/kg bw/d). Limited *in-vitro* genotoxicity data indicate no concern. The feeding of 13 or 26 mg/rat/d over a period of five months did not induce adverse effects on the number of offspring and did not induce developmental toxicity. In view of the transient nature of this compound no further need for a toxicological investigation was identified.

Mechanistic studies with nicobifen have shown that the test substance induces a liver weight increase with concomitant histopathological changes in zone 3 hepatocytes (proliferation/accumulation of smooth endoplasmatic reticulum (SER) and glycogen depletion). These changes are considered to be related to the induction of hepatic metabolising enzymes. Nicobifen induced both phase I (oxidative) enzymes, as demonstrated by increased cytochrome P450 content, as well as phase II (conjugation) enzymes. The latter was shown by increased activities of p-nitrophenol-glucuronyltransferase, 4-methylumbelliferone-glucuronyltransferase, and 4-hydroxybiphenyl-glucuronyltransferase. The increases in phase II (conjugation) hepatic activity were accompanied by the reduction of thyroid hormone T3 and T4 levels and by a concomitant increase in TSH. While the decrease in serum T3 and T4 concentrations appear to have resulted from increased elimination via nicobifen-mediated induction of phase II hepatic enzyme activities, the increased TSH levels were obviously the result of a feedback mechanism induced by decreased in serum T3 and T4 levels.

B.6.8.1 Toxicity studies of metabolites

Para-chlorobenzoic acid (CAS No. 74-11-3, *syn.* 4-chlorobenzoic acid, PCBA, *p*-carboxy-chlorobenzene; also referred to as metabolite M510F64 in the applicant's dossier) was found to be a degradation product of nicobifen in the aquatic environment.

Figure B.6.8-1: Molecular structure of para-chlorobenzoic acid



A literature research on the toxicological properties of this compound resulted in a number of findings regarding acute toxicity in several species, studies with repeated administration and

mutagenicity. A useful summary is provided in the German MAK documentation on chlorobenzoic acid and its isomers [TOX2001-737; MAK: "Chlorbenzoesäure und ihre Isomeren"; dated June 14, 1985] and the results presented below have been excerpted from this documentation.

Acute oral toxicity:
Studies on acute oral toxicity testing are shown in Table B.6.8-1 below.

Table B.6.8-1: Para-chlorobenzoic acid: summary of acute toxicity data

Species tested	LD_{50} (mg/kg bw)
Mouse	661 (515 – 925)
Guinea pig	1050
Rat	4170 (3723 – 4670)

Repeated dose administration:
The oral administration of 1500 mg/d of para-chlorobenzoic acid to rabbits for a period of six months did not result in adverse effects. Likewise the feeding of 13 or 26 mg/rat/d over a period of five months also did not induce adverse effects. In a drinking water study, the administration of 0.3 mg/kg bw/d to rats over a period of six months did not induce toxic changes in the animals.

The feeding of 13 or 26 mg/rat/d over a period of five months did not induce adverse effects on the number of offspring and did not induce developmental toxicity.

Monochlorinated benzoic acids did not induce mutagenicity in the Ames test in several strains. Moreover, a negative response was observed in sister chromatid exchange tests performed in lymphocytes of rabbits.

In view of the transient nature of this compound no further need for a toxicological investigation has been identified.

B.6.8.2 Supplementary studies

Report: Mellert W. et al. 1999 (TOX2001-738)
BAS 510 F - Hepatic enzyme induction study in Wistar rats - Administration in the diet for 2 weeks
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 1999/10522, unpublished
(Experimental work from October 1997 - November 1997)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: No test guidelines exist for this type of study.

Deviations: Not applicable (no guideline).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch No. N 26, purity: 95.3%.
Test animals: Male and female Wistar rats (Chbb:THOM (SPF))

The potential of nicobifen to induce hepatic metabolising enzymes was investigated in rats.

The test substance was administered to groups of 5 male and 5 female Wistar rats at dietary concentrations of 0 ppm and 15000 ppm for 2 weeks. Additional groups of 3 males and 3 females were also treated for 2 weeks with 0 ppm and 15000 ppm and subjected to perfusion fixation for subsequent electron microscopy of the liver. The following parameters regarding enzyme induction were examined:

- Cytochrome P450-content (Cyt.P450)
- Ethoxyresorufin-O-deethylase (EROD)
- Pentoxyresorufin-O-depentylase (PROD)
- Light and Electron microscopy of liver
- Glutathione (GSH)
- Cyanide-insensitive Palmitoyl-CoA-oxidation (PalCoA)
- Lipidperoxidation ("TBA-reactive material")
- Liver weight

Findings:
The stability of the test substance over the study period was demonstrated. The stability of the test substance in the vehicle was verified. The homogeneity of the mixtures was verified. The correctness of the concentrations was demonstrated. Compared to controls the following findings were obtained at 15000 ppm (see Table B.6.8-2):

Table B.6.8-2: Effects of nicobifen on liver-specific parameters

Parameter	Effect (15000 ppm vs. control)
Cyanide-insensitive palmitoyl-CoA-oxidation (PalCoA):	No treatment-related changes in both sexes
Cytochrome P450-content (Cyt.P450):	Increased in both sexes; by 124% in males and 74% in females
Ethoxyresorufin-O-deethylase (EROD):	No treatment-related changes in both sexes
Pentoxyresorufin-O-depentylase (PROD):	No treatment-related changes in both sexes
Glutathione (GSH):	No treatment-related changes in both sexes
Lipidperoxidation ("TBA-reactive material"):	Slightly increased in males but not in females; In absence of any change in the glutathione levels this change (in one sex only) is of questionable toxicological significance
Light and Electron microscopy of liver:	Proliferation / accumulation of smooth endoplasmatic reticulum (SER) in zone 3 hepatocytes. Glycogen depletion in hepatocytes exhibiting severe SER accumulation
Liver weights	Increased by 32% in males and 23% in females

Conclusion:
Thus, nicobifen can be regarded as an inducer of rat liver cytochrome P450. The observed structural changes and the increased liver weights are in-line with the induction of hepatic metabolising enzymes.

Report: Mellert W. et al., 2001 (TOX2001-739)
Hormone and Enzyme induction Study in Wistar rats
Administration in the diet for 4 weeks
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000141, unpublished
(Experimental work from July – August 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: No test guidelines exist for this type of study.

Deviations: Not applicable (no guideline).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Nicobifen; batch No. N 46, purity: 96.3%.
Test animals: Male and female Wistar rats (Chbb:THOM (SPF))

Nicobifen was administered to groups of 5 male and 5 female Wistar rats at dietary concentrations of 0 ppm and 15000 ppm for 4 weeks. Food consumption and body weights were determined once a week, and the animals were examined for signs of toxicity or mortality at least once a day.

Hormone analyses (T3, T4, TSH) were carried out on study days -3, 2, 4, 7, 14, 21 and 28.

Phase II enzyme examinations in liver tissue (p-nitrophenol-glucuronyltransferase, 4-methylumbelliferon-glucuronyltransferase, 4-hydroxybiphenyl-glucuronyltransferase) were carried out at the end of the study.

Findings:
The stability of the test substance over the study period was demonstrated. The stability of the test substance in the vehicle was verified. The homogeneity of the mixtures was verified. The correctness of the concentrations was demonstrated.

The daily test substance intake at 15000 ppm was 957 mg/kg bw for males and 1197 mg/kg bw for females.

There were no clinical signs of toxicity nor effects on body weight or food consumption at 15000 ppm.

Total triiodothyronine (T3):
In the treated males decreased serum T3 concentrations (84% to 69% of control) were observed throughout the study. With the exception of day 4 all these changes were seen as a trend toward reduced values. In the treated females, slight, non-statistically significant decreases (99% to 76% of control) in serum T3 levels were found from day 4 onward.

Total thyroxine (T4):
Significantly decreased T4 concentrations (approximately 87% to 73% of control) were measured in the sera of the treated males from day 4 onward. On day 2 this finding was also seen as a trend toward reduced values. In the treated females serum T4 concentrations were slightly reduced (approximately 97% to 87% of control) from day 7 onward, without being statistically significantly different to the controls.

Thyroid stimulating hormone (TSH):
TSH levels were statistically significantly increased (168% to 283% of control) in the treated males from day 14 onward. From day 2 through day 7 this finding occurred as a trend toward increased values in the males. With the exception of day 7 statistically significantly increased TSH concentrations (180% to 277% of control) were found in the sera of the treated females throughout the study.

Liver weights:
Liver weights were statistically significantly increased in treated males and females. The liver weight was 25% in males and 22% in females above control.

p-nitrophenol-glucuronyltransferase (pNP-GT):
pNP-GT activities were statistically significantly increased in treated males and females. The increase in pNP-GT activity was almost two-fold in males and 1.25 fold in females.

4-methylumbelliferone-glucuronyltransferase (MUF-GT):
MUF-GT activities were statistically significantly increased in treated males and females. The increase in MUF-GT activity was 2-fold in males and 2.4 fold in females.

4-hydroxybiphenyl-glucuronyltransferase (HOBI-GT):
HOBI-GT activities were statistically significantly increased in treated males and females. The increase in HOBI-GT activity was about 3-fold in males and females.

Conclusion:
Administration of nicobifen to male and female rats at a dose level of 15000 ppm for a period of 4 weeks caused decreases in the circulating T3 and T4 levels and increases in serum TSH concentrations. Increased metabolism of T4 via hepatic enzyme conjugation (as shown by increased activities of p-nitrophenol-glucuronyltransferase, 4-methylumbelliferone-glucuronyltransferase, and 4-hydroxybiphenyl-glucuronyltransferase) appeared to be responsible for the increased TSH.

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Medical surveillance on manufacturing plant personnel

Since industrial production has not yet commenced no data on medical surveillance of the manufacturing personnel is available. The personnel which is handling developmental compounds is surveyed by regular medical examinations. This surveillance programme is not aimed to specifically identify nicobifen-related symptoms or diseases.

B.6.9.2 Direct observation, e.g. clinical cases and poisoning incidents

No clinical cases or poisoning incidents are known to us.

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 known to us.

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; symptomatic and supportive treatment, no specific antidote known.

B.6.9.6 Expected effects of poisoning

Effects of poisoning are not known.

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

Toxicokinetics and metabolism
Following oral administration to rats, radiolabelled nicobifen was rapidly but incompletely absorbed from the gastrointestinal tract, widely distributed and rapidly eliminated from the body. Based on recovery of the radiolabel in bile from bile-duct cannulated rats within 48 h and in urine from non-cannulated rats within 12 h of application, gastrointestinal absorption of an administered low and high dose was estimated to be approx. 44 % and 12 %, respectively. Blood/plasma kinetics revealed initial half-lives of approx. 8 h and terminal half-lives ranging between 20 and 40 h. AUC values of both dose levels indicated a sublinear kinetics. Tissue distribution determined 8 h after administration revealed highest amounts of radioactivity in the GI tract, liver and adipose tissue in low-dose rats. In the high-dose group, a similar distribution was observed in males, while in females, highest concentrations were found in the GI-tract, liver, thyroid and kidney. There was no evidence of a cumulative potential of nicobifen. The administered low dose was completely recovered in excreta within 2 days (approx. 20 % via urine and 80 % via faeces). At the high dose level of 500 mg/kg bw, total excretion within 7 days was in the range of 93–106 % AD), while only 3–5 % AD was eliminated via the urine. There were no significant differences in the excretory pattern with regard to sex, radiolabel used or frequency of application.

The systemically available portion of nicobifen was rapidly and intensively metabolised to a large number of biotransformation products. The hydroxylation of the diphenyl moiety was the quantitatively most important pathway. Second important was the substitution of the Cl in the 2-chloropyridine moiety against SH by conjugation with glutathione. Partial cleavage of the glutathione moiety afforded the cysteine conjugate and finally the SH-compound, which was subsequently methylated or oxidised. In addition, the introduction of glutathione and a second hydroxy group into the diphenyl part of the molecule was observed. Combinations of these reactions and the conjugation of the OH-groups with glucuronic acid or sulphate and the conjugation of the SH-group with glucuronic acid led to the large number of metabolites. The cleavage of the amide bond is negligible because the 2-chloronicotinic acid was detected only in trace amounts. No major differences were observed with regard to label, sex, and dose level.

The absorption, distribution and excretion of radioactivity was studied in male Wistar rats following a single dermal administration of ^{14}C-radiolabelled nicobifen (using concentrations of 10, 100 and 1000 µg/cm²) in the blank commercial formulation BAS 510 01 F. Relative absorption was highest at the low dose level. Taking into account a worst case situation consisting of a working day of 10 h and assuming that the operator will not wash exposed skin for up to 10 hours, the highest values for dermal penetration were obtained at the low concentration level: 8.07 % (10 h exposure/10 h sacrifice) and 6.26 % (10 h exposure/24 h sacrifice), resulting in an average of approx. 7 %. Since there was no evidence for the assumption that the skin compartment serves as a reservoir for further bioavailability after the end of exposure, the amounts of radiolabel remaining in the skin were not taken into account.

In *in vitro* investigations with rat and human epidermal membranes exposed to radiolabelled nicobifen for 24 h, the comparison of total radiolabel recovery in receptor fluid and epidermal membranes did not provide evidence for a notable difference in the extent of bioavailability between species at concentration levels relevant for human exposure settings.

Therefore, it is proposed to use 7 % as estimate for human dermal absorption for risk assessment calculation purposes.

Acute toxicity

Nicobifen is characterised by a very low acute oral (LD_{50} > 5000 mg/kg bw) dermal (LD_{50} > 2000 mg/kg bw) and inhalation (LC_{50} > 6.7 mg/l) toxicity. The substance is neither irritating to the skin nor to the eyes. It is not a skin sensitiser in the Maximisation Test.

Short term toxicity

The short-term toxicity of nicobifen was studied in dietary 3-month studies in rats and mice, and in 3- and 12-month studies in dogs. In addition the short-term toxicity following dermal exposure was determined in a 28-day study in rats. The overall NOAELs and LOAELs from short-term studies are shown in Table B.6.10-1:

Table B.6.10-1: NOAELs and LOAELs obtained in short-term toxicity studies

Study **Dose levels** **Nicobifen purity**	**NOAEL** **males / females** **mg/kg bw/d**	**LOAEL** **males / females** **mg/kg bw/d**
Oral studies		
Rat 90-day 0–100–500–2000–5000–15000 ppm purity: 95.3 %	34 / 40 (500 ppm)	137 / 159 (2000 ppm)
Mouse 90-day 0–150–1000–4000–8000 ppm purity: 95.3 %	29 / 42 (150 ppm)	197 / 277 (1000 ppm)
Dog 90-day 0–250–2500–25000 ppm purity: 94.4 %	7.6 / 8.1 (250 ppm)	78 / 82 (2500 ppm)
Dog 12-month 0–200–800–2000–20000 ppm purity: 94.4 %	22 / 22 (800 ppm)	57 / 58 (2000 ppm)
Dermal studies		
Rat 28-day 0–10–250–1000 mg/kg bw/d purity: 96.3 %	1000	–

Nicobifen has a very low toxic potential as demonstrated by the high dose levels which were administered. For all studies the high dose level was in the range of 1000 mg/kg bw/d. Even at this dose level, clinical signs of toxicity or adverse effects on food consumption or body weight gain were very rarely seen. The signs of toxicity observed in the three species tested were overall similar and consisted mainly of altered clinical-chemical changes. Main target organ was the liver. Weight increases of the liver were observed in all three species. Histopathological changes, however, were minor (hypertrophy and fatty change) and suggest an adaptation of this organ to increased functional demand. In rats and dogs the thyroids were identified as a second target organ as evidenced by weight increases (rats and dogs) and histopathologically by follicular cell hyperplasia (only rats).

In a 28-day dermal toxicity study in rats no substance-related systemic adverse effects were detected up to the highest dose level tested of 1000 mg/kg bw/d. There were no signs of local irritation in this study.

Genotoxicity

The potential genotoxicity of nicobifen was investigated in a series of both *in vitro* and *in vivo* studies. All regular end points for genetic damage (point mutations, chromosome damage and DNA-damage and repair) were assessed: In *in vitro* investigations, nicobifen was evaluated for its potential genotoxicity using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis 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 negative test result of this study corroborated the evidence obtained *in vitro* that nicobifen has no chromosome-damaging potential. In conclusion, there is no evidence from the available *in vitro* and *in vivo* data to assume mutagenic or genotoxic properties of nicobifen.

Long-term toxicity and carcinogenicity

The results of a two-year chronic toxicity study and a two-year carcinogenicity study in rats indicate that a dose of 15000 ppm exceeded the criteria for a maximum tolerated dose. At the

next lower concentration (2500 ppm), however, there were still sufficient signs of toxicity (reduced body weight in females, indications of an anaemic effect in females, clinical chemical changes indicating liver toxicity in both sexes as well as pathological changes to the liver and the thyroid).

In the carcinogenicity study there was a slight increase in the incidence of thyroid follicular cell adenomas at the high dose. A similar increase was not observed in the chronic toxicity study, possibly because the effect is so weak that the number of animals were not high enough to detect it. The concomitant changes to the thyroid (hypertrophy and hyperplasia), however, suggest that the slight increase in the carcinogenicity study is likely to be treatment related.

From a mechanistic point of view the thyroid changes can be linked to an increased metabolism of thyroid hormones (T3 and T4) due to increased phase II (conjugation) hepatic activity. The reduced thyroid hormone levels trigger, by means of a feedback mechanism, the release of increased amounts of TSH, in an attempt to restore homeostatic conditions. Due to continued treatment with nicobifen, the metabolic activity of the liver, however, remains elevated resulting in a continuously increased breakdown of thyroid hormone and continuously increased TSH levels.
Chronic stimulation of the thyroid due to increased TSH levels is well known to results in follicular cell hypertrophy, hyperplasia and ultimately in benign thyroid tumours in rats.

According to various publications the rat is particularly sensitive to this secondary mechanism.

Nicobifen does not need to be classified with respect to its tumourigenic potential in rats of the following reasons:

- Nicobifen is clearly non genotoxic.
- For non genotoxic agents, the mechanism of action must be determined. Nicobifen was shown to enhance the metabolism of thyroid hormones.
- Nicobifen has a very low potency of the tumourigenic effect, as indicated by the marginal increase of (thyroid follicular cell) adenomas in rats. There was no increase in the incidence of carcinomas. There was no tumourigenic response in the thyroid in mice.

In conclusion, the marginal increase of thyroid follicular cell adenomas in rats is not considered to be relevant to man.

A carcinogenicity study in mice was conducted up to a maximum tolerated dose as evidenced by significant body weight depression (8 - 10 % in both sexes) at 8000 ppm. Liver weights were increased at the high dose level, histopathology revealed hypertrophy. Moderate effects on body weights were seen at 2000 ppm (both sexes) and in 400 ppm males. Liver weights were also increased in 2000 ppm females, histopathology revealed hypertrophy. There was no evidence of a carcinogenic effect of nicobifen in mice at any dose level.

The overall combined (males and females) NOAELs and LOAELs obtained in long-term studies are shown in Table B.6.10-2:

Table B.6.10-2: NOAELs and LOAELs obtained in long-term toxicity studies

Study **Dose levels** **Purity**	**NOAEL** **males / females** **mg/kg bw/d**	**LOAEL** **males / females** **mg/kg bw/d**
Rat 24-mo oral diet (combined chronic toxicity and carcinogenicity) 0–100–500–2500–15000 ppm purity: 94.4 %	4.4 / 5.9 (100 ppm)	22 / 30 (500 ppm)
Mouse 18-mo oral diet (carcinogenicity) 0–80–400–2000–8000 ppm purity: 94.4 %	13 / 90 (80 / 400 ppm)	65 / 443 (400 / 2000 ppm)

Reproductive toxicity

The reproduction toxicity of nicobifen was investigated in a two-generation reproduction study as well as in developmental toxicity studies in rats and rabbits. Nicobifen had no adverse effects on reproductive performance or fertility of the F0 or F1 parental animals of all substance-treated groups up to a dose of 10000 ppm (1165 mg/kg bw/d). Signs of general toxicity/systemic effects occurred in both parental generations at 1000 and 10000 ppm. The effects at 10000 ppm were characterised by decreased food consumption and reduced body weights during parts of the administration period. Pathology showed statistically significantly increased liver weights, centrilobular hypertrophy of liver cells and centrilobular liver cell degeneration in single or all male and/or female animals. Systemic effects at 1000 ppm were confined to an increased incidence of centrilobular hepatocellular hypertrophy, which occurred in few F0 and F1 parental animals. No substance-related effects were noted at 100 ppm. Substance-induced signs of developmental toxicity were observed in progeny of the F0 and F1 parents at 1000 and 10000 ppm. At 10000 ppm a slightly increased pup mortality of the F2 litters was noted between days 0 and 4 post partum only. Pup body weight development was impaired in both F1 and F2 litters. At 1000 ppm, slightly decreased body weight gains were recorded for the male F2 pups only. 100 ppm did not induce any indication of developmental toxicity. The NOAEL for parental toxicity of the test substance was established at 100 ppm (11 mg/kg bw/d) for the F0 and F1 parental males and females. The NOAEL for developmental toxicity was 1000 ppm (113 mg/kg bw/d) for the male and female F1 and female F2 progeny and 100 ppm (11 mg/kg bw/d) for the male F2 progeny.

In the developmental toxicity study in rats, incomplete ossification of the thoracic centrum was observed at the highest dose tested (1000 mg/kg bw/d) in the absence of overt maternal toxicity. At this limit dose level there were also no signs of maternal toxicity. However, results from the 90-day oral feed study in rats indicate that liver toxicity would have been detected in dams at 1000 mg/kg bw/d.

In the rabbit developmental toxicity study, incomplete ossification of the thoracic centrum was also observed at significantly increased incidences at the highest dose level (1000 mg/kg bw/d). At this dose level there was overt maternal toxicity (clinical signs of toxicity, reduced body weight and body weight gain). At 300 mg/kg bw/d clinical signs (abortion and discoloured/reduced faeces) were observed in a single animal only. Thus, the NOAELs for maternal and for developmental toxicity were 100 mg/kg bw/d and 300 mg/kg bw/d, respectively.

Table B.6.10-3: NOAELs and LOAELs obtained in reproductive toxicity studies

Study dose levels purity	Target	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d
Rat 2-generation study 0–100–1000–10000 ppm purity: 94.4 %	Parental toxicity	11 (100 ppm)	113 (1000 ppm)
	Fertility	1165 (10000 ppm)	–
	Offspring toxicity	11 (100 ppm)	113 (1000 ppm)
Rat teratogenicity 0–100–300–1000 mg/kg bw/d purity: 94.4 %	Maternal toxicity	1000	–
	Developmental toxicity	300	1000
Rabbit teratogenicity 0–100–300–1000 mg/kg bw/d purity: 94.4 %	Maternal toxicity	100	300
	Developmental toxicity	300	1000

Neurotoxicity

Three oral neurotoxicity studies with nicobifen were conducted in rats. In the acute neurotoxicity study, piloerection observed on the day of treatment in 2 of 20 rats was the only clinical sign of toxicity to be seen at the top dose level (2000 mg/kg bw). This finding was considered to reflect an unspecific reaction of the animals to excessive dosing and was therefore not regarded as a substance-related effect. No adverse reaction to treatment was observed in any animals at 1000 mg/kg bw or lower dose levels. No signs of neurotoxicity were observed at any dose level.

In the 90-day oral neurotoxicity study in rats, there were no test substance related adverse effects at any dose level and there were no signs of neurotoxicity at any dose level. The no observed effect level was 15000 ppm, i.e. 1050 mg/kg bw/d in males and 1272 mg/kg bw/d in females.

In a developmental neurotoxicity study, the slight reduction on pup body weight (at 10000 and 1000 ppm) were in line with similar effects seen in the 2-generation study in rats, where parental toxicity was noted in form of hepatotoxicity at these dose levels. No signs of developmental neurotoxicity were noted up to the highest concentration of 10000 ppm (1442 mg/kg bw/d), which was clearly above a recommended limit dose of 1000 mg/kg bw/d.

The NOAELs and LOAELs obtained from the neurotoxicity studies are summarised in Table B.6.10-4. In conclusion, nicobifen is neither neurotoxic to adult rats nor to the developing rat. As there were no neurotoxic effects observed in any of the studies with nicobifen, studies on the delayed neurotoxicity in hens were not triggered.

Table B.6.10-4: NOAELs and LOAELs obtained in neurotoxicity studies

Study dose levels purity	Target	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d
Rat acute oral neurotoxicity 0–500–1000–2000 mg/kg bw purity: 96.3 %	Neurotoxicity	2000	–
	General toxicity	1000	2000
Rat 90-day oral neurotoxicity 0–150–1500–15000 ppm purity: 96.3 %	Neurotoxicity	1050 / 1273 (15000 ppm)	–
	General toxicity	1050 / 1273 (15000 ppm)	–
Rat developmental neurotoxicity 0–100–1000–10000 ppm purity: 96.3 %	Maternal toxicity	1442 (10000 ppm)	–
	General toxicity in offspring	14 (100 ppm)	147 (1000 ppm)
	Neurotoxicity in offspring	1442 (10000 ppm)	–

Further toxicological studies

Para-chlorobenzoic acid was identified in the aquatic environment as degradation product of nicobifen. Results from a literature survey indicate that para-chlorobenzoic acid is more toxic than nicobifen after acute oral intake (LD_{50}: 4170 mg/kg bw vs. > 5000 mg/kg bw, respectively). No adverse effects were reported in oral subchronic toxicity studies in rabbits (NOAEL: 1500 mg/rabbit/d) and rats (NOAELs: 26 mg/rat/d and 0.3 mg/kg bw/d). Limited *in-vitro* genotoxicity data indicate no concern. The feeding of 13 or 26 mg/rat/d over a period of five months did not induce adverse effects on the number of offspring and did not induce developmental toxicity. In view of the transient nature of this compound no further need for a toxicological investigation was identified.

Mechanistic studies with nicobifen have shown that the test substance induces a liver weight increase with concomitant histopathological changes in zone 3 hepatocytes (proliferation/accumulation of smooth endoplasmatic reticulum (SER) and glycogen depletion). These changes are considered to be related to the induction of hepatic metabolising enzymes. Nicobifen induced both phase I (oxidative) enzymes, as demonstrated by increased cytochrome P450 content, as well as phase II (conjugation) enzymes. The latter was shown by increased activities of p-nitrophenol-glucuronyltransferase, 4-methylumbelliferone-glucuronyltransferase, and 4-hydroxybiphenyl-glucuronyltransferase. The increases in phase II (conjugation) hepatic activity were accompanied by the reduction of thyroid hormone T3 and T4 levels and by a concomitant increase in TSH. While the decrease in serum T3 and T4 concentrations appear to have resulted from increased elimination via nicobifen-mediated induction of phase II hepatic enzyme activities, the increased TSH levels were obviously the result of a feedback mechanism induced by decreased in serum T3 and T4 levels.

Human toxicological data

Since industrial production has not yet commenced no data on medical surveillance of the manufacturing personnel is available. However, the personnel which is handling developmental compounds is surveyed by regular medical examinations.

B.6.10.2 Risk assessment for operator, worker and bystanders

Nicobifen is of low acute oral, dermal and inhalation toxicity. If the rules of good agricultural practice are followed, the risk of an acute intoxication by nicobifen can be ruled out.

Acceptable Operator Exposure Level (AOEL):
For the definition of the AOEL and the risk assessment to be made thereof, the results of the short-term toxicity and reproduction/developmental toxicity studies are considered to be of relevance. The overall combined short term NOAELs are shown in Table B.6.10-5.

Table B.6.10-5: Overall combined short-term NOAELs and LOAELs

Study	NOAEL mg/kg bw/d		LOAEL mg/kg bw/d	
Oral studies				
Rat 90-day	34	(500 ppm)	137	(2000 ppm)
Rat 2-gen. study, parental toxicity	11	(100 ppm)	113	(1000 ppm)
Rat teratogenicity	300		1000	
Mouse 90-day	29	(150 ppm)	197	(1000 ppm)
Dog 90-day	7.6	(250 ppm)	78	(2500 ppm)
Dog 1-year	22	(800 ppm)	57	(2000 ppm)
Rabbit maternal tox., teratogenicity	100		300	
Dermal				
Rat 28-day	1000		–	

From the above studies, those with the overall highest NOAELs were determined for each species and subsequently compared. By this approach, the 90-day studies in rats and mice, and the 1-year dog study were identified to be the most relevant. The corresponding NOAELs were in the same range (22–34 mg/kg bw/d), the dog appearing to be the most sensitive species with both the lowest NOAEL and LOAEL. However, it can not be excluded that the "sensitivity" of the dog was only a consequence of the choice of doses used in the different studies. Nevertheless, for setting the AOEL the most relevant study was considered to be the dog 1-year oral feed study (NOAEL of 22 mg/kg bw/d, corresponding to 800 ppm).

The systemic AOEL ($AOEL_{(SYS)}$) is derived from the NOAEL of the dog 1-year oral feed study by applying a standard safety factor of 100. Since gastrointestinal absorption was estimated to be approx. 44 % based on toxicokinetic investigations with rats, an additional correction factor was employed for calculation of the systemic AOEL:

$$\mathbf{AOEL_{(SYS)}} = \frac{22\ \text{mg/kg bw/d}}{100} \times 44\ \% = \mathbf{0.10\ mg/kg\ bw/d}$$

In addition, a 28 -day dermal toxicity study in rats was employed in the assessment. No systemic toxicity was detected up to the highest dose level tested of 1000 mg/kg bw. A 90-day dermal study, which could be compared with the oral 90-day study, was not submitted. A dermal AOEL was therefore not established.

The risk assessment for the operator, worker and bystander is based on the specific exposure scenarios and calculations for the plant protection products containing nicobifen. These calculations can be found in section B.6.14.

B.6.10.3 Risk assessment for consumers

For the long-term risk assessment determination for consumers (ADI calculation), long-term toxicity studies and developmental/reproduction toxicity studies are considered the most relevant. For the calculation of an acute reference dose the result from acute or short-term feeding studies should be considered.

Acute reference dose (ARfD)
From the evaluation of the available toxicological database of nicobifen, there is no need to establish an ARfD. Acute oral studies demonstrate the low toxicity of nicobifen. No adverse clinical signs were observed early in repeated-dose studies at dose levels that were relevant for human exposure. No developmental toxicity was induced by nicobifen treatment. Slight alterations of thyroid hormone levels and induction of liver enzymes were observed within several days after repeated administration of 15000 ppm (approx. 1000 mg/kg bw/d). However, these effects are not considered relevant since the expected human exposure is lower by several orders of magnitude. In conclusion, due to low toxicity of nicobifen, it is not necessary to derive an ARfD.

Acceptable daily intake (ADI)
Chronic feeding studies with nicobifen in dogs, rats and mice demonstrated that liver and the thyroid are the target organs. The long-term toxicity is rather similar to the short-term effects. The NOAELs obtained in long-term studies are shown in Table B.6.10-6:

Table B.6.10-6: Overall combined long-term NOAELs

Study	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d
Dog 12-month oral	22 (800 ppm)	57 (2000 ppm)
Rat 24- month oral	4.4 (100 ppm)	22 (500 ppm))
Mouse 18-month oral	13 (80 ppm)	65 (400 ppm)

Thus, the lowest NOAEL in the long-term studies was approximately 4 mg/kg bw/d in the chronic rat study. The slight increase in the incidence of thyroid follicular adenomas at a dose of 2500 ppm in the carcinogenicity study in rats is considered to be a rat specific phenomenon and is not relevant for humans. Nicobifen was not carcinogenic in mice. Nicobifen has been investigated in a series of studies both in vitro and in vivo designed to measure the major endpoints of genotoxicity. The results of these studies clearly demonstrate that nicobifen has no genotoxic potential. Nicobifen has no reproduction toxicity potential, and the NOAELs obtained were higher than the 4 mg/kg bw from the long-term rat study. A full and current toxicology database evaluating all major endpoints of toxicity has been developed for nicobifen, and clear no effect levels have been determined for all treatment-related effects. Therefore, the standard assessment factor of 100 is considered appropriate.

The proposed ADI is:

$$\mathbf{ADI} = \frac{4\ \mathrm{mg/kg\ bw}}{100} = \mathbf{0.04\ mg/kg\ bw}$$

B.6.11 Acute toxicity including irritancy and skin sensitization of preparations (Annex IIIA 7.1)

BAS 510 01 F is formulated as a waterdispersible granule (WG, syn. DF) containing 50 % nicobifen.
BAS 510 01 F has low acute toxicity after oral, dermal and inhalative exposure. The formulation is neither irritating to the skin nor to the eyes, nor is there evidence of a skin sensitising effect as shown in a modified Buehler test (Table B.6.11-1).

Table B.6.11-1: Acute toxicity of BAS 510 01 F

Study type	Results
Rat acute oral toxicity	LD_{50}: >2000 mg/kg bw
Rat acute dermal toxicity	LD_{50}:> 2000 mg/kg bw
Rat acute inhalation toxicity	LC_{50}:> 5.2 mg/l
Rabbit skin irritation	Not irritating
Rabbit eye irritation	Not irritating
Guinea pig skin sensitisation (modified Buehler test: 9 inductions)	Not sensitising

Classification:
With respect to the prevailing EU classification schemes, a classification of BAS 510 01 F is not required.

B.6.11.1 Oral

Report: Gamer A. O., Hoffmann H. D., 2001(a)
BAS 510 01 F - Acute oral toxicity study in Wistar rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000119; Feb. 09, 2001; unpublished
(Experimental work: May / June, 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 423, EPA/OPPTS 870.1100

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: BAS 510 01 F; Batch/purity: 2000-1;
formulation: 51.3 % BAS 510 F = Reg. No. 300 355.
Test animals: Male and female rats (Rat / Wistar (SPF) / Crl:WI (GLX/BRL/HAN)IGS BR)

The test substance preparation was administered as a dispersion in *aqua bidest.* by single gavage to three male and three female fasted Wistar rats at a dose level of 2000 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 the study is shown in Table B.6.11-2.

Table B.6.11-2: Cumulative mortality

Effects at 2000 mg/kg bw	Males	Females
No. of animals tested	3	3
Mortality at 1 h	0	0
2 h	0	0
Day 2 – 15	0	0
Cumulative mortality	0 / 3	0 / 3

Signs of toxicity noted in the male animals comprised impaired and poor general state, dyspnoea, apathy, staggering, easily frightened state and piloerection. The symptoms were observed until including Study Day 1.

Signs of toxicity noted in the female animals comprised poor general state, dyspnoea, apathy, staggering, twitching and piloerection. The symptoms were observed until including 5 hours after test substance application.

The mean body weights of the test groups increased throughout the study.

No abnormalities were noted at necroscopy of animals sacrificed at the end of the study.

Conclusion:
The oral LD_{50} of BAS 510 01 F was found to be > 2000 mg/kg bw for male and female rats.

B.6.11.2 Percutaneous

Report: Gamer A. O., Hoffmann H. D., 2001(b)
BAS 510 01 F - Acute dermal toxicity study in rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000120; Feb. 08, 2001; unpublished
(Experimental work: July / Aug., 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, OECD 402, EPA/OPPTS 870.1200

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: BAS 510 01 F; Batch/purity: 2000-1;
formulation: 51.3 % BAS 510 F = Reg. No. 300 355.

Test animals: Male and female rats (Rat / Wistar (SPF) / Crl:WI (GLX/BRL/HAN)IGS BR)

The test material was applied as a preparation in 0.5 % carboxymethylcellulose in *aqua bidest.* dermally to five male and five female Wistar rats for 24 hours under semi-occlusive dressing at a dose level of 2000 mg/kg bw. The application area was about 40 cm² (corresponds to at least 10 % of the body surface area). The animals were observed for 14 days.

Findings:
The test substance formulation was demonstrated to be stable. The homogeneity was analytically confirmed.

No mortality occurred during the 14 days post application observation period. The female animals did not gain body weight throughout the study period. The mean body weights of all animals increased throughout the study period. Clinical signs of systemic toxicity were not observed. No local effects were observed in male animals. In two female animals very slight or well defined erythema and eczematoid skin changes were observed on Study Day 7. No pathological findings were detected in the animals.

Conclusion:
The dermal LD_{50} of BAS 510 01 F was found to be > 2000 mg/kg bw for male and female rats.

B.6.11.3 Inhalation

Report: Gamer A. O., Hoffman H. D., 2000
BAS 510 01 F - Acute inhalation toxicity study in Wistar rats – 4 hour dust exposure
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000121; Dec. 08, 2000; unpublished
(Experimental work: May 29 to June 13, 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, EEC 93/21, OECD 403, EPA/OPPTS 870.1300

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: BAS 510 01 F; Batch/purity: 2000-1;
formulation: 51.3 % BAS 510 F = Reg. No. 300 355.

Test animals: Male and female rats (Rat / Wistar (SPF) / Crl:WI (GLX/BRL/HAN)IGS BR)

Five male and five female Wistar rats were exposed to a dust aerosol of the test material for four hours in a head/nose inhalation system at a mean analytical concentration of 5.2 mg/l. The observation time was 14 days.

Findings:
The test substance is a formulation which was homogeneous and stable over the test period. The homogeneous distribution of atmospheres in the inhalation system has been proven in technical tests with model dust aerosols.

Cascade impactor measurements resulted in a particle size distribution with mass median aerodynamic diameters (MMAD) of 4.5 µm, which was at the upper end of the respirable range.

No mortality occurred in the study. Clinical signs of toxicity comprised visually accelerated respiration, piloerection and smeared fur. Clinical signs were observed until and including the day after exposure. Body weight development of the animals exposed was not influenced. In the animals examined at the end of the post exposure observation period, no gross pathological abnormalities were detected.

Conclusion:
The inhalation LC_{50} of BAS 510 01 F was found to be > 5.2 mg/l (4h) for male and female rats.

B.6.11.4 Skin irritation

Report: Wiemann C., Hellwig J., 2001(a)
BAS 510 01 F - Acute dermal irritation/corrosion in rabbits
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000122; Jan. 29, 2001; unpublished
(Experimental work: May, 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, OECD 404, EPA/OPPTS 870.2500

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: BAS 510 01 F; Batch/purity: 2000-1;
formulation: 51.3 % BAS 510 F = Reg. No. 300 355.
Test animals: Female rabbits (White New Zealand HsdIf: NZW; SPF)

The test substance was applied as a single topical dose of 0.5 g to the intact skin of 3 female White New Zealand rabbits for 4 hours on a 6.25 cm2 test patch under semi-occlusive dressing. After the exposure period the test substance was removed with Lutrol® and Lutrol®/water (1:1). The observation period was 72 hours after test material application. Skin readings were performed about 1 h, 24 h, 48 h and 72 h after removal of the patch.

Findings:
The stability of the test substance was guaranteed for the duration of the study. The homogeneity of the test substance was confirmed by analysis.

Skin findings are summarised in Table B.6.11-3.

Table B.6.11-3: Skin irritation values (erythema/oedema)

Animal number	Time after patch removal				
	1 h	24 h	48 h	72 h	mean
1	1 / 0	0 / 0	0 / 0	0 / 0	0 / 0
2	1 / 0	0 / 0	0 / 0	0 / 0	0 / 0
3	1 / 0	0 / 0	0 / 0	0 / 0	0 / 0

The average score (24 to 72 hours) for irritation was calculated to be 0 for both erythema and for oedema. Under the test conditions chosen and considering the findings the test substance gives no indication of an irritant property to the skin.

Conclusion:
BAS 510 01 F was found to be not irritant to the skin.

B.6.11.5 Eye irritation

Report: Wiemann C., Hellwig J., 2001(b)
BAS 510 01 F - Acute eye irritation in rabbits
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000123; Jan. 29, 2001; unpublished
(Experimental work: June, 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, OECD 405, EPA/OPPTS 870.2400

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: BAS 510 01 F; Batch/purity: 2000-1;
formulation: 51.3 % BAS 510 F = Reg. No. 300 355.
Test animals: Female and male rabbits (White New Zealand HsdIf: NZW; SPF)

The test substance was applied in a single dose to the conjunctival sac of two female and one male New Zealand White rabbits. The application volume was about 0.1 ml. The test substance was washed out with tap water about 24 hours after the application. Readings were carried out at 1 hour and 1, 2 and 3 days after application of the test substance on all animals.

Findings:
The test substance is an unchanged commercial formulation which was demonstrated to be stable for the duration of the study. The homogeneity was analytically confirmed.

The mean values (readings of 24 h, 48 h and 72 h) for all animals are given in Table B.6.11-4).

Table B.6.11-4: Eye irritation; mean readings and symptoms

Animal No.	Opacity	Iris	Conjunctiva	
			Redness	Swelling
1	0.0	0.0	0.3	0.0
2	0.0	0.0	1.0	0.3
3	0.0	0.0	0.7	0.0
Mean	**0.0**	**0.0**	**0.7**	**0.1**

The findings were reversible within 72 hours. Under the test conditions chosen the test substance does not give indication of an irritant property to the eye.

Conclusion:
Only very slight effects were seen on the conjunctivae. According to the EC criteria, BAS 510 01 F is not irritant to the eye.

B.6.11.6 Skin sensitisation

Report: Wiemann C., Hellwig J., 2001(c)
BAS 510 01 F – Modified Buehler test (9 inductions) in guinea pigs
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000124; Jan. 29, 2001; unpublished
(Experimental work: pretest - May, 2000; main test - Nov. / Dec., 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 406, EPA/OPPTS 870.2600; JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: BAS 510 01 F; Batch/purity: 2000-1;
formulation: 51.3 % BAS 510 F = Reg. No. 300 355.

Test animals: Guinea pigs (Hsd Poc:DH; SPF)

BAS 510 01 F was tested for its sensitising effect on the skin of the guinea pig in the Modified Buehler Test with nine inductions. The study consists of a pre-test and a main test. The concentrations for the main experiment were determined in the pre-test.
The main study was performed in 20 guinea pigs in the test group and 10 animals in each of the control groups 1 and 2. All inductions were performed with 50 % test substance preparation in *aqua bidest.* (9 inductions: three applications per week on the same application area). For the induction, 2 x 2 cm gauze patches containing 0.5 ml of the test substance preparation were applied to the skin of the flank under occlusive dressing. 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 hours after the beginning of application.

A challenge was performed 13 days after the last induction with a 25 % test substance preparation. A volume of 0.5 ml of the test substance formulation was applied to each animal. The test group and control group 1 were treated with the test substance (control group 2 remained untreated). The duration of exposure was 6 hours, the test substance was applied on the posterior right flank. Readings were performed at 24 and 48 hours after removal of the patch.

A positive control (reliability check) with a known sensitiser was not included in this study. However, a separate study is performed twice a year in the laboratory. The positive controls with α-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 diluted commercial formulation. The stability was guaranteed for the duration of the study, the homogeneity was confirmed by analysis. The stability of the test substance in water over a time period of 96 hours was confirmed by analysis.

After the first until eighth induction discrete or patchy erythema could be observed in 2–12 animals of the test group. After the ninth induction discrete or patchy erythema was observed in 8 out of 20 test group animals, in one animal additionally swelling. Moderate and confluent erythema was noticed in two test animals.

The number of animals with skin findings after the challenge is summarised in Table B.6.11-5.

Table B.6.11-5: Skin findings after challenge

	Challenge (x/y: number of animals with skin finding/number of animals tested)
Control group 1	0 / 10
Test group	0 / 20

Based on the results of this study it is concluded that BAS 510 01 F does not have a sensitising effect on the skin of the guinea pig in the Modified Buehler Test.

Conclusion:
BAS 510 01 F does not have a sensitising potential to the skin of the guinea pig in the Modified BUEHLER Test (9 inductions).

B.6.12 Dermal absorption (Annex IIIA 7.3)

For estimation of in-vivo human dermal absorption of nicobifen contained in the commercial formulation BAS 510 01 F, an in-vivo dermal absorption study in rats and an in-vitro comparison of the penetration of radiolabelled nicobifen through rat and human epidermal membranes was performed using different test concentrations.

On the basis of worst-case assumptions, an overall dermal absorption value of 7 % was derived from the results of the rat in-vivo study. In in-vitro investigations with rat and human epidermal membranes exposed to radiolabelled nicobifen for 24 h, the comparison of total radiolabel recovery in receptor fluid and epidermal membranes did not provide evidence for a notable difference in the extent of bioavailability between species at concentration levels relevant for human exposure settings.

In conclusion, in-vivo human dermal absorption is estimated to be approx. 7 %. It is proposed to use this dermal absorption estimate for risk assessment calculation purposes.

B.6.12.1 In-vivo dermal absorption study

Report: Leibold E., Hoffmann H. D., 2001 (TOX2001-704)
^{14}C-Nicobifen - Study of the dermal absorption in rats
BASF AG, Ludwigshafen/Rhein, Germany
BASF RegDoc# 2001/1000111, unpublished
(Experimental work from 2 May – 20 December 2000)

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 416; EEC 87/302, EPA/OPPTS 870.7600

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Radiolabelled nicobifen (diphenyl-U-^{14}C); batch 641-2017; radiochemical purity: > 95 %. Non radiolabelled nicobifen; batch 01183-190; purity: > 99 %

Test animals: Male Wistar rats [CrI:WI (GLXIBRL/HAN) IGS BR]; age during application: 14–17 wk
Source: Charles River Laboratories, Sulzfeld, Germany

The absorption, distribution and excretion of radioactivity was studied in male Wistar rats following a single dermal administration of ^{14}C-nicobifen mixed with the blank commercial formulation (BAS 510 01 F) and taken up in water at nominal dose levels of 0.01, 0.10 and 1.00 mg/cm2 corresponding to 0.1, 1.0 and 10 mg/animal or about 0.4, 4 and 40 mg/kg body weight. Groups of four animals were exposed according the following regimen (see Table B.6.12-1):

Table B.6.12-1: Rat in vivo: Experimental design

Duration of exposure and sacrifice times						
Duration of exposure (h)	1	4	10	24	10*	10
Sacrifice after (h)	1	4	10	24	24*	72

*only at the low dose level

Twenty-four hours prior to dosing the back shoulders of the rats were clipped free of hair and the area (about 10 cm²) was washed with acetone. A silicone ring was glued to the skin, the test substance preparation (about 10 µl/cm²) was 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.

The animals were dosed and then placed in metabolism cages in order to collect excreta up to 72 hours. For each dose group four animals were used. After the respective exposure period the protective cover was removed and the exposed skin was 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 was analytically verified. The recovery of the administered radiolabel in different compartments is summarised in Table B.6.12-2, Table B.6.12-3 and Table B.6.12-4.

Table B.6.12-2: Rat in-vivo dermal absorption: % radioactivity absorbed, low dose

Dose: 0.01 mg/cm²		% of applied dose at time point					
	Exposure	1 h	4 h	10 h	10 h	10 h	24 h
	Sacrifice	1 h	4 h	10 h	24 h	72 h	24 h
Sample							
Urine		0.00	0.06	0.84	0.96	1.66	1.10
Faeces		0.00	0.00	0.25	1.19	2.98	2.80
Excreted (urine, faeces, cage wash)		0.01	0.07	1.16	2.20	4.67	4.01
Carcass, kidney, liver, blood		0.51	1.95	6.91	4.06	1.05	6.92
Total Absorbed		**0.52**	**2.02**	**8.07**	**6.26**	**5.72**	**10.93**
Skin at/surrounding application site		3.39	3.86	4.34	1.18	0.62	4.63
Potentially absorbed		**3.91**	**5.88**	**12.41**	**7.44**	**6.34**	**15.56**
Protective cover and skin washes		101.52	99.44	100.07	92.92	97.37	93.47
Total Recovery		**105.42**	**105.33**	**112.49**	**109.04**	**100.36**	**113.98**

Table B.6.12-3: Rat in-vivo dermal absorption: % radioactivity absorbed, mid dose

Dose: 0.1 mg/cm²		% of applied dose at time point					
	Exposure	1 h	4 h	10 h	10 h	10 h	24 h
	Sacrifice	1 h	4 h	10 h	24 h	72 h	24 h
Sample							
Urine		0.02	0.01	0.05	–	0.49	0.33
Faeces		0.00	0.00	0.01	–	1.07	0.30
Excreted (urine, faeces, cage wash)		0.03	0.02	0.06	–	1.61	0.74
Carcass, kidney, liver, blood		0.34	0.24	0.57	–	0.48	1.92
Total Absorbed		**0.37**	**0.26**	**0.63**	**–**	**2.07**	**2.63**
Skin at/surrounding application site		2.03	1.87	3.06	–	5.14	1.07
Potentially absorbed		**2.40**	**2.13**	**3.69**	**–**	**7.21**	**3.70**
Protective cover and skin washes		106.72	107.39	113.04		91.82	93.32
Total Recovery		**109.11**	**109.50**	**116.74**	**–**	**96.90**	**100.99**

Table B.6.12-4: Rat in-vivo dermal absorption: % radioactivity absorbed, high dose

Dose: 1.00 mg/cm²		% of applied dose at time point					
	Exposure	1 h	4 h	10 h	10 h	10 h	24 h
	Sacrifice	1 h	4 h	10 h	24 h	72 h	24 h
Sample							
Urine		0.00	0.00	0.02	–	0.25	0.04
Faeces		0.00	0.02	0.01	–	0.79	0.03
Excreted (urine, faeces, cage wash)		0.00	0.02	0.05	–	1.10	0.08
Carcass, kidney, liver, blood		0.17	0.31	0.37	–	0.38	0.33
Total Absorbed		**0.17**	**0.33**	**0.42**	**–**	**1.48**	**0.41**
Skin at/surrounding application site		3.92	10.37	2.55	–	0.48	3.67
Potentially absorbed		**4.09**	**10.70**	**2.97**	**–**	**1.96**	**4.08**
Protective cover and skin washes		97.11	89.33	113.05	–	93.58	94.09
Total Recovery		**101.20**	**100.03**	**116.02**	**–**	**95.54**	**98.04**

Mean recoveries of radioactivity were in the range of 95.5–116.7 % AD independent of dose level. The largest proportion of radioactivity was recovered from the dressing and skin wash. In general, the total amount of radioactivity absorbed (calculated from recoveries in excreta, cage wash, tissues/organs and carcass) increased with increasing exposure duration, whilst a similar correlation to the time point of sacrifice was evident only for the mid- and high-dose

groups. The relative amount of radioactivity absorbed increased with decreasing dose levels, indicating saturation of absorption at the higher dose levels.

By 72 h after onset of exposure, the amount of absorbed radioactivity eliminated via the faeces was slightly higher than by the urinary route.

In the low dose group (0.01 mg/cm²), the highest level of bioavailability for a 10-hour exposure period was 8.07 % AD, which was obtained in animals killed immediately at the end of exposure. Although not significantly lower than the initial bioavailability estimate, the corresponding 24-h and 72-h values (6.26 % AD and 5.72 % AD, respectively) were suggestive of a time-dependent decrease. Recoveries in skin at and surrounding the application also showed a time-dependent decrease. As a result, the amount of radiolabel potentially available for absorption was not constant but decreased from 12.41 % AD to 6.34 % AD (overall mean: 8.7 % AD).

The maximum amount of bioavailable radiolabel determined for the mid-dose group (0.1 mg/cm²) was approx. 2 % AD, obtained 72 h after onset of the 10-h exposure; the corresponding mean amount of potentially absorbed nicobifen was 7.21 % AD.

In the high dose group (1.00 mg/cm²), the highest estimate for actual bioavailability following 10-h exposure was established at 72 h post dosing (1.48 % AD). Taking into account the amount retained in and surrounding the application site skin, the amount of radiolabel potentially available for absorption was 2–3 %. The value 10.7 % AD established in skin 4 h after dosing is considered to be an outlier.

Discussion:
Taking into account a worst case situation, consisting of a working day of 10 hours and assuming that the operator will not wash exposed skin for up to 10 hours, the corresponding rat in-vivo data can be used as basis for estimation of the human dermal absorption. Based on the assumption that virtually all of the dermal exposure occurs on the hands and forearms (*ca*. 1600 cm²), preliminary human exposure estimations indicate that the low and intermediate concentration levels tested in the in-vitro study would realistically fall in the range of operator exposure settings. Subsequent to a 10-h dermal exposure period, the highest percentages of administered radiolabelled nicobifen entering the systemic circulation were obtained at the low-dose level, amounting to 8.07, 6.26, and 5.72 % AD for the 10, 24 and 72-h termination time points, respectively. At the low-dose level, no further absorption occurred after washing performed at the end of the 10-h exposure period; a time-dependent increase in bioavailability was not observed. For this reason it is considered justified to use an average value of approx. 7 % for estimation of rat in-vivo dermal absorption. At least at the low-dose level (where the highest extent of absorption was observed), the assumption that the skin compartment serves as a reservoir for further absorption is not supported by the available data, because the time-dependent reduction of radiolabel observed in the skin compartment did not result in increased bioavailability. Even so, the proposed dermal absorption estimate of 7 % is similar to the corresponding overall mean estimate for potentially bioavailable nicobifen (8.7 % AD).

Conclusion:
The in vivo dermal absorption of nicobifen in rats is approximately 7 % or less depending on the duration of exposure and concentration. It is proposed to use this estimate for extrapolation to human dermal absorption of nicobifen.

B.6.12.2 In-vitro dermal penetration study

Report: Thornley K. and Bryson S., 2001 (TOX2001-705)
^{14}C-nicobifen - Rates of penetration through rat and human skin using an in vitro system
Covance Laboratories (formerly Corning Hazleton), Harrogate, North Yorkshire HG3 1PY, UK
BASF RegDoc# 2001/1000112, unpublished
(Experimental work from 22 September – 15 November 2000)

GLP: Yes
(laboratory certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Guideline: Principles of draft OECD guideline on in vitro dermal penetration

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material: Radiolabelled nicobifen; Batch: 641-2017, radiochem. purity: >99 %.
Non radiolabelled nicobifen; batch 01183-190; purity: 99.3 %

Test system: *in vitro* epidermal membranes prepared from:
a) Female Wistar (Crl: CD WI) rats aged 3–5 wk;
b) Human Caucasian skin from dorsal region of cadavers obtained from Pennsylvania Regional Tissue Bank (USA). Donors had not received medical treatment that could have compromised the validity of the study. Tissue was transported in the presence of solid CO_2, and stored at less than -10 °C.

The in vitro dermal absorption of (^{14}C)-nicobifen, from a commercial formulation (BAS 510 01 F) was determined at three dose levels (10, 100 and 1000 µg active ingredient/cm²), through rat and human epidermal membranes.

Preparation of rat skin samples: The epidermis from female Wistar rats was isolated from intact dorso-lumbar skin samples via separation of epidermis and dermis (acantholysis in sodium bromide solution) followed by washing and subsequent incubation in de-ionised water at 1–10 °C for up to 24 hours. Thereafter, rat epidermis was mounted in the diffusion cell and assessed for membrane integrity.

Preparation of human skin samples: Intact Dorsal skin samples were excised from human Caucasian cadavers or plastic surgery patients. The donors had not received medical treatment that could have compromised the integrity of the study. No further information regarding tissue quality (time elapsed between surgery and start of experiment, storage conditions, etc.) was provided. Excess subcutaneous fat was removed. The excised skin was blistered by placing in hot water (60 ± 2 °C) for ca. 1 min to separate dermis and epidermis. The isolated epidermis was incubated in de-ionised water at 1–10 °C for up to 24 hours. Thereafter, the isolated human epidermis was mounted in the diffusion cell and assessed for membrane integrity.

Determination of membrane integrity: On the day prior to ^{14}C-nicobifen/BAS 501 01F application, tritiated water was applied to the epidermal surface of the skin and penetration was measured.

Epidermal membranes were only used for calculation of dermal penetration if the following criteria were fulfilled:
a) visual inspection shows no sign of physical damage to the epidermal membranes during the experiment
b) Permeability constants for tritiated water below a threshold of 5 and 2 µl $^{3}H_2O$/cm²/(2 h) for rat and human epidermal membranes, respectively. (Epidermal membranes were not rejected on the basis of membrane integrity checks alone)
c) Radioactivity recovery in the range of 85–115 % AD
d) Time-dependent absorption profile for ^{14}C-nicobifen established for epidermal membranes

Test procedure: At termination of the membrane integrity check an prior to dose application, the receptor chamber, containing a magnetic stirrer bar, was filled 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 diffusion cells were placed on a magnetic stirrer in a water bath maintained at 32 ± 2 °C throughout the exposure period. The dose formulation was applied to the upper surface of the epidermal membranes using a positive displacement pipette. The epidermal membranes were left unoccluded throughout the 24-h exposure period. Duplicate aliquots (0.1 ml) of receptor fluid were taken at 0 (i.e., pre-dose time point), 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 (approx. 10 % v/v) containing no organic solvent and rinsed with de-ionised 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 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 establish the overall mass balance of radioactivity. The percentage of the applied dose in each sample and the rate of penetration (µg eq./cm²/h) was determined.

Following review of data generated for the high dose groups it was clear that there was little if any lag phase associated with the absorption of ^{14}C-nicobifen. To obtain more precise information on the initial rate of absorption, an additional sample was taken at the 0.5 h time point during the intermediate and low dose group experiments.

Findings:
The stability, homogeneity and correctness of the test substance preparation was analytically verified. An overall material balance of 100.45 % was obtained for the rat epidermal membranes, the one for human epidermal membranes being 97.44 % at study termination (24 h). The table below summarises the key mean absorption parameters of nicobifen formulated in the BAS 510 01 F vehicle, through rat and human epidermal membranes. The results of the in-vitro dermal penetration study differences are shown in Table B.6.12-5. Time-dependent differences in skin penetration through rat and human skin can be best compared by

comparison of time-concentration curved depicted in Figure B.6.12-1, Figure B.6.12-2 and Figure B.6.12-3.

Penetration of ^{14}C-nicobifen through rat epidermal membranes

The onset of epidermal penetration was fast, with the lag time at all concentration levels of less than 14 minutes. Independent of the concentration level tested, the penetration of radioactivity through rat epidermal membranes was initially rapid at or around the 1-h time point of investigation, but decreased significantly thereafter: the rate of penetration measured between 10 and 24 hours was less than 14 % of the initial rate. At study termination, absorbed radioactivity accounted for 33 %, 4 % and 3 % at the low, medium and high dose levels respectively. At the low, intermediate and high doses, 31 %, 73 % and 96 % of applied radioactivity was recovered in the skin washings respectively and was unabsorbed. For a 10 and a 100 fold increase in the concentration of the formulation applied to rat, a corresponding 2.18 and 12.63 fold increase in the initial rate of absorption was apparent.

Penetration of ^{14}C-nicobifen through human epidermal membranes

Penetration of administered radioactivity through human epidermal membranes was detectable within 14 minutes of application, regardless of the concentration of formulation. A linear relationship between the concentration of radioactivity in the receptor fluid and exposure time was established throughout the 24-h investigation period for each of the three dose groups. The rate of absorption increased 3.3 and 33.2 times for a 10 and 100 fold increase in the concentration. Washing was an effective method of removing the test material from epidermal membranes, particularly at the high application rate.

Figure B.6.12-1: In-vitro dermal penetration study: Absorption/time curves for rat and human epidermal membranes exposed to (^{14}C)-nicobifen at 0.01 mg/cm² (1 mg/ml)–Groups A and D

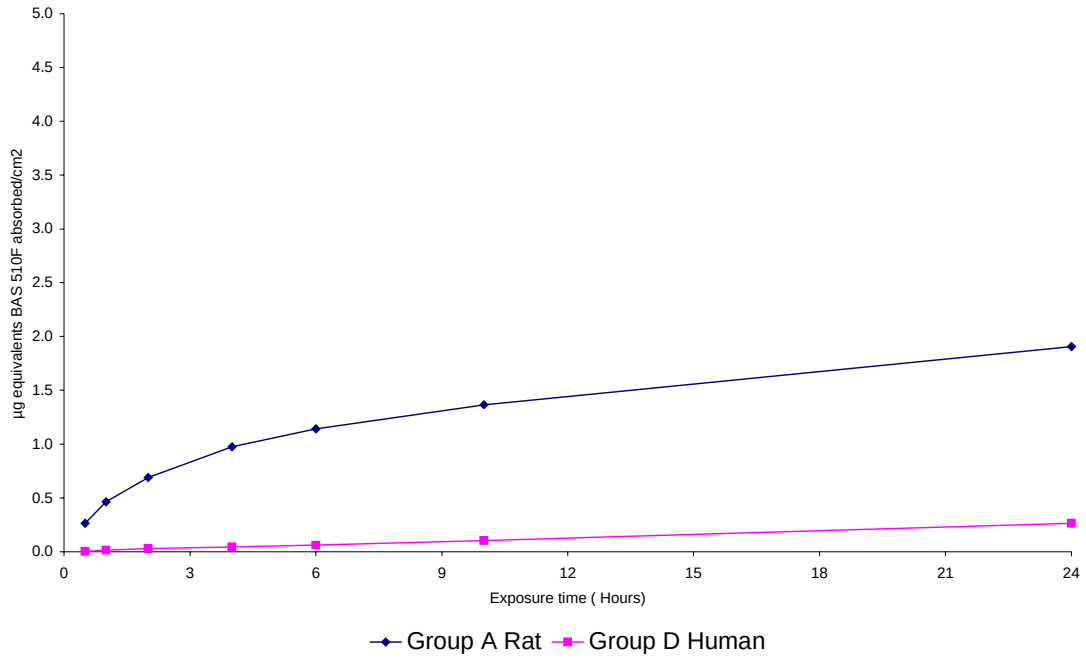


Figure B.6.12-2: In-vitro dermal penetration study: Absorption/time curves for rat and human epidermal membranes exposed to (^{14}C)-nicobifen at 0.1 mg/cm² (10 mg/ml) – Groups B and E

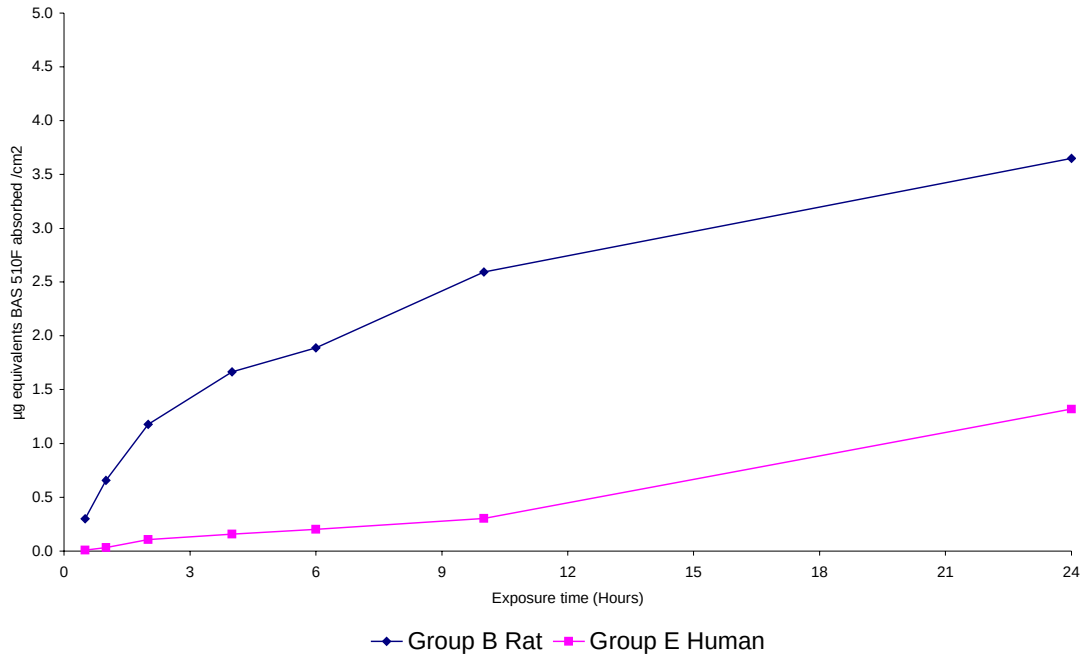


Figure B.6.12-3: In-vitro dermal penetration study: Absorption/time curves for rat and human epidermal membranes exposed to (^{14}C)-nicobifen at 1.00 mg/cm²(100 mg/ml) – Groups C and F

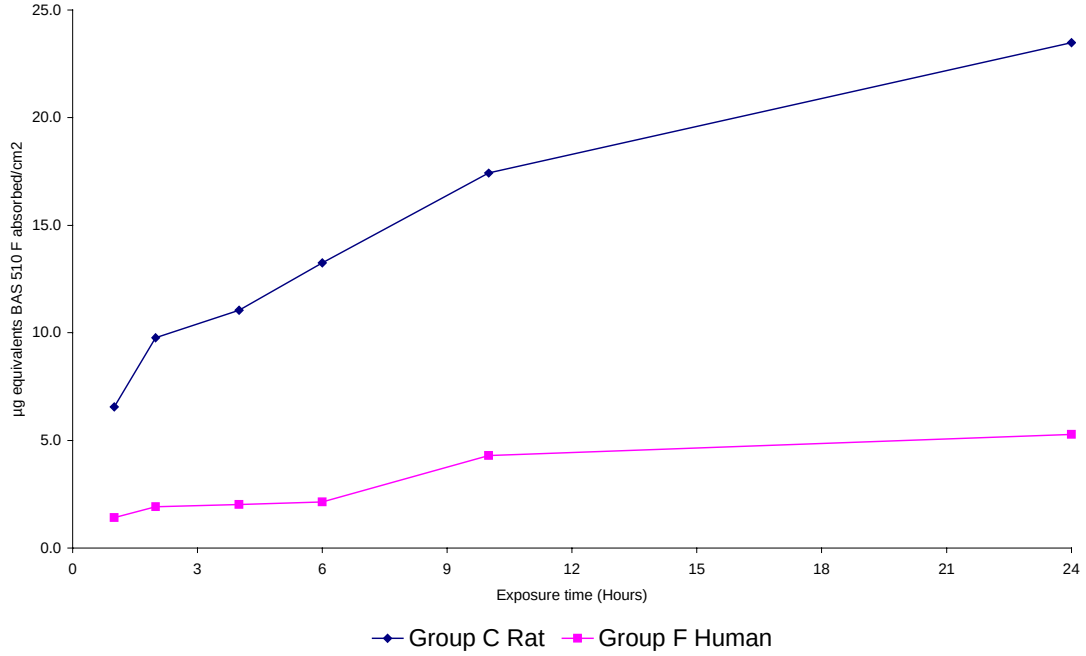


Table B.6.12-5: In-vitro dermal penetration study: Summary of test results

Administered dose		Mean cumulative absorption of (^{14}C)-nicobifen (µg/cm² skin)								
		0.01 mg/cm² (1 mg/ml)			0.1 mg/cm² (10 mg/ml)			1.00 mg/cm² (100 mg/ml)		
Sample time (h)		Rat	Human	Ratio	Rat	Human	Ratio	Rat	Human	Ratio
0.5		0.264	0.002	132.0	0.302	0.009	33.6	NA	NA	–
1		0.462	0.014	33.0	0.657	0.032	20.5	7.703	1.404	5.5
2		0.691	0.029	23.8	1.178	0.108	10.9	11.47	1.929	5.9
4		0.977	0.046	21.2	1.667	0.157	10.6	13.03	2.026	6.4
6		1.143	0.061	18.7	1.890	0.204	9.3	15.63	2.143	7.3
10		1.366	0.103	13.3	2.593	0.304	8.5	20.64	4.295	4.8
24		1.906	0.264	7.2	3.650	1.320	2.8	27.87	5.283	5.3
Lag time (h):	Mean	0.055	0.185	0.3	0.110	0.227	0.5	0.219	0.115	1.9
	SD	± 0.089	± 0.153		± 0.125	± 0.367		± 0.314	± 0.230	
Penetration rate	Mean	0.302	0.015	20.1	0.685	0.050	13.7	3.813	0.498	7.7
(µg/cm²/h)	SD	± 0.298	± 0.007		± 0.226	± 0.019		± 2.831	± 0.486	
Kp*	Mean	51.6	2.572		7.188	0.53		6.571	0.858	
(x 10^{-5} cm/h)	SD	± 50.8	± 1.127		± 2.375	± 0.20		± 4.879	± 0.837	

*Kp = Permeability coefficient; NA – Not applicable

Based on comparison of penetration rates or permeability coefficients, it appears that there is at least a 7.7-fold higher penetration rate through rat epidermis than through human epidermis.

The recovery of radiolabel was determined at the end of the experiment and summarised in Table B.6.12-6.

Table B.6.12-6: In-vitro dermal penetration study: recovery of radioactivity in through rat and human skin

Administered dose	Recovery of (^{14}C)-radiolabel (% applied dose) at 24 h								
	0.01 mg/cm² (1 mg/ml)			0.1 mg/cm² (10 mg/ml)			1.00 mg/cm² (100 mg/ml)		
	Rat	Human	Ratio	Rat	Human	Ratio	Rat	Human	Ratio
Receptor fluid	32.54	4.545	7.2	3.954	1.416	2.8	3.482	0.641	5.4
Receptor cell wash	1.809	0.485	3.7	0.368	2.596	0.1	1.150	0.509	2.3
Total penetrated	**34.35**	**5.03**	**6.8**	**4.322**	**4.012**	**1.1**	**4.632**	**1.15**	**4.0**
Skin	33.07	61.59	0.5	17.94	17.29	1.0	4.366	1.086	4.0
Total penetrated + skin	**67.42**	**66.62**	**1.0**	**22.26**	**21.3**	**1.0**	**8.998**	**2.236**	**4.0**
Surface washings	31.28	31.16	1.0	73.06	73.45	1.0	96.33	95.30	1.0
Donor cell wash	0.635	0.309	2.1	1.014	1.806	0.6	0.376	0.299	1.3
Total	99.34	98.09	1.0	96.33	96.39	1.0	105.70	97.84	1.1

The total amount of radiolabel that fully penetrated the skin membrane ("absorbed radiolabel") was recovered in the receptor fluid and in the wash of the receptor cell. At the low and high concentration levels, the amount of absorbed radiolabel was approx. 7-fold and 4-fold higher in the rat than in the human specimens, while at the intermediate concentration level, the absorption ratio was approx. 1. When the amount of radioactivity remaining associated to the skin was additionally taken into account, no differences in the "potentially absorbable" percentages of administered radiolabel were observed between species in the low and intermediate dose groups, while a 4-fold higher recovery was established in the high-dose group.

Discussion:
The notifier has based its evaluation on the present Draft Guidance document of the European Commission (Sanco/222/2000 rev.4; 11/04/2001). For the correction factor regarding results generated by in-vivo studies *"...preferably maximum flux values should be used from the in-vitro studies..."* (chapter 4.4, page 7)

In the opinion of the RMS, this quoted part of the current EU Draft Guidance document is in itself inconsistent and also in contradiction to the OECD Draft Guidelines and should therefore be revised: The quoted paragraph of the current EU Draft Guidance Document continues as follows:

> *"... Alternatively, the dermal absorption percentage (receptor medium plus skin dose) may be used. Because by definition, the permeation constant (Kp in cm/hr) is established at inifinite dose levels, the usefulness of the Kp for dermal risk assessment is limited".*

This last sentence is in direct contradiction to the recommendation of using maximum flux values (µg/cm²/h) from in-vitro studies. According to the current OECD draft guideline 428 and the corresponding draft guidance for the conduct of skin absorption studies (both December 2000), finite doses are usually encountered under normal conditions of human exposure. Therefore, applications mimicking human exposures (doses up to 10 µl/cm² or 10 mg/cm² for liquids) are recommended. Flux values or permeability coefficients (Kp) should be determined only in the case of infinite dose conditions (e.g. human exposure to a water pollutant while bathing), whereas in the case of finite dose experiments the estimation of dermal penetration should be based on recovery determinations. The experimental design of the in-vitro dermal penetration study with nicobifen fully complied with the recommendation of applying finite dose conditions, since the choice of concentrations was based on preliminary human exposure estimations.

The epidermal penetration rates through rat and human samples showed considerable time-dependent differences: For the rat, independent of the dose level tested, the epidermal penetration rate was initially high at or around the 1-h time point, but slowed rapidly thereafter. The assessment of the human skin penetration kinetics revealed, on the other hand, a linear relationship between the amount of radioactivity absorbed and exposure time throughout the whole of the 24-h investigation period for each of the three dose groups. For this reason, it is considered to be inappropriate to perform a rat-to-human extrapolation for estimation of absorption through human skin based on a ratio of permeability constants because wrong conclusions will be drawn with respect to the actual extent of human dermal absorption.

For the reasons discussed above, the estimation of in-vitro dermal penetration through rat and human epidermal membranes was performed on the basis of recovery determinations.

According to OECD draft guideline 428, the substance remaining in the skin should be considered as absorbed unless it can be demonstrated that absorption can be determined from receptor fluid alone. When calculations were based on recovery in receptor fluid alone, the rat/human extent of dermal penetration ratio was not dose-dependent. Therefore, it was considered to be justified to additionally take into account the amount of radiolabel associated to the epidermal membranes. In this case, the rat/human dermal penetration ratios were approx. 1 for both low and intermediate concentration levels, and 4 for the high concentration

level (which, based on preliminary operator exposure calculations, was too high to reflect realistic human exposure settings).

Conclusion:
The in-vitro absorption through rat epidermal membranes was the same as through human epidermal membranes at low and intermediate dose levels (0.01 and 0.1 mg/cm²) and approx. 4-fold greater at the high concentration level (1.00 mg/cm²). The results obtained for the low and intermediate concentrations were considered to be the most relevant, because these concentration levels fall in the range of the expected operator exposure. Thus, based on the results of the in-vitro dermal penetration study, it is concluded that there is no notable difference in the extent of bioavailability following dermal exposure to nicobifen between rat and humans species.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

BAS 510 01 F is formulated as a waterdispersible granule (WG, syn. DF) containing 50% nicobifen.

Besides its active ingredient nicobifen, BAS 510 01 F contains different co-formulants. The respective data are given in Safety Data Sheets. The possibly acute toxic properties of all co-formulants are covered by the studies with the preparation.

B.6.14 Exposure data (Annex IIIA 7.2)

Information on product and use
BAS 510 01 F is formulated as a waterdispersible granule (WG, *syn.* DF) containing 50% nicobifen.

The intended use is as a fungicide in grapes and in field crops such like oilseed rape, peas and field beans. The maximum recommended application rate is 0.6 kg ai/ha for grapes, 0.5 kg ai/ha for peas and beans and 0.25 kg ai/ha in oilseed rape. For all use situations water will be the diluent/carrier. The spray volume will be 1000–1600 l/ha for grapes, about 400 l/ha for peas and about 300 l/ha for beans and oilseed rape.

Applications of BAS 510 01 F will be primarily performed by using vehicle-mounted or trailed sprayers, however, uses with knapsack sprayers may also occur in grapes and are thus considered in this evaluation as well.

B.6.14.1 Operator exposure

B.6.14.1.1 Estimation of operator exposure; risk assessment

On the basis of the data submitted by the notifier, 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.

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 assessed via the absorption rates on the basis of the systemic AOEL derived for the active ingredient.

Determination of the acceptable operator exposure level (systemic AOEL)
The NOAELs of the 90-day studies in rats and mice, and the 1-year dog study were in the same range (22–34 mg/kg bw/day). For setting the AOEL the most relevant study was considered to be the dog 1-year oral feed study (NOAEL of 22 mg/kg bw/d, corresponding to 800 ppm) applying a standard safety factor of 100. Since absorption was only 44 % of the administered dose by the oral route, the AOEL is corrected (B.6.10.2):

$$\mathrm{AOEL}_{(SYS)} = \frac{22\ mg/kg\ bw/d}{100} \times 44\ \% = 0.0968\ \ rounded\ \ 0.1\ \mathrm{mg/kg\ bw/d}$$

Dermal absorption rate
On the basis of the results of an in-vivo dermal absorption study in rats and an in-vitro comparison of the penetration of radiolabelled nicobifen through rat and human epidermal membranes an overall dermal absorption value of 7 % is proposed to use for risk assessment calculation purposes (B.6.12).

B.6.14.1.1.1 Operator exposure (German model)

The following assessment is made on the basis of the German model where the risk assessment is based on the comparison of the potential exposure and the tolerable exposure for each exposure route. The subsequent addition according to the following formula results in the "total degree of exposure" E:

$$E = \frac{D_{M(H)}}{D^{tol}} + \frac{D_{A(H)}}{D^{tol}} + \frac{D_{A(B)}}{D^{tol}} + \frac{D_{A(C)}}{D^{tol}} + \frac{I_M}{I^{tol}} + \frac{I_A}{I^{tol}}$$

If, according to this equation, the total degree of exposure E exceeds 1 - corresponding to 100% - (i.e. if the estimated or potential exposure is higher than the tolerable exposure or the AOEL) instructions for additional protective measures to reduce E are required.

The assessment was made with consideration of three individual levels of personal protective equipment (PPE) used by operators subsequently referred to as scenario 1 to 3.

Scenario 1:
No personal protective equipment is used when handling both the undiluted product (handling of product during mixing/loading) and the diluted product during application.

Scenario 2:
Protective gloves are used when handling the undiluted product.

Scenario 3:
PPE with gloves, standard protective garment and sturdy footwear is used when handling the undiluted and the diluted product (handling of product during mixing/loading and application).

Determination of the tolerable dermal and inhalation exposures for nicobifen
For the determination of the **tolerable dermal exposure** (**D^{tol}**) the NOAEL (1yr-oral-dog: 22 mg/kg bw/d) as considered to be relevant for the derivation of the systemic AOEL (corrected by 44% oral absorption), a dermal absorption rate of 7%, an assessment factor (AF) of 100 and an average body weight of 70 kg/person are to be taken into account. The tolerable dermal exposure is then determined as:

$$D^{tol} = \frac{\text{NOAEL x \% (oral abs.rate) x bw (man)}}{\text{AF x \% (dermal abs. rate)}} = \frac{22\ \text{mg/kg bw x 44\% x 70 kg bw/person}}{100\ \text{x}\ 7\%}$$

D^{tol} = 97.02 mg/person/d (equiv. to a dermal AOEL of 1.386 mg/kg bw/d).

For the determination of the **tolerable inhalation exposure (I^{tol})** assuming a 100% pulmonary absorption, the tolerable inhalation exposure is calculated as follows on the basis of the dog 1-year oral feed study and the oral absorption as mentioned above:

$$I^{tol} = \frac{\text{NOAEL}_0\ \text{x \% (oral abs. rate) x bw (man)}}{\text{AF}} = \frac{22\ \text{mg/kg bw x 44\% x 70 kg bw/person}}{100}$$

I^{tol} = 6.79 mg/person (equiv. to an inhal. AOEL of 0.097 rounded 0.1 mg/kg bw).

Estimation of operator exposure in high crops, tractor mounted

Assumptions used for the calculation:

Formulation type:	Waterdispersible granule (WG)
Application technique:	Tractor mounted application/high crops
Area treated per day:	8 ha
Application rate:	0.6 kg as/ha
Dermal absorption rate:	7%
Body weight of an operator:	70 kg
Penetration rate:	Gloves: 1%; standard protective garment: 5%

A summary of the expected/potential operator exposure for the different levels of PPE is provided in Table B.6.14-1. More detailed information as to how these results have been generated is presented in appendix 1.

Table B.6.14-1: Summary of the estimated operator exposure (mg/person/d) for nicobifen in high crops – tractor operated system

Route of exposure and type of work	Scenario 1 No PPE	Scenario 2 PPE, mixing/loading	Scenario 3 PPE, all operations
Dermal exposure			
- Mixing/loading	9.60	0.096	0.096
- Application	55.20	55.200	8.098
Total dermal (D)	64.80	55.296	8.194
Inhalation exposure			
- Mixing/loading	0.0384	0.0384	0.0384
- Application	0.0864	0.0864	0.0864
Total inhalation (I)	0.1248	0.1248	0.1248

* all figures in mg ai/operator

The results of a comparison of estimated and tolerable exposures are presented in Appendix 1 and summarised as follows:

Degree of exposure for scenario 1 (No PPE): E = 0.686
Degree of exposure for scenario 2 (PPE during mixing/loading): E = 0.588
Degree of exposure for scenario 3 (PPE during all operations): E = 0.103

It is thus concluded that the estimated degree of exposure for tractor operated application systems in grapes is in the range of 68.6% to 10.3% of the tolerable exposures (AOELs) depending on the level of PPE used.

Estimation of operator exposure in high crops, knapsack application

Assumptions used for the calculation:

Formulation type:	Waterdispersible granule (WG)
Application technique:	Knapsack application/high crops
Area treated per day:	1 ha
Application rate:	0.6 kg as/ha
Dermal absorption rate:	7%
Body weight of an operator:	70 kg
Penetration rate:	Gloves: 1%; standard protective garment: 5%

A summary of the expected/potential operator exposure for the different levels of PPE is provided in Table B.6.14-2. More detailed information as to how these results have been generated is presented in appendix 2.

Table B.6.14-2: Summary of the estimated operator exposure (mg/person/d) for nicobifen in high crops – knapsack application

Route of exposure and type of work	Scenario 1 No PPE	Scenario 2 PPE, mixing/loading	Scenario 3 PPE, all operations
Dermal exposure			
- Mixing/loading	12.60	0.126	0.126
- Application	24.24	24.240	3.694
Total dermal (D)	36.84	24.366	3.820
Inhalation exposure			
- Mixing/loading	0.012	0.012	0.012
- Application	0.180	0.180	0.180
Total inhalation (I)	0.192	0.192	0.192

The results of a comparison of estimated and tolerable exposures are presented in Appendix 2 and summarised as follows:

Degree of exposure for scenario 1 (No PPE): E = 0.408
Degree of exposure for scenario 2 (PPE during mixing/loading): E = 0.279
Degree of exposure for scenario 3 (PPE during all operations): E = 0.068

It is thus concluded that the estimated degree of exposure for the knapsack application in high crops is in the range of 40.8% to 6.8% of the tolerable exposures (AOELs) depending on the level of PPE used.

Estimation of operator exposure for nicobifen in field crops, tractor mounted

Assumptions used for the calculation:

Formulation type:	Waterdispersible granule (WG)
Application technique:	Tractor mounted application/high crops
Area treated per day:	20 ha
Application rate:	0.5 kg as/ha
Dermal absorption rate:	7%
Body weight of an operator:	70 kg
Penetration rate:	Gloves: 1%; standard protective garment: 5%

A summary of the expected/potential operator exposure for the different levels of PPE is provided in Table B.6.14-3. More detailed information as to how these results have been generated is presented in appendix 3.

Table B.6.14-3: Summary of the estimated operator exposure (mg/person/d) for nicobifen in field crops, tractor mounted

Route of exposure and type of work	Scenario 1 No PPE	Scenario 2 PPE, mixing/loading	Scenario 3 PPE, all operations
Dermal exposure			
- Mixing/loading	20.0	0.2	0.200
- Application	20.4	20.4	1.438
Total dermal (D)	40.4	20.6	1.638
Inhalation exposure			
- Mixing/loading	0.08	0.08	0.08
- Application	0.01	0.01	0.01
Total inhalation (I)	0.09	0.09	0.09

The results of a comparison of estimated and tolerable exposures are presented in Appendix 3 and summarised as follows:

Degree of exposure for scenario 1 (No PPE): E = 0.430
Degree of exposure for scenario 2 (PPE during mixing/loading): E = 0.226
Degree of exposure for scenario 3 (PPE during all operations): E = 0.030

It is thus concluded that the degree of exposure in field crops (tractor mounted) is in the range of 43.0% to 3.0% of the tolerable exposures (AOELs) depending on the level of PPE used.

Summary of risk assessments according to the German model

As a summary the results of the risk assessment in terms of degree of exposure (E) are shown in Table B.6.14-4.

Table B.6.14-4: German model: Summary of risk assessments degrees of exposure (E)

Scenario	Scenario 1 (no PPE)	Scenario 2 (PPE, mixing/loading)	Scenario 3 (PPE, all operations)
High crops (tractor)	0.686	0.588	0.103
High crops (knapsack)	0.408	0.279	0.068
Field crops	0.430	0.226	0.030

Following the results of this risk assessment, no additional protective measures are needed to stay below the proposed AOELs.

B.6.14.1.1.2 Operator exposure (UK POE-Model)

As given by the notifier, the operator exposure estimates were done using the UK-POEM for tractor application in high crops and field crops as well as for knapsack application (low level).

The assessment was made with consideration of three individual levels of personal protective equipment (PPE) used by operators subsequently referred to as scenario 1 to 3.

Scenario 1:
No personal protective equipment is used when handling both the undiluted and the diluted product (handling of the product during mixing/loading and application)

Scenario 2:
Protective gloves are used when handling the undiluted product.

Scenario 3:
Protective gloves are used when handling the undiluted as well as the diluted product (handling of the product during mixing/loading and application).

All calculations of total predicted operator exposures are presented at the end of this evaluation paper (see appendices 4 to12).

Estimation of operator exposure for nicobifen in high crops, tractor application
Assumptions used for the calculation:

Formulation type:	Waterdispersible granule (WG)
Packaging	5 kg containers
Application technique:	Tractor mounted application/high crops
Area treated per day:	30 ha
Application rate:	1.2 kg/ha (i.e. 0.6 kg as/ha)
Water volume used	1000 l/ha
Dermal absorption rate:	7%
Body weight of an operator:	60 kg
Penetration rate:	Gloves during mixing/loading 1%; gloves during application: 10%

A summary of the expected/potential operator exposure for the different levels of PPE is provided in Table B.6.14-5 for tractor operated equipment. More detailed information as to how these results have been generated is presented in appendices 4 to 6.

Table B.6.14-5: UK POEM estimates of exposure to nicobifen in high crops with tractor operated sprayer

dermal exposure[1)] (mg/person/d)			inhalation exposure[1)] (mg/person/d)	systemic* expos./absorbed dose (mg/person/d)		
mix/load	**spray**	**total**	**spray**	**mix/load**	**spray****	**total****
No personal protective equipment worn						
40.0	72.72	112.72	0.18	2.800	5.270	8.070
Gloves during mixing/loading only						
0.4	72.72	73.12	0.18	0.028	5.270	5.298
Gloves during mixing/loading and application						
0.4	51.12	51.52	0.18	0.028	3.758	3.786

1) Source: Appendices 4 to 6.
* dermal absorption 7%; inhal. absorption 100%.
** dermal and inhalation exposure.

Table B.6.14-6: Predicted exposure as a proportion of the systemic AOEL achieved for nicobifen in high crops with tractor operated sprayer

PPE	Total systemic exposure		% of AOEL
	mg/person/d	mg/kg bw/d[1)]	(0.1 mg/kg bw/d)
No PPE	8.070	0.135	134.5
Gloves during mixing	5.298	0.088	88.3
Gloves during mixing and appl.	3.786	0.063	63.1

1) body weight: 60 kg/person

It is thus concluded that the estimated exposure for the tractor mounted application in high crops is in the range of 134.5% to 63.1% of the proposed systemic AOEL depending on the level of PPE used.

Estimation of operator exposure for nicobifen; knapsack application, low level

Assumptions used for the calculation:

Formulation type:	Waterdispersible granule (WG)
Packaging	1 kg containers
Application technique:	Knapsack application/low level
Area treated per day:	1 ha
Application rate:	1.2 kg/ha (i.e. 0.6 kg as/ha)
Water volume used	600 l/ha
Dermal absorption rate:	7%
Body weight of an operator:	60 kg
Penetration rate:	Gloves during mixing/loading 1%; gloves during application: 10%

A summary of the expected/potential operator exposure for the different levels of PPE is provided in the following Table B.6.14-7 for knapsack application, low level. More detailed information as to how these results have been generated is presented in appendices 7 to 9.

Table B.6.14-7: UK POEM estimates of exposure to nicobifen for knapsack sprayers, low level

dermal exposure[1)] (mg/person/d)			inhalation exposure[1)] (mg/person/d)	systemic* expos./absorbed dose (mg/person/d)		
mix/load	spray	total	spray	mix/load	spray**	total**
No personal protective equipment worn						
200.0	102.0	302.0	0.12	14.00	7.260	21.260
Gloves during mixing/loading only						
2.0	102.0	104.0	0.12	0.14	7.260	7.400
Gloves during mixing/loading and application						
2.0	49.5	51.5	0.12	0.14	3.585	3.725

1) Source: Appendices 7 to 9.

* dermal absorption 7%; inhal. absorption 100%.

** dermal and inhalation exposure.

Table B.6.14-8: Predicted exposure as a proportion of the systemic AOEL achieved for nicobifen for knapsack sprayers, low level

PPE	Total systemic exposure		% of AOEL
	mg/person/d	mg/kg bw/d[1)]	(0.1 mg/kg bw/d)
No PPE	21.260	0.354	354.3
Gloves during mixing	7.400	0.123	123.3
Gloves during mixing and appl.	3.725	0.062	62.1

1) body weight: 60 kg/person

It is thus concluded that the estimated exposure for the knapsack application at low level is in the range of 354.3% to 62.1% of the proposed systemic AOEL depending on the level of PPE used.

Estimation of operator exposure for nicobifen, field crops, tractor application

Assumptions used for the calculation:

Formulation type:	Waterdispersible granule (WG)
Packaging	5 kg containers
Application technique:	Field crops, tractor application
Area treated per day:	50 ha
Application rate:	1.0 kg/ha (i.e. 0.5 kg as/ha)
Water volume used	300 l/ha
Dermal absorption rate:	7%
Body weight of an operator:	60 kg
Penetration rate:	Gloves during mixing/loading 1%; gloves during application: 10%

A summary of the expected/potential operator exposure for the different levels of PPE is provided in the following Table B.6.14-9 for knapsack application, low level. More detailed information as to how these results have been generated is presented in appendices 10 to 12.

Table B.6.14-9: UK POEM estimates of exposure to nicobifen in field crops, tractor mounted

dermal exposure[1)] (mg/person/d)			inhalation exposure[1)] (mg/person/d)	systemic* expos./absorbed dose (mg/person/d)		
mix/load	spray	total	spray	mix/load	spray**	total**
No personal protective equipment worn						
50.0	69.264	119.264	0.1	3.5	4.948	8.448
Gloves during mixing/loading only						
0.5	69.264	69.764	0.1	0.035	4.948	4.983
Gloves during mixing/loading and application						
0.5	10.752	11.252	0.1	0.035	0.853	0.888

1) Source: Appendices 10 to 12.

* dermal absorption 7%; inhal. absorption 100%.

** dermal and inhalation exposure.

Table B.6.14-10: Predicted exposure as a proportion of the systemic AOEL achieved for nicobifen in field crops, tractor mounted

PPE	Total systemic exposure		% of AOEL
	mg/person/d	mg/kg bw/d[1)]	(0.1 mg/kg bw/d)
No PPE	8.449	0.141	140.8
Gloves during mixing	4.984	0.083	83.1
Gloves during mixing and appl.	0.888	0.015	14.8

1) body weight: 60 kg/person

It is thus concluded that the estimated exposure for the tractor mounted application in field crops is in the range of 140.8% to 14.8% of the proposed systemic AOEL depending on the level of PPE used.

Summary of risk assessments according to the UK-POEM

As a summary the results of the risk assessment in terms of percentage of systemic AOEL are shown in Table B.6.14-11.

Table B.6.14-11: UK-POEM: Summary of risk assessments – percentage of systemic AOEL (0.1 mg/kg bw/d)

Scenario	Scenario 1 (no PPE)	Scenario 2 (PPE, mixing/loading)	Scenario 3 (PPE, all operations)
High crops (tractor)	134.5	88.3	63.1
Knapsack, low level	354.3	123.3	62.1
Field crops (tractor)	140.8	83.1	14.8

Following the results of this risk assessment, additional protective measures are needed to stay below the proposed systemic AOEL.

Final conclusion of the risk evaluation for operators:

In the German model (see Table B.6.14-12), the results show that the operator exposure for all proposed uses is acceptable even if no personal protection is used (exposures < 66.6% of the systemic AOEL).

Table B.6.14-12: Results of the German model calculations and a comparison with the proposed systemic AOEL

Application technique	treated area (ha/d)	PPE	Systemic exposure* (mg/kg bw/d)	% of AOEL (0.1 mg/kg bw/d)
High crops (tractor)	8	none	0.067	66.6
		m/l: gloves	0.057	57.1
		m/l: gloves; appl.: gloves + garment	0.010	10.0
High crops, knapsack	1	none	0.040	39.6
		m/l: gloves	0.027	27.1
		m/l: gloves; appl.: gloves + garment	0.007	6.6
Field crops (tractor)	20	none	0.042	41.7
		m/l: gloves	0.022	21.9
		m/l: gloves; appl.: gloves + garment	0.003	2.9
* In the calculations a body weight of 70 kg and a dermal absorption rate of 7% were used.				

Calculating with the UK-POEM (see Table B.6.14-13), PPE are needed to get an estimated operator exposure which is acceptable.

Table B.6.14-13: Results of the UK-POEM calculations and a comparison with the proposed systemic AOEL

Application technique	treated area (ha/d)	PPE	Systemic exposure* (mg/kg bw/d)	% of AOEL (0.1 mg/kg bw/d)
High crops (tractor)	30	none	0.135	134.5
		m/l: gloves	0.088	88.3
		m/l: gloves; appl.: gloves	0.063	63.1
Knapsack, low level	1	none	0.354	354.3
		m/l: gloves	0.123	123.3
		m/l: gloves; appl.: gloves	0.062	62.1
Field crops (tractor)	50	none	0.141	140.8
		m/l: gloves	0.083	83.1
		m/l: gloves; appl.: gloves	0.015	14.8
* In the calculations a body weight of 60 kg and a dermal absorption rate of 7% were used.				

Considering the results of the risk assessments based on the German model as well as the UK-POEM it is concluded that BAS 510 01 F can be handled safely under the recommended conditions of use.

B.6.14.1.2 Measurement of operator exposure

Since the risk assessment carried out indicates that the health-based limit value (AOEL) will not be exceeded under practical conditions of use, a study to provide a measurement of operator exposure to nicobifen under field conditions, was not necessary and was, therefore, not carried out.

B.6.14.2 Worker exposure

B.6.14.2.1 Estimation of worker exposure

BAS 510 01 F will be applied in grapes with a maximum application rate of 1.2 kg BAS 510 01 F/ha corresponding to 0.6 kg ai/ha.

Applications in other crops will be performed with lower application rates, re-entry situations in these crops are likely to occur less frequently, are considered to comprise shorter periods of re-entry and the extent of exposure to leaf surfaces (in terms of transfer factors, TF) is considered to be less compared to the grapes scenario.

For the subsequent risk assessment the re-entry situation in grapes is therefore used as a worst case scenario. The estimation will be based on the model as developed by the German BBA (Biologische Bundesanstalt) and the US EPA:
- 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
- EPA, Science Advisory Council for Exposure; 1998; Agricultural Default Transfer Coefficients, Policy #98/11675

The following parameters will be considered:

Dislodgeable foliar residue	DFR	=	1 µg/cm² x kg ai applied
Transfer factor, according to EPA	TF	=	15000 cm²/h x person
Working period	A	=	8 h/day
Penetration of protective material	P	=	5% (= factor 0.05)
Rate of application	R	=	0.6 kg nicobifen/ha

Calculation of potential dermal exposure:

Dermal exposure (D) of the unprotected worker

D = DFR x TF x A x R
= 1 x 15000 x 8 x 0.60
= 72000 µg/person x day
= 72 mg/person x day

considering a body weight of 70 kg

D = 1.029 mg/kg bw/d

Dermal exposure (D(PPE)) for the protected worker

D (PPE) = D x P
= 1.029 x 0.05
D (PPE) = 0.051 mg/kg bw/d

Estimated absorbed dose (considering a dermal absorption rate of 7%)**:**

Potential dermal exposures			x 0.07
without PPE	:	1.029	x 0.07
			= 0.0720 mg/kg bw/d
protected worker	:	0.051	x 0.07
			= 0.0036 mg/kg bw/d

Risk assessment (comparison of the absorbed dose and the systemic AOEL)

	unprotected	protected
Potential exposure (mg/kg bw/d)	1.029	0.051
Estimated absorbed dose (mg/kg bw/d)	0.0720	0.0036
systemic AOEL (mg/kg bw/d)	0.1	0.1
AOEL covered (%):	72.03	3.57

The results of the risk assessment (systemic AOEL covered: 72.03% unprotected; 3.57% protected) indicates that re-entry of treated vineyards and other field crops is possible after the spray solution has dried up.

Final remarks by the notifier

In the risk assessment by the notifier a specific dermal AOEL of 10 mg/kg bw/d was used. On this basis values for AOEL covered of 10% (unprotected) and 0.5% (protected) were calculated. Nevertheless the notifier stated that workers should wear clothing and gloves to avoid dermal exposure of the active ingredients deposited on the foliage.

For the protection of workers the label should carry the following instruction:

Do not re-enter treated areas/crops before the spray deposit is completely dry. Avoid dermal exposure. Re-entry should be done with working clothing and gloves.

B.6.14.2.2 Measurement of worker exposure

It is concluded that the measurement of worker exposure is not necessary and was therefore not performed.

B.6.14.3 Bystander exposure

As outlined by the notifier, BAS 510 01 F is used in grapes and field crops. The usual form of application is by tractor-mounted sprayers without bystanders. The spray solution will contain a maximum of 1.2 kg product in a maximum of 1,600 l water.

In view of the recommended application technique in combination with Good Agricultural Practice (GAP) bystanders may be exposed briefly and to relatively low quantities of spray compared to an operator.

A possible situation in which bystander exposure may occur would be a person walking on a footpath alongside an area which is being treated at the same time. Even under these conditions the bystander will never walk directly next to the outer spraying nozzle. A distance of some meters from the downwind edge of the treated swath can always be expected.

To estimate exposure to bystanders, a comparison with the operator exposure can be helpful. In high crops, tractor application, the following points are of prime importance:

a) Bystanders walking alongside a field which is being treated, are exposed only for the few seconds when the sprayer moves along the person. Assumed that passing a bystander takes a minute, the exposure time is only the 360th part of the exposure time of the operator, spraying 6 hours a day.

b) Repeated exposure is unlikely, since the sprayer is considered to only pass once along the edge of a field for each spraying swath.

c) Bystanders always stand at a larger distance from the edge of the spray boom and thus from the spraying nozzle, than the operator. For example, an operator (the tractor driver) may be at 2 to 4 m distance to the nearest spraying nozzles, bystanders at least at about 8 m.

These factors even outweigh the reduction of exposure for the operator caused by garment worn during application. Therefore, during application in vineyards bystander exposure is no subject of special concern.
Due to considerations as indicated above estimation of bystander exposure was not performed.

Taking into account the results from the assessment of the estimated operator exposure which indicate that the exposure is always below the proposed systemic AOEL in the German model without PPE, the rapporteur agreed with the risk assessment for bystanders as given by the notifier (see above).

Appendix 1:
BBA model: Operator exposure for nicobifen in high crops – tractor mounted

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	WG (DF)	$\mathbf{D_{M(H)}}$	= 2.0 mg/person x kg ai
Application technique:	tractor-mounted	$\mathbf{D_{A(H)}}$	= 0.7 mg/person x kg ai
Application rate:	0.6 kg nicobifen/ha	$\mathbf{D_{A(B)}}$	= 9.6 mg/person x kg ai
Area treated per day (Ar):	8 ha	$\mathbf{D_{A(C)}}$	= 1.2 mg/person x kg ai
		$\mathbf{I_M}$	= 0.008 mg/person x kg ai
		$\mathbf{I_A}$	= 0.018 mg/person x kg ai

Route of exposure			Scenario 1 (No PPE)	Scenario 2 PPE, mix./load.	Scenario 3 PPA, all operations
Dermal/mixing					
exposure (hands):	$\mathbf{D_{M(H)}}$	=	2.00 x 0.60 x 8		
		=	9.6 mg/person	0.096 mg/person*1	0.096 mg/person*1
Dermal/application					
exposure (hands, body, head)	$\mathbf{D_{A(H)}}$	=	0.70 x 0.60 x 8		
		=	3.36 mg/person	3.36 mg/person	0.034 mg/person*1
	$\mathbf{D_{A(B)}}$	=	9.6 x 0.60 x 8		
		=	46.08 mg/person	46.08 mg/person	2.304 mg/person*2
	$\mathbf{D_{A(C)}}$	=	4.8 x 0.60 x 1		
		=	5.76 mg/person	5.76 mg/person	5.76 mg/person
Total dermal exposure		=	**64.80 mg/person**	**55.30 mg/person**	**8.19 mg/person**
Inhalation/mixing					
	$\mathbf{I_M}$	=	0.008 x 0.60 x 8		
		=	0.0384 mg/person	0.0384 mg/person	0.0384 mg/person
Inhalation/application					
	$\mathbf{I_A}$	=	0.018 x 0.60 x 8		
		=	0.0864 mg/person	0.0864 mg/person	0.0864 mg/person
Total inhalation exposure		=	**0.125mg/person**	**0.125mg/person**	**0.125mg/person**

*1 reduction factor of gloves = 0.01
*2 reduction factor of protective clothing = 0.05

Calculation of degree of exposure (E):

$$E = \frac{D_{M(H)}}{D^{tol}} + \frac{D_{A(H)}}{D^{tol}} + \frac{D_{A(B)}}{D^{tol}} + \frac{D_{A(C)}}{D^{tol}} + \frac{I_M}{I^{tol}} + \frac{I_A}{I^{tol}}$$

For tolerated exposures see B.6.14.1.1.1:

D^{tol} = 97.02 mg/person/d (i.e.1.386 mg/kg bw/d)
I^{tol} = 6.79 mg/person/d (i.e.0.097, rounded = 0.1 mg/kg bw/d)

Scenario 1: without PPE:

$$E = \frac{9.6}{97.02} + \frac{3.36}{97.02} + \frac{46.08}{97.02} + \frac{5.76}{97.02} + \frac{0.0384}{6.79} + \frac{0.0864}{6.79} = \mathbf{0.686}$$

Scenario 2: considering PPE (gloves during mixing/loading):

$$E = \frac{55.20}{97.2} + \frac{0.125}{6.79} = \mathbf{0.588}$$

Scenario 3: considering PPE (gloves and garment during all operations):

$$E = \frac{8.19}{97.2} + \frac{0.125}{6.79} = \mathbf{0.103}$$

Appendix 2:
BBA model: Operator exposure for nicobifen in high crops –knapsack application

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	WG (DF)	$\mathbf{D_{M(H)}}$	= 21.0 mg/person x kg ai
Application technique:	knapsack operated	$\mathbf{D_{A(H)}}$	= 10.6 mg/person x kg ai
Application rate:	0.6 kg nicobifen/ha	$\mathbf{D_{A(B)}}$	= 25.0 mg/person x kg ai
Area treated per day Ar):	1 ha	$\mathbf{D_{A(C)}}$	= 4.8 mg/person x kg ai
		$\mathbf{I_M}$	= 0.02 mg/person x kg ai
		$\mathbf{I_A}$	= 0.3 mg/person x kg ai

Route of exposure			Scenario 1 (No PPE)	Scenario 2 PPE, mix./load.	Scenario 3 PPA, all operations
Dermal/mixing					
exposure (hands):	$\mathbf{D_{M(H)}}$	=	21.0 x 0.60 x 1		
		=	12.6 mg/person	0.126 mg/person*1	0.126 mg/person*1
Dermal/application					
exposure (hands, body, head)	$\mathbf{D_{A(H)}}$	=	10.6 x 0.60 x 1		
		=	6.36 mg/person	6.36 mg/person	0.064 mg/person*1
	$\mathbf{D_{A(B)}}$	=	25.0 x 0.60 x 1		
		=	15.0 mg/person	15.0 mg/person	0.75 mg/person*2
	$\mathbf{D_{A(C)}}$	=	4.8 x 0.60 x 1		
		=	2.88 mg/person	2.88 mg/person	2.88 mg/person
Total dermal exposure		=	**36.84 mg/person**	**24.37 mg/person**	**3.82 mg/person**
Inhalation/mixing					
	$\mathbf{I_M}$	=	0.02 x 0.60 x 1		
		=	0.012 mg/person	0.012 mg/person	0.012 mg/person
Inhalation/application					
	$\mathbf{I_A}$	=	0.3 x 0.60 x1		
		=	0.18 mg/person	0.18 mg/person	0.18 mg/person
Total inhalation exposure		=	**0.192mg/person**	**0.192 mg/person**	**0.192 mg/person**

*1 reduction factor of gloves = 0.01
*2 reduction factor of protective clothing = 0.05

Calculation of degree of exposure (E):

$$E = \frac{D_{M(H)}}{D^{tol}} + \frac{D_{A(H)}}{D^{tol}} + \frac{D_{A(B)}}{D^{tol}} + \frac{D_{A(C)}}{D^{tol}} + \frac{I_M}{I^{tol}} + \frac{I_A}{I^{tol}}$$

For tolerated exposures see B.6.14.1.1.1:

D^{tol} = 97.02 mg/person/d (i.e.1.386 mg/kg bw/d)
I^{tol} = 6.79 mg/person/d (i.e.0.097, rounded = 0.1 mg/kg bw/d)

Scenario 1: without PPE:

$$E = \frac{12.6}{97.02} + \frac{6.36}{97.02} + \frac{15.0}{97.02} + \frac{2.88}{97.02} + \frac{0.012}{6.79} + \frac{0.18}{6.79} = \mathbf{0.408}$$

Scenario 2: considering PPE (gloves during mixing/loading):

$$E = \frac{24.37}{97.02} + \frac{0.192}{6.79} = \mathbf{0.279}$$

Scenario 3: considering PPE (gloves and garment during all operations):

$$E = \frac{3.82}{97.02} + \frac{0.192}{6.79} = \mathbf{0.068}$$

Appendix 3
BBA model: Operator exposure for nicobifen in field crops – tractor mounted

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	WG (DF)	$\mathbf{D_{M(H)}}$	= 2.0 mg/person x kg ai
Application technique:	knapsack operated	$\mathbf{D_{A(H)}}$	= 0.38 mg/person x kg ai
Application rate:	0.5 kg nicobifen/ha	$\mathbf{D_{A(B)}}$	= 1.6 mg/person x kg ai
Area treated per day Ar):	20 ha	$\mathbf{D_{A(C)}}$	= 0.06 mg/person x kg ai
		$\mathbf{I_M}$	= 0.008 mg/person x kg ai
		$\mathbf{I_A}$	= 0.001 mg/person x kg ai

Route of exposure			Scenario 1 (No PPE)	Scenario 2 PPE, mix./load.	Scenario 3 PPA, all operations
Dermal/mixing					
exposure (hands):	$\mathbf{D_{M(H)}}$	=	2.0 x 0.5 x 20		
		=	20 mg/person	0.2 mg/person*1	0.2 mg/person*1
Dermal/application					
exposure (hands, body, head)	$\mathbf{D_{A(H)}}$	=	0.38 x 0.5 x 20		
		=	3.8 mg/person	3.8 mg/person	0.038 mg/person*1
	$\mathbf{D_{A(B)}}$	=	1.6 x 0.5 x 20		
		=	16.0 mg/person	16.0 mg/person	0.8 mg/person*2
	$\mathbf{D_{A(C)}}$	=	0.06 x 0.5 x 20		
		=	0.6 mg/person	0.6 mg/person	0.6 mg/person
Total dermal exposure		=	**40.4 mg/person**	**20.6 mg/person**	**1.64 mg/person**
Inhalation/mixing					
	$\mathbf{I_M}$	=	0.008 x 0.5 x 20		
		=	0.08 mg/person	0.08 mg/person	0.08 mg/person
Inhalation/application					
	$\mathbf{I_A}$	=	0.001 x 0.5 x 20		
		=	0.01 mg/person	0.01 mg/person	0.01 mg/person
Total inhalation exposure		=	**0.09 mg/person**	**0.09 mg/person**	**0.09 mg/person**

*1 reduction factor of gloves = 0.01
*2 reduction factor of protective clothing = 0.05

Calculation of degree of exposure (E):

$$E = \frac{D_{M(H)}}{D^{tol}} + \frac{D_{A(H)}}{D^{tol}} + \frac{D_{A(B)}}{D^{tol}} + \frac{D_{A(C)}}{D^{tol}} + \frac{I_M}{I^{tol}} + \frac{I_A}{I^{tol}}$$

For tolerated exposures see B.6.14.1.1.1:

D^{tol} = 97.02 mg/person/d (i.e.1.386 mg/kg bw/d)
I^{tol} = 6.79 mg/person/d (i.e.0.097, rounded = 0.1 mg/kg bw/d)

Scenario 1: without PPE:

$$E = \frac{20.0}{97.02} + \frac{3.8}{97.02} + \frac{16.0}{97.02} + \frac{0.6}{97.02} + \frac{0.08}{6.79} + \frac{0.01}{6.79} = \mathbf{0.430}$$

Scenario 2: considering PPE (gloves during mixing/loading):

$$E = \frac{20.6}{97.02} + \frac{0.09}{6.79} = \mathbf{0.226}$$

Scenario 3: considering PPE (gloves and garment during all operations):

$$E = \frac{1.64}{97.02} + \frac{0.09}{6.79} = \mathbf{0.030}$$

Appendix 4

UK POEM: TRACTOR DRAWN AIR ASSISTED ORCHARD SPRAYER			PPE: None	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	0.6	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	kg		
Hand contamination/operation	0.01	ml		
Application dose	1.2	kg product/ha		
Work rate	30	ha/day		
Number of operations	8	/day		
Hand contamination	0.08	g/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to formulation	0.08	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor drawn orchard sprayer with hydraulic nozzles			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	None	Permeable	Permeable	
Penetration	100	2	5	%
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.08	g/day	121.2	ml/day
Concentration of ai	500	mg/ml	0.6	mg/ml
Dermal exposure to ai	40	mg/day	72.72	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	2.8	mg/day	5.0904	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.05	ml/h		
Duration of exposure	6	h		
Concentration of ai	0.6	mg/ml		
Inhalative exposure to ai	0.18	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.18	mg/day		

PREDICTED EXPOSURE				
Total absorbed dose	8.0704	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.1345**	**mg/kg bw/day**		

Appendix 5

UK POEM: TRACTOR DRAWN AIR ASSISTED ORCHARD SPRAYER			Gloves during mixing/loading	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	0.6	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	kg		
Hand contamination/operation	0.01	ml		
Application dose	1.2	kg product/ha		
Work rate	30	ha/day		
Number of operations	8	/day		
Hand contamination	0.08	g/day		
Protective clothing	Gloves			
Transmission to skin	1	%		
Dermal exposure to formulation	0.0008	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor drawn orchard sprayer with hydraulic nozzles			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	None	Permeable	Permeable	
Penetration	100	2	5	%
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.0008	g/day	121.2	ml/day
Concentration of ai	500	mg/ml	0.6	mg/ml
Dermal exposure to ai	0.4	mg/day	72.72	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	0.028	mg/day	5.0904	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.05	ml/h		
Duration of exposure	6	h		
Concentration of ai	0.6	mg/ml		
Inhalative exposure to ai	0.18	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.18	mg/day		

PREDICTED EXPOSURE				
Total absorbed dose	5.2984	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.0883**	**mg/kg bw/day**		

Appendix 6

UK POEM: TRACTOR DRAWN AIR ASSISTED ORCHARD SPRAYER			Gloves during mixing/loading and appl.	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	0.6	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	kg		
Hand contamination/operation	0.01	ml		
Application dose	1.2	kg product/ha		
Work rate	30	ha/day		
Number of operations	8	/day		
Hand contamination	0.08	g/day		
Protective clothing	Gloves			
Transmission to skin	1	%		
Dermal exposure to formulation	0.0008	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor drawn orchard sprayer with hydraulic nozzles			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	Gloves	Permeable	Permeable	
Penetration	10	2	5	%
Dermal exposure	4	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85.2	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.0008	g/day	85.2	ml/day
Concentration of ai	500	mg/ml	0.6	mg/ml
Dermal exposure to ai	0.4	mg/day	51.12	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	0.028	mg/day	3.5784	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.05	ml/h		
Duration of exposure	6	h		
Concentration of ai	0.6	mg/ml		
Inhalative exposure to ai	0.18	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.18	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	3.7864	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.0631**	**mg/kg bw/day**		

Appendix 7

UK POEM: LOW LEVEL HYDRAULIC KNAPSACK SPRAYER MODEL			PPE: None	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	1	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	1	kg		
Hand contamination/operation	0.01	ml		
Application dose	1.2	kg product/ha		
Work rate	1	ha/day		
Number of operations	40	/day		
Hand contamination	0.4	g/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to formulation	0.4	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Knapsack, hydraulic nozzle, low level			
Application volume	600	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
Clothing	None	Permeable	Permeable	
Penetration	100	20	18	%
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.4	g/day	102	ml/day
Concentration of ai	500	mg/ml	1	mg/ml
Dermal exposure to ai	200	mg/day	102	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	14	mg/day	7.14	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.02	ml/h		
Duration of exposure	6	h		
Concentration of ai	1	mg/ml		
Inhalation exposure to ai	0.12	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.12	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	21.26	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.3543**	**mg/kg bw/day**		

Appendix 8

UK POEM: LOW LEVEL HYDRAULIC KNAPSACK SPRAYER MODEL			Gloves during mixing/loading	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WDG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	1	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	1	kg		
Hand contamination/operation	0.01	ml		
Application dose	1.2	kg product/ha		
Work rate	1	ha/day		
Number of operations	40	/day		
Hand contamination	0.4	g/day		
Protective clothing	Gloves			
Transmission to skin	1	%		
Dermal exposure to formulation	0.004	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Knapsack, hydraulic nozzle, low level			
Application volume	600	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25	25	50	%
Clothing	None	Permeable	Permeable	
Penetration	100	20	18	%
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.004	g/day	102	ml/day
Concentration of ai	500	mg/ml	1	mg/ml
Dermal exposure to ai	2	mg/day	102	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	0.14	mg/day	7.14	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.02	ml/h		
Duration of exposure	6	h		
Concentration of ai	1	mg/ml		
Inhalation exposure to ai	0.12	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.12	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	7.4	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.1233**	**mg/kg bw/day**		

Appendix 9

UK POEM: LOW LEVEL HYDRAULIC KNAPSACK SPRAYER MODEL			Gloves during mixing/loading and appl.	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	1	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	1	kg		
Hand contamination/operation	0.01	ml		
Application dose	1.2	kg product/ha		
Work rate	1	ha/day		
Number of operations	40	/day		
Hand contamination	0.4	g/day		
Protective clothing	Gloves			
Transmission to skin	1	%		
Dermal exposure to formulation	0.004	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Knapsack, hydraulic nozzle, low level			
Application volume	600	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25	25	50	%
Clothing	Gloves	Permeable	Permeable	
Penetration	10	20	18	%
Dermal exposure	1.25	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	49.5	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.004	g/day	49.5	ml/day
Concentration of ai	500	mg/ml	1	mg/ml
Dermal exposure to ai	2	mg/day	49.5	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	0.14	mg/day	3.465	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.02	ml/h		
Duration of exposure	6	h		
Concentration of ai	1	mg/ml		
Inhalation exposure to ai	0.12	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.12	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	3.725	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.0621**	**mg/kg bw/day**		

Appendix 10

UK POEM: TRACTOR-MOUNTED DRAWN FIELD CROP SPRAYER WITH HYDRAULIC NOZZLES.			PPE: None	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	1.667	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	kg		
Hand contamination/operation	0.01	ml		
Application dose	1	kg product/ha		
Work rate	50	ha/day		
Number of operations	10	/day		
Hand contamination	0.1	g/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to formulation	0.1	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor drawn field crop sprayer with hydraulic nozzles			
Application volume	300	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65	10	25	%
Clothing	None	Permeable	Permeable	
Penetration	100	5	15	%
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.1	g/day	41.55	ml/day
Concentration of ai	500	mg/ml	1.667	mg/ml
Dermal exposure to ai	50	mg/day	69.264	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	3.5	mg/day	4.8485	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	ml/h		
Duration of exposure	6	h		
Concentration of ai	1.667	mg/ml		
Inhalation exposure to ai	0.1	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.1	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	8.4485	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.1408**	**mg/kg bw/day**		

Appendix 11

UK POEM. TRACTOR-MOUNTED/DRAWN FIELD CROP SPRAYER WITH HYDRAULIC NOZZLES.			Gloves during mixing/loading	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	1.667	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	kg		
Hand contamination/operation	0.01	ml		
Application dose	1	kg product/ha		
Work rate	50	ha/day		
Number of operations	10	/day		
Hand contamination	0.1	g/day		
Protective clothing	Gloves			
Transmission to skin	1	%		
Dermal exposure to formulation	0.001	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor drawn field crop sprayer with hydraulic nozzles			
Application volume	300	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65	10	25	%
Clothing	None	Permeable	Permeable	
Penetration	100	5	15	%
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.001	g/day	41.55	ml/day
Concentration of ai	500	mg/ml	1.667	mg/ml
Dermal exposure to ai	0.5	mg/day	69.264	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	0.035	mg/day	4.8485	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	ml/h		
Duration of exposure	6	h		
Concentration of ai	1.667	mg/ml		
Inhalation exposure to ai	0.1	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.1	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	4.9835	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.0831**	**mg/kg bw/day**		

Appendix 12

UK POEM: TRACTOR-MOUNTED/DRAWN FIELD CROP SPRAYER WITH HYDRAULIC NOZZLES.			Gloves during mixing/loading and appl.	
PRODUCT DATA				
Product	BAS 510 01 F			
Active substance	Nicobifen			
Concentration	500	mg/g		
Formulation type	WG			
Main solvent				
Concentration of solvent		w/w		
Maximum in-use ai concentration	1.667	mg/ml		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	kg		
Hand contamination/operation	0.01	ml		
Application dose	1	kg product/ha		
Work rate	50	ha/day		
Number of operations	10	/day		
Hand contamination	0.1	g/day		
Protective clothing	Gloves			
Transmission to skin	1	%		
Dermal exposure to formulation	0.001	g/day		
EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor- drawn field crop sprayer with hydraulic nozzles			
Application volume	300	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65	10	25	%
Clothing	Gloves	Permeable	Permeable	
Penetration	10	5	15	%
Dermal exposure	0.65	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	6.45	ml/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.001	g/day	6.45	ml/day
Concentration of ai	500	mg/ml	1.667	mg/ml
Dermal exposure to ai	0.5	mg/day	10.752	mg/day
Percent absorbed	7	%	7	%
Absorbed dose	0.035	mg/day	0.7527	mg/day
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	ml/h		
Duration of exposure	6	h		
Concentration of ai	1.667	mg/ml		
Inhalation exposure to ai	0.1	mg/day		
Percent absorbed	100	%		
Absorbed dose	0.1	mg/day		
PREDICTED EXPOSURE				
Total absorbed dose	0.8877	mg/day		
Operator body weight	60	kg		
Operator exposure	**0.0148**	**mg/kg bw/day**		

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[5]
AIIA-5.1	Grosshans, F. and Knoell, H.-E.	2001	The metabolism of 14C-BAS 510F (reg. no. 300355) in rats. 41855 ! 2000/1017220 GLP, unpublished TOX2001-706	Y	BAS
AIIA-5.1	Leibold, E. and Hoffmann, H.D.	2001	14C-BAS 510 F - Study of the dermal absorption in rats. 01B0426/976047 ! 2001/1000111 GLP, unpublished TOX2001-704	Y	BAS
AIIA-5.1	Leibold, E., Hoffmann, H.D. and Hildebrand, B.	2000	14C-BAS 510 F - Study of the biokinetics in rats. 02B0426/976030 ! 2000/1014183 GLP, unpublished TOX2001-703	Y	BAS
AIIA-5.1	Thornley, K. and Bryson, S.	2001	(14C)-BAS 510F: Rates of penetration through rat and human skin using an in vitro system. 729/204 ! 53H0179/979094 ! 2001/1000112 GLP, unpublished TOX2001-705	Y	BAS
AIIA-5.2.1	Wiemann, C.	2000	Amendment no. 1: BAS 510 F - Acute oral toxicity in rats. 10A0179/971052 ! 2000/1018715 GLP, unpublished TOX2001-708	Y	BAS
AIIA-5.2.1	Wiemann, C. and Hellwig, J.	1998	BAS 510 F - Acute oral toxicity in rats. 10A0179/971052 ! #BASF 98/10643 GLP, unpublished TOX2001-707	Y	BAS
AIIA-5.2.2	Wiemann, C.	2000	Amendment no. 1: BAS 510 F - Acute dermal toxicity in rats. 11A0179/971053 ! 2000/1018711 GLP, unpublished TOX2001-710	Y	BAS
AIIA-5.2.2	Wiemann, C. and Hellwig, J.	1998	BAS 510 F - Acute dermal toxicity in rats. 11A0179/971053 ! #BASF 98/10642 GLP, unpublished TOX2001-709	Y	BAS
AIIA-5.2.3	Gamer, A.O. and Hoffmann, H.D.	1998	BAS 510 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure. 13I0179/977011 ! #BASF 98/10803 GLP, unpublished TOX2001-711	Y	BAS

[5] 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[5]
AIIA-5.2.4	Wiemann, C.	2000	Amendment no. 1: BAS 510 F - Acute dermal irritation / corrosion in the rabbit. 14H0179/972089 ! 2000/1018712 GLP, unpublished TOX2001-713	Y	BAS
AIIA-5.2.4	Wiemann, C. and Hellwig, J.	1998	Study on the acute dermal irritation/corrosion of BAS 510 F in the rabbit. 14H0179/972089 ! #BASF 98/10640 GLP, unpublished TOX2001-712	Y	BAS
AIIA-5.2.5	Wiemann, C.	2000	Amendment no. 1: BAS 510 F - Acute eye irritation in the rabbit. 13H0179/972090 ! 2000/1018713 GLP, unpublished TOX2001-715	Y	BAS
AIIA-5.2.5	Wiemann, C. and Hellwig, J.	1998	Study on the acute eye irritation of BAS 510 F in the rabbit. 13H0179/972090 ! #BASF 98/10641 GLP, unpublished TOX2001-714	Y	BAS
AIIA-5.2.6	Wiemann, C.	2000	Amendment no. 1: BAS 510 F - Maximization test in guinea pigs. 30H0179/972091 ! 2000/1018714 GLP, unpublished TOX2001-717	Y	BAS
AIIA-5.2.6	Wiemann, C. and Hellwig, J.	1998	BAS 510 F - Maximization test in guinea pigs. 30H0179/972091 ! #BASF 98/10638 GLP, unpublished TOX2001-716	Y	BAS
AIIA-5.3.1	Mellert, W., Deckardt, K., Kaufmann, W. and Hildebrand, B.	2000	BAS 510 F - Repeated dose dermal toxicity study in Wistar rats administration for 4 weeks. 33C0179/97151 ! 2000/1013240 GLP, unpublished TOX2001-718	Y	BAS
AIIA-5.3.2	Mellert, W., Deckardt, K., Kaufmann, W. and Hildebrand, B.	2000	BAS 510 F - Subchronic oral toxicity study in C57BL mice administration in the diet for 3 months. 60C0179/97060 ! 2000/1000188 GLP, unpublished TOX2001-720	Y	BAS
AIIA-5.3.2	Mellert, W., Deckardt, K., Kaufmann, W. and Hildebrand, B.	2000	BAS 510 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months. 50S0179/97058 ! 2000/1012190 GLP, unpublished TOX2001-719	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[5]
AIIA-5.3.2	Schilling, K., Deckardt, K., Kaufmann, B. and Hildebrand, B.	2000	BAS 510 F - Subchronic oral toxicity study in beagle dogs administration in the diet for 3 months. 31D0179/97101 ! 2000/1012306 GLP, unpublished TOX2001-721	Y	BAS
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	1999	In vitro chromosome aberration assay with BAS 510 F in V79 cells. 32M0179/974076 ! 1999/10978 GLP, unpublished TOX2001-724	Y	BAS
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	2000	In vitro unscheduled DNA synthesis (UDS) assay with BAS 510 F in primary rat hepatocytes. 81M0179/974096 ! 2000/1011413 GLP, unpublished TOX2001-725	Y	BAS
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	2000	In vitro gene mutation test with BAS 510 F in CHO cells (HPRT locus assay). 50M0179/974097 ! 2000/1000180 GLP, unpublished TOX2001-723	Y	BAS
AIIA-5.4.1	Engelhardt, G. and Hoffmann, H.D.	1998	Salmonella typhimurium/escherichia coli reverse mutation assay (standard plate test and preincubation test) with BAS 510 F (reg. no. 300 355). 40M0179/974089 ! 98/11440 GLP, unpublished TOX2001-722	Y	BAS
AIIA-5.4.2	Engelhardt, G. and Hoffmann, H.D.	1999	Cytogenetic study in vivo with BAS 510 F in the mouse micronucleus test after two intraperitoneal administrations. 26M0179/974095 ! 1999/11048 GLP, unpublished TOX2001-726	Y	BAS
AIIA-5.5	Mellert, W., Deckardt, K., Kaufmann, W., Heider, K. and Van Ravenzwaay, B.	2001	BAS 510 F - Carcinogenicity study in Wistar rats administration in the diet for 24 months. 82C0179/97090 ! 2001/1000115 GLP, unpublished TOX2001-729	Y	BAS
AIIA-5.5	Mellert, W., Deckardt, K., Kaufmann, W., Heider, K. and Van Ravenzwaay, B.	2001	BAS 510 F - Chronic toxicity study in Wistar rats administration in the diet for 24 months. 82C0179/97091 ! 2001/1000114 GLP, unpublished TOX2001-728	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[5]
AIIA-5.5	Mellert, W., Deckardt, K., Küttler, K. and Van Ravenzwaay, B.	2001	BAS 510 F - Carcinogenicity study in C57BL mice administration in the diet for 18 months. 76C0179/97103 ! 2001/1000116 GLP, unpublished TOX2001-730	Y	BAS
AIIA-5.5	Wiemann, C., Deckardt, K., Kaufmann, W., Kolling, A. and Hildebrand, B.	2000	BAS 510 F - Chronic oral toxicity study in beagle dogs administration in the diet for 12 months. 33D0179/97118 ! 2000/1016881 GLP, unpublished TOX2001-727	Y	BAS
AIIA-5.6.1	Schilling, K., Gembardt, C. and Van Ravenzwaay, B.	2001	BAS 510 F - Two-generation reproduction toxicity study in Wistar rats continuous dietary administration. 70R0179/97136 ! 2001/1000117 GLP, unpublished TOX2001-731	Y	BAS
AIIA-5.6.2	Schilling, K. and Hellwig, J.	2000	BAS 510 F - Prenatal developmental toxicity study in Himalayan rabbits oral administration (gavage). 40R0179/97127 ! 2000/1013425 GLP, unpublished TOX2001-733	Y	BAS
AIIA-5.6.2	Schilling, K. and Hellwig, J.	2000	BAS 510 F - Prenatal developmental toxicity study in Wistar rats oral administration (gavage). 30R0179/97140 ! 2000/1015001 GLP, unpublished TOX2001-732	Y	BAS
AIIA-5.7	Kaufmann, W., Schilling, K., Mellert, W. and Van Ravenzwaay, B.	2001	BAS 510 F - Developmental neurotoxicity study in Wistar rats administration in the diet. 67R0179/97167 ! 2001/1000118 GLP, unpublished TOX2001-736	Y	BAS
AIIA-5.7	Mellert, W., Kaufmann, W. and Hildebrand, B.	2000	BAS 510 F - Acute oral neurotoxicity study in Wistar rats. 20C0179/97144 ! 2000/1018638 GLP, unpublished TOX2001-734	Y	BAS
AIIA-5.7	Mellert, W., Kaufmann, W. and Van Ravenzwaay, B.	2001	BAS 510 F - Subchronic oral neurotoxicity study in Wistar rats administration in the diet for 3 months. 50C0179/97148 ! 2001/1000113 GLP, unpublished TOX2001-735	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[5]
AIIA-5.8.1	Anonym	1985	Chlorbenzoesäure und ihre Isomeren. German MAK Dokumentation, 1985, 1-4 1985/1000225 not GLP,published TOX2001-737	N	-
AIIA-5.8.2	Mellert, W., Deckardt, K., Leibold, E. and Van Ravenzwa-ay, B.	2001	BAS 510 F - Hormone and enzyme induction study in Wistar rats administration in the diet for 4 weeks. 99C0179/97174 ! 2001/1000141 GLP, unpublished TOX2001-739	Y	BAS
AIIA-5.8.2	Mellert, W., Kaufmann, W., Leibold, E., Deckardt, K. and Hildebrand, B.	1999	BAS 510 F - Hepatic enzyme induction study in Wistar rats administration in the diet for 2 weeks. 99C0179/97063 ! 1999/10522 GLP, unpublished TOX2001-738	Y	BAS
AIIA-5.10	Anonym	1999	Draft summary record: Commission group of specialised experts in the fields of carcinogeni-city, mutagenicity and reprotoxicity. Meeting at Arona, 1-2 September 1999. Euro-pean Chemicals Bureau., 1999, 1-12 1999/1004397 not GLP,published TOX2001-743	N	-
AIIA-5.10	Anonym	1979	Carbimazole and other antithyroid agents. Martindale The Extra Pharmacopoeia. Edited by N. W. Blacow and A. Wade, London The Pharmaceutical Press., 26, 1979, 379-385 1979/1000181 not GLP,published TOX2001-741	N	-
AIIA-5.10	Costigan, M.	1998	The relevance of rat thyroid gland tumours to humans. HSE Toxicology Unit Bootle., 1998, 1-14 1998/1001262 not GLP,published TOX2001-742	N	-
AIIA-5.10	Odell, W.D., Wilber, J.F. and Utiger, R.D.	1967	Studies of thyrotropin physiology by means of radioimmunoassay. Recent Progress in Hormone Research. Edited by Gregory Pincus, Academic Press, New York and London., 23, 1967, 47-85 #BASF 67/10025 not GLP,published TOX2001-740	N	-

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[5]
AIIIA-7.1.1	Gamer, A.O. and Hoffmann, H.D.	2001	BAS 510 01 F - Acute oral toxicity study in Wistar rats. 10A0295/001046 ! 2001/1000119 GLP, unpublished TOX2001-696	Y	BAS
AIIIA-7.1.2	Gamer, A.O. and Hoffmann, H.D.	2001	BAS 510 01 F - Acute dermal toxicity study in rats. 11A0295/001047 ! 2001/1000120 GLP, unpublished TOX2001-697	Y	BAS
AIIIA-7.1.3	Gamer, A.O. and Hoffmann, H.D.	2000	BAS 510 01 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure. 13A0295/007008 ! 2001/1000121 GLP, unpublished TOX2001-698	Y	BAS
AIIIA-7.1.4	Wiemann, C. and Hellwig, J.	2001	BAS 510 01 F - Acute dermal irritation / corrosion in rabbits. 18H0295/002086 ! 2001/1000122 GLP, unpublished TOX2001-699	Y	BAS
AIIIA-7.1.5	Wiemann, C. and Hellwig, J.	2001	BAS 510 01 F - Acute eye irritation in rabbits. 11H0295/002087 ! 2001/1000123 GLP, unpublished TOX2001-700	Y	BAS
AIIIA-7.1.6	Wiemann, C. and Hellwig, J.	2001	BAS 510 01 F - Modified Buehler Test (9 inductions) in guinea pigs. 33H0295/002088 ! 2001/1000124 GLP, unpublished TOX2001-701	Y	BAS
AIIIA-7.2.3.1	Anonym	1998	Policy science advisory council for exposure. From US EPA. #BASF 98/11675 not GLP,published TOX2001-702	N	-

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

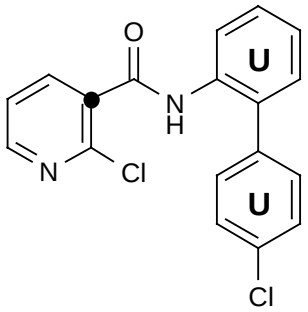
Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

B-7: Residue data

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 in plants of nicobifen was investigated using [diphenyl-U-^{14}C]-nicobifen and [pyridine-3-^{14}C]-nicobifen.



U = diphenyl label
● = 3-pyridine label

B.7.1.1 Grape

Report:	Rabe U., Schlueter H. 2001 Metabolism of 14C-BAS 510 F in grapevine BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. unpublished BASF RegDoc# 2000/1014860
Test material:	[diphenyl-U-^{14}C]-nicobifen, batch no. 641-1018, radiochemical purity: > 99 %, specific activity: 5.23 MBq/mg [pyridine-3-^{14}C]-nicobifen, batch no. 640-1026, radiochemical purity: > 99 %, specific activity: 5.81 MBq/mg
Guidelines:	EPA 860.1300, EPA 860.1000, BBA IV 3-2
GLP:	Yes
Acceptability:	Yes

Material and Methods

In the plant-uptake part of the study, ^{14}C-nicobifen was applied in the form of a nicobifen SC-formulation to grapevines (variety: Mueller-Thurgau). Three post-emergence applications at use rates of 800 g as/ha each were performed. The first application was performed at growing stage 68 – 69 (BBCH code, 80% flowerhoods fallen to end of flowering), the second with an interval of 12 days at growth stage 71 and the third 41 days after the second application at growth stage 81 (beginning of ripening).

An intermediate sampling of leaves was done 15 days after the third application on occasion of thinning out the foliage. No further work was done with this sample. The final harvest was at growth stage 89 (PHI 45 days). The samples were separated into grapes, stalks and leaves

and stored in a freezer until analysis. An overview of the plant-uptake study design is given in Table B.7.1-1.

Table B.7.1-1: Design of the plant uptake part – grape

	Diphenyl label	Pyridine label
Total application rate [g as/ha/year]	2400	2400
Number of applications	3	3
Interval between applications [days]	12, 41	12, 41
Comparison to the maximum recommended use rate	4 x	4 x
Sampling [days after last application]	45 (15)	45 (15)

The samples from both labels were sequentially extracted with methanol and water and the radioactivity was determined by LSC. The radioactive residues in the post extraction solid were determined by combustion. The TRR was calculated as the sum of extractable radioactivity and residual radioactivity in the residue.

In order to classify the extracted radioactivity into organosoluble and water-soluble components, a liquid/liquid partition between cyclohexane/water, followed by ethyl acetate/water was carried out. For a more detailed determination of the nature of the residues the methanol extracts, in some cases, the water extracts and the phases after partition were analysed by radio HPLC. Metabolite identification was performed by comparison with reference substances or by LC/MS after isolation by HPLC.

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

The residues in grapes were much lower compared to the rest of the plant. For the diphenyl label a TRR of 1.2 mg/kg was detected for grapes whereas the TRR in stalks was 12.4 mg/kg and in leaves 43.7 mg/kg. For the pyridine label the TRR in grapes was 2.1 mg/kg whereas a TRR of 19.6 mg/kg in stalks and of 63.4 mg/kg in leaves was found.

For all sample materials, the solvent extractability was very high. For both labels it was about 93 % of the TRR in grapes, about 96% - 97 % in stalks and about 98 % in leaves. Due to the low level of non extractable residues (≤ 7.3 %), no further investigations with the residual residues were performed.

The extraction behaviour of nicobifen is summarised in Table B.7.1-2.

In order to classify the metabolites into organo-soluble and water-soluble ones, liquid/liquid partition experiments were carried out. For both labels most of the radioactive residues in the methanol extracts of all matrices were organo-soluble and only a small amount was detected in the water phase (0.2 – 1.1 %TRR) after partition.

Table B.7.1-2: Total radioactive residues and extractability of radioactivity in grape matrices 45 days after last treatment with ^{14}C-Nicobifen

Matrix	TRR (ERR + RRR)		Methanol		Water		ERR		RRR	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Diphenyl label										
Grapes	1.181	100	1.095	92.7	0.005	0.4	1.100	93.2	0.081	6.8
Stalks	12.356	100	11.810	95.6	0.104	0.8	11.914	96.4	0.442	3.6
Leaves	43.672	100	42.801	98.0	n.d.		42.801	98.0	0.871	2.0
Pyridine label										
Grapes	2.066	100	1.905	92.2	0.009	0.5	1.916	92.7	0.150	7.3
Stalks	19.637	100	19.054	97.0	0.108	0.5	19.162	97.6	0.475	2.4
Leaves	63.359	100	62.031	97.9	n.d.		62.031	97.9	1.328	2.1

n. d. = not determined

HPLC analysis of the methanol and water extracts showed that most of the radioactive residues were unchanged parent. In grapes 92.7% of the TRR were represented by the unchanged parent nicobifen for the diphenyl label and 92.2% for the pyridine label. In stalks 96.4% for the diphenyl label and 97.5% for the pyridine label were found to be the parent substance.

In leaves nicobifen amounted to 95.6% TRR and to 96.1% TRR after treatment with the diphenyl label and the pyridine label, respectively. Additionally, small amounts of a metabolite more polar than nicobifen were found in the methanol extract of leaves. This metabolite amounted to approximately 2% TRR in leaves of both labels (diphenyl and pyridine). As grapevine leaves are neither a food nor a feed item, this metabolite was not further investigated.

Table B.7.1-3: Summary of identified and unidentified components after treatment with diphenyl labelled and pyridine labelled nicobifen

Metabolite Code	Grapes		Stalks		Leaves	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Diphenyl label						
Nicobifen	1.095	92.7	11.914	96.4	41.752	95.6
Unidentified					1.049	2.4
Non extractable residue	0.081	6.8	0.442	3.6	0.871	2.0
Total	1.176	99.5	12.356	100.0	43.672	100.0
Pyridine label						
Nicobifen	1.905	92.2	19.152	97.5	60.859	96.1
Unidentified			0.010	0.1	1.172	1.8
Non extractable residue	0.150	7.3	0.475	2.4	1.328	2.1
Total	2.055	99.5	19.637	100.0	63.359	100.0

Storage stability

The storage stability investigations in grapes showed that within a storage time of 16 months for both labels, no major differences in the extractability were found. During HPLC analysis minor traces of radioactivity more polar than nicobifen were found, indicating a slight degra-

dation during the storage period. Nevertheless, the storage stability was sufficient over the period of 16 months.

Conclusion

The investigation of the metabolism of nicobifen in grapevines using material labelled in either the diphenyl or the pyridine ring showed that the parent compound itself is the only relevant residue in grapes, grape stalks and leaves of grapevines following three applications and a pre-harvest interval of 45 days. The good extractability and the low level of non-extractable residues in all the matrices indicated a slow metabolisation and conjugation to plant assimilates.

B.7.1.2 Lettuce

Report: Hamm R.T. 1999
Metabolism of BAS 510 F in lettuce
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/11240

Test material: [diphenyl-U-^{14}C]-nicobifen, batch no. 641-1018, radiochemical purity: > 99 %, specific activity: 5.23 MBq/mg
[pyridine-3-^{14}C]-nicobifen, batch no. 640-1026, radiochemical purity: > 99 %, specific activity: 5.81 MBq/mg

Guidelines: EPA 860.1300, BBA IV 3-2

GLP: Yes

Acceptability: Yes

Material and Methods

In the plant-uptake part of the study, ^{14}C-nicobifen was applied in the form of a SC-formulation to lettuce (variety: Nadine). Three post-emergence applications at use rates of 700 g as/ha each were performed. The first application was performed 8 days after planting, the second and third application 14 days later, respectively.

The study was performed in a greenhouse and a vegetation hall and only one sampling was done 18 days after the third application at growth stage BBCH 49. An overview of the plant-uptake study design is given in Table B.7.1-4.

Table B.7.1-4: Design of the plant uptake part - lettuce

	Diphenyl label	Pyridine label
Total application rate [g as/ha/year]	2100	2100
Number of applications	3	3
Comparison to the maximum recommended use rate	2.6 x	2.6 x
Interval between applications [days]	14	14
Sampling [days after last application]	18	18

The samples from both labels were extracted with methanol and the radioactivity was determined by LSC. The radioactive residues in the post extraction solid were determined by combustion. The total radioactive residues were calculated as the sum of extractable and non-extractable radioactivity.

For a more detailed determination of the nature of the residues the methanol extracts were analysed by radio HPLC. Identification was performed by comparison with reference substance and by LC/MS after isolation by HPLC.

Because of the shortness of analysis time (64 - 109 days after sampling) a storage stability analysis was not performed.

Findings

The calculated TRR levels of leaves treated with the two labels corresponded well amounting to 17.5 mg/kg (diphenyl label) and 17.6 mg/kg (pyridine label). The residue levels were relatively high because the pre-harvest interval was only 18 days.

About 99% of the TRR (diphenyl and pyridine label) could be extracted by a threefold methanol extraction. Nicobifen showed almost no metabolisation to plant assimilates. The extraction behaviour of nicobifen is summarised in Table B.7.1-5.

Because of the low residual radioactive residues (RRR < 1 %) no further investigation with these residues were performed.

Table B.7.1-5: Total radioactive residues and extractability of radioactivity in lettuce matrices after treatment with ^{14}C-Nicobifen

Matrix	TRR		Methanol		ERR		RRR	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Diphenyl label								
Leaves	17.541	100	17.412	99.3	17.412	99.3	0.129	0.7
Pyridine label								
Leaves	17.622	100	17.507	99.3	17.507	99.3	0.115	0.7

n. d. = not determined

In the methanol extracts analysed by radio - HPLC, the only peak was unchanged parent. The structure was confirmed by LC/MS/MS.

Conclusion

The investigation of the metabolism of nicobifen in lettuce using material labelled in either the diphenyl or the pyridine ring showed that the parent compound itself is the only relevant residue following three applications and a pre-harvest interval of 18 days.

The good extractability and the low level of non-extractable residues indicated a slow metabolisation and conjugation to plant assimilates.

B.7.1.3 Beans

Report:	Veit P. 2001 Metabolism of 14C-BAS 510 F in beans BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. unpublished BASF RegDoc# 2000/1014861
Test material:	[diphenyl-U-^{14}C]-nicobifen, batch no. 641-2017, radiochemical purity: > 99 %, specific activity: 6.27 MBq/mg [pyridine-3-^{14}C]-nicobifen, batch no. 640-1026, radiochemical purity: > 99 %, specific activity: 5.81 MBq/mg
Guidelines:	EPA 860.1300, BBA IV 3-10, EPA 860.1000
GLP:	Yes
Acceptability:	Yes

Material and Methods

Green beans (variety: Hild´s Maxi) were grown in plastic pots in a growth chamber or in a glass house. The bean plants were separately treated with SC-formulations of [diphenyl-U-^{14}C]-nicobifen or [pyridine-3-^{14}C]-nicobifen. Three applications of the test substance were performed, each at an application rate of 500 g as/ha. The first application was carried out at the beginning of flowering, the second application 8 – 10 days later and the third application 8 – 10 days after the second. The tests with the different labels were not done in the same time period. The pyridine label was applied at the end of February and the plants were grown in a growth chamber. The diphenyl label was applied in the beginning of March of the following year and the plants were grown in a glasshouse.

Bean plant samples were collected directly after the last treatment. At 14/15 days after the last treatment, bean forage and green beans were harvested. The green beans were separated into pods and seeds and analysed individually to cover other bean varieties. At 53/51 days after the last treatment, bean straw, dry pods and dry seeds were harvested. An overview of the design of the in-life part is given in Table B.7.1-6.

Table B.7.1-6: Design of the plant uptake part – beans

	Diphenyl label	Pyridine label
Application rate [g as/ha]	500	500
Number of applications	3	3
Interval between applications [days]	8 - 10	8 – 10
Comparison to the maximum recommended use rate	1,5 x	1.5 x
Sampling [days after last application]	0 plant 14 forage green beans 53 bean straw, bean pots, bean seeds	0 plant 15 forage green beans 51 bean straw, bean pots, bean seeds

The samples from both labels were sequentially extracted with methanol and water and the radioactivity was determined by LSC. The radioactive residues in the post extraction solid were determined by combustion. The total radioactive residues were calculated as the sum of extractable and non-extractable radioactivity. In order to classify the extracted radioactivity into organosoluble and water-soluble components, a liquid/liquid partition between n-hexane/ water, followed by ethyl acetate/water or only ethyl acetate/water was carried out.

For a more detailed determination of the nature of the residues the methanol extracts, in some cases, the water extracts and the phases after partition were analysed by radio HPLC. Metabolite identification was performed by comparison with reference substances or, where possible, by LC/MS/MS after isolation by HPLC. Chloronicotinic acid was identified by comparison with pure substance in regard to retention times and behaviour after derivatisation. Chlorophenylaminobenzene was identified by co-chromatography.

For characterisation of the radioactivity in the post extraction solid, the residues after methanol and water extraction were treated with an aqueous ammonia solution. In some cases the ammonia extract was analysed by HPLC. The residues from bean straw (diphenyl label) were examined for radioactivity associated with cellulose and starch. Different methods, such as enzymes treatments, refluxing with sodium hydroxide and treatment with dimethylsulfoxide were applied.

Findings

Total radioactive residues

The TRR values for the diphenyl label were, in all cases, higher than for the pyridine label. The reason might be that the two parts of the study were conducted a year apart. The pattern and the ratio of the TRR values of the two labels were similar for the different matrices.

The residues in the edible parts of the crop were much lower compared to the rest of the plant. The total radioactive residues (TRR) in green beans 14/15 DALT amounted to 1.03 mg/kg (diphenyl label) and to 0.09 mg/kg (pyridine label). After separation of green beans into pods and seeds, the major part of radioactivity was found in the pods (diphenyl/pyridine label pods 14/15 DALT: 0.90 mg/kg/ 0.11 mg/kg and seeds 14/15 DALT: 0.20 mg/kg/ 0.07 mg/kg).

In bean forage 14/15 DALT, the radioactive residues were 66.24 mg/kg for the diphenyl label and for 16.97 mg/kg for the pyridine label.. The highest amount of residues were found in bean straw 53/51 DALT (diphenyl label: 127.3 mg/kg and pyridine label: 93.8 mg/kg).

The residue levels in dry seeds 53/51 DALT were lower than in dry pods 53/51 DALT (dry seeds: 0.21/0.13 mg/kg diphenyl/pyridine label; dry pods: 6.12/1.37 mg/kg diphenyl/ pyridine label), as already seen for green pods and seeds. This comparison indicated that there was only a minor translocation of the parent compound and its degradation products into the edible part of the crop. The total radioactive residues are summarised in Table B.7.1-7.

Extractability and organo solubility

The different matrices were extracted with methanol followed by water. In all sample materials, the solvent extractability was high. Methanol could release the major part of the radioactive residues (≥ 89/75 % TRR diphenyl/pyridine label; for dry pods: 75.0/47.6 % TRR diphenyl/pyridine label). The radioactivity levels in the water extracts were low (0.3 – 2.4/ 0.4 – 14.6 % TRR diphenyl/pyridine label; for dry seeds: 5.5/21.3 % TRR diphenyl/pyridine label).The results are summarised in Table B.7.1-7.

For further characterisation, a liquid/liquid partition was carried out with the methanol extracts. Most of the radioactive residues in the methanol extracts of bean plant, forage and straw were organosoluble and only a small amount was detected in the water phase (0.4 – 2.2% TRR both labels) after partition. For the phenyl label, the concentration in the water phase of green beans, pods, seeds, dry pods and dry seeds ranged from 1.1 – 2.2 % TRR and the rest of the radioactive residues in the methanol extracts was organosoluble. For the pyridine label the concentration in the water phase of green beans, pods, dry pods and dry seeds was higher and ranged from 11.0 – 17.4% TRR. The highest value in % TRR in the water phase was detected in seeds (51.4% TRR).

Residual residues

The remaining radioactive residues were in most cases very low. Except for bean seeds, they were higher in pyridine label than in diphenyl label. In the green plant matrices: plant, forage and green beans the residue levels ranged from 0.5 – 1.9/1.5 – 2.1 % TRR diphenyl/pyridine label. In bean pots and seeds the residue levels ranged from 1.9 - 8.6/2.0 – 4.1 % TRR diphenyl/pyridine label. In the dry matrices the residue levels were slightly higher for straw (3.3/5.6 % TRR) and dry pods (4.6/9.7% TRR). The highest levels of residual radioactive residues in % TRR were found in dry seeds (19.5/31.1 % TRR diphenyl/pyridine label). The extraction results are shown in Table B.7.1-7.

Part of the remaining radioactivity was released by extraction with an aqueous ammonia solution. For bean plant, forage and straw the radioactivity in the ammonia extract ranged from 0.2 – 0.7 % TRR (for both labels) and for dry pods and dry seeds it ranged from 1.2 – 10.4 % TRR.. The results are shown in Table B.7.1-8 and Table B.7.1-9.

In bean straw diphenyl label, the residues after ammonia extraction (3.06 mg/kg / 2.4 % TRR) were further investigated. Aliquots were taken and separately treated with enzymes, NaOH and DMSO. Treatment with macerozyme and cellulase released a portion of the radioactive residues corresponding to cellulose (0.53 mg/kg / 0.4 % TRR). Extraction with NaOH released 0.95 mg/kg / 0.7 % TRR and with DMSO released 1.87 mg/kg / 1.4 % TRR. After precipitation with ethanol, almost no radioactive starch could be detected.

Table B.7.1-7: Total radioactive residues and extractability of radioactivity in bean matrices after treatment with ^{14}C-nicobifen

Crop parts Days after last treatment DALT	TRR ERR + RRR		Methanol		Water		ERR		RRR	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Diphenyl label										
Bean Plant (0)	49.091	100	48.714	99.2	0.137	0.3	48.851	99.5	0.239	0.5
Bean Forage (14)	66.236	100	65.117	98.3	0.525	0.8	65.642	99.1	0.595	0.9
Green Beans (14)	1.027	100	1.003	97.6	0.007	0.7	1.010	98.3	0.017	1.7
Bean Pods (14)	0.903	100	0.878	97.3	0.008	0.9	0.886	98.2	0.017	1.9
Bean Seeds (14)	0.198	100	0.176	89.0	0.005	2.4	0.181	91.4	0.017	8.6
Bean Straw (53)	127.285	100	122.037	95.9	1.101	0.9	123.138	96.8	4.147	3.3
Bean Dry Pods (53)	6.118	100	5.772	94.3	0.068	1.1	5.840	95.4	0.279	4.6
Bean Dry Seeds (53)	0.205	100	0.154	75.0	0.011	5.5	0.165	80.5	0.040	19.5
Pyridine label										
Bean Plant (0)	21.249	100	20.704	97.4	0.158	0.7	20.862	98.1	0.387	1.8
Bean Forage (15)	16.967	100	16.579	97.7	0.133	0.8	16.712	98.5	0.255	1.5
Green Beans (15)	0.090	100	0.088	97.4	<0.001	0.5	0.088	97.9	0.002	2.1
Bean Pods (15)	0.108	100	0.106	97.6	<0.001	0.4	0.106	98.0	0.002	2.0
Bean Seeds (15)	0.067	100	0.063	94.7	0.001	1.2	0.064	95.9	0.003	4.1
Bean Straw (51)	93.775	100	79.801	85.1	8.708	9.3	88.509	94.4	5.265	5.6
Bean Dry Pods (51)	1.369	100	1.037	75.8	0.199	14.6	1.236	90.4	0.132	9.7
Bean Dry Seeds (51)	0.126	100	0.060	47.6	0.027	21.3	0.087	68.9	0.039	31.1

Metabolites

In all methanol extracts analysed with HPLC, parent nicobifen was the main peak. The cleavage products chlorophenylaminobenzene (M510F62) and chloronicotinic acid (M510F47) were detected in most of the samples. Furthermore in almost all samples, a range of polar peaks were detected in the ethyl acetate and water phases after partition of the methanol extracts. Some of those peaks were identified in bean straw by LC/MS as hydroxy parent and sugar conjugates of nicobifen.

In *bean plant and forage* ≥ 98.1 % TRR (both labels) were identified as parent. In bean plant of the pyridine label only parent was detected in all phases (methanol, hexane, ethyl acetate, water). In the other bean plant and forage samples, also a range of small peaks were found in the ethyl acetate phase and the water phase after partition.

In *green beans and bean pods* from the diphenyl label, the situation was very similar and 97.2/ 96.7% TRR were identified as parent. In *seeds*, parent accounted for 87.5 % TRR. In *green beans* and in *seeds*, chlorophenylaminobenzene (M510F62) was identified in the n-hexane phase. Additionally, some minor peaks were detected in green beans and in pods.

In *green beans, pods and seeds* from the pyridine label, the parent concentration was lower than from the diphenyl label (78.1 % TRR in green beans, 87.0 % TRR in pods and 64.9 % TRR in seeds). In addition, a very polar degradation product was detected with highest levels in green beans at 0.014 mg/kg / 15.3 % TRR. After partition of the methanol extract, this peak was found in the water phase and resolved into more than one peak. Also, chloronicotinic acid (M510F47) was identified in very low concentrations. After partition of the methanol extract, chloronicotinic acid was found in the water phase.

In *bean straw*, ≥ 93.6 % TRR (both labels) was identified as parent in the methanol and in the water extract. In addition, a wide range of polar peaks were detected. For the diphenyl label, most of those peaks were seen in both the ethyl acetate and the water phase, after partition of the methanol extract. Some of those peaks were isolated and identified by LC/MS as hydroxy-metabolites of nicobifen, where the OH-group was attached either to the phenyl-ring or the pyridine-ring, or as sugar conjugates of nicobifen (see Figure B.7.1-1). Also a cleavage between the both ring systems was observed but this reaction was less pronounced.

In *dry pods*, 79.7 % TRR were identified as parent for the pyridine label and 94.5 % TRR as parent for the diphenyl label. For the pyridine label, chloronicotinic acid was found in a concentration of 1.1 % TRR and there was, in addition, a range of small peaks. Also for the diphenyl label, a number of small peaks were detected, mainly in the ethyl acetate and water phase.

In *dry seeds* from the diphenyl label, 72 % TRR were identified as parent and, in addition, some small polar and non-polar peaks were seen. In dry seeds from the pyridine label, the concentration of parent was much lower and amounted to 36.9 % TRR in the methanol extract. In addition, chloronicotinic acid and some other polar peaks were detected.

The *ammonia extracts* of bean plant, forage, straw and dry pods of the diphenyl label (0.2 – 1.2 % TRR) were analysed by HPLC. Parent was found in a concentration range of 0.05 – 0.25 % TRR. Also, a wide range of polar peaks were detected with a major one in all the samples under investigation. The concentration of this peak ranged from 0.09 – 0.55 % TRR. In the ammonia extracts of bean plant, forage and straw from the pyridine label (0.5 – 3.4 % TRR), ≥ 95.4% of the radioactivity and for dry pods 75.7 % was identified as parent.

Table B.7.1-8: Summary of identified and unidentified components in beans after treatment with diphenyl labelled nicobifen

Metabolite Code	Bean plant 0 DALT	Forage 14 DALT	Green beans 14 DALT	Pods 14 DALT	Seeds 14 DALT	Straw 53 DALT	Dry pods 53 DALT	Dry seeds 53 DALT
	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Identified:								
Nicobifen	48.724 (99.3)	66.269 (98.6)	0.999 (97.2)	0.872 (96.7)	0.173 (87.5)	120.968 (95.1)	5.788 (94.5)	0.148 (72.0)
M510F62 (chlorophenylaminoben-zene)		+	+		+	0.610 (0.5)		
Characterised:								
Extractable residues	0.122 (0.3)	0.373 (0.6)	0.011 (1.1)	0.014 (1.5)	0.008 (3.9)	1.560 (0.08)	0.052 (0.9)	0.015 (8.5)
Ammonia extract of non extractable residues	0.077 (0.2)	0.175 (0.3)	n.d.	n.d.	n.d.	0.641 (0.5)	0.075 (1.2)	0.021 (10.4)
Total identified or characterised	48.923 (99.8)	65.817 (99.5)	1.010 (98.3)	0.886 (98.2)	0.181 (91.4)	123.779 (96.2)	5.915 (96.6)	0.184 (90.9)
Final residue	0.14 (0.3)	0.37 (0.6)	0.017 (1.7)	0.017 (1.9)	0.017 (8.6)	3.060 (2.4)	0.201 (3.3)	0.018 (8.9)
Total	49.07 (100.1)	66.19 (100.1)	1.03 (100.0)	0.90 (100.1)	0.20 (100.0)	126.84 (98.6)	6.12 (99.9)	0.20 (99.8)

\+ identified but not quantified
n.d. not determined

Table B.7.1-9: Summary of identified and unidentified components in beans after treatment with pyridine labelled nicobifen

Metabolite Code	Bean plant 0 DALT	Forage 15 DALT	Green beans 15 DALT	Pods 15 DALT	Seeds 15 DALT	Straw 51 DALT	Dry pods 51 DALT	Dry seeds 51 DALT
	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Identified:								
Nicobifen	20.860 (98.1)	16.695 (98.4)	0.071 (78.1)	0.095 (87.0)	0.043 (64.9)	87.78 (93.6)	1.090 (79.7)	0.047 (36.9)
M510F47 (chloronicotinic acid)			0.003 (2.8)	0.002 (2.2)	0.007 (10.0)		0.015 (1.1)	0.002 (1.7)
Characterised:								
From extractable residues	0.002 (0.01)	0.016 (0.1)	0.015 (17.0)	0.008 (8.8)	0.015 (21.1)	0.737 (0.8)	0.130 (9.6)	0.039 (30.2)
Ammonia extract	0.109 (0.5)	0.079 (0.5)	n.d.	n.d.	n.d.	0.622 (0.7)	0.046 (3.4)	0.011 (8.5)
Total identified or characterised	20.971 (98.6)	16.79 (99.0)	0.089 (97.9)	0.105 (98.0)	0.065 (95.9)	89.139 (95.1)	1.181 (93.8)	0.099 (77.4)
Loss from ammonia extract	0.133 (0.6)	0.068 (0.4)				0.849 (0.9)	0.005 (0.4)	0.011 (9.1)
Final residue	0.145 (0.7)	0.108 (0.6)	0.002 (2.1)	0.002 (2.0)	0.003 (4.1)	3.794 (4.0)	0.081 (5.9)	0.017 (13.5)
Total	21.25 (99.9)	16.97 (100.1)	0.09 (100.0)	0.11 (100.0)	0.07 (100.0)	93.78 (100.0)	1.37 (100.1)	0.13 (100.0)

n.d. not determined

Storage stability

Storage stability investigations were carried out in green beans (both labels) and bean straw (pyridine label) with stored samples and stored extracts. Green beans from the diphenyl label were worked up again after a storage time of 5 months. Radioactive residues, extractability and metabolite pattern compared well with the original sample.

For the pyridine label, green beans and bean straw were worked up again after 28 and 29 months. Total radioactive residues were found at 68 – 78 % of the amount of the original samples. Post extraction residues were slightly higher than were found by the extraction procedure at the beginning of the study. Only minor changes were observed in metabolite pattern of stored samples and stored extracts in comparison to the original chromatograms.

Under the chosen conditions, the radioactive residues, which were mostly parent, showed a sufficient stability in extracts and in the corresponding original sample material over the time period of the study.

Conclusion

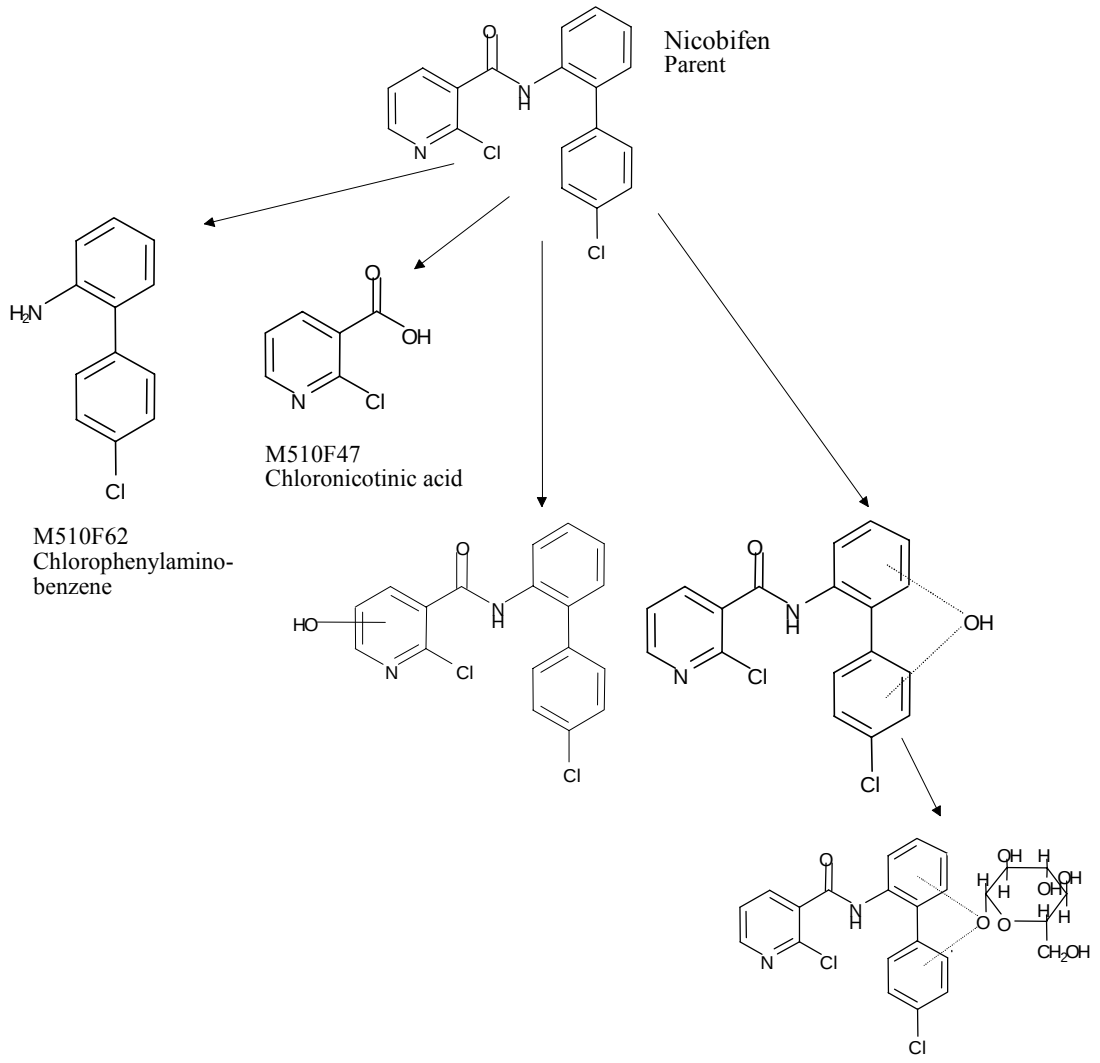
The total radioactive residues in the edible parts of beans were low. A separation of green beans into pods and seeds showed, that the radioactivity levels were higher in the pods than in the seeds which was also the case for dry pods and dry seeds. Most of the radioactivity was

found in plant/forage samples and in straw samples. The translocation of nicobifen from leaves into fruits and from pods into seeds was small.

The extractability with methanol was very good and the extractable radioactive residues (methanol and water) were ≥ 90.4 % except for dry seeds of both labels. The low level of non-extractable residues indicated a slow metabolisation and conjugation to plant assimilates.

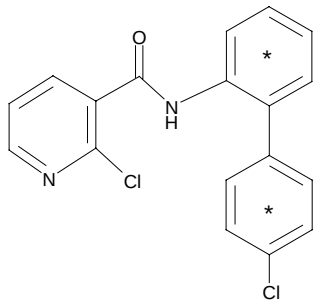
In all bean matrices, the unchanged parent compound was the dominant residue. Besides the cleavage products chloronicotinic acid (M510F47) and chlorophenylaminobenzene (M510F62), a range of mainly peaks were detected. Some of these peaks were identified as hydroxy-parent and sugar conjugates. All metabolites identified or characterized were clearly below 10% TRR and therefore of minor importance.

Figure B.7.1-1: Metabolic pathway in beans



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 was investigated using [diphenyl-U-^{14}C] labelled nicobifen. Based on the findings that the amide bond of nicobifen is stable under metabolic conditions in goats and hens it was not necessary to conduct an additional ^{14}C-Pyridine ring label nicobifen study.



* ^{14}C-labelled position [diphenyl-U-^{14}C]

B.7.2.1 Lactating goats

Report: Leibold E.; Hoffmann, H.D. (2000)
^{14}C-BAS 510 F - Absorption, distribution and excretion after repeated oral administration in lactating goats
BASF AG, Ludwigshafen/Rhein, Germany
Unpublished
BASF RegDoc# 2000/1012353

GLP: Yes (laboratory certified)

Guideline: U.S. EPA, Residue Chemistry Test Guidelines, Nature of the Residue: Plants, Livestock; OPPTS 860.1300; August 1996

Acceptability: The study is considered to be acceptable.

Objectives of the study:
This study was designed to investigate the excretion of the test compound as well as the distribution in milk and edible tissue of lactating goats, and to quantify the total radioactive residues.
To investigate the nature of the residues that occur in organs, tissues and milk, and to obtain a biotransformation pathway of nicobifen in lactating goats, a seperate study was performed.

Report: Fabian E.; Grosshans, F. (2000)
The metabolism of ^{14}C-BAS 510 F in lactating goat
BASF AG, Agrarzentrum Limburgerhof, Germany
Unpublished
BASF RegDoc# 2000/1017221

GLP: Yes (laboratory certified)

Guideline: U.S. EPA, Residue Chemistry Test Guidelines, Nature of the Residue: Plants, Livestock; OPPTS 860.1300; August 1996

Acceptability: The study is considered to be acceptable

Material and Methods:

Test material and animals:

The metabolism and distribution of nicobifen was investigated using [diphenyl-U-^{14}C] labelled material (99% purity) in two lactating goats (strain Bunte deutsche Edelziege, about 15-18 month old) following repeated oral administration at one dose level. The test compound was administered once daily after the morning milking on 5 consecutive days at a nominal dose level of 35 mg/kg feed, which was an exaggerated dose of nicobifen with regard to residues in feed stuff produced under normal agricultural conditions. The test substance was administered orally with a syringe connected to an intubation catheter through the mouth of the test-animals.

Sampling and sample storage:

Urine and faeces were collected once daily, milk was collected twice daily in the afternoon and in the morning. 23 hours after the last dose, the animals were sacrified and muscle, fat, liver, kidney, blood and GI tract with content were collected for analysis. All samples were stored at $\leq$ -15 °C until processed and analysed. Prior to analysis, the tissue samples and milk samples of both goats were pooled.

Analysis:

The total radioactive residues of organs, tissues and faeces were determined by combustion analysis. The limit of quantitation (LOQ) was at 0.002 mg/kg for each matrix. Aliquotes of liquid samples e.g. urine and milk were radioassayed directly. LOQ was 0.0007 mg/kg for liquids.

The samples were extracted with methanol. Urine samples were directly injected into HPLC without sample preparation steps. In order to defat it, the fat sample was extracted with acetonitrile iso-hexane mixture prior to methanol extraction.

The extracted radioactivity was analysed using radio HPLC. Major metabolites present in the extracts were identified by cochromatography with compounds isolated from urine of the test animals. The identification of these urine metabolites based on MS and NMR experiments.

Beside conventional extractions, several methods were applied to characterise non-released radioactivity in liver. The most successful method was a Microwave treatment with mixtures of formic or acetic acid and acetonitrile, followed by SPE-C18 clean up. The Microwave method was successfully applied to milk extracts, too, and allowed a characterisation of minor metabolites in milk.

Findings:

Animal health:

The health status of the animals was checked daily. Feed consumption and milk production remained consistent and did not seem to be affected by the test substance. Body weights slightly decreased during administration period.

Recovery and concentrations of radioactivity:

Following administration of ^{14}C-nicobifen to two lactating goats, the radioactivity was rapidly and almost completely excreted [see Table B.7.2-1]. Excretion mainly occurred via the faeces, which contained 46.4 and 64.3% of the total dose, respectively. 23.7 and 44.6% of the radioactivity were found in urine, respectively. Radioactivity recovered from urine, faeces and cage wash amounted to 88.3 and 93.4% of the total applied radioactivity, respectively. Overall recovery of radioactivity was calculated as 94.5 and 98.3%, respectively.

Table B.7.2-1: Material balance after administration of ^{14}C-nicobifen to lactating goats

Matrix	Recovery of radiolabel (% administered dose)	
	Goat 1	Goat 2
Milk and tissues		
Liver [1)]	0.61	0.43
Kidney [1)]	0.01	0.01
Muscle [1)]	0.02	0.01
Fat [1)]	0.02	0.01
Milk [2)]	0.06	0.15
Blood	0.01	0.03
Bile, stomach and gut + contents	4.96	4.27
Excrements and cage wash		
Cage wash	0.90	2.44
Urine	23.68	44.63
Faeces	64.25	46.37
Total	**94.53**	**98.34**

[1)] Recalculated from pooled sample values to individual organ/tissue weight

[2)] Average from Day 1 to 5 samples

Determination of radioactivity in liver, kidney, fat and muscles was performed in a pooled sample of both animals. The total radioactive residues (TRR) are summarized in Table B.7.2-2.

The radioactive residues were low in muscle and fat with levels of 0.012 and 0.036 mg/kg, respectively. The TRR in liver was accounted for 2.593 mg/kg and in kidney for 0.270 mg/kg.

Concentrations of radioactivity in milk were relatively constant for both goats and did not increase over the application period. In the pooled milk samples the TRR ranged between 0.034 and 0.044 mg/kg. The average values in the pooled samples was 0.039 mg/kg.

Table B.7.2-2: Total radioactive residues in edible matrices after dosing of lactating goats with ^{14}C-nicobifen

Pooled Matrix	TRR [mg / kg]
Milk [1)]	0.039
Muscle	0.012
Fat	0.036
Kidney	0.270
Liver	2.593

[1)] Pooled sample of aliquots from Day 1 to 5 milk of both goats

Extractability:
For most of the samples, the extractability with organic solvents was good. About 80% to 99% of the TRR could be extracted in case of muscle, fat, kidney and milk. For liver the extractability was not sufficient, less than 20 % were extractable with methanol. Therefore a Microwave extraction was performed, using mixtures of formic or acetic acid and acetonitrile heated at 170°C for 30 min.
A summary of the extraction behaviour is given in Table B.7.2-3.

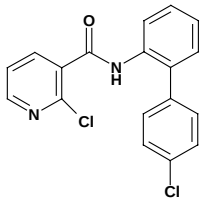
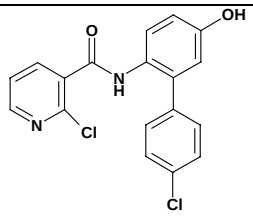
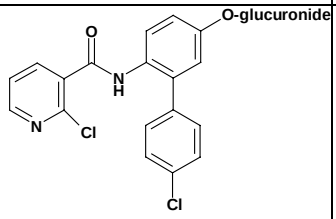
Table B.7.2-3: Extractability of goat matrices with methanol after dosing of lactating goats with ^{14}C-nicobifen

	TRR	Extractable radioactivity		Non extractable radioactivity	
Matrix	mg/kg	mg/kg	% TRR	mg/kg	% TRR
Milk, pool	0.037	0.037	99.3	0.002	5.8
Muscle	0.012	0.010	79.7	0.003	24.1
Fat	0.036	0.031	87.1	0.001	4.0
Fat [1)]	0.036	0.024	62.8	0.009	24.9
Kidney	0.270	0.219	81.3	0.044	16.3
Liver	2.593	0.430	16.6	1.833	70.7
Liver [2)]	2.593	2.593	100	N/A.	N/A.

[1)] Extraction with acetonitrile iso-hexane mixture
[2)] Results from Microwave extraction
N/A. not analysed

Metabolites and metabolic pathway:
The metabolism of nicobifen in goats is characterised by a hydroxylation of nicobifen to form the metabolite M510F01, followed by a glucuronidation to create the glucuronic acid M510F02. The sulfatation of M510F01 leads to the sulfate M510F03. Further hydroxy and thiol substitutions of the biphenyl system occur, followed by methylation. The substitution of the chlorine atom in the pyridine ring system by thiol groups of biomolecules leads to the formation of the cysteine conjugates, as M510F05 and M510F22, detected in urine. Considerable quantities of radioactivity were bound in liver, based on this substitution (most likely SH-groups from cysteine containing protein).
The amide bond of nicobifen was very stable under metabolic conditions in goats. The summary of all identified metabolites in the edible portions is shown in Table B.7.2-4.

Table B.7.2-4: Summary of metabolite identities and quantities in extractable radioactivity of goat tissues and milk after dosing with ^{14}C-nicobifen

Metabolite code (Reg.-No. of reference substance)	Structure	Milk[2] (pool)	Muscle	Fat	Kidney	Liver
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
nicobifen		0.001 (3.2)	0.002 (20.4)	0.012 (34.6)	0.007 (2.5)	0.129 (5.0)
M510F01		0.006 (14.9)	0.003 (20.6)	0.009 (26.3)	0.023 (8.6)	0.074 (2.9)
M510F02		0.002 (6.4)	0.001 (11.9)	n.d.	0.136 (50.3)	n.d.
Total identified in extractable radioactivity		0.009 (24.5)	0.006 (52.9)	0.022 (60.9)	0.166 (61.4)	0.203 (7.9)
Total characterised in extractable radioactivity [1]		0.023 [15] [3] (58.7)	0.002 [1] (19.8)	0.004 [2] (10.9)	0.052 [8] [4] (19.4)	0.170 [13] [5] (6.6)
Non extractable residues		0.002 (4.1)	0.003 (24.1)	0.003 (8.0)	0.044 (16.3)	2.201 (84.9)
Grand Total Recovery		**0.034 (87.3)**	**0.017 (96.7)**	**0.011 (79.8)**	**0.262 (97.1)**	

n.d. not detected
[1] Characterised by HPLC Retention times; number of peaks is given in brackets
[2] Day 1 to 5, both goats
[3] Each peak less than 0.004 mg/kg
[4] Each peak less than 0.019 mg/kg
[5] Each peak less than 0.067 mg/kg

The parent nicobifen and its hydroxylated metabolite M510F01, including the conjugate M510F02 were the main residues in nearly all samples under investigation. The main residues in muscle were nicobifen, the hydroxylated compound M510F01 and the glucuronic acid conjugate M510F02. These three metabolites were also detected in kidney. The main residues

in fat were identified to be parent nicobifen and M510F01. The main residues in milk extracts were nicobifen, M510F01 and M510F02. In the methanol extract of liver, nicobifen and M510F01 could be identified.

For further characterisation of non-extractable residues in liver, a Microwave method was applied. The Microwave treatment dissolved the whole liver material and allowed the characterisation and identification of residues after SPE-C18 clean up. The hydrolysis was carried out either with formic acid or acetic acid and acetonitrile heated at 170°C for 30 min. After this time a stable pattern of analytes was formed. The results of both treatments were comparable. [See Table B.7.2-5]
From detailed experiments with reference compounds it was discovered that the metabolites M510F01, M510F49, M51F51 and nicobifen originate from extractable residues. Bound residues were cleaved under Microwave treatment at the amide bond to form M510F52 (N-(4'-Chloro-biphenyl)formamide) as a product from reaction with formic acid and M510F53 (N-(4'-Chloro-biphenyl)acetamide) from reaction with acetic acid. Hence it could be demonstrated that the major residues in liver were bound.
The application of the Microwave method to milk extracts reduced the diversity of metabolites and allowed a further characterisation of residues in milk. It was possible to detect the metabolites M51F51 and M510F01, representing hydroxylated residues. M510F49 and nicobifen are representing analytes that are due to unchanged parent compound. The method was applied to an acetonitrile extract of milk, and although no bound residues were contained, the analyte M510F53 was detected in small amounts. Therefore, it has to be concluded, that is due to soluble sulfur linked residues in milk.

Table B.7.2-5: Summary of analyte identities and quantities in goat milk and liver after Microwave treatment with formic and acetic acid

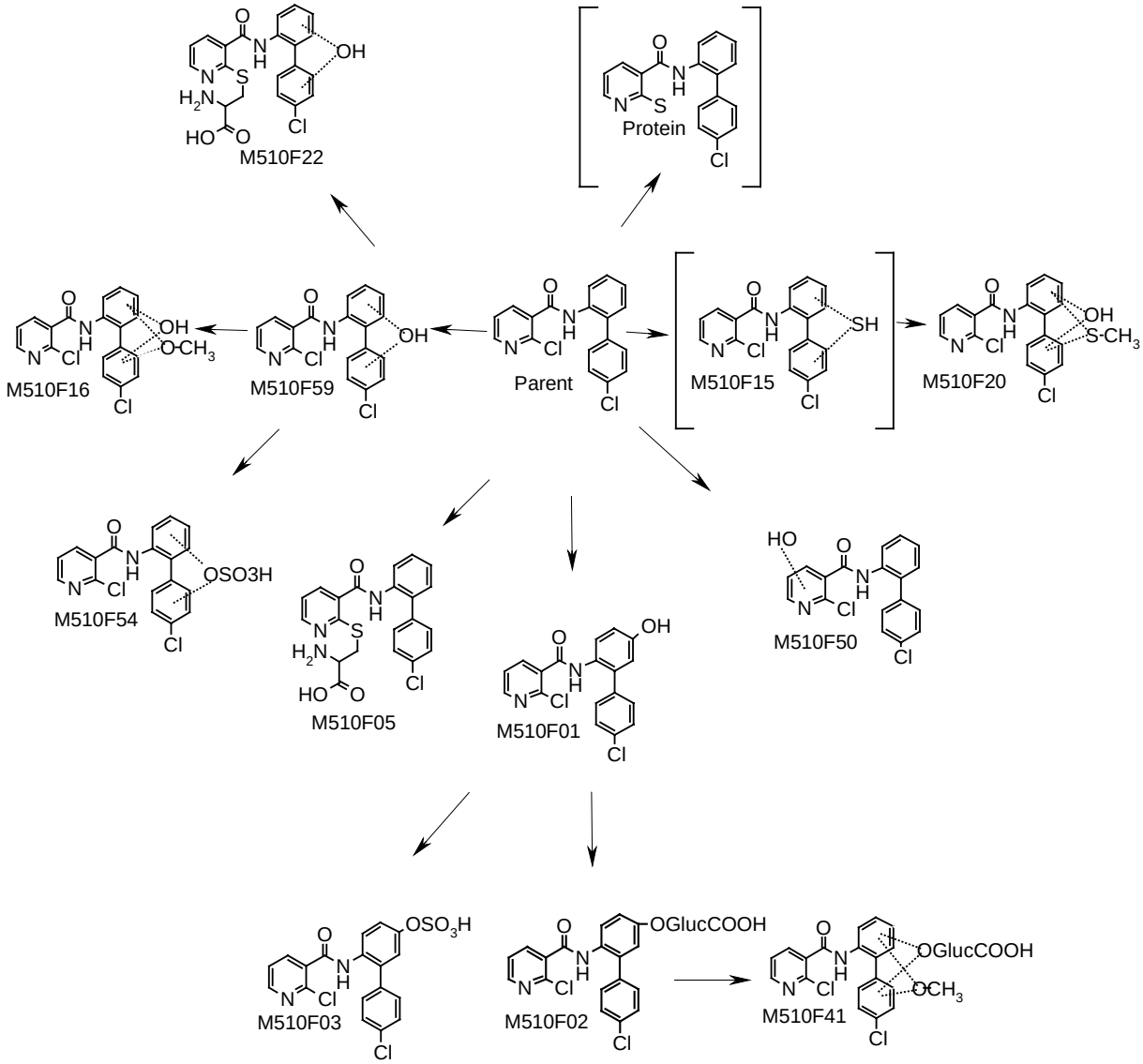
Metabolite code	Liver Formic acid Mw extract		Liver Acetic acid Mw extract		Milk Acetic acid Mw extract	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
nicobifen	0.148.	5.7	n.d.	n.d.	0.003	7.9
M510F01	0.109	4.2	0.166	6.4	0.007	19.0
M510F49	0.296	11.4	0.285	11.0	0.003	7.7
M510F51	0.171	6.6	0.062	2.4	0.005	12.2
M510F52 [1)]	0.918	35.4	n.d.	n.d.	n.d.	n.d.
M510F53 [1)]	n.d.	n.d.	1.130	43.6	0.004	11.2
Total identified in the hydrolysate	0.1641	63.3	0.1641	63.4	0.023	58.0
Total characterised by solubility [2)]	0.952	36.7	0.952	36.6	0.012	30.5
Grand Total Recovery	**2.593**	**100**	**2.593**	**100**	**0.035**	**88.5**

[1)] Formed during Microwave treatment, released from bound residues
[2)] Values were obtained by subtraction from theoretical value, for milk 88.5% TRR were extracted
Mw Microwave
n.d. not detected

Storage Stability:
A comparison of the extractability and the HPLC metabolites profiles directly after sampling and close to the end of the experimental part indicated no noticeable change in the nature of the radioactive residues,. Therefore it has been proven that all residues are stable during sample storage of more than one year.

Conclusion:
The metabolism and distribution of ^{14}C-nicobifen was investigated in two lactating goats using material labelled in the biphenyl system. The proposed metabolic pathway is shown in Figure B.7.2-1.
After 5 consecutive daily oral administrations of ^{14}C-nicobifen at a nominal dose level of 35 mg/kg feed, there was a rapid and almost complete excretion from the gastrointestinal tract. There was no indication of accumulation of radioactivity in fat and milk. The total radioactive residues in the edible portions were low. The parent compound and the hydroxylated metabolite M510F01, including its glucuronic acid conjugate were the main residues in all samples under investigation. The same key transformation steps had been also observed in rat and in hen.

Figure B.7.2-1: Metabolic pathway of nicobifen in the goat



B.7.2.2 Laying hens

Report: Nietschmann, D.A.; Lam, W.W. (2000)
Nature of residues of ^{14}C-BAS 510 F in laying hens
BASF Corporation Agricultural Products Center, Research Triangle Park, NC 27709, USA
unpublished
BASF RegDoc# 2000/5154

GLP: Yes (laboratory certified)

Guideline: U.S. EPA, Residue Chemistry Test Guidelines, Nature of the Residue: Plants, Livestock; OPPTS 860.1300

Acceptability: The study is considered to be acceptable.

Objectives of the study:
The study was designed to investigate the excretion and distribution of the test compound as well as the concentration and the identity of the biotransformation residues in tissues and eggs of laying hens.

Material and Methods:
Test animals and material:
The metabolism and distribution of ^{14}C-nicobifen in laying hens (Leg Horn, approx. 74 weeks old) was investigated following repeated oral administration of ^{14}C-nicobifen at one dose level. The 10 test animals were administered encapsulated ^{14}C-diphenyl-labelled nicobifen once per day for 10 consecutive days at a nominal rate of 12.5 mg / kg feed. The actual dose level was 12.1 mg /kg feed.
Sampling and sample storage:
Excreta and eggs were collected daily. Each cage was rinsed following the excreta collection. Within 24 hours after the last dose, test animals were sacrified and muscle, fat, liver and GI tract samples were taken for analysis. All samples were stored at $\leq$ -15 °C until processed and analysed. Prior to analysis, the eggs and the tissue samples were pooled.
Radioactivity measurements and analysis:
The total radioactive residues in eggs, muscle, fat, liver and GI tract were determined by combustion of aliquotes to $^{14}CO_2$ prior to liquid scintillation counting. The limit of quantitation was at 0.001 mg/kg for each matrix. Aliquotes of liquid samples were radioassayed directly.
For further investigations the samples were extracted with acetonitrile or acetonitrile hexane mixture. The extractable radioactivity was characterised, identified and quantified using radio HPLC, TLC methods and LC/MS analysis. For liver, multiple methods were attempted to extract and characterise the protein bound residues. Beside conventional extractions, several methods like hydrolysis and protease digest were applied to characterise non released residues in liver. However, the most successful method was a Microwave extraction with acetonitril formic acid mixture followed by SPE-C18 clean up.

Findings:

Animal health:

All animals tolerated the dosing procedure without any problems and were observed to be healthy and normal throughout the study. Feed consumption, body weights and egg production remained consistent and did not seem to be affected by the test substance.

Recovery and concentrations of radioactivity:

The overall recovery of ^{14}C-nicobifen equivalent residues was 98.25% of the total applied radioactivity with only 0.30 % coming from the tissues and GI tract. Transfer of radioactivity into eggs was low, 0.11% of the total dose were excreted via eggs. The test material was eliminated primarily via the excreta, the recovery of ^{14}C-nicobifen equivalent residues in the excreta across all days was 97.68 % of the total applied radioactivity.

Details are summarized in Table B.7.2-6.

Table B.7.2-6: Material balance and total radioactive residues (TRR) in edible matrices after administration of ^{14}C-nicobifen to laying hens

Matrix	TRR (mg/kg)	Percent Total Dose
Muscle	0.0025	0.003
Fat	0.0250	0.004
Liver	0.1687	0.039
GI tract	0.3074	0.258
Eggs [1)]	0.0580	0.115
Excreta	4.1675	97.68
Cage wash	0.3416	0.154
Total		**98.25**

[1)] Average from Days 2 to10 samples; Day 1 eggs had no radioactivity

Very low residue levels were detected in muscle with less than 0.01 mg/kg and in fat with 0.025 mg/kg. A higher residue level was measured in liver with 0.1687 mg/kg.

The residue in eggs varied from 0 mg/kg (Day 1) to 0.0805 mg/kg (Day 10). The residue level reached a plateau of 0.0739 mg/kg starting at Day 6 and continuing to Day 10 (0.0805 mg/kg).

Details are summarized in Table B.7.2-7.

Table B.7.2-7: Total radioactive residues (TRR) in eggs after administration of ^{14}C-nicobifen to laying hens

Eggs	TRR (mg/kg)
Day 1	0.0000
Day 2	0.0239
Day 3	0.0305
Day 4	0.0447
Day 5	0.0521
Day 6	0.0739
Day 7	0.0677
Day 8	0.0752
Day 9	0.0739
Day 10	0.0805
Average (Day 2-Day10)	**0.0580**

Extractability:

The extractability with organic solvents was above 90% of TRR for fat, egg and excreta samples. For liver the extractability was not sufficient, only about 12 % of TRR were

extractable. Therefore a Microwave extraction was performed, using an acetonitrile formic acid mixture heated at 170°C for 30 min. A summary of the extraction behaviour is given in Table B.7.2-8.

Table B.7.2-8: Extractability of hen matrices after dosing of laying hens with ^{14}C-nicobifen

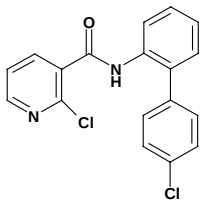
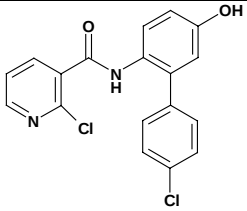
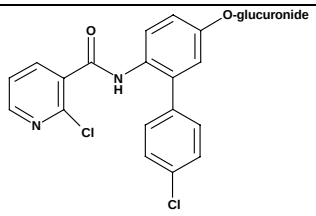
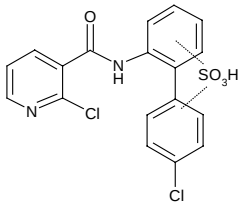
	TRR	Extractable radioactivity		Non extractable radioactivity	
Matrix	**mg/kg**	**mg/kg**	**(% TRR)**	**mg/kg**	**(% TRR)**
Eggs [1)]	0.0580	0.0572	98.98	0.0017	2.71
Muscle [2)]	0.0025	n.a.	n.a.	n.a.	n.a.
Fat	0.0250	0.0233	93.32	0.0023	9.32
Liver	0.1687	0.0203	12.04	n.a.	n.a.
Liver [3)]	0.1687	0.1687	100	n.a.	n.a.
Excreta [4)]	n.a.	n.a.	95.35	n.a.	8.25

[1)] Average from Days 2 to10 samples; Day 1 eggs had no radioactivity and were not extracted
[2)] Muscle was not extracted because it was below 0.01 mg/kg
[3)] Results from Microwave extraction
[4)] Average from 10 sampling days
n.a. not analysed

Metabolites and metabolic pathway:
The metabolic pathway of nicobifen in hen is first the formation of the hydroxylated nicobifen (M510F01), followed by the glucuronidation of M510F01 to form the glucuronic acid (M510F02). The substitution of the chlorine atom in the pyridine ring system by thiol-groups of biomolecules could be observed in the goat metabolism and leads to bound residues in liver. Considerable quantities of nicobifen were also bound in hen liver based on this substitution (most likely SH-groups from cysteine containing protein). The amide bond of nicobifen was very stable under metabolic conditions in hens.
A proposed metabolic pathway is given in Figure B.7.2-2.

The summary of all identified metabolites in the edible portions is shown in Table B.7.2-9.

Table B.7.2-9: Summary of metabolite identities and quantities in eggs, fat and excreta of hens after dosing with ^{14}C-nicobifen

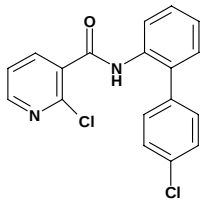
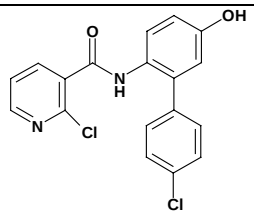
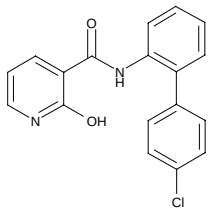
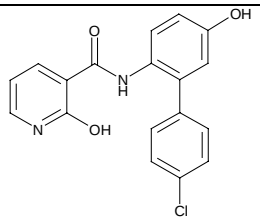
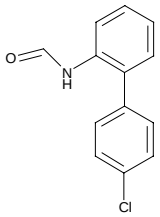
Metabolite code	Structure	Eggs [1)]		Fat		Excreta [2)]
		mg/kg	% TRR	mg/kg	% TRR	% TRR
nicobifen		0.0196	35.48	0.0233	93.32	4.05
M510F01		0.0149	26.90	n.d.	n.d.	75.47
Glucuronic acid M510F02		0.0108	17.32	n.d.	n.d.	0.82
Sulfate M510F54		0.0013	1.89	n.d.	n.d.	2.11
Total identified in extractable radioactivity		0.0466	81.59	0.0233	93.32	82.45
Total characterised in extractable radioactivity		0.0059	8.11	-	-	7.99
Non extractable residue		0.0016	2.71	0.0023	9.32	8.25
Grand Total Recovery		**0.0541**	**92.41**	**0.0256**	**102.64**	**98.69**

[1)] Average values for egg sample analysis between Day 2 and Day 10
[2)] Average values for excreta sample analysis between Day 1 and Day 10
n.d. not detected

There were four compounds identified in both egg and excreta samples and they were parent nicobifen, its hydroxylated metabolite M510F01 and its sulfate M510F54, as well as the glucuronic acid of M510F01 (M510F02). For fat, only parent nicobifen was present and identified. Muscle had a very low residue level of 0.0025 mg/kg and therefore it was not further investigated.
Liver was different than the other tissue. Metabolites were bound extensively in liver protein. Because conventional extraction only allowed a small amount of residue characterisation, a Microwave method was applied. Four analytes could be detected after Microwave treatment of liver samples using acetonitrile and formic acid. It could be demonstrated in detailed

experiments that the detected analytes M510F01, M510F49 and M510F51 originate from extractable residues. Residues not extractable with organic solvents are cleaved under Microwave treatment at the amide bond to form M510F52. Hence the major residues in liver were bound residues that correspond to M510F52. Detailed data are shown in Table B.7.2-10.

Table B.7.2-10: Summary of analyte identities and quantities in hen liver after Microwave treatment with acetonitrile and formic acid

Metabolite code	Structure	Liver Microwave Extract	
		mg/kg	% TRR
nicobifen		n.d.	n.d.
M510F01		0.0094	5.55
M510F49		0.0214	12.71
M510F51		0.0366	21.69
N-(4'-Chloro-biphenyl)formamide M510F52		0.0710	42.09
Total identified in extractable radioactivity		0.1384	82.04
No further analysed fractions from SPE [1)]		0.0303	17.96
Total		**0.1687**	**100**

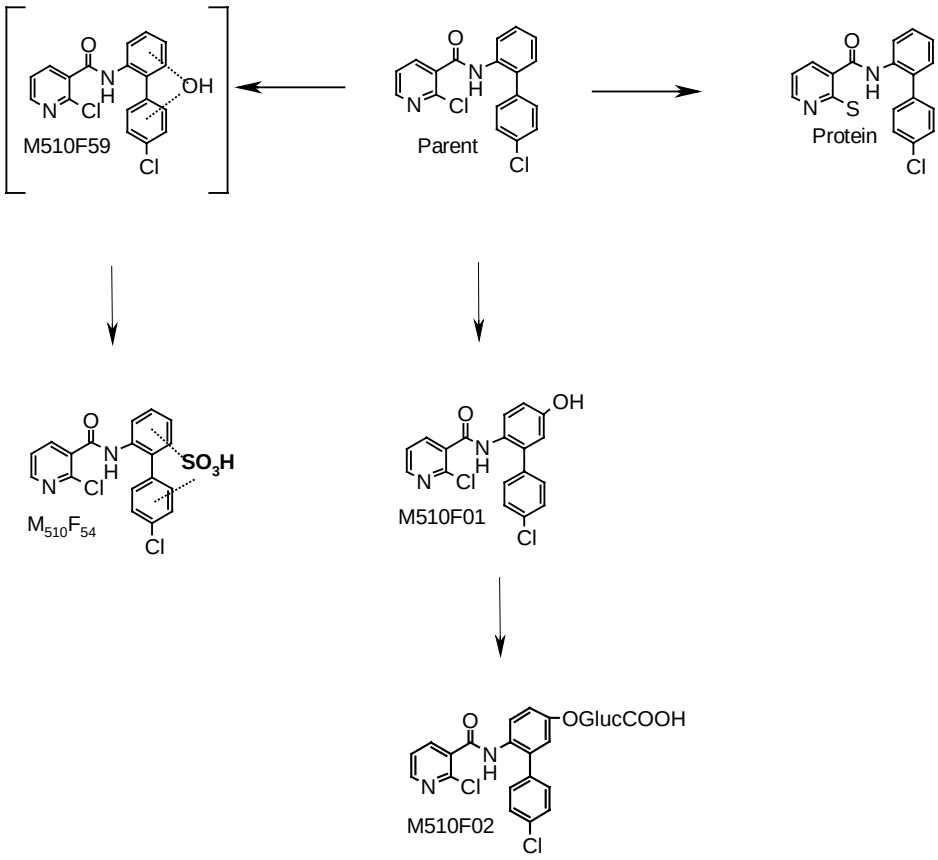
[1)] Values were obtained by subtraction from theoretical value

Storage stability:

There was no noticeable change in the nature of the radioactive residues during extract and sample storage of more than one year.

Conclusion:
The metabolism and distribution of ^{14}C-nicobifen in laying hens has been investigated using test compound labelled in the biphenyl system. The proposed metabolic pathway is shown in Figure B.7.2-2.
Laying hens received 10 consecutive daily oral administrations of ^{14}C-biphenyl-nicobifen at a nominal dose level of 12.5 mg / kg feed, which was an exaggerated dose.
After administration of the test compound the radioactivity was completely excreted. There was no indication of accumulation of ^{14}C-nicobifen neither in eggs nor in fat. The total radioactive residues in the edible portions were low. The parent compound and the hydroxylated metabolite M510F01, including its glucuronic acid conjugate were the main residues. The same key transformation steps had been also observed in rat and in goat.

Figure B.7.2-2: Metabolic pathway of nicobifen in the hen



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
Leaf vegetables:	lettuce
Pulses:	bean

In these three studies, parent compound formed the major part of the residue in almost all of the plant samples under investigation.

Proposed residue definition for plants: Parent Nicobifen (BAS 510 F)

B.7.3.2 Animal matrices

Metabolism studies performed on goats and hens (see B.7.2) show that residues in products of animal origin derive from the parent compound as well as from the hydroxylated metabolite M510F01 including its conjugates. Further metabolites result from a substitution of the chlorine of the 2-chloropyridine moiety by the thiol group of glutathione to create metabolites as the cysteine conjugate. Nicobifen derived residues were also bound in liver based on this substitution (most likely SH-groups from cysteine containing protein). The amide bond of nicobifen was very stable under metabolic conditions in goats and hens.

An individual metabolite is considered to be relevant if

- relative amount > 10 % of total radioactive residue (TRR) in a matrix, or
- absolute concentration > 0.05 mg / kg in the same matrix

Applying these criteria to the results of the goat and hen meatbolism studies selects the following compounds that are listed differentiated in residues for risk assessment and residues for monitoring purposes:

Residues for risk assessment:
- Nicobifen
- M510F01 (including its conjugates)
- M510F53 (for bound residues in liver and minor metabolites in milk)

Residues for monitoring:
- Nicobifen
- M510F01 (including its conjugates)

Three analytical methods were developed for products of animal origin. A LC/MS/MS method was used for data generation for risk assessment. The method focused on nicobifen and M510F01 including its conjugates. Since it could be demonstrated in the goat and hen metabolism studies these compounds formed the major parts of extractable residues in edible tissues and hence data obtained by this method represent reasonable representative residues for risk assessment in all matrices.

A second data generation method for risk assessment allowed the detection of bound nicobifen derived residues in liver and minor soluble metabolites in milk (as M510F53) by Microwave treatment. Residue data obtained by this method represent reasonable worst case estimates for risk assessment esp. in liver.

Based on requirements for monitoring purposes, an modified multi-residue method DFG S19 was developed. The method allows the monitoring of nicobifen and its major metabolite M510F01 (present also as conjugate).

Proposed residue definition for monitoring:
Nicobifen, M510F01 and its conjugates, sum expressed as Nicobifen

B.7.4 Use pattern

The use of nicobifen containing products is intended in grapes, peas, beans and oilseed rape in the northern and southern region of Europe. Other uses in vegetable and fruit crops are planned with different formulations 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 and details to the planned uses are given in Table B.7.4-2 to Table B.7.4-6.

Table B.7.4-1: Nicobifen containing formulations

BAS-code	Formulation type	Content of nicobifen [g/kg or g/L]	Other active substance [g/kg or g/L]
BAS 510 01 F*	WG	500	
BAS 516 00 F	WG	267	pyraclostrobin, 67
BAS 517 00 F	SC	200	kresoxim-methyl, 100
BAS 516 01 F	SE	200	pyraclostrobin, 100
BAS 538 00 F	WG	79	mancozeb, 632

- formulation used for residue trials

Table B.7.4-2: Summary of intended uses BAS 510 01 F

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grape	**Germany**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 - 81	1	-	0.038	1600	0.600	28	
Grape	**France**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 – 81	1	-	0.060	1000	0.600	28	
Grape	**Italy**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 - 81	1	-	0.060	1000	0.600	28	
Grape	**Austria**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 – 81	1	-	0.060	1000	0.600	28	
Grape	**Spain**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 – 81	1	-	0.060	1000	0.600	28	
Oil seed rape	**Denmark**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.062 0.125	200 400	0.250	-	
Oil seed rape	**Sweden**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.062 0.125	200 400	0.250	-	
Oil seed rape	**Norway**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.062 0.125	200 400	0.250	-	
Oil seed rape	**Finland**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.062 0.125	200 400	0.250	-	
Oil seed rape	**Germany**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.062 0.125	200 400	0.250	-	
Oil seed rape	**France**	BAS 510 01 F	F	*Alternaria Sclerotinia, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.100 0.050	200 400	0.200	-	

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Oil seed rape	**UK**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.083	300	0.250	-	
Peas	**Denmark**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 - 10	0.125	400	0.500	7	
Peas	**Finland**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 - 10	0.125	400	0.500	7	
Peas	**France**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 - 10	0.125	400	0.500	7	
Beans	**Germany**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 – 10	0.166	300	0.500	7	
Beans	**Spain**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 – 10	0.166	300	0.500	7	

Table B.7.4-3: Summary of intended uses BAS 516 00 F

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Lettuce	**Germany**	BAS 516 00 F	F, G	*Botrytis, Sclerotinia*	WG	267	spraying	1-2 weeks after planning	2	7 – 10	0.080	500	0.400	14	
Lettuce	**France**	BAS 516 00 F	F, G	*Botrytis, Sclerotinia*	WG	267	spraying	1-2 weeks after planning	2	7 – 10	0.080	500	0.400	14	

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Lettuce	**Spain**	BAS 516 00 F	F, G	*Botrytis, Sclerotinia*	WG	267	spraying	1-2 weeks after planning	2	7 – 10	0.080	500	0.400	14	
Lettuce	**Netherlands**	BAS 516 00 F	F, G	*Botrytis, Sclerotinia*	WG	267	spraying	1-2 weeks after planning	2	7 – 10	0.080	500	0.400	14	
Lettuce	**Belgium**	BAS 516 00 F	F, G	*Botrytis, Sclerotinia*	WG	267	spraying	1-2 weeks after planning	2	7 – 10	0.080	500	0.400	14	
Carrotts	**Germany**	BAS 516 00 F	F	*Alternaria, powdery mildew*	WG	267	spraying	41 - 49	2	7 - 10	0.050	400	0.200	28	
Carrotts	**France**	BAS 516 00 F	F	*Alternaria, powdery mildew*	WG	267	spraying	41 – 49	2	7 – 10	0.027	400	0.107	28	
Carrotts	**Denmark**	BAS 516 00 F	F	*Alternaria, powdery mildew*	WG	267	spraying	41 – 49	2	7 - 10	0.050	400	0.200	28	
Carrotts	**Netherlands**	BAS 516 00 F	F	*Alternaria, powdery mildew*	WG	267	spraying	41 – 49	2	7 - 10	0.050	400	0.200	28	
Carrotts	**Belgium**	BAS 516 00 F	F	*Alternaria, powdery mildew*	WG	267	spraying	41 – 49	2	7 - 10	0.050	400	0.200	28	
Cabbage	**France**	BAS 516 00 F	F	*Alternaria Sclerotinia, Botrytis*	WG	267	spraying	41 - 89	3	7 - 10	0.053	500	0.267	14	
Cabbage	**United Kingdom**	BAS 516 00 F	F	*Alternaria, Sclerotinia, Botrytis*	WG	267	spraying	41 – 89	3	7 - 10	0.053	500	0.267	14	
Cabbage	**Denmark**	BAS 516 00 F	F	*Alternaria, Sclerotinia, Botrytis*	WG	267	spraying	41 – 89	3	7 - 10	0.053	500	0.267	14	
Cabbage	**Netherlands**	BAS 516 00 F	F	*Alternaria, Sclerotinia,*	WG	267	spraying	41 – 89	3	7 - 10	0.053	500	0.267	14	

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
				Botrytis											
Cabbage	**Belgium**	BAS 516 00 F	F	*Alternaria, Sclerotinia, Botrytis*	WG	267	spraying	41 – 89	3	7 - 10	0.053	500	0.267	14	
Tomato	**Spain**	BAS 516 00 F	F	*Leveillula Botrytis*	WG	267	spraying	50 – 85	3	7 – 10	0.040	1000	0.400	3	
Pepper	**Spain**	BAS 516 00 F	F	*Leveillula Botrytis*	WG	267	spraying	50 – 85	3	7 - 10	0.040	1000	0.400	3	
Leek	**Netherlands**	BAS 516 00 F	F	*Stemphylium Cladosporiun Phytophthora*	WG	267	spraying	41 – 89	3	10	0.080	500	0.400	14	
Leek	**Belgium**	BAS 516 00 F	F	*Stemphylium Cladosporiun, Phytophthora*	WG	267	spraying	41 – 89	3	10	0.080	500	0.400	14	
Stonefruit	**Germany**	BAS 516 00 F	F	*Monilinia*	WG	267	spraying	60 – 67, 77 – 81	3	5 – 10	0.020	1000	0.200	7	
Stonefruit	**France**	BAS 516 00 F	F	*Monilinia*	WG	267	spraying	60 – 67, 77 – 81	3	5 – 10	0.020	1000	0.200	7	
Stonefruit	**Denmark**	BAS 516 00 F	F	*Monilinia*	WG	267	spraying	60 – 67, 77 - 81	3	5 – 10	0.020	1000	0.200	7	
Strawberry	**Germany**	BAS 516 00 F	F	*Botrytis, powdery mildew*	WG	267	spraying	60 - 81	2	5 – 7	0.024 0.048	1000 2000	0.481	3	
Strawberry	**United Kingdom**	BAS 516 00 F	F	*Botrytis, powdery mildew*	WG	267	spraying	60 – 81	2	5 - 7	0.048	1000	0.481	3	
Strawberry	**France**	BAS 516 00 F	F	*Botrytis, powdery mildew*	WG	267	spraying	60 – 81	2	5 - 7	0.048	1000	0.481	3	
Strawberry	**Denmark**	BAS 516 00 F	F	*Botrytis, powdery mildew*	WG	267	spraying	60 – 81	2	5 - 7	0.048	1000	0.481	3	
Strawberry	**Spain**	BAS 516 00 F	F	*Botrytis, powdery mildew*	WG	267	spraying	60 – 81	2	5 - 7	0.048	1000	0.481	3	

Crop and/ or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks:
(a)			(b)	(c)	Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (days)	kg as/hL min max	water L/ha min max	kg as/ha min max	(l)	(m)
Strawberry	**Netherlands**	BAS 516 00 F	F	*Botrytis, powdery mildew*	WG	267	spraying	60 – 81	2	5 - 7	0.048	1000	0.481	3	
Strawberry	**Belgium**	BAS 516 00 F	F	*Botrytis, powdery mildew*	WG	267	spraying	60 – 81	2	5 - 7	0.048	1000	0.481	3	

Table B.7.4-4: Summary of intended uses BAS 516 01 F

Crop and/ or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks:
(a)			(b)	(c)	Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (days)	kg as/hL min max	water L/ha min max	kg as/ha min max	(l)	(m)
Apple	**Belgium**	BAS 516 01 F	F	*Venturia, powdery mildew*	SE	200	spraying	54 – 81	4	8 - 14	0.020	1000	0.200	14	
Apple	**France**	BAS 516 01 F	F	*Venturia, powdery mildew*	SE	200	spraying	54 – 81	4	8 - 14	0.020	1000	0.200	14	
Apple	**Italy**	BAS 516 01 F	F	*Venturia, powdery mildew*	SE	200	spraying	54 – 81	4	8 - 14	0.013 0.020	1000 1500	0.200	14	
Apple	**Netherlands**	BAS 516 01 F	F	*Venturia, powdery mildew*	SE	200	spraying	54 – 81	4	8 - 14	0.020	1000	0.200	14	
Hops	**Germany**	BAS 516 01 F	F	*powdery mildew*	SE	200	spraying	27 – 79	3	8 - 30	0.018	2300 2700	0.420 0.500	21	

Table B.7.4-5: Summary of intended uses BAS 517 00 F

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grape	**Germany**	BAS 517 00 F	F	*powdery mildew*	SC	200	spraying	55 - 81	3	12 – 14	0.008	1600	0.128	28	
Grape	**France**	BAS 517 00 F	F	*powdery mildew*	SC	200	spraying	55 - 81	3	12 – 14	0.008	1000	0.080	28	
Grape	**Italy**	BAS 517 00 F	F	*powdery mildew*	SC	200	spraying	55 -81	3	12 – 14	0.008 0.010	1000	0.080 0.100	28	
Grape	**Spain**	BAS 517 00 F	F	*powdery mildew*	SC	200	spraying	55 - 81	3	12 – 14	0.008 0.010	1000	0.080 0.100	28	
Grape	**Austria**	BAS 517 00 F	F	*powdery mildew*	SC	200	spraying	55 - 81	3	12 - 14	0.008	1600	0.128	28	
Grape	**Portugal**	BAS 517 00 F	F	*Powdery mildew*	SC	200	Spraying	55 - 81	3	12 - 14	0.008 0.010	1000	0.080 0.100	7	
Stonefruit	**Spain**	BAS 517 00 F	F	*powdery mildew*	SC	200	spraying	10 - 81	2 4	7 - 14	0.015	1000	0.150	7	
Cucurbits	**Spain**	BAS 517 00 F	F, G	*powdery mildew*	SC	200	spraying	first symptoms visible	3	7 - 10	0.010	1000	0.100	3	
Cucurbits	**Denmark**	BAS 517 00 F	F, G	*powdery mildew*	SC	200	spraying	first symptoms visible	3	7 – 10	0.010	1000	0.100	3	
Cucurbits	**Netherlands**	BAS 517 00 F	F, G	*powdery mildew*	SC	200	spraying	first symptoms visible	3	7 – 10	0.010	1500	0.150	3	
Roses	**Netherlands**	BAS 517 00 F	F, G	*powdery mildew*	SC	200	spraying	first symptoms visible	9	7 - 10	0.020 0.060	1000 3000	0.200 0.600	-	

Table B.7.4-6: Summary of intended uses BAS 538 00 F

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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Ornamental bulbs	**Netherlands**	BAS 538 00 F	F	*Botrytis*	WG	79	spraying	-	4	weekly	0.020 0.040	200 400	0.079	-	
Tulips	**Netherlands**	BAS 538 00 F	F	*Botrytis*	WG	79	spraying	-	4	weekly	0.020 0.040	200 400	0.079	-	
Lillis / Gladiolus	**Netherlands**	BAS 538 00 F	F	*Botrytis*	WG	79	spraying	-	6	weekly	0.025 0.050	200 400	0.099	-	

(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

WG: waterdispersible granule
SE: suspoemulsion
SC: suspoconcentrate

B.7.5 Identification of critical GAPs

The critical GAP for the use of nicobifen is at the latest possible developing stage. It is summarised 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 (days)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Grape	**EU (North & South)**	BAS 510 01 F	F	*Botrytis*	WG	500	spraying	68 - 81	1	-	0.038 - 0.060	1000 - 1600	0.600	28	
Oil seed rape	**EU (North)**	BAS 510 01 F	F	*Sclerotinia, Alternaria, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.062 0.125	200 400	0.250	-	
Oil seed rape	**EU (South)**	BAS 510 01 F	F	*Alternaria Sclerotinia, Phoma*	WG	500	spraying	30, 63-65	2	4-6 weeks	0.100 0.050	200 400	0.200	-	
Peas	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 - 10	0.125	400	0.500	7	
Beans	**EU (North & South)**	BAS 510 01 F	F	*Botrytis, Sclerotinia*	WG	500	spraying	60 – 69	2	7 – 10	0.166	300	0.500	7	

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)

B.7.6.1 Analytical method

The samples were analysed with BASF method no. 445/0. Nicobifen was extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of nicobifen was performed by LC/MS/MS. For details see chapter B.5. The method quantifies the parent compound nicobifen with a limit of quantitation of 0.05 mg/kg.

All recoveries (fortification level 0.05, 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:	Funk H., Mackenroth Ch. 2001(a) Investigation of the stability of residues of BAS 510 F in plant matrices under normal storage conditions BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep. unpublished BASF RegDoc# 2001/1015028
Guidelines:	IVA Guideline Residue Chemistry Part II Storage Stability 1992, EPA 171-4(e)
GLP:	Yes
Acceptability:	Yes

Materials and methods:

The deep freeze stability of nicobifen in various plant matrices was investigated over a period of two years. Untreated samples were fortified with 0.5 mg/kg nicobifen. The samples were stored under the usual storage conditions for field samples (polyethylene bottle, -20 °C). The samples were analysed with BASF method no. 445/0.

Findings:

Samles were taken and analysed after about 1, 3, 6, 12, 19 and 24 months storage at –20 °C. The results showed that nicobifen is stable in various plant matrices under the chosen storage conditions. Storage stability is guaranteed over the whole testing period of two years. Thus, the results obtained in the residue trials, with intervals between sampling and analysis of 5 - 13 months for rape, beans and peas and of 11 - 24 months for grapes, are regarded as valid.

Table B.7.6-1: Storage stability of nicobifen fortified at 0.50 mg/kg in various plant matrices

Month	Nicobifen found[1) 2)] (mg/kg)							
	Wheat plant	Wheat grain	Wheat straw	Oilrape seed	Sugar beet	White cabbage	Peach	Pea
0	0.52	0.48	0.49	0.40	0.50	0.51	0.53	0.49
1	0.45	0.41	0.53	0.40	0.48	0.47	0.46/0.50[3)]	0.49
3	0.49	0.45	0.50	0.45	0.48	0.52	-	0.55
6	0.47	0.46	0.46	0.44	0.57	0.52	0.50	0.50
12	0.54	0.47	0.50	0.46	0.52	0.50	0.52	0.51
19	0.43	0.48	0.47	0.47	0.46	0.48	0.46	0.44
24	0.44	0.53	0.43	0.38	0.54	0.49	0.51	0.52

1) Corrected for individual procedural recovery
2) Mean of two replicates
3) Two replicates each, analysed on different days

B.7.6.3 Residues in grapes

Material and methods

During the growing seasons 1998 and 1999, a total of 39 field trials were conducted in different representative wine growing areas in Germany, Spain, France and Italy (18 N, 21 S) to determine the residue levels of nicobifen. In 1998, only the SC formulation BAS 510 00 F (formerly called BAS 510 KAF) was tested. In 1999, this formulation as well as the WG formulation BAS 510 01 F were used in parallel. Both products were applied three times at growth stages of about 67 and 77 (BBCH code) as well as about 28 days before expected harvest at growth stage 80 - 85. Different varieties of both white and red wine were used. In case of BAS 510 00 F, the application rate was 1.4 L/ha, BAS 510 01 F was applied in 1.2 kg/ha corresponding to 600 – 700 g as/ha. The application volume was either 100 L/ha or 600 –1000 L/ha.

In all trials, grape samples were taken directly after the last application (0 DALA) as well as about 3, 4, 5, 6, and 7 weeks thereafter.

Findings

The study design differed in the number of treatments (3 in the residue trials) from the indented use with only one treatment (see chapter B.3.3). The results of the residue trials in grapes are given in detail in Table B.7.6-3 for formulation BAS 510 00 F and in Table B.7.6-4 for BAS 510 01 F.

Comparable results were found for the trials conducted in parallel with the two different formulations and residue levels were slightly higher in 1998. Table B.7.6-2 gives an overview of the ranges found in the residue trials.

Table B.7.6-2: Overview over results from residue trials in grapes

	1998 BAS 510 00 F mg/kg	**1999 BAS 510 01 F** mg/kg	**1999 BAS 510 01 F** mg/kg
PHI 0	1.35 – 5.76	0.32 – 2.94	0.26 - 1.85
PHI 28	0.41 – 3.69	0.52 – 2.33	0.26 - 3.44
PHI 49	0.21 – 3.26	0.27 - 1.82	0.18 - 2.10

Table B.7.6-3: Residue trials in grapes with formulation BAS 510 00 F

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						
Northern Europe											
# 2000/1000250 (BSF/594-1) FR - 69480 Lachassagne (EU North) 02.05.2001	Pinot blanc	1.) 1979 2.) mid-end 06/98 3.) from 15.09.98	0.700	100	0.700	30.06.98 18.07. 21.08.	83 - 85	grapes, fruit	2.01 2.52 <u>3.69</u> 2.81	0 21 28 35	RIP2001-333
# 2000/1000250 (BSF/594-2) FR -69480 Lachassagne (EU North) 02.05.2001	Gamay	1.) 1976 2.) mid-end 06/98 3.) from 15.09.98	0.700	100	0.700	30.06.98 18.07. 21.08.	83 - 85	grapes, fruit	2.68 1.93 3.08 <u>3.49</u> 1.65 3.12	0 21 28 35 41 48	RIP2001-333
# 2000/1012410 (AGR/15/98) DE - 54518 Kesten (EU North) 02.05.2001	Kerner	1.) 1983 2.) 16.-29.6.98 3.) 11.10.98	0.700	600 to 800	0.117 to 0.088	24.06.98 30.07. 01.09.	83	grapes, fruit	2.43 2.77 2.25 2.20 2.60 <u>3.26</u>	0 21 28 35 42 49	RIP2001-334
# 2000/1012410 (AGR/16/98) DE - 53474 Bad Neuenahr- Ahrweiler (EU North) 02.05.2001	Dornfelder	1.) 1993 2.) 18.6.-6.7.98 3.) 30.9.98	0.700	600 to 800	0.117 to 0.088	24.06.98 30.07. 01.09.	83	grapes, fruit	5.76 2.32 2.95 <u>3.40</u> 2.09 2.35	0 21 28 35 42 49	RIP2001-334
# 2000/1012410 (DU3/07/98) DE - 76833 Böchingen (EU North) 02.05.2001	Müller-Thurgau	1.) 1975 2.) 13.-23.6.98 3.) 2.10.98	0.700	600 to 800	0.117 to 0.088	19.06.98 28.07. 26.08.	81	grapes, fruit	2.81 1.40 1.04 1.34 <u>2.01</u> 1.90	0 21 29 35 42 49	RIP2001-334

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						
# 2000/1014881 (X 98 62 58) FR - 51200 Brimont (EU North) 02.05.2001	Pinot Noir 41B	1.) 1985 2.) 8.-22.6.98 3.) 18.-24.09.98	0.700	600 to 800	0.117 to 0.088	19.06.98 22.07. 25.08.	83-85	grapes, fruit	2.42 0.761 0.407 0.737 0.230 0.858	0 20 27 35 41 49	RIP2001-335
# 2000/1000251 (BSF/606-1) FR - 51220 Brimont (EU North) 02.05.2001	Pinot meunier	1.) 1968 2.) 21.6.99 3.) 20.9.99	0.700	100	0.700	28.06.98 19.07. 24.08.	85	grapes, fruit	2.16 1.99 1.90 1.64 2.01 1.82	0 21 28 35 42 49	RIP2001-336
# 2000/1000251 (BSF/606-2) FR - 37210 Roche Corbon (EU North) 02.05.2001	Chenin blanc	1.) 1982 2.) 10.-19.6.99 3.) 6.-9.10.99	0.700	100	0.700	28.06.99 20.07. 26.08.	85	grapes, fruit	1.97 0.950 1.40 0.760 1.33 1.04	0 21 28 35 42 49	RIP2001-336
# 2000/1012411 (AGR/04/99) DE - 54518 Kesten (EU North) 02.05.02001	Kerner	1.) 1983 2.) 12.-28.6.99 3.) 29.09.99	0.700	600 to 800	0.117 to 0.088	23.06.99 21.07. 01.09.	83	grapes, fruit	1.39 1.18 1.29 1.25 1.45 1.39	0 21 28 35 42 48	RIP2001-337
# 2000/1012411 (DU2/05/99) DE – 69168 Wiesloch (EU North) 02.05.2001	Müller-Thurgau	1.) 1978 2.) 7.-21.6.99 3.) 21.09.99	0.700	600 to 800	0.117 to 0.088	17.06.99 02.08. 24.08.	81	grapes, fruit	0.910 1.51 1.79 1.00 1.11 1.24	0 21 28 35 41 48	RIP2001-337

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						
# 2000/1012411 (DU4/13/99) DE - 76831 Eschbach (EU North) 02.05.20001	Portugieser	1.) 1984 2.) 10.-18.6.99 3.) 30.09.99	0.700	600 to 800	0.117 to 0.088	21.06.99 20.07. 02.09.	83	grapes, fruit	1.16 1.37 1.71 1.69 0.810 1.17	0 22 28 35 42 49	RIP2001-337
# 2000/1014880 (X 99 62 10) FR - 51220 Merfy (EU North) 02.05.2001	Pinot Noir	1.) 1975 2.) 4.-14.6.99 3.) 15.-27.09.99	0.700	600 to 1000	0.117 to 0.070	11.06.99 12.07. 20.08.	83	grapes, fruit	1.45 1.15 0.952 1.76 1.09 0.987	0 20 27 34 41 49	RIP2001-338
Southern Europe											
# 2000/1000250 (BSF/594-3) FR - 84190 Beaumes de Venise (EU South) 02.05.2001	Uniblanc	1.) 1958 2.) mid 06/98 3.) from 15.09.98	0.700	100	0.700	23.06.98 12.07. 20.08.	83 - 85	grapes, fruit	4.76 2.82 2.89 3.22 2.87 2.28	0 21 28 35 43 50	RIP2001-333
# 2000/1000250 (BSF/594-4) FR - 84190 Beaumes de Venise (EU South) 02.05.2001	Carignan	1.) 1958 2.) mid 06/98 3.) from 15.09.98	0.700	100	0.700	23.06.98 12.07. 20.08.	83 - 85	grapes, fruit	1.35 2.53 3.15 2.82 2.39 2.33	0 21 28 35 43 50	RIP2001-333
# 2000/1012410 (AC/16/98) ES - 11500 Jerez de la Frontera, Cadiz (EU South) 02.05.2001	Palomino fino	1.) 1967 2.) 10.5.-5.6.98 3.) 17.9.98	0.700	600 to 800	0.117 to 0.088	26.05.98 03.07. 04.08	79 - 81	grapes, fruit	2.35 2.32 1.48 1.07 1.16	21 28 35 42 49	RIP2001-334

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						
# 2000/1014881 (9842R) IT – 47020 S. Giorgio di Cesane (EU South) 02.05.2001	Lambrusco	1.) 1980 2.) 1.-15.6.98 3.) 26.08.98	0.700	600 to 800	0.117 to 0.088	12.06.99 10.07. 29.07.	80	grapes, fruit	3.78 2.88 <u>1.72</u> 1.07 (g) 0.661 1.15	0 21 27 35 42 50	RIP2001-335
# 2000/1014881 (9843R) IT - 44020 Vaccolino (EU South) 02.05.2001	Fortana	1.) 1958 2.) 1.-15.6.98 3.) 26.08.98	0.700	600 to 800	0.117 to 0.088	10.06.98 08.07. 29.07.	80	grapes, fruit	5.52 3.22 <u>1.07</u> 0.826 0.302 0.207	0 20 27 35 42 50	RIP2001-335
# 2000/1000251 (BSF/606-3) FR - 84190 Beaumes de Venise (EU South) 02.05.2001	Clairette	1.) 1987 2.) 28.5.-11.6.99 3.) end 09/.99	0.700	100	0.700	11.06.99 02.07. 24.08.	83	grapes, fruit	1.59 1.70 1.14 <u>1.39</u> 0.630 1.33	0 21 28 35 41 48	RIP2001-336
# 2000/1000251 (BSF/606-4) FR - 30210 Pouzilhac (EU South) 02.05.2001	Syrah	1.) 1973 2.) 10.-20.6.99 3.) end 09/.99	0.700	100	0.700	22.06.99 10.07. 28.08.	84	grapes, fruit	0.320 0.600 <u>0.520</u> 0.410	0 21 28 34	RIP2001-336
# 2000/1012411 (AC/07/99) ES -21830 Bonares (AC/07/99) (EU South) 02.05.2001	Zalema	1.) 1961 2.) 20.4.-25.5.99 3.) 18.09.99	0.700	600 to 800	0.117 to 0.088	26.05.99 02.07. 10.08.	81	grapes, fruit	1.54 1.11 0.960 <u>1.21</u> 0.580 0.270	0 21 28 35 42 49	RIP2001-337

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						
# 2000/1012411 (AC/08/99) ES -21840 Niebla Huelva (EU South) 02.05.2001	Zalema	1.) 1969 2.) 15.4.-29.5.99 3.) 22.09.99	0.700	600 to 800	0.117 to 0.088	26.05.99 02.07. 10.08.	81	grapes, fruit	0.920 2.16 <u>1.54</u> 1.41 1.13 1.31	0 21 28 35 42 49	RIP2001-337
# 2000/1014880 (X 99 62 08) FR - 33210 Saint Pardon de Conques (EU South) 02.05.2001	Merlot	1.) 1992 2.) 1.-20.6.99 3.) 21.09.99	0.700	600 to 1000	0.117 to 0.070	10.06.99 08.07. 01.09.	85	grapes, fruit	0.970 0.561 0.526 0.561 <u>0.843</u> 0.639	0 21 28 35 42 48	RIP2001-338
# 2000/1014880 (X 99 62 09) FR – 84150 Caumont (EU South) 02.05.2001	Carignan	1.) 1970 2.) 25.5.-10.6.99 3.) 9.-17.09.99	0.700	600 to 1000	0.117 to 0.070	02.06.99 01.07. 06.08.	81 - 83	grapes, fruit	2.94 1.32 1.38 <u>1.74</u> 1.66 1.09	0 21 28 35 42 49	RIP2001-338
# 2000/1014880 (9938R) IT - 40024 Castel San Pietro Terme 02.05.2001 (EU South)	Trebbiano	1.) 1986 2.) 31.5.-8.6.99 3.) 23.-29.09.99	0.700	600 to 1000	0.117 to 0.070	31.05.99 28.06. 18.08.	83	grapes, fruit	0.844 1.22 <u>1.45</u> 1.04 1.10 0.783	0 20 28 35 42 49	RIP2001-338
# 2000/1014880 (9939R) IT – 40055 Castenaso (EU South) 02.05.20001	Trebbiano	1.) 1962 2.) 31.5.-5.6.99 3.) 22.-23.09.99	0.700	600 to 1000	0.117 to 0.070	31.05.99 28.06. 18.08.	85	grapes, fruit	1.08 1.89 <u>2.33</u> 1.25 0.919 1.65	0 21 28 35 42 49	RIP2001-338

Table B.7.6-4: Residue trials in grapes with formulation BAS 510 01 F (parallel to trials with BAS 500 00F)

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						
Northern Europe											
# 2000/1000251 (BSF/606-1) FR - 51220 Brimont (EU North) 02.05.2001	Pinot meunier	1.) 1968 2.) 21.6.99 3.) 20.9.99	0.600	100	0.600	28.06.99 19.07. 24.08.	85	grapes, fruit	1.49 1.07 0.620 0.790 0.770 1.00	0 21 28 35 42 49	RIP2001-336
# 2000/1000251 (BSF/606-2) FR - 37210 Roche Corbon (EU North) 02.05.2001	Chenin blanc	1.) 1982 2.) 10.-19.6.99 3.) 6.-9.10.99	0.600	100	0.600	28.06.99 20.07. 26.08.	85	grapes, fruit	1.84 1.00 0.860 0.980 1.38 0.590	0 21 28 35 42 49	RIP2001-336
# 2000/1012411 (AGR/04/99) DE - 54518 Kesten (EU North) 02.05.2001	Kerner	1.) 1983 2.) 12.-28.6.99 3.) 29.09.99	0.600	600 to 800	0.100 to 0.075	23.06.99 21.07. 01.09.	83	grapes, fruit	1.32 0.710 1.09 1.01 0.830 0.770	0 21 28 35 42 48	RIP2001-337
# 2000/1012411 (DU2/05/99) D E- 69168 Wiesloch (EU North) 02.05.2001	Müller-Thurgau	1.) 1978 2.) 7.-21.6.99 3.) 21.09.99	0.600	600 to 800	0.100 to 0.075	17.06.99 02.08. 24.08.	81	grapes, fruit	1.03 1.42 1.39 1.34 1.30 2.10	0 21 28 35 41 48	RIP2001-337
# 2000/1012411 (DU4/13/99) DE - 76831 Eschbach (EU North) 02.05.2001	Portugieser	1.) 1984 2.) 10.-18.6.99 3.) 30.09.99	0.600	600 to 800	0.100 to 0.075	21.06.99 20.07. 02.09.	83	grapes, fruit	0.910 1.54 0.710 0.990 0.860 1.17	0 22 28 35 42 49	RIP2001-337

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						
# 2000/1014880 (X 99 62 10) FR - 51220 Merfy (EU North) 02.05.2001	Pinot Noir	1.) 1975 2.) 4.-14.6.99 3.) 15.-27.09.99	0.700	600 to 1000	0.117 to 0.070	11.06.99 12.07. 20.08.	83	grapes, fruit	1.21 0.947 <u>1.16</u> 0.757 1.02 0.883	0 20 27 34 41 49	RIP2001-338
Southern Europe											
# 2000/1000251 (BSF/606-3) FR - 84190 Beaumes de Venise (EU South) 02.05.2001	Clairette	1.) 1987 2.) 28.5.-11.6.99 3.) end 09/.99	0.600	100	0.600	11.06.99 02.07. 24.08.	83	grapes, fruit	1.02 0.510 0.680 0.900 <u>0.930</u> 0.890	0 21 28 35 41 48	RIP2001-336
# 2000/1000251 (BSF/606-4) FR - 30210 Pouzilhac (EU South) 02.05.2001	Syrah	1.) 1973 2.) 10.-20.6.99 3.) end 09/.99	0.600	100	0.600	22.06.99 10.07. 28.08.	84	grapes, fruit	0.260 0.520 <u>0.260</u> 0.220	0 21 28 34	RIP2001-336
# 2000/1012411 (AC/07/99) ES -21830 Bonares (EU South) 02.05.2001	Zalema	1.) 1961 2.) 20.4.-25.5.99 3.) 18.09.99	0.600	600 to 800	0.100 to 0.075	26.05.99 02.07. 10.08.	81	grapes, fruit	1.85 1.34 0.640 <u>1.60</u> 0.380 0.180	0 21 28 35 42 49	RIP2001-337
# 2000/1012411 (AC/08/99) ES -21840 Niebla Huelva (EU South) 02.05.2001	Zalema	1.) 1969 2.) 15.4.-29.5.99 3.) 22.09.99	0.600	600 to 800	0.100 to 0.075	26.05.99 02.07. 10.08.	81	grapes, fruit	1.09 1.22 <u>0.970</u> 0.810 0.430 0.540	0 21 28 35 42 49	RIP2001-337

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						
# 2000/1014880 (X 99 62 08) FR - 33210 Saint Pardon de Conques (EU South) 02.05.2001	Merlot	1.) 1992 2.) 1.-20.6.99 3.) 21.09.99	0.700	600 to 1000	0.117 to 0.070	10.06.99 08.07. 01.09.	85	grapes, fruit	0.698 0.652 <u>0.671</u> 0.650 0.537 0.604	0 21 28 35 42 48	RIP2001-338
# 2000/1014880 (X 99 62 09) FR - 84150 Caumont (EU South) 02.05.2001	Carignan	1.) 1970 2.) 25.5.-10.6.99 3.) 9.-17.09.99	0.700	600 to 1000	0.117 to 0.070	02.06.99 01.07. 06.08.	81 - 83	grapes, fruit	1.25 1.39 <u>1.10</u> 0.796 0.844 0.659	0 21 28 35 42 49	RIP2001-338
# 2000/1014880 (9938R) IT - 40024 Castel San Pietro Terme (EU South) 02.05.2001	Trebbiano	1.) 1986 2.) 31.5.-8.6.99 3.) 23.-29.09.99	0.700	600 to 1000	0.117 to 0.070	31.05.99 28.06. 18.08.	83	grapes, fruit	1.32 0.427 <u>1.16</u> 0.959 0.465 0.416	0 20 28 35 42 49	RIP2001-338
# 2000/1014880 (9939R) IT - 40055 Castenaso (EU South)	Trebbiano	1.) 1962 2.) 31.5.-5.6.99 3.) 22.-23.09.99	0.700	600 to 1000	0.117 to 0.070	31.05.99 28.06. 18.08.	85	grapes, fruit	1.38 1.09 3.44* 0.796 <u>1.18</u> 0.329	0 21 28 35 42 49	RIP2001-338 *possible outliner

MRL calculation grapes

In 1998 and 1999, residue trials with nicobifen were conducted in Germany, France, Spain and Italy with two different formulations. SC-formulation BAS 510 00 F was used in both years (12 N, 13 S) while product BAS 510 01 F (WG formulation) was applied in 1999 only, in parallel to the other trials. The results of the parallel trials are comparable. For calculation of the MRL the results obtained with formulation BAS 510 00 F are sufficient. Results for formulation BAS 510 01 F are given as supplementary data. The highest residue values at or after a PHI of 28 days were chosen for calculation of the MRL.

Differing from the intended use with only one application at growth stage 68 – 81 (BBCH), the residue trials were conducted with three applications at growth stages of about 67, 77 and 80 - 85 (BBCH).

Northern Europe

Supporting residue data:
12 trials with formulation BAS 510 00 F in 1998 and 1999 to grapes
Fruit, PHI 28 – 49 days: 0.86, 1.40, 1.45, 1.71, 1.76, 1.79, 2.01, 2.01, 3.26, 3.40, 3.49, 3.69

Supplementary data, 6 parallel trials with formulation BAS 510 01 F in 1999 to grapes
Fruit, PHI 28 – 49 days: 1.00, 1.09, 1.16, 1.17, 1.38, 2.10

MRL calculation

Formulation:	BAS 510 00 F	BAS 510 01 F
R_{max}:	4.86	2.82
R_{ber} (2 x R0.75):	6.73	3.12
STMR:	1.90	1.17

Southern Europe

Supporting residue data:
13 trials with formulation BAS 510 00 F in 1998 and 1999 to grapes
Fruit, PHI 28 – 42 days: 0.52, 0.84, 1.07, 1.21, 1.39, 1.45, 1.54, 1.72, 1.74, 2.32, 2.33, 3.15, 3.22

Supplementary data: 8 parallel trials with formulation BAS 510 01 F in 1999 to grapes
Fruit, PHI 28 – 41 days: 0.26, 0.67, 0.93, 0.97, 1.10, 1.16, 1.18, 1.60

MRL calculation

Formulation:	BAS 510 00 F	BAS 510 01 F
R_{max}:	3.93	2.24
R_{ber} (2 x R0.75):	4.65	2.35
STMR:	1.54	1.04

Due to the higher residues in the trials from Northern Europe the MRL proposal is calculated on the basis of these results.

MRL proposal for grapes: 5 mg/kg

B.7.6.4 Residues in oilseed rape

Material and methods

During the growing seasons 1999 and 2000, a total of 12 field trials were conducted in different representative areas for winter rape cultivation in Germany, Denmark, France, Great Britain and Sweden (10 N, 2 S) to determine the residue levels of nicobifen. In 1999, the fungicide was applied only once (at GS 63 – 65 BBCH code), whereas in 2000, two applications were performed (GS 53 –55 and 65). In both years the formulation BAS 510 01 F was tested at an application rate of 0.5 kg/ha (0.250 kg as/ha) in a water volume of 300 L/ha.

In all trials, plant without roots were collected directly after the last application (0 DALA). Oilrape grain samples were taken 38 – 93 (GS 89) days thereafter.

Findings

The residues of nicobifen found directly after the last application ranged between 0.71 and 3.21 mg/kg for plant without root. At time of harvest, 38 – 93 days after last application, the residues in oilrape grain did not exceed the limit of quantitation of 0.05 mg/kg. Due to the early application, no difference between one or two applications can be observed.

The results are summarised in Table B.7.6-5.

Table B.7.6-5: Residue trials in winter rape

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
Northern Europe											
# 2000/1012409 (D05/04/99) DE - 24625 Großharrie (EU North) 02.05.2001	Artus	1.) 3.9.98 2.) 2.-26.5.99 3.) 27.07.99	0.250	300	0.083	10.05.99	65	plant without root grain	2.02 < 0.05	0 78	RIP2001-339
# 2000/1012409 (D07/03/99) DE - 85445 Oberding (EU North) 02.05.2001	Express	1.) 26.8.98 2.) 8.-31.5.99 3.) 23.07.99	0.250	300	0.083	15.05.99	65	plant without root grain	2.75 < 0.05	0 66	RIP2001-339
# 2000/1014851 (D05/03/00) DE - 24625 Großharrie (EU North) 02.05.2001	Artus	1.) 1.9.99 2.) 29.4.-16.5.00 3.) 24.07.00	0.250	300	0.083	11.04.00 04.05.	65	plant without root grain	2.67 < 0.05	0 81	RIP2001-340
# 2000/1012409 (ALB/07/99) DK - 5500 Middelfart (EU North) 02.05.2001	Elite	1.) 4.8.98 2.) 1.-25.5.99 3.) 28.07.99	0.250	300	0.083	12.05.99	64	plant without root grain	0.71 < 0.05	0 77	RIP2001-339
# 2000/1012409 (FR2/06/99) FR - 62580 Neuvireuil (EU North) 02.05.2001	Adelie	1.) 26.8.98 2.) 7.-30.4.99 3.) 17.07.99	0.250	300	0.083	15.04.99	63	plant without root grain	2.30 < 0.05	0 93	RIP2001-339

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
# 2000/1012409 (FR4/07/99) FR - 21310 Beze (EU North) 02.05.2001	Columbus	1.) 28.8.98 2.) 10.4.-15.5.99 3.) 15.07.99	0.250	300	0.083	22.04.99	63	plant without root grain	1.11 < 0.05	0 91	RIP2001-339
# 2000/1012409 (OAT/11/99) GB - Leaminton Spa CU33 9QB (EU North) 02.05.2001	Apex	1.) 25.8.98 2.) 22.4.-5.5.99 3.) 21.07.99	0.250	300	0.083	27.04.99	65	plant without root grain	3.21 < 0.05	0 84	RIP2001-339
# 2000/1012409 (OAT/12/99) GB - Radclive MK18 4AB Buckingham (EU North) 02.05.2001	Contact	1.) 12.9.98 2.) 12.4.-10.5.99 3.) 20.07.99	0.250	300	0.083	28.04.99	65	plant without root grain	2.41 < 0.05	0 83	RIP2001-339
# 2000/1014851 (OAT/03/00) GB - Leaminton Spa CV33 9QB (EU North) 02.05.2001	Apex	1.) 4.9.99 2.) 13.4.-9.5.00 3.) 21.07.00	0.250	300	0.083	06.04.00 27.04.	65	plant without root grain	2.16 < 0.05	0 83	RIP2001-340
# 2000/1014851 (HUS/05/00) SE - 23791 Bjärred (EU North) 02.05.2001	Capitol	1.) 28.8.99 2.) 5.5.-1.6.00 3.) 20.07.00	0.250	300	0.083	30.04.00 13.05.	65	plant without root grain	2.27 < 0.05	0 69	RIP2001-340

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
Southern Europe											
# 2000/1014877 (A0034 BD1) FR - 26750 St. Paul les Romans (EU South) 02.05.2001	Ebonite	1.) 31.8.99 2.) n. a. 3.) n. a.	0.250	300	0.083	14.04.00 02.05.	69	plant without root grain	2.32 < 0.05	0 48	RIP2001-341
# 2000/1014877 (A0034 TL1) FR – 31330 Aussonne (EU South) 02.05.2001	Constant	1.) 10.9.99 2.) n. a. 3.) n. a.	0.250	300	0.083	21.04.00 13.05.	69	plant without root grain	2.03 < 0.05	0 38	RIP2001-341

MRL calculation rape

In rape, 12 residue trials were conducted (10 N, 2 S) with either one or two applications at the intended application rates. Since the treatments are performed during flowering, no residues above the LOQ (0.05 mg/kg) were found and are to be expected in seeds. Therefore, the data presented are sufficient to set a MRL for the intended uses in oilseed rape with two applications and for both regions of Europe.

MRL proposal for rape seeds: 0.05 mg/kg

B.7.6.5 Residues in beans

Material and methods

During the growing seasons 1999 and 2000, a total of 11 field trials were conducted in different representative bean growing areas in Germany, Denmark and France (9 N, 2 S) And 8 glasshouse trials in Spain to determine the residue levels of nicobifen. In 15 trials bush beans were chosen, the remaining four were performed with climbing beans. In all cases, the WG formulation BAS 510 01 F was tested with an application rate of 1.0 kg/ha (0.5 kg as/ha) in a spray volume of 300 L/ha and 3 applications.

From all trials, plant samples without roots were taken directly after the last application (0 DALA). After about 3, 7 and 14 days pods with seed as well as shoots without pods were collected.

Findings

The nicobifen residues on plants without roots taken directly after the last application ranged between 10.6 and 30.1 mg/kg in the field trials and between 26.0 and 114.2 mg/kg in the glasshouse ones. These differences between the indoor and outdoor results could be due to the absence of wind which prevents a drift of the fungicide.

Seven days after the last application which is the intended PHI of the crop, the residues in pods of bush beans from field trials were between 0.12 and 0.95 mg/kg. In the glasshouse trials, the bush bean pods showed residues between 0.29 and 1.67 mg/kg and the climbing bean pods between 0.06 and 0.61 mg/kg. Results are given in Table B.7.6-7, Table B.7.6-8 and Table B.7.6-9.

Data for outdoor application in South Europe are not sufficient (2 trials), further trials are required.

In 2 of the glasshouse trials, significantly higher residues were found (1.65 and 1.67 mg/kg). It is not clear from the data whether only late treatments or low temperatures as well are the reason for these high values. Further trials according to GAP are required to get a realistic worst case for glasshouse uses. Different regions and seasons should be covered. A summary of the indoor trials in relation to growth stage and temperature is given in Table B.7.6-6.

Table B.7.6-6: Residues in beans in relation to growth stage and temperature

Growth season	Number of trials	Growth stage at last treat-ment	Air temp. at treatments °C	Soil temp. at treatments °C	Air temp. during last month °C	Highest residue at or after PHI 7 mg/kg
Bush beans						
4/99 – 6/99	2	69 –71	26 - 36	23.8 – 29.8	13 - 47	0.29, 0.69
9/00 – 12/00	2	75, 87	5 - 16	14.6 – 15.8	0 - 37	1.65, 1.67
Climbing beans						
3/00 – 5/00	1	69	21 - 27	19.5 – 22.5	8 - 35	0.06
9/99 – 11/99	2	69 – 73	18 - 25	16.4 – 20.7	11 - 33	0.28, 0.28
8/00 – 12/00	1	83	12 - 22	15.0 – 19.9	7 - 36	0.61

Table B.7.6-7: Field trials in bush beans

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						
Northern Europe											
# 2000/1014846 (ACK/03/99) DE - 16818 Wustrau (EU North) 02.05.2001	Berggold	1.) 23.6.99 2.) 10.-20.8.99 3.) 15.8.99	0.500	300	0.167	10.08.99 18.08. 24.08.	71 - 73	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	20.2 43.0 0.710 29.0 0.471 27.4 0.472	0 3 3 7 7 15 15	RIP2001-342
# 2000/1014846 (DU2/07/99) DE - 67667 Studernheim (EU North) 02.05.2001	Primel	1.) 27.4.99 2.) 10.6.-12.7.99 3.) 5.7.99	0.500	300	0.167	14.06.99 21.06. 28.06.	66	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	14.0 28.5 0.632 12.8 0.262 6.06 0.181	0 3 3 7 7 14 14	RIP2001-342
# 2000/1014846 (D08/02/99) DE - 59505 Bad Sassendorf- Sieningsen (EU North) 02.05.2001	Scuba	1.) 2.5.99 2.) 21.6.-1.7.99 3.) 15/16.7.99	0.500	300	0.167	22.06.99 30.06. 06.07.	73	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	14.5 20.4 0.479 22.2 0.219 6.50 0.077	0 3 3 8 8 14 14	RIP2001-342
# 2000/1014850 (DU2/10/00) DE - 74613 Öhringen Büttelbrunn (EU North) 02.05.2001	Paulista	1.) 20.4.00 2.) 10.-20.6.00 3.) 4.7.00 - n.a.	0.500	300	0.167	07.06.00 14.06. 20.06.	71	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	30.1 43.2 0.893 45.6 0.666 44.5 0.529	0 3 3 8 8 14 14	RIP2001-345

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
# 2000/1014846 (ALB/04/99) DK - 5500 Middelfart (EU North) 02.05.2001	Bonbon	1.) 19.5.99 2.) 18.-30.7.99 3.) 15.8.99	0.500	300	0.167	23.07.99 30.07. 05.08.	75	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	16.4 10.5 0.145 11.4 0.118 6.75 0.126	0 4 4 8 8 15 15	RIP2001-342
# 2000/1014850 (ALB/02/00) DK - 5580 Nr. Aaby (EU North) 02.05.2001	Bon-Bon	1.) 16.5.00 2.) 24.7.-8.8.00 3.) 22.8.-4.9.00	0.500	300	0.167	01.08.00 08.08. 15.08.	75	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	22.9 23.5 0.842 19.8 0.498 16.7 0.284	0 3 3 7 7 14 14	RIP2001-345
# 2000/1014850 (ALB/03/00) DK - 5500 Middelfart (EU North) 02.05.2001	Bon-Bon	1.) 16.5.00 2.) 20.7.-4.8.00 3.) 14.-24.8.00	0.500	300	0.167	24.07.00 01.08. 07.08.	75	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	24.7 23.7 0.805 29.7 0.834 22.5 0.425	0 3 3 7 7 14 14	RIP2001-345
# 2000/1014850 (FR2/02/00) FR - 62123 Gouves (EU North) 02.05.2001	Flagrano	1.) 5.6.00 2.) 1.-8.8.00 3.) 24.-25.8.00	0.500	300	0.167	03.08.00 10.08. 17.08.	76	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	18.1 17.7 0.534 20.1 0.255 16.5 0.292	0 4 4 7 7 14 14	RIP2001-345
# 2000/1014876 (A0033 AN1) FR - 67112 Breuschwickers- heim (EU North) 02.05.2001	Masai	1.) 16.6.00 2.) n. a. 3.) n. a.	0.500	300	0.167	01.08.00 08.08. 15.08.	71	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	18.4 < 0.05 * 0.582 21.5 0.531 15.4 0.314	0 3 3 7 7 14 14	RIP2001-346 *sample was proba- bly identical with untreated sample

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						
Southern Europe											
# 2000/1014876 (A0033 TL2) FR - 31330 Saint Caprais (EU South) 02.05.2001	Booster	1.) 8.7.00 2.) n. a. 3.) n. a.	0.500	300	0.167	23.08.00 30.08. 06.09.	73	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	13.9 30.3 0.710 22.6 0.950 19.9 0.645	0 3 3 7 7 15 15	RIP2001-346
# 2000/1014876 (A0033 SA1) FR - 40260 Castets (EU South) 02.05.2001	Pissos	1.) 10.5.00 2.) n. a. 3.) n. a.	0.500	300	0.167	20.06.00 27.06. 04.07.	69	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	10.6 8.71 0.740 7.73 0.617 2.72 0.286	0 3 3 6 6 13 13	RIP2001-346

[1)] shoots without pods

Table B.7.6-8: Indoor residue trials in bush beans

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						
# 2000/1014847 (AC/11/99) ES - 11549 La Algaida (EU South) 02.05.2001	Primel	1.) 22.4.99 2.) 25.5.99 - n.a. 3.) 4.6.99	0.500	300	0.167	20.05.99 25.05. 02.06.	69	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	78.3 51.8 3.10 30.3 0.693 18.5 0.059	0 2 2 7 7 14 14	RIP2001-343
# 2000/1014847 (AC/12/99) ES - 11549 La Algaida (EU South) 02.05.2001	Primel	1.) 3.5.99 2.) 5.6.99 - n.a. 3.) 16.6.99	0.500	300	0.167	04.06.99 09.06. 16.06.	69-71	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	40.1 27.9 2.63 36.3 0.289 17.5 0.063	0 2 2 8 8 15 15	RIP2001-343
# 2000/1014849 (AC/11/00) ES - 11160 Barbate Cadiz (EU South) 02.05.2001	Primel	1.) 30.9.00 2.) 4.11.00 - n.a. 3.) 4.-18.12.00	0.500	300	0.167	21.11.00 28.11. 04.12.	75	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	34.6 83.3 3.80 71.8 1.29 121.3 1.65	0 3 3 7 7 14 14	RIP2001-344
# 2000/1014849 (AC/12/00) ES - 11160 Barbate Cadiz (EU South) 02.05.2001	Primel	1.) 26.9.00 2.) 31.10.00 - n.a. 3.) 27.11.- 18.12.00	0.500	300	0.167	21.11.00 28.11. 04.12.	87	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	40.9 54.3 1.46 76.5 1.67 104.0 1.13	0 3 3 7 7 14 14	RIP2001-344

[1)] shoots without pods

Table B.7.6-9: Indoor residue trials in climbing beans

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						
# 2000/1014847 (AC/13/99) ES – 41720 Los Palacios (EU South) 02.05.2001	Festival RZ	1.) 11.9.99 2.) 19.10.- 2.11.99 3.) 1.11.99	0.500	300	0.167	19.10.99 26.10. 02.11.	69 - 73	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	64.0 38.1 0.485 44.0 0.277 38.8 0.142	0 3 3 7 7 14 14	RIP2001-343
# 2000/1014847 (AC/23/99) ES - 41720 Los Palacios (EU South) 02.05.2001	Emerite	1.) 11.9.99 2.) 19.10.- 2.11.99 3.) 1.11.99	0.500	300	0.167	19.10.99 26.10. 02.11.	69 - 71	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	114.2 57.4 0.562 104.0 0.281 29.0 0.103	0 3 3 7 7 14 14	RIP2001-343
# 2000/1014849 (AC/13/00) ES - 41720 Los Palacios Sevilla (EU South) 02.05.2001	Elda	1.) 9.3.00 2.) 17.4.00 - n.a. 3.) 2.5.00 - n.a.	0.500	300	0.167	11.04.00 17.04. 25.04.	69	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	50.5 26.5 0.946 14.1 0.062 15.1 < 0.05	0 3 3 8 8 14 14	RIP2001-344
# 2000/1014849 (AC/14/00) ES - 11549 Sanlucar de Barrameda Cadiz (EU South) 02.05.2001	Judia Dulce	1.) 25.8.00 2.) 30.10.00 - n.a. 3.) 17.11.- 29.12.00	0.500	300	0.167	21.11.00 28.11. 04.12.	83	pl. w/o root shoots [1)] pods w. seed shoots [1)] pods w. seed shoots [1)] pods w. seed	26.0 110.2 0.960 91.9 0.612 92.6 0.457	0 3 3 7 7 14 14	RIP2001-344

[1)] shoots without pods

MRL calculation beans

In 1999 and 2000, 11 field trials with nicobifen were conducted in Denmark, Germany and France (9 N, 2 S) and 8 indoor trials in Spain. The data are sufficient to derive a MRL for North Europe. Further trials are required for Southern Europe and for indoor. The highest residue values at or after a PHI of 7 days were chosen for calculation of the MRL.

Northern Europe

Supporting residue data:
9 trials with formulation BAS 510 01 F in 1999 and 2000 to bush beans, out door.
Pods with seeds, PHI 7 – 14 days: 0.13, 0.22, 0.26, 0.29, 0.47, 0.50, 0.53, 0.67, 0.83

MRL calculation

R_{max}:	1.13
R_{ber} (2 x R0.75):	1.20
STMR:	0.47

In the glasshouse trials higher residue levels of up to 1.67 mg/kg were found, but further trials are required. Therefore, the MRL proposal is

MRL proposal for beans (with pods): 2 mg/kg

B.7.6.6 Residues in peas

Material and methods

During the growing seasons 1999 and 2000, a total of 11 field trials were conducted in different representative pea growing areas in Germany, Denmark, France and Sweden (9 N, 2 S) to determine the residue levels of nicobifen. In all trials, the WG formulation BAS 510 01 F was applied two times at an application rate of 1.0 kg/ha (0.5 kg as/ha) in spray volumes between 300 and 400 L/ha.

Plant samples without roots were taken directly after the last application (0 DALA). After about 3, 7 and 14 days green seed, pods without seed and the rest of the plant were sampled.

Findings

The residues of nicobifen found directly after the last application ranged between 4.38 and 13.3 mg/kg. After about seven days at the proposed PHI, 7 out of 11 samples of green seeds did not show residues above the limit of quantitation (0.05 mg/kg). In the remaining four seed samples residues between 0.064 and 0.090 mg/kg were found.

Data for application in South Europe are not sufficient (2 trials), further trials are required.

Table B.7.6-10: Residue trials in peas

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						
Northern Europe											
# 2000/1014848 (DU4/10/99) DE - 67227 Frankenthal (EU North) 02.05.2001	Progress	1.) 13.3.99 2.) 19.5.-6.6.99 3.) 21.6.99	0.500	400	0.125	07.06.99 14.06.	81	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	5.78 11.49 1.54 < 0.05 10.81 1.16 < 0.05 7.57 1.27 < 0.05	0 3 3 3 7 7 7 14 14 14	RIP2001-347
# 2000/1014852 (D08/02/00) DE - 59494 Soest- Hiddingsen (EU North) 02.05.2001	DUELL	1.) 8.4.00 2.) 1.-18.6.00 3.) 10.-11.7.00	0.500	400	0.125	16.06.00 23.06.	73	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	4.38 3.64 0.504 0.122 2.93 0.433 < 0.05 1.15 0.208 < 0.05	0 3 3 3 7 7 7 14 14 14	RIP2001-349
# 2000/1014848 (ALB/14/99) DK - 5500 Middelfart (EU North) 02.05.2001	Polar	1.) 28.4.99 2.) 20.6.-8.7.99 3.) 23.7.99	0.500	400	0.125	09.07.99 16.07.	73	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	8.24 12.02 1.24 0.102 1.07* 10.54* < 0.05 7.60 1.27 < 0.05	0 3 3 3 7 7 7 14 14 14	RIP2001-347 * values probably mixed up

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						
# 2000/1014852 (ALB/04/00) DK - 5500 Middelfart (EU North) 02.05.2001	Polar	1.) 8.5.00 2.) 1.-17.7.00 3.) 1.-4.8.00	0.500	400	0.125	17.07.00 24.07.	77	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	13.3 22.6 3.89 0.334 21.6 3.62 0.090 22.4 4.71 0.230	0 3 3 3 7 7 7 14 14 14	RIP2001-349
# 2000/1014879 (X 99 62 14) FR - 62116 Ablainzevelle (EU North) 02.05.2001	Bonette	1.) 9.4.99 2.) 10.-25.6.99 3.) 14.7.99	0.500	300	0.167	01.07.99 08.07.	77 - 79	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	8.97 12.0 1.86 0.060 15.2 2.38 0.070 22.1 3.88 < 0.05	0 4 4 4 7 7 7 14 14 14	RIP2001-348
# 2000/1014852 (FR2/03/00) FR - 62580 Neuville Saint Vaast (EU North) 02.05.2001	Modena	1.) 13.3.00 2.) 23.5.-2.6.00 3.) 22.6.00	0.500	400	0.125	09.06.00 16.06.	77	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	11.0 17.5 1.76 0.064 15.9 2.47 0.064 22.6 2.68 0.067	0 3 3 3 7 7 7 14 14 14	RIP2001-349
# 2000/1014878 (A0035 AN1) FR - 67112 Breuschwickers-heim (EU North) 02.05.2001	Merveille de Kelvedon	1.) 8.6.00 2.) n. a. 3.) n. a.	0.500	400	0.125	21.07.00 28.07.	72	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	8.70 3.53 0.60 < 0.05 1.60 0.274 < 0.05 1.52 0.231 < 0.05	0 3 3 3 7 7 7 14 14 14	RIP2001-350

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						
# 2000/1014848 (HUS/08/99) SE - 23791 Bjärred (EU North) 02.05.2001	F8	1.) 17.4.99 2.) 28.6.-6.7.99 3.) 18.7.99	0.500	400	0.125	06.07.99 14.07.	76	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	5.83 1.51 12.1 0.057 2.26 10.7 < 0.05 7.49 34.1 * 0.061	0 3 3 3 7 7 7 15 15 15	RIP2001-347 * result was reana-lysed
# 2000/1014852 (HUS/06/00) SE - 23791 Bjärred (EU North) 02.05.2001	40 453	1.) 21.4.00 2.) 13.-25.6.00 3.) 6.-8.7.00	0.500	400	0.125	21.06.00 28.06.	76	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	6.32 2.06 0.354 < 0.05 1.81 0.308 < 0.05 1.83 0.183 < 0.05	0 4 4 4 7 7 7 14 14 14	RIP2001-349
Southern Europe											
# 2000/1014879 (X 99 62 13) FR - 86120 Curcay sur Dive (EU South) 02.05.2001	Alladin	1.) 19.3.99 2.) 25.5.-8.6.99 3.) 16.6.99	0.500	300	0.167	01.06.99 08.06.	69	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	6.59 10.0 1.19 0.130 11.5 0.800 0.070 9.02 0.500 < 0.05	0 3 3 3 8 8 8 14 14 14	RIP2001-348
# 2000/1014878 (A0035 DR1) FR - 47120 Duras (EU South) 02.05.2001	Cisca	1.) 20.4.00 2.) n. a. 3.) n. a.	0.500	400	0.125	26.06.00 03.07.	69 - 72	pl. w/o root rest of plant pods w/o seed seed rest of plant pods w/o seed seed rest of plant pods w/o seed seed	5.03 10.6 3.06 0.082 8.78 3.86 < 0.05 11.9 7.14 < 0.05	0 3 3 3 8 8 8 14 14 14	RIP2001-350

MRL calculation peas

In 1998 and 1999, 11 residue trials with nicobifen were conducted in Denmark, Germany, France and Sweden (9 N, 2 S). The data are sufficient to derive a MRL for North Europe. Further trials are required for Southern Europe. The highest residue values at or after a PHI of 7 days were chosen for calculation of the MRL.

Northern Europe

Supporting residue data:
9 trials with formulation BAS 510 01 F in 1999 and 2000 to peas, out door.
Seeds, PHI 7 – 15 days: 0.05, 0.05, 0.05, 0.05, 0.05, 0.06, 0.07, 0.07, 0.23

MRL calculation

R_{max}:	0.25
R_{ber} (2 x R0.75):	0.14
STMR:	0.05

MRL proposal for peas without pods: 0.3 mg/kg

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 residues

Report:	Scharf J. 1998 Hydrolysis of BAS 510 F at 90°C, 100°C, and 120°C BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep. unpublished BASF RegDoc# 1998/10878
Test material:	[diphenyl-U^{14}C]-nicobifen
Guidelines:	91/414/EEC
GLP:	Yes
Acceptability:	Yes

Material and methods

To estimate the degradation behaviour of the test substance during industrial processing or household preparation, different processes (pasteurisation, baking, brewing, boiling and sterilisation) were simulated. The test was performed with the diphenyl labelled compound. The test substances were dissolved in aqueous buffer solutions of different pH-values. To avoid an influence of light, the glassware was wrapped. The test conditions are given in Table B.7.7-1.

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. By means of HPLC-analysis it could be shown that nicobifen remained unchanged during all the different tests.

Table B.7.7-1: Recovery data after the simulation of processing

Process	Test conditions	Diphenyl-label % TAR* after test
Pasteurisation	pH 4, 90 °C, 20 min	99.3
Baking, brewing and boiling	pH 5, 100 °C, 60 min	100.2
Sterilisation	pH 6, 120 °C, 20 min	99.1

* Total applied radioactivity

Conclusion

Nicobifen 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). Therefore, no degradation products were observed.

Residues in peel/pulp

No distribution of residues in peel/pulp had to be analysed as BAS 510 01 F is not intended to be used in plant products with inedible portions.

B.7.7.2 Effects on the residue level

B.7.7.2.1 Residues in grapes

Report: Meumann H. et al. 2000(c)
Study on the residue behaviour of BAS 510 F in grape process fractions after treatment with BAS 510 01 F under field conditions in Germany, 1999
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep.
unpublished
BASF RegDoc# 2000/1012412

Test material: BAS 510 01 F

Guidelines:	FAO Guidelines on producing pesticide residue data from supervised trials, Rome 1990 BBA Guideline Part IV, 3-3, "Testing of residue behaviour - General information on design, preparation and realization of residue tests" BBA Guideline Part IV, 3-4, "Testing the residue behaviour - Residue tests on processed plant products (processing guideline)", BBA Guideline Part IV, 3-3.4, "Testing the residue behaviour - Studies on grape must and wine", IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992
GLP:	Yes
Acceptability:	Yes

Material and methods

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 nicobifen in grapes and grape process fractions (must, wine, pomace).

The fungicidal test product BAS 510 01 F, which contains nominal 500 g/kg of nicobifen as a WG-formulation, was applied 3 times at growth stage (GS) BBCH 67 and 77 as well as 28 +/- 2 days before normal harvest at application rates of about 1.2 kg/ha in spray volumes of 600, 700 and 800 l/ha respectively.

For the analysis grape samples were collected within 3 hours after the last application as well as 28 days later. Fruit samples for processing of juice, wine and pomace were taken 28 days after the last application.

The samples were analysed with BASF method no. 445/0. The method quantifies the residues of nicobifen with a limit of quantitation of 0.05 mg/kg in all sample materials. The results of procedural recovery experiments obtained with each analytical series were about 88 % at fortification levels between 0.05 mg/kg and 5.0 mg/kg.

Findings

The residue level directly after the application ranged between 0.56 and 1.49 mg/kg. After 28 days, the residues of nicobifen in grapes were between 0.50 and 1.58 mg/kg. These grapes were processed according to the normal practice of wine making producing must, wet pomace and wine.

The residues in must and wine were lower than in grapes and lowest after mash heating (0.06 – 0.14 mg/kg). Wet pomace, the waste product obtained after the filter step, contained residues of nicobifen between 1.30 and 3.79 mg/kg.

Table B.7.7-2: Residues of nicobifen in grape process fractions

Portion analysed	DALA[1)]	Range of	
		residues (mg/kg)	concentration factors[2)]
fruit	0	0.56 - 1.49	-
fruit	28	0.50 - 1.58	-
wet pomace	28	1.30 - 3.79	1.95 – 3.41
must, cold	28	0.23 - 0.68	0.32 – 0.52
wine, from must, cold	28	0.18 - 0.66	0.26 – 0.47
must, after short time heating	28	0.26 - 0.67	0.45 – 0.48
wine from must, after short time heating	28	0.21 - 0.64	0.36 – 0.46
must, after mash heating	28	0.09 - 0.14	0.09 – 0.18
wine from must, after mash heating	28	0.06 - 0.13	0.08 – 0.12

1) days after last application
2) Concentration factor = residue in process fraction / residue in RAC

Conclusion

In none of the consumer products like must or wine a concentration of the nicobifen residues can be observed which is expressed by concentration factors < 1. However, in the waste product wet pomace a concentration was observed, expressed by concentration factors ranging between 1.95 and 3.41.

B.7.7.2.2 Residues in peas

Report: Scharm M. 2001
Determination of the residue of Reg. No. 300 355 in peas and processed products following tretment with BAS 510 01 F under field conditions in Germany 2000
Fresenius, Chem. und biolog. Laboratorien, Taunusstein-Neuhof, Germany Fed. Rep.
unpublished
BASF RegDoc# 2000/1014885

Guidelines: BBA Guideline Part IV, 3-3, "Testing of residue behaviour - General information on design, preparation and realization of residue tests"
BBA Guideline Part IV, 3-4, "Testing the residue behaviour - Residue tests on processed plant products (processing guideline)"
IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992, EEC 91/414

GLP: Yes

Acceptability: Yes

Material and methods

During the 2000 growing season 4 field trials were conducted in different pea cultivation areas to determine the residue levels of nicobifen in peas and process fractions involved in canned pea production.

The fungicidal test product BAS 510 01 F, which contains nominal 500 g/kg of BAS 510 F as a WG-formulation, was applied two times at growth stage (GS) BBCH 67 to 77 (14 to 15 days before expected harvest) and at GS 75 to 81 (7 to 8 days before expected harvest) at application rates of about 2 kg/ha (1 kg as/ha) in spray volumes of 300 to 400 l/ha. This application rate is the twofold of the recommended rate in order to increase the probability of finding residues.

For the analysis shoots (whole plant without roots) were collected directly after the last application. In three trials, green seeds were sampled at GS 79 (7 - 8 DALA) and manually separated from the pods. In one trial, the seeds were ripe earlier than expected. The almost dry seeds were harvested using a combine harvester at GS 81 (7 DALA). All seed samples were used for residue analysis and processing.

During the processing of peas in order to produce canned peas, the following fractions are obtained: washed peas, wash water, cooked peas, boiled water, canned peas and vegetable stock.

The samples were analysed with BASF method no. 445/0. The method quantifies the residues of nicobifen with a limit of quantitation of 0.05 mg/kg in all sample materials. The results of procedural recovery experiments obtained with each analytical series averaged at about 92% at fortification levels between 0.05 mg/kg and 0.5 mg/kg.

Findings

The residue level in shoots sampled directly after the application ranged between 5.09 and 20.27 mg/kg. After 7 - 8 days, only the sample of green seeds which were harvest almost dry showed a residue of nicobifen of 0.14 mg/kg. The other three samples did not show residues above the limit of quantitation.

Washing the peas gave the following result: The washed peas obtained from the one sample containing nicobifen showed a residue of 0.07 mg/kg. The wash water therefrom contained 0.06 mg/kg nicobifen. All other washed pea or wash water samples did not show any residues of nicobifen.

Subsequently, the peas were cooked. The fraction of cooked peas as well as the boiled water from all trials did not contain any residues of nicobifen. Finally, the peas were canned according to the normal practice. All canned peas as well as their vegetable stock did not show any residues of nicobifen.

Table B.7.7-3: Residues of nicobifen in peas and process fractions thereof

Portion analysed	DALA[1)]	Range of	
		residues (mg/kg)	concentration factors [2)]
Shoots	0	5.09 – 20.27	
Green seed (RAC)	7 – 8	< 0.05 – 0.14	
Washed peas	-	< 0.05 – 0.07	0.50[3)]
Wash water	-	< 0.05 – 0.06	0.43[3)]
Cooked peas	-	< 0.05	
Boiled water	-	< 0.05	
Canned peas	-	< 0.05	
Vegetable stock	-	< 0.05	

[1)] Days after last application
[2)] Concentration factor = residue in process fraction / residue in RAC
[3)] Result from single sample with residues above LOQ

Conclusion

The one trial in which residues in green seeds were found, allows the following conclusion: The nicobifen residues from the RAC are partly washed off by the washing process and therefore are found in both the washed peas and the washing water. After the subsequent process of cooking no residues of nicobifen above the LOQ were seen in both cooked peas and boiled water. Accordingly, no residues were found in the consumer products canned peas and vegetable stock.

No concentration of nicobifen took place during the process of canned pea production, expressed by a concentration factor < 1.

B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)

B.7.8.1 Ruminants

Report: Tilting N. 2001
Investigation of residues of BAS 510 F (Reg. No. 300355) in tissues and milk of dairy cows
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep.
unpublished
BASF RegDoc# 2000/1017228

Guidelines: EPA 860.1480

GLP: Yes

Acceptability: Yes

Material and methods

Fourteen lactating Holstein cows (Bos taurus) in the weight range from 480 kg to 735 kg before treatment and a milk yield approximately 15 kg or above per day were used in the study. The feeding and dosing was performed by ADAS Bridgets in Martyr Worthy (Winchester) UK. Animals were selected from the dairy herd of ADAS Bridgets.

Feeding and husbandry

The animals were group housed in a stall typical for dairy cattle. They were kept according to usual dairy cow husbandry conditions. The cows were offered a total mixed ration mainly consisting of grass and maize silage from automatic feeding stations and they had access to water ad libitum. Feed consumption was recorded for individual animals.

Selection of dose levels

The dietary burden was calculated using data from residue trials performed in Europe and the US. The main contributions to the dietary burden were vegetables. As data were based on a limited number of available results, values may deviate from actual tolerance proposals, but still provide a valid picture of the exposure of cattle to nicobifen residues. While US and EU models gave slightly different feeding levels a value reflecting a true US 10x level was used as highest feeding level.

The calculations performed for the estimation of the feeding levels according to US EPA guidelines and EU proposals are summarised in Table B.7.8-1 and Table B.7.8-2.

Table B.7.8-1: Calculation according to US EPA Guidelines

Feed Item	%Dry Matter	% in Diet	Maximum Residue Level (mg/kg)	Feed Burden (mg/kg)
Carrot (culls)	12	25	0.50	1.00
Almond hulls	90	10	2.50	0.30
Potato waste	15	65	0.10	0.50
Total diet (mg/kg)				1.80

Table B.7.8-2: Calculation for the EU

Feed Item	%Dry Matter	% in Diet	Maximum Residue Level (mg/kg)	Feed Burden (mg/kg)
Dairy Cow				
Carrots	10	30	0.25	0.750
Cale/cabbage	14	35	0.10	0.250
Oilseed	86	30	0.15	0.052
Pulses	86	5	0.05	0.003
Total diet (mg/kg)				**1.06**
Beef Cattle				
Carrots	10	60	0.25	1.500
Cale/cabbage	14	35	0.10	0.250
Oilseed	86	5	0.15	0.009
Total diet (mg/kg)				**1.76**

Anticipated residues were estimations. Extreme residue values from early growth stages of the crop or extremely short PHIs were not taken into account. Therefore, it was concluded from the results that a feeding level of 1.5 mg/kg feed (dry matter) would reflect a realistic 1 x residue situation in feed, leading to feeding levels of 4.5 mg/kg feed (dry matter) for the 3 x level and 18 mg/kg feed (dry matter) for the highest dose level (US =10 x level, EU = 12x).

Dose preparation and administration

The test substance was dissolved in ethanol once a week, diluted to appropriate concentrations and filled into glass vials. Concentration was verified by HPLC analysis and the stability of the test compound in solution was confirmed.

Animals were dosed twice daily. 20 ml of the solution containing the active substance was added to molassed sugar beet (appr. 0.5 kg) and offered to the animals. The achieved daily intake is calculated in terms of mg/kg feed, (nominal and actual), absolute intake in mg/day and animal and mg/kg body weight. The calculations were based on average weight and feed intake data during the dosing period. The results are listed in Table B.7.8-3.

The dosing period was of four weeks duration. Three cows from each dose group were slaughtered after 28 or 29 days (on the day of last dosing) and one cow from group D (high dose group) was kept on normal diet for another 7 days to monitor the residue decline after withdrawal of the test compound. One of the 5 animals in group D had to be removed from the study, because the cow developed mastitis, which did not respond to antibiotic treatment and milk yield and food intake dropped to zero.

Table B.7.8-3: Dose level achieved

Cow (BASF Number)	Nominal Dose m/kg feed	Actual Dose: mg/animal/day	Actual Concentration: mg/kg-bw	Actual Concentration:** mg/kg-feed
1068 (1)	0	0	0	0
3191 (2)	0	0	0	0
5337 (3)	0	0	0	0
Average group A	**0**	**0**	**0**	**0**
996 (4)	1.5	30	0.047	1.583
3541 (5)	1.5	30	0.058	2.058
5180 (6)	1.5	30	0.044	1.765
Average group B	**1.5**	**30**	**0.050**	**1.802**
3137 (7)	4.5	90	0.161	5.56
3253 (8)	4.5	90	0.130	7.15
3555 (9)	4.5	90	0.176	5.02
Average group C	**4.5**	**90**	**0.156**	**5.91**
1226 (10)	18	360	0.612	20.50
1938 (11)	18	360	0.611	19.18
5070* (12)	18	360	-	-
3048 (13)	18	360	0.507	20.61
3083 (14)	18	360	0.495	20.36
Average group D	**18**	**360**	**0.556**	**20.16**

* Animal withdrawn from study due to mastitis
** Based on average feed intake during dosing period

Milking and sampling

Cows were milked twice daily in an automatic milking parlour. Representative aliquots of milk from each milking were taken by a proportional sample collector. Milk yields were recorded, no treatment related effects upon milk yield were observed. Aliquots from morning and evening milk were pooled for analysis. On day 21 of the study a larger amount of milk was taken and separated into cream and skim milk by centrifugation.

Terminal procedures

Animals were killed at the Central Veterinary Laboratory (Weybridge, UK) by using a captive bolt, followed by use of a pithing rod and exsanguination.

Residue analysis

Analysis of milk and tissue samples was carried out according to BASF method 471/0 to determine residues of the parent substance as well as its metabolites M510F01 and M510F02. These compound form the relevant residue in animals. Method 471/0 is based on LC/MS/MS quantification and the limit of quantitation is 0.01 mg/kg for each analyte in milk and milk products and 0.025 mg/kg for tissues.

To allow a risk assessment for non extractable residues, liver and selected milk samples were analysed with a method using microwave treatment for extraction and derivatisation according to method 476/0. BASF Method No. 476/0 was developed to determine residues of nicobifen in liver (bound residues) and milk (minor metabolites) after microwave treatment. The chosen microwave conditions lead to the formation of M510F53, a characteristic analyte that is representative for bound residues of nicobifen in liver and a sum of minor metabolites in milk. Method 476/0 is a GC-MS method with a limit of quantitation of 0.01 mg/kg in milk and 0.05 mg/kg in liver.

During the study, procedural recoveries were analysed along with each set of samples. High average recoveries for method 471/0 and method 476/0 were found for all matrices and spiking levels. All mean recoveries ranged from 84.4 % to 90.0 % in case of nicobifen, from 76.6 % to 90.8 % for M510F01, with relative standard deviations from 2.4 to 13 and a recovery from 93.2 % to 103.1% for M510F53.

Findings

Animal Observations

Daily observations and necropsy revealed no treatment related health problem of the animals. For most of the cows a gain in bodyweight was observed during the study. This effect was considered not to be treatment related.

Residues in whole milk, skim milk and cream

As shown in Table B.7.8-4 no residues were detected in samples taken from the control and the 1 x dose groups. In few samples from the 3 x dose group residues close above the limit of quantitation of 0.01 mg/kg for the active substance nicobifen were detected, but no residues of M510F01 or M510F02 were observed. For calculation of the group average, residues below the limit of quantitation were set to ½ of the LOQ. Therefore, the group average is < 0.02 mg/kg.

In the 10 x dose group residues of the active substance occurred regularly from day 1 on and a plateau was reached after two weeks with average residues between 0.04 mg/kg and 0.05 mg/kg.

Analysis of the samples from the withdrawal animal showed that no residues in milk could be observed three days after dosing had stopped. Therefore, it can be concluded that residues are rapidly excreted.

On day 21 of the study, milk samples were also separated into skim milk and cream. In one case in group D (10 x does) a residue of 0.01 mg/kg was detected in skim milk. In cream, residues of parent nicobifen were found at all dose levels with good dose-linear correlation.

No residues that form M510F53 on microwave treatment were found in selected milk samples from all dose groups. The results are summarised in Table B.7.8-5.

Table B.7.8-4: Summary of Group Mean Milk Results (Sum of nicobifen, M510F02 and M510F01 determined by method no. 471/0) expressed as parent equivalent

Day of Study	Group A (control) (mg/kg)	Group B 1 x (mg/kg)	Group C 3 x (mg/kg)	Group D 10 x (mg/kg)
-3	< 0.02	< 0.02	< 0.02	< 0.02
1	n.a.	n.a.	< 0.02	0.02
3	n.a.	n.a.	< 0.02 (0.022*)	0.04
6	< 0.02	< 0.02	< 0.02	0.02
9	< 0.02	< 0.02	< 0.02	0.03
12	< 0.02	< 0.02	< 0.02	0.03
15	n.a.	n.a.	< 0.02 (0.021*)	0.04
18	n.a.	n.a.	< 0.02 (0.023*)	0.05
21	< 0.02	n.a.	< 0.02 (0.021*)	0.04
24	n.a.	n.a.	< 0.02	0.04
28	< 0.02	< 0.02	< 0.02 (0.02*)	0.04
29	n.a.	n.a.	< 0.02	n.a.
32**	n.a.	n.a.	n.a.	< 0.02**
36**	n.a.	n.a.	n.a.	< 0.02**
Skim Milk	< 0.02	< 0.02	< 0.02	< 0.02 (0.021*)
Cream	< 0.02	0.04	0.12	0.34

n.a. = not analysed
* Single value higher than LOQ
** Individual results cow 14 (3 and 7 days after dosing stopped)
For calculation of means, results below the LOQ were set to ½ of the LOQ

Table B.7.8-5: Summary of Individual and Group Mean Milk Results forming Metabolite M510F53 (Total Method no. 476/0) expressed as parent equivalent in mg/kg

	Group B, Cow #			Group C, Cow #			Group D. Cow #				
Day	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
3	< 0.01	n.a.	n.a.	< 0.01	n.a.	n.a.	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
9	n.a.	< 0.01	n.a.	n.a.	<0.01.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
18	n.a.	n.a.	< 0.01	n.a.	n.a.	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
28	< 0.01	n.a.	n.a.	< 0.01	n.a.	n.a.	< 0.01	< 0.01	n.a.	< 0.01	< 0.01
Mean	< 0.01			< 0.01			< 0.01				

n.a. = not analysed
For calculation of means, results below the LOQ were set to ½ of the LOQ

Tissues

Only in liver and kidney residues consisting of parent and the hydroxylated metabolites (M510F01 and M510F02) could be quantified. In all other matrices the parent molecule was the only quantifiable residue.

In muscle, only one sample out of the 10 x dose group showed residues of nicobifen close above the LOQ, all others were < 0.05 mg/kg. In fat, one sample of the 1 x dosing group showed a residue higher than LOQ, while in the 3x and 10x quantifiable residues of nicobifen were found.

In liver and kidney of group C, only metabolite M510F01 and M510F02 were found. In group D, parent as well as metabolites were determined. Additionally, liver was analysed for bound residues by microwave treatment. Only the 10 x dose group contained quantifiable residues in the range of 0.08 to 0.09 mg/kg.

Seven days after withdrawal, no residues above the LOQ could be detected in tissues. The results are summarised in Table B.7.8-6 and Table B.7.8-7.

Table B.7.8-6: Summary of Residue Levels in Tissues (Sum of nicobifen, M510F01 and M510F02, determined by method 471/0)

Treatment Group	Group Mean Nicobifen Residue (mg/kg) Sum of Nicobifen and Metabolites			
	Muscle	Fat	Liver	Kidney
A (Control)	< 0.05	< 0.05	< 0.05	< 0.05
B (1 x)	< 0.05	< 0.05 (0.078*)	< 0.05	< 0.05
C (3 x)	< 0.05	0.11	0.06	0.07
D (10 x)	< 0.05 (0.058*)	0.27	0.18	0.24
D (10 x) (7 days withdrawal)	< 0.05	< 0.05	< 0.05	< 0.05

For calculation of mean, results below the LOQ were set ½ of the LOQ
* Single value higher than LOQ

Table B.7.8-7: Summary of Residue Levels of Nicobifen (Method 471/0) and its non Extractable Metabolites in Liver (Microwave Treatment Method 476/0)

Treatment Group	BAS 510 F and M510F01 Method 471/0	M510F53 Method 476/0	Total Residue
A (Control)	< 0.05	< 0.05	< 0.1
B (1 x)	< 0.05	< 0.05	< 0.1
C (3 x)	0.06	< 0.05	0.11
D (10 x)	0.18	0.09	0.27
D (7 days withdrawal)	< 0.05	< 0.05	< 0.1

Conclusion

A residue transfer study with nicobifen was conducted in cows. The animals were dosed with 1.5, 4.5 and 18 mg/kg feed (dry matter) equal to 30, 120 and 360 mg/animal/day for a minimum period of 28 days.

In the dose group relevant under normal agricultural conditions (1.5 mg/kg feed), no residues could be detected in milk, meat, liver and kidney. In fat, the group average was zero though a single residue above the limit of quantitation was detected. In cream, residues were found at all dose levels.

At higher dose levels, residues were detected in milk, fat, liver and kidney.. No residues of non extractable nicobifen metabolite residues occurred in liver samples from the 1 x and 3 x dose groups. In the 10 x group (18 mg/kg feed) non extractable residues could be detected.

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.

Both prerequisites are not fulfilled:

Residues may occur in oilseed rape grain, which is the only part of oilseed crops that is fed to poultry with a proposed MRL of 0.05 mg/kg. Additional crops will be part of future project like cale, but do not add significantly to the feed burden. Up to now, submission in peas and beans is not for fodder crops.

The worst case scenario is the assumption that chicken are fed with 10 % oilseed rape grains in the total diet. The calculated dose in terms of mg/kg feed is very low [see Table B.7.8-8].

Table B.7.8-8: Calculation of daily intake of nicobifen in poultry

	% dry matter in feed	Intake of fresh feed	Residues in fresh feed	Residue intake mg/kg feed
Poultry: bw 1.9 kg, feed intake 0.12 kg				
Oilseed rape	86	10 % (0.01 kg)	0.05 mg/kg	0.005
Total:				**0.005**

The metabolism study in laying hens was performed at dose levels of approximately 12 mg per kg feed. This corresponds to approximately the 2400 x dose [see Table B.7.8-9]. 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 (see chapter B.7.2). 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.7.8-10]. It can also be concluded that additional crops can be registered without exceeding the trigger values for a hen feeding study.

Table B.7.8-9: Calculation of overdosing factor (nominal values)

	Hen metabolism	Potential intake poultry
mg/kg body weight	0.76	0.0005
mg/kg feed	12	0.005
Overdosing factor: dose in metabolism study / potential intake		
mg/kg feed		2400

Table B.7.8-10: Potential transfer of residues to poultry tissues and eggs

Matrix	Total radioactive residue (mg/kg) from metabolism studies	Extrapolated total residue from actual intake (mg/kg)
Eggs	0.058	$2.4\ 10^{-5}$
Muscle	0.0025	$1.0\ 10^{-6}$
Liver	0.1687	$7.0\ 10^{-5}$
Fat	0.025	$1.0\ 10^{-5}$

B.7.8.3 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.7.8.4 Storage Stability

Report: Grosshans F. 2001(c)
Investigation of the stability of residues of BAS 510 F and M510F01 in sample materials of animal origin under usual storage conditions
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep.
unpublished
BASF RegDoc# 2000/1017229

Guidelines: EPA 860.1380, EEC 91/414 (7032/VI/95)

GLP: Yes

Acceptability: Yes

Material and methods

The deep freeze stability of nicobifen and the metabolite M510F01 in animal matrices was under investigation over a period of 170 days. Untreated samples of muscle, liver and milk from the cow were fortified with 0.5 mg/kg with nicobifen and M510F01. These matrices were representative for the samples stored during the residue transfer study in cows. All samples were stored under the usual storage conditions for samples (polyethylene bottles, < -18° C). After 0 and approx. 60, 100 and 170 days, samples were analysed with BASF method no. 471. The average procedural recoveries and their relative standard deviations obtained with BAS method 471 fulfilled the requirements with regard to limit of quantification for the

analytes nicobifen and M510F01 (0.01 mg/kg in milk, cream and eggs and 0.025 mg/kg for tissues), repeatability and recoveries.

Findings

The results show no significant decrease in concentration of the analytes for milk, muscle and liver. Detailed results are summarised in Table B.7.8-11 and Table B.7.8-12.

Table B.7.8-11: Recovery of nicobifen and M510F01 after storage of samples fortified with 0.5 mg/kg

Day	Nicobifen*		M510F01*	
	mg/kg	%	mg/kg	%
Milk				
0	0.526	105.2	0.498	99.6
62	0.507	101.4	0.481	96.2
103	0.467	93.4	0.500	100.0
167	0.499	99.8	0.506	101.2
Muscle				
0	0.507	101.4	0.495	99.0
62	0.501	100.2	0.467	93.4
103	0.456	91.2	0.470	94.0
167	0.521	104.2	0.449	89.8
Liver				
0	0.509	101.8	0.500	100.0
63	0.507	101.4	0.504	100.8
103	0.488	97.6	0.514	102.8
167	0.482	96.4	0.465	93.0

* Recoveries corrected for procedural recovery

Table B.7.8-12: Degradation of nicobifen and M510F01 after 166 / 167 days assuming a linear degradation

	Nicobifen [%]	M510F01 [%]
Milk	7.7	-2.9 *
Muscle	1.8	8.5
Liver	5.2	4.2

* a negative value indicates a calculatory increase

Conclusion

The residues of nicobifen and M510F01 proved to be stable in all matrices for at least 5 months. All samples from the cow feeding study were analysed within 5 months. Thus the residues were sufficiently stable during the period of storage.

B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6; Annex IIIA 8.5)

B.7.9.1 Confined Rotational Crop

Report:	Hamm R.T.,Veit P. 2001 Confined rotational crop study with 14C-BAS 510 F BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep. unpublished BASF RegDoc# 2000/1014862
Test material:	[diphenyl-U-^{14}C]-nicobifen, batch no. 641-2017, radiochemical purity: > 99 %, specific activity: 6.27 MBq/mg [pyridine-3-^{14}C]-nicobifen, batch no. 640-1026, radiochemical purity: > 99 %, specific activity: 5.81 MBq/mg
Guidelines:	BBA Guideline Part IV, 3-10, "Testing of residue behaviour of plant protection products in rotational crops (crop rotation guideline)" EPA 860.1850, Residue Chemistry Test Guidelines - Confined Accumulation in Rotational Crops
GLP:	Yes
Acceptability:	Yes

Materials and Methods

The residue levels and the nature of the residues in three different succeeding crops were investigated following application of ^{14}C-nicobifen (pyridine and diphenyl 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 2100 g as/ha.

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), 270 days (simulating a plant back for vegetables; 270 DAT) and 365 days (365 DAT). After 30 days, ploughing was simulated by mixing the treated and untreated soil layers (about 20 cm). Afterwards, the crops: radish, lettuce and wheat were sowed or planted and grown either in growth chambers, where natural climatic conditions were simulated, or in a vegetation hall or in a green house.

After each harvest, the top layer of 20 cm was digged up again and the next plants were sowed or planted correspondent to the ageing periods. The roots of wheat and lettuce remained in the soil after harvest. Decrease of TRR levels in soil due to plant up take of crops grown during the ageing period was negligible.

Food and feed items of mature crops were harvested, processed and analysed by combustion and subsequent radioactivity measurement for the determination of the total radioactive residues in the raw agricultural commodities (RAC's). In addition soil samples were taken after application, ploughing and after each harvest of mature crops.

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 aqueous ammonia extraction was added. The remaining post extraction solids from the wheat matrices: forage,

straw and grain and in addition from radish leaf and root (diphenyl label, 120 DAT) were treated with sodium hydroxide or DMSO to release part of the remaining radioactivity. Methanol extracts of all samples under investigation were analysed by HPLC.

Findings

Soil

The total radioactive residues in soil after ageing and ploughing decreased at longer ageing intervals. After the 1st ageing period (30 DAT), the TRR level in the diphenyl treated soil was higher than in the pyridine treated soil (1.112 to 0.716 mg/kg) but the levels were close after an ageing interval of 365 days (0.429 to 0.356 mg/kg).

The TRR levels in soils after harvest varied within the crops and the plant back intervals. A tendency could not be detected. Soils after harvest were extracted with methanol and analysed by HPLC. Only parent was detected.

Table B.7.9-1: Total radioactive residues in soil samples after treatment with ^{14}C-nicobifen (pyridine and diphenyl label)

Soil Samples	Pyridine label TRR [mg/kg]	Diphenyl label TRR [mg/kg]
After application		
Plant back intervals (after soil ageing, ploughing)		
30 DAT	0.716	1.112
120 DAT	0.648	0.813
270 DAT	0.647	n.d.
365 DAT	0.356	0.429
After harvest of ripe crops		
Plant back interval: 30 DAT		
Radish	n.d.	0.731
Lettuce	0.545	0.747
Wheat	0.379	0.393
Plant back interval: 120 DAT		
Radish	0.548	0.585
Lettuce	0.484	0.409
Wheat	0.386	0.506
Plant back interval: 270 DAT		
Radish	0.377	0.521
Lettuce	0.321	0.436
Wheat	0.537	0.551
Plant back interval: 365 DAT		
Radish	n.d.	0.460
Lettuce	n.d.	0.434
Wheat	0.125	0.343

n.d. = not determined

Total radioactive residue

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.

In *lettuce leaves* (both labels) highest residue levels were found after an ageing period of 120 days and decreased afterwards (pyridine label 120 DAT: 0.161 mg/kg; 365 DAT: 0.022 mg/kg; diphenyl label 120 DAT: 0.084 mg/kg; 365 DAT: 0.028 mg/kg).

The residue levels in *radish roots* (pyridine label) did not change significantly after longer ageing periods (30 DAT: 0.048 mg/kg; 365 DAT: 0.066 mg/kg). The residue levels in radish roots of the diphenyl label were highest at plant back interval 270 DAT and lowest at 365 DAT. (270 DAT: 0.098 mg/kg; 365 DAT: 0.030 mg/kg).

The residue level in *wheat grain* (pyridine label) was highest after an ageing period of 120 days and decreased again afterwards (120 DAT: 0.285 mg/kg; 365 DAT: 0.148 mg/kg). The residue levels in wheat grain of the diphenyl label decreased over the four ageing periods (30 DAT: 0.166 mg/kg; 365 DAT: 0.048 mg/kg).The TRR levels at plant back intervals of 270 DAT and 365 DAT were significantly higher in pyridine label compared to diphenyl label.

Also in *wheat straw* (pyridine label) the residue level was highest after an ageing period of 120 days and decreased afterwards (120 DAT: 4.008 mg/kg; 365 DAT: 1.925 mg/kg). The residue levels in wheat straw of the diphenyl label were very high and decreased over the four ageing periods (30 DAT: 9.826 mg/kg; 365 DAT: 1.404 mg/kg).

Extractability

Methanol extraction could release 56.4 – 97.3 % TRR for the pyridine label and 62.8 – 96.1 % TRR for the diphenyl label, with the exception of wheat grain. The extractability with methanol was low for wheat grain and ranged from 4.0 – 11.7 % TRR for the pyridine label and 12.3 – 35.4 % TRR for the diphenyl label.

Table B.7.9-2: Quantitative distribution of radioactive residues in rotational crops after treatment with pyridine labelled ^{14}C-nicobifen

Crop parts / DAP	TRR ERR + RRR	ERR Methanol		RRR	
	mg/kg	mg/kg	%TRR	mg/kg	%TRR
Plant back interval: 30 DAT					
Lettuce Leaf 71	0.035	0.028	81.2	0.007	18.8
Radish Leaf 86	0.343	0.317	92.2	0.027	7.8
Radish Root 86	0.048	0.039	80.7	0.009	19.3
Wheat Forage 97	0.690	0.643	93.2	0.047	6.8
Wheat Straw 163	3.609	3.258	90.3	0.351	9.7
Wheat Grain 163	0.147	0.017	11.7	0.130	88.3
Plant back interval: 120 DAT					
Lettuce Leaf 99	0.161	0.146	90.8	0.015	9.2
Radish Leaf 112	0.211	0.187	88.8	0.024	11.2
Radish Root 112	0.038	0.031	81.6	0.007	18.4
Wheat Forage 111	0.433	0.379	87.5	0.054	12.5
Wheat Straw 191	4.008	2.715	67.7	1.293	32.3
Wheat Grain 191	0.285	0.025	8.9	0.260	91.1

Crop parts / DAP	TRR ERR + RRR	ERR Methanol		RRR	
	mg/kg	mg/kg	%TRR	mg/kg	%TRR
Plant back interval: 270 DAT					
Lettuce Leaf 89	0.031	0.023	74.5	0.008	25.5
Radish Leaf 89	0.125	0.108	86.1	0.017	13.9
Radish Root 89	0.017	0.013	77.1	0.004	22.9
Wheat Forage 87	0.230	0.224	97.3	0.006	2.7
Wheat Straw 154	1.614	0.911	56.4	0.703	43.6
Wheat Grain 154	0.271	0.011	4.0	0.260	96.0
Plant back interval: 365 DAT					
Lettuce Leaf 58	0.022	0.017	76.1	0.005	23.9
Radish Leaf 58	0.113	0.103	91.1	0.010	8.9
Radish Root 58	0.066	0.060	91.0	0.006	9.0
Wheat Forage 55	0.255	0.213	83.5	0.042	16.5
Wheat Straw 161	1.925	1.488	77.3	0.437	22.7
Wheat Grain 161	0.148	0.010	6.8	0.138	93.2

Table B.7.9-3: Quantitative distribution of radioactive residues in rotational crops after treatment with diphenyl labelled ^{14}C-nicobifen

Crop parts	TRR ERR + RRR	ERR Methanol		RRR	
	mg/kg	mg/kg	%TRR	mg/kg	%TRR
Plant back interval: 30 DAT					
Lettuce Leaf 77	0.050	0.047	93.8	0.003	6.2
Radish Leaf 89	0.337	0.324	96.1	0.013	3.9
Radish Root 89	0.072	0.067	93.1	0.005	6.9
Wheat Forage 92	1.575	1.504	95.5	0.071	4.5
Wheat Straw 156	9.826	8.414	85.6	1.412	14.4
Wheat Grain 156	0.166	0.031	18.4	0.135	81.6
Plant back interval: 120 DAT					
Lettuce Leaf 101	0.084	0.075	89.2	0.009	10.8
Radish Leaf 114	0.294	0.248	84.4	0.046	15.6
Radish Root 114	0.052	0.041	78.7	0.011	21.3
Wheat Forage 92	0.980	0.867	88.5	0.113	11.5
Wheat Straw 150	3.912	3.498	89.4	0.414	10.6
Wheat Grain 150	0.243	0.030	12.3	0.213	87.8
Plant back interval: 270 DAT					
Lettuce Leaf 60	0.067	0.063	94.1	0.004	5.9
Radish Leaf 60	0.150	0.141	94.3	0.009	5.7
Radish Root 60	0.098	0.091	92.8	0.007	7.2
Wheat Forage 55	0.562	0.496	88.3	0.066	11.7
Wheat Straw 124	3.226	2.487	77.1	0.739	22.9
Wheat Grain 124	0.023	0.008	35.4	0.015	64.6

Crop parts	TRR ERR + RRR	ERR Methanol		RRR	
	mg/kg	mg/kg	%TRR	mg/kg	%TRR
Plant back interval: 365 DAT					
Lettuce Leaf 60	0.028	0.018	62.8	0.010	37.2
Radish Leaf 93	0.207	0.197	95.2	0.018	4.8
Radish Root 93	0.030	0.027	89.9	0.003	10.1
Wheat Forage 63	0.265	0.247	93.1	0.018	6.9
Wheat Straw 158	1.404	1.253	89.3	0.151	10.7
Wheat Grain 158	0.048	0.012	25.1	0.036	79.4

Residual residues

The remaining residues after extraction (RRR) varied significantly in the three crops. They were low in lettuce and in radish root and ranged between 0.003 and 0.015 mg/kg. In radish leaves the results were similar and ranged between 0.009 and 0.046 mg/kg. In wheat straw the values were significantly higher with 0.151 to 1.412 mg/kg, and in grains the corresponding values were 0.015 to 0.260 mg/kg.

In *wheat forage and straw*, further characterisation by an aquatic ammonia extraction, followed by an NaOH-extraction showed, that smaller parts of the organo-insoluble radioactive residues belong to the protein-, cellulose- or lignin-fraction. Extractability with ammonia was better for diphenyl label than for pyridine label.

In *wheat grain*, the organo-insoluble residues were extracted with ammonia and afterwards with DMSO followed by starch precipitation with ethanol. Ammonia extraction released 13.0 – 22.9 % TRR (0.005 – 0.049 mg/kg) for both labels. For the pyridine label, 36.2 – 48.4 % TRR (0.061 – 0.118 mg/kg) was found in the starch fraction. For the diphenyl label, the concentrations in the starch fraction were significantly lower, they ranged from 0.6 - 4.3 % TRR (0.001 – 0.004 mg/kg).

Results on the nature of the residual residues are summarised in Table B.7.9-4 and Table B.7.9-5.

Table B.7.9-4: Summary of released organo-insoluble radioactivity in rotational crops after treatment with pyridine labelled ^{14}C-nicobifen

Crop parts	RRR	NH3	Cellulose	Lignin solid	Lignin liquid	Protein precipita-tion*	Starch
	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)
Plant back interval: 30							
Wheat forage	0,047 (6,8)	0,011 (1,5)	0,008 (1,2)	0,007 (1,0)	0,018 (2,7)	-	-
Wheat straw	0,351 (9,7)	0,089 (2,5)	0,054 (1,5)	0,176 (4,9)	0,124 (3,4)	0,004 (0,1)	
Wheat grain	0,130 (88,3)	0,019 (13,0)	-	-	-	0,005 (3,1)	0,071 (48,4)
Plant back interval: 120							
Wheat forage	0,054 (12,5)	0,005 (1,1)	0,007 (1,5)	0,008 (1,9)	0,025 (5,8)	<0,001 (0,1)	-
Wheat straw	1,293 (32,3)	0,167 (4,2)	0,076 (1,9)	0,110 (2,7)	0,405 (10,1)	0,012 (0,4)	-
Wheat grain	0,260 (91,1)	0,049 (17,3)	-	-	-	0,027 (9,6)	0,118 (41,4)
Plant back interval: 270							
Wheat straw	0,703 (43,6)	0,087 (5,4)	0,041 (2,6)	0,041 (2,5)	0,164 (10,1)	0,007 (0,4)	-
Wheat grain	0,260 (96,0)	0,038 (13,9)	-	-	-	0,016 (5,9)	0,098 (36,2)
Plant back interval: 365							
Wheat straw	0,437 (22,7)	0,094 (4,8)	0,030 (1,6)	0,057 (3,0)	0,178 (9,2)	0,008 (0,4)	-
Wheat grain	0,138 (93,2)	0,019 (12,9)	-	-	-	0,008 (5,7)	0,061 (41,0)

* part of the ammonia extract

Table B.7.9-5: Summary of released organic insoluble radioactivity in rotational crops after treatment with diphenyl labelled ^{14}C-nicobifen

Crop parts	RRR	NH3	Cellulose	Lignin solid	Lignin liquid	Protein precipita-tion*	Starch
	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)
Plant back interval: 30							
Wheat forage	0,071 (4,5)	0,027 (1,7)	0,014 (0,9)	0,009 (0,6)	0,015 (0,9)	0,021 (1,3)	
Wheat straw	1,412 (14,4)	0,800 (8,2)	0,121 (1,2)	0,216 (2,2)	0,177 (1,8)	0,019 (0,2)	
Wheat grain	0,135 (81,6)	0,028 (16,9)				0,011 (6,5)	0,001 (0,6)

Crop parts	RRR	NH3	Cellulose	Lignin solid	Lignin liquid	Protein precipita-tion*	Starch
	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)	mg/kg (%TRR)
Plant back interval: 120							
Wheat forage	0,113 (11,5)	0,042 (4,3)	0,018 (1,8)	0,015 (1,5)	0,023 (2,4)	0,003 (0,3)	
Wheat straw	0,414 (10,6)	0,206 (5,3)	0,040 (1,0)	0,052 (1,3)	0,054 (1,4)	0,014 (0,4)	
Wheat grain	0,213 (87,8)	0,032 (13,0)				0,012 (4,9)	0,004 (1,6)
Plant back interval: 270							
Wheat straw	0,739 (22,9)	0,378 (11,7)	0,114 (3,5)	0,150 (4,6)	0,066 (2,0)	0,044 (1,4)	
Wheat grain	0,015 (64,6)	0,005 (22,9)				0,002 (9,6)	0,001 (4,3)
Plant back interval: 365							
Wheat forage	0,018 (6,9)	0,008 (3,0)	0,002 (0,7)	0,004 (1,6)	0,008 (2,9)	0,001 (0,3)	
Wheat straw	0,151 (10,7)	0,082 (5,8)	0,014 (1,0)	0,036 (2,5)	0,019 (1,4)	0,007 (0,5)	
Wheat grain	0,036 (79,4)	0,007 (15,2)				0,003 (6,2)	0,001 (2,1)

* part of the ammonia extract

Metabolism

The methanol extractable ^{14}C-residues were characterised by different HPLC methods. In all extracts, the most prominent peak was ^{14}C-nicobifen.

In *lettuce*, the ^{14}C-nicobifen concentration ranged from 0.014 – 0.072 mg/kg/ 55.6 – 94.1 % TRR for the both labels. One higher value was detected after 120 days of soil ageing for the pyridine label at 0.146 mg/kg. One polar peak at low concentrations was detected in both labels.

In *radish root*, the concentration of ^{14}C-nicobifen varied between 0.009 - 0.091 mg/kg (52.6 – 92.8 % TRR) for both labels. In addition, the metabolite M510F61, a sugar conjugate of the parent compound, was detected in a concentration of ≤ 0.006 mg/kg (diphenyl label) after a soil ageing period of 120 and 365 days and a polar peak was detected.

In *radish leaves* the concentration of ^{14}C-nicobifen varied between 0.088 to 0.304 mg/kg for both labels. The metabolite M510F61 was found in most of the radish leave samples (0.004 – 0.032 mg/kg; with the highest values for the diphenyl label). Additionally, in the sample at 120 DAT of the diphenyl label a polar peak in a concentration of 0.039 mg/kg / 13.2% TRR was found.

In *wheat straw*, the concentration of ^{14}C-nicobifen varied between 0.808 to 3.156 mg/kg (50.0 – 87.5 % TRR) for the pyridine label and between 1.088 – 7.991 mg/kg (70.8 – 84.6 % TRR) for the diphenyl label. In addition, the metabolite M510F61 was detected in a concentration of ≤ 0.117 mg/kg for the pyridine label and with a concentration of 0.025 – 0.423 mg/kg for the diphenyl label. At plant back intervals of 270 DAT and 365 DAT two peaks were detected in concentrations of ≤ 0.032 mg/kg / 2.0 % TRR (pyridine label) and 0.140 – 0.174 mg/kg / 5.4 – 10.0 % TRR (diphenyl label).

In *wheat grain*, the concentration of ^{14}C-nicobifen ranged from 0.005 to 0.015 mg/kg for the pyridine label and from 0.008 – 0.028 mg/kg for the diphenyl label. The metabolite M510F61 was not detected in grain. A polar peak amounted to ≤ 0.010 mg/kg (≤ 3.8% TRR) for both labels.

Table B.7.9-6: Investigation of the nature of the residues in rotational crops after treatment with pyridine labelled ^{14}C-nicobifen

Crop parts	TRR	ERR	RRR	Nicobifen	M510F61	Unidentified Metabolites	
	mg/kg	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg	% TRR
Plant back interval: 30 DAT							
lettuce leaf	0.035	0.028 (81.2)	0.007 (18.8)	0.020 (58.5)	-	1 peak 0.008	22.7%
radish leaf	0.343	0.317 (92.2)	0.027 (7.8)	0.301 (87.6)	0.016 (4.6)	-	
radish root	0.048	0.039 (80.7)	0.009 (19.3)	0.030 (62.7)	-	1 peak: 0.009	18.0%
wheat forage	0.690	0.643 (93.2)	0.047 (6.8)	0.619 (89.8)	0.024 (3.4)	-	
wheat straw	3.609	3.258 (90.3)	0.351 (9.7)	3.156 (87.5)	0.102 (2.8)	-	
wheat grain	0.147	0.017 (11.7)	0.130 (88.3)	0.009 (6.1)	-	2 peaks: 0.006 0.003	3.8% 1.8%
Plant back interval: 120 DAT							
lettuce leaf	0.161	0.146 (90.8)	0.015 (9.2)	0.146 (90.8)	-	-	
radish leaf	0.211	0.187 (88.8)	0.024 (11.2)	0.172 (81.8)	0.015 (7.0)	-	
radish root	0.038	0.031 (81.6)	0.007 (18.4)	0.023 (60.1)	-	1 peak: 0.008	21.5%
wheat forage	0.433	0.379 (87.5)	0.054 (12.5)	0.379 (87.5)	-	-	
wheat straw	4.008	2.715 (67.7)	1.293 (32.3)	2.598 (64.8)	0.117 (2.9)	-	
wheat grain	0.285	0.025 (8.9)	0.260 (91.1)	0.015 (5.3)	-	1 peak: 0.010	3.6%

Crop parts	TRR	ERR	RRR	Nicobifen	M510F61	Unidentified Metabolites	
	mg/kg	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg	% TRR
Plant back interval: 270 DAT							
lettuce leaf	0.031	0.023 (74.5)	0.008 (25.5)	0.020 (65.1)	-	1 peak: 0.003	9.4%
radish leaf	0.125	0.108 (86.1)	0.017 (13.9)	0.104 (82.5)	0.004 (3.6)	-	
radish root	0.017	0.013 (77.1)	0.004 (22.9)	0.009 (52.6)	-	1 peak: 0.004	24.5%
wheat forage	0.230	0.224 (97.3)	0.006 (2.7)	0.214 (92.8)	0.005 (2.3)	1 peak 0.005	2.2%
wheat straw	1.614	0.911 (56.4)	0.703 (43.6)	0.808 (50.0)	0.071 (4.4)	1 peak: 0.032	2.0%
wheat grain	0.271	0.011 (4.0)	0.260 (96.0)	0.005 (1.9)	-	1 peak: 0.006	2.1%
Plant back interval: 365 DAT							
lettuce leaf	0.022	0.017 (76.1)	0.005 (23.9)	0.014 (61.6)	-	1 peak: 0.003	14.5%
radish leaf	0.113	0.103 (91.1)	0.010 (8.9)	0.088 (78.2)	0.013 (11.2)	1 peak: 0.002	1.7%
radish root	0.066	0.060 (91.0)	0.006 (9.0)	0.060 (91.0)	-	-	
wheat forage	0.255	0.213 (83.5)	0.042 (16.5)	0.191 (74.7)	0.008 (2.9)	2 peaks: 0.005 0.010	1.8% 4.0%
wheat straw	1.925	1.488 (77.3)	0.437 (22.7)	1.488 (77.3)	-	-	
wheat grain	0.148	0.010 (6.8)	0.138 (93.2)	0.006 (4.2)	-	1 peak: 0.004	2.6%

Table B.7.9-7: Investigation of the nature of the residues in rotational crops after treatment with diphenyl labelled ^{14}C-nicobifen

Crop parts	TRR	ERR	RRR	Nicobifen	M510F61	Unidentified Metabolites	
	mg/kg	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg	% TRR
Plant back interval: 30 DAT							
lettuce leaf	0.050	0.047 (93.8)	0.003 (6.2)	0.047 (93.8)	-	-	
radish leaf	0.337	0.324 (96.1)	0.013 (3.9)	0.304 (90.2)	0.020 (5.9)	-	
radish root	0.072	0.067 (93.1)	0.005 (6.9)	0.064 (89.6)	-	1 peak: 0.003	3.5%
wheat forage	1.575	1.504 (95.5)	0.071 (4.5)	1.472 (93.5)	0.032 (2.0)	-	
wheat straw	9.826	8.414 (85.6)	1.412 (14.4)	7.991 (81.3)	0.423 (4.3)	-	
wheat grain	0.166	0.031 (18.4)	0.135 (81.6)	0.028 (16.8)	-	1 peak: 0.003	1.6%
Plant back interval: 120 DAT							
lettuce leaf	0.084	0.075 (89.2)	0.009 (10.8)	0.072 (85.2)	-	1 peak: 0.003	4.0%
radish leaf	0.294	0.248 (84.4)	0.046 (15.6)	0.209 (71.2)	-	1 peak: 0.039	13.2%
radish root	0.052	0.041 (78.7)	0.011 (21.3)	0.035 (67.8)	0.006 (10.9)	-	
wheat forage	0.980	0.867 (88.5)	0.113 (11.5)	0.846 (86.4)	0.021 (2.1)	-	
wheat straw	3.912	3.498 (89.4)	0.414 (10.6)	3.311 (84.6)	0.187 (4.8)	-	
wheat grain	0.243	0.030 (12.3)	0.213 (87.8)	0.023 (9.6)	-	1 peak: 0.007	2.7%
Plant back interval: 270 DAT							
lettuce leaf	0.067	0.063 (94.1)	0.004 (5.9)	0.063 (94.1)	-	-	
radish leaf	0.150	0.141 (94.3)	0.009 (5.7)	0.109 (73.1)	0.032 (21.2)	-	
radish root	0.098	0.091 (92.8)	0.007 (7.2)	0.091 (92.8)	-	-	
wheat forage	0.562	0.496 (88.3)	0.066 (11.7)	0.352 (62.8)	0.102 (18.1)	2 peaks: 0.024 0.018	4.2% 3.3%
wheat straw	3.226	2.487 (77.1)	0.739 (22.9)	2.283 (70.8)	0.030 (0.9)	1 peak: 0.174	5.4%
wheat grain	0.023	0.008 (35.4)	0.015 (64.6)	0.008 (35.4)	-	-	

Crop parts	TRR	ERR	RRR	Nicobifen	M510F61	Unidentified Metabolites	
	mg/kg	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg	% TRR
Plant back interval: 365 DAT							
lettuce leaf	0.028	0.018 (62.8)	0.010 (37.2)	0.016 (55.6)	-	1 peak: 0.002	7.2%
radish leaf	0.207	0.197 (95.2)	0.018 (4.8)	0.144 (69.4)	0.032 (15.5)	2 peaks: 0.018 0.004	8.5% 1.7%
radish root	0.030	0.027 (89.9)	0.003 (10.1)	0.024 (78.4)	0.001 (4.0)	1 peak: 0.002	7.5%
wheat forage	0.265	0.247 (93.1)	0.018 (6.9)	0.199 (75.0)	0.026 (9.8)	1 peak: 0.002	8.3%
wheat straw	1.404	1.253 (89.3)	0.151 (10.7)	1.088 (77.6)	0.025 (1.8)	1 peak: 0.140	10.0%
wheat grain	0.048	0.012 (25.1)	0.036 (74.9)	0.011 (23.6)	-	1 peak: 0.001	1.5%

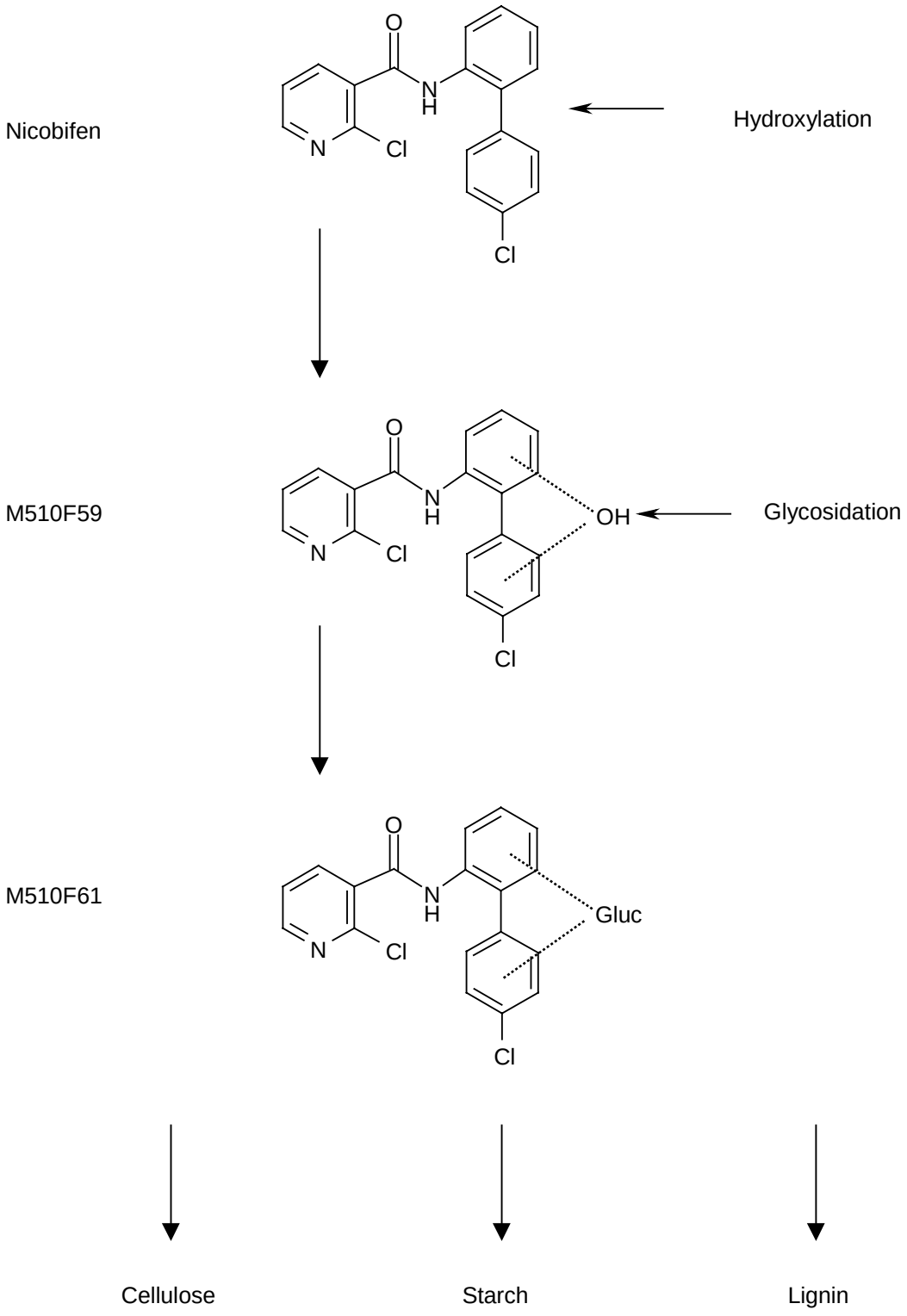
Storage stability

Wheat straw samples (DAT 30, both labels) were worked up again after 20 – 22 months. HPLC analysis showed that nicobifen and metabolite M510F61 were sufficiently stable in original matrix and in an organic solution of wheat straw after storage in a freezer.

Metabolic pathway

For succeeding crops, the proposed metabolic pathway involves hydroxylation and conjugation reactions. Part of the residues was also incorporated and/or associated into/with natural products, such as starch, cellulose and lignin.

Figure B.7.9-1: Metabolic pathway in succeeding crops



Conclusion

This study was conducted with an application rate of 2.1 kg as/ha to bare soil. The application rate according to GAP is 2 x 500 g as/ha for beans and peas.

With the exception of wheat grain the major part of the residues in all other matrices was identified as parent. The concentrations of nicobifen were relatively low in lettuce leaf (0.014

– 0.072 mg/kg, one sample = 0.146 mg/kg) and radish root (0.009 – 0.09 mg/kg). Higher residues were found in radish leaves (0.09 – 0.30 mg/kg) and wheat forage (0.19 – 1.47 mg/kg) and very high residues in wheat straw (0.81 – 7.99 mg/kg).

In wheat grain, the concentration of parent was low (≤ 0.028 mg/kg). The greater portion of the TRR were non extractable residues and part of these radioactive residues, especially for the pyridine label, could be detected in the starch fraction (36.2 – 48.4 % TRR for pyridine label, 0.6 – 4.3 % TRR for diphenyl label). Ammonia solubility of the residual residues in wheat grain was in the range of 12.9 – 22.9 % TRR.

Besides parent one metabolite (M510F61) could be identified in low concentrations in radish leaves/roots and in wheat straw/forage. This metabolite was a sugar conjugate of the parent compound.

Although only some of the lettuce leaf and radish root samples exceed the LOQ of the enforcement method (0.05 mg/kg) which could be assigned to an exaggerated application rate, significantly higher levels were found in radish leaves and wheat forage and very high levels in straw even after plant back intervals of 270 and 365 days. This indicates that residues of nicobifen could occur above the LOQ of 0.05 mg/kg in edible parts of other crops than investigated.

B.7.9.2 Field tests

It was found in the confined rotational crop study that wheat grain contained total residues above the limit of quantitation of the residue methods. However, the part of the parent compound was clearly below the LOQ (≤ 0.028 mg/kg). Since for this crop no data from residue trials were available (because of the intended use pattern of nicobifen), wheat samples were analysed grown as succeeding crop after nicobifen application in order to confirm these data under normal practical conditions.

Report:	Funk H., Mackenroth C. 2001 Determination of the residues of BAS 510 F in wheat obtained from the trial year 2000 BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep. unpublished BASF RegDoc# 2000/1014853
Guidelines:	BBA Guideline Part IV, 3-3, "Testing of residue behaviour - General information on design, preparation and realization of residue tests" IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992, Guidelines of Producing Pesticide Residue Data from Supervised Trials FAO Rome,1990
GLP:	Yes, except for one field part (indicated in the text)
Acceptability:	Yes, combined with addendum

Addendum: Funk H., Mackenroth C. 2001(d)
Determination of the residues of BAS 510 F in wheat obtained from the trial year 2000
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed. Rep.
unpublished
BASF RegDoc# 2001/1000989

GLP: Yes

Material and methods

In 1998 and 1999 respectively, two trials were performed applying nicobifen to either vegetables (accumulation study) or winter rape (field residue study see B.7.6, RIP 2001-339) in order to investigate the residue situation. In both cases, wheat was planted on those plots in the succeeding season. Wheat samples were taken in 2000 to check the behaviour of nicobifen in rotational crop under normal practical conditions.

In the accumulation study (BOD 2001-296, see B.8), nicobifen was applied in 1998 onto lettuce (2 x 300 g as/ha) and green beans (3 x 500 g as/ha) and in 1999 onto carrots (3 x 300 g as/ha) and cauliflower (2 x 400 g as/ha). The total amounts of nicobifen applied were 2.1 kg in 1998 and 1.7 kg in 1999. In 2000 spring wheat was grown on the plots and no product containing nicobifen was applied to the plots. Growth of vegetables in 2 succeeding years followed by cultivation of cereals during the third year is typical agricultural practice in Germany. It represents a reasonable worst case for the application of nicobifen in a crop rotation.

In the residue trial, BAS 510 01 F was applied once onto winter rape at an application rate of about 0.5 kg/ha (1 x 250 g as/ha). In 2000 wheat was grown on the plots and no product containing nicobifen was applied to the plots. Sampling of wheat was not performed under GLP.

For the analysis grain and straw were sampled at a growth stage of about 92 (BBCH code). In one trial also plant without root was taken at an earlier stage. The samples were analysed with BASF method no. 445/0. The method quantifies the relevant residue of nicobifen with a limit of quantitation of 0.05 mg/kg in all sample materials. The results of procedural recovery experiments obtained with each analytical series were about 90% at fortification levels of 0.05 mg/kg and 5.0 mg/kg.

Findings

Planting wheat as a succeeding crop, no residues of nicobifen above the limit of quantification were found in the food item wheat grain under practical conditions.

For wheat succeeding treated vegetables, residues of nicobifen were found in plant with out root (0.10 mg/kg) and wheat straw (0.75 mg/kg). The concentrations of nicobifen in soil before sowing as well as for forage and straw were at about half of those found in the rotational crop study for a plant back interval of 365 days. A comparison of the results is shown in Table B.7.9-8.

No residues were found in straw (< 0.05 mg/kg) succeeding treated rape. The results of the field test are summarised in Table B.7.9-9.

Table B.7.9-8: Comparison of residues of nicobifen found in the rotational crop study and the field test

	Soil before sowing mg/kg	**Wheat plant** mg/kg	**Straw** mg/kg
Rotational crop study			
Pyridine label (365 DAT)	0.356	0.19	1.49
Diphenyl label (365 DAT)	0.429	0.20	1.09
Field test	0.24*	0.10	0.75

* average of three plots and of layers from 0 to 25 cm after ploughing

Table B.7.9-9: Summary of the residue of nicobifen in wheat grown as succeeding crop after nicobifen application

Crops planted in preceding years	**Total application rate** (g as /ha)	**Succeeding crop**	**Portion analysed**	**Nicobifen** (mg/kg)
Lettuce, green beans	2100	Spring wheat	plant without root	0.10
Carrots, cauliflower	1700		straw	0.75
			grain	< 0.05
Rape	250	Winter wheat*	straw	< 0.05
			grain	< 0.05

* sampling not performed under GLP

Conclusion

Residues were found in wheat forage and wheat straw after two years treatment of vegetables. Although the applied amounts of nicobifen were higher than would be according to GAP and future intended uses, it can be seen from the accumulation study that under practical conditions concentrations of nicobifen remain in the soil which lead to residues in green plant parts and straw (for details see B.8.1).

Thus, taking into account the results of the rotational crop study as well as the results obtained in the field test it can not generally be guaranteed that no residues above the LOQ of 0.05 mg/kg occur in succeeding crops. Therefore, further trials regarding the residue situation in succeeding crops are necessary. (Authorengespräch: Abschätzung von Rückständen im Boden)

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)

Taking into account the intended uses (cf. B.3.3) and the results of the supervised residue trials, the following PHI are proposed:

Grapes: 28 days
Peas: 7 days
Beans: 7 days
Rape: The PHI for is covered by the normal vegetation period between application and harvest.

B.7.11 Community MRLs and MRLs in EU Member States (Annex IIIA 12.2)

Nicobifen is a new active substance which is not authorised in any EU Member State and no MRL has been set in the EU yet.

B.7.12 Proposed EU MRLs and justification for the acceptability of those residues (Annex IIA 6.7; Annex IIIA 8.6)

B.7.12.1 MRL proposal for plants

Proposed MRL's based on an assessment of the GAP and residue data submitted		
Commodity	**proposed MRL [mg/kg]**	**Data requirements**
grapes	5	none
rape seed	0.05	none
beans (with pods)	2	further glasshouse trials
peas (without pods)	0.3	none

B.7.12.2 MRL proposal for animal products

The dietary burden was calculated using data from residue trials performed in Europe and the US. The main contributions to the dietary burden were vegetables. The following table shows the calculations performed for the estimation of the feeding levels. As data were based on a limited number of available results, values may deviate from actual tolerance proposals, but still provide a valid picture of the exposure.

Table B.7.12-1: Estimation of potential residues in livestock feed

	% DM	Chicken	Dairy Cattle	Beef Cattle	Pig	Residue (mg/kg)	Intake (mg/kg, dry feed basis)			
							Chicken	Dairy Cattle	Beef Cattle	Pig
Body weight		1,9 kg	550 kg	350 kg	75 kg					
Daily Maximum Feed (DM))		0,120 kg	20 kg	15 kg	3 kg					
Maximum Percentage		% DM	% DM	% DM	% DM					
I. Green Forage (incl. Hay)										
Kale/Cabbage	14	5	35	35	15	0.10	**0.036**	**0.250**	**0.250**	**0.107**
Fruit Pomace (Apples, Citrus)	23	0	10	30	0	0.10	0.000	0.043	0.130	0.000
IV. Pulses	86	30	20	20	40	0.05	**0.017**	**0.012***	0.012	**0.023***
V. Root and Tubers										
Carrots	10	20	30	60	60	0.25	**0.500**	**0.750**	**1.500**	**1.500**
VI. Oil Seed (Meal, Cake)										
(e.g. Soya bean, Peanuts, Rape seed, Sunflower seed, Linseed)	86	10	30	30	20	0.15	**0.017**	**0.052**	**0.052***	**0.035**
Maximum intake (mg/kg feed)							**0.571**	**1.055**	**1.759**	**1.645**
Maximum intake (mg/kg bw)							**0.036**	**0.038**	**0.075**	**0.066**
Maximum intake (mg/animal)							**0.068**	**21.105**	**26.381**	**4.935**

For calculation all values printed fat were taken
* these values were only taken partly to add up to 100 %

Thus, for the worst case the doses to be used when estimating the residues in products of animal origin are for:

Chicken	0.036 mg/kg bw/d
Dairy cattle	0.038 mg/kg bw/d
Beef cattle	0.075 mg/kg bw/d
Pigs	0.066 mg/kg bw/d

In the cow feeding study the lowest nominal dose level had been chosen to be 30 mg/d/animal which corresponded to 0.05 mg/kg bw/d for dairy cattle and an actual feed intake of 1.8 mg/kg (DM). No residues were detected in milk, muscle, liver and kidney in the 1x dose group. In fat a single residue of 0.078 mg/kg was detected. In cream, residues above the limit of quantitation were found (0.04 mg/kg). No quantifiable amounts of bound residues of nicobifen in liver could be detected.

As the calculated dietary burden for beef cattle is 1.5 x the concentration used in the feeding study the results of the 3 x dose group also have to be taken into consideration. In this group residues in fat (0.11 mg/kg), liver (0.06 mg/kg) and kidney (0.07 mg/kg) were quantifiable. No quantifiable amounts of bound residues of nicobifen in liver could be detected. Therefore, MRLs above the LOQ of 0.05 mg/kg are proposed.

In pigs, no feeding study was performed as the metabolic pathway in rats and goats were similar. The residue situation in pig products can be extrapolated from the cow feeding study instead. The dietary burden of pigs and cattle are comparable. Therefore the same MRLs are proposed.

No feeding study with chicken was performed due to a low dietary burden derived from rape (see B.7.8.2). As can be seen in Table B.7.12-1, with more crops containing nicobifen a feed intake of 0.57 mg/kg feed (DM) can be calculated. In the metabolism study 12 mg/kg feed was fed. Comparison with the results of the metabolism study show that no detectable residues of nicobifen are to be expected and the MRL is proposed at the limit of quantification.

Table B.7.12-2: Potential transfer of residues to poultry tissues and eggs

Matrix	Total radioactive residue (mg/kg) from metabolism studies	Extrapolated total residue from calculated* feed intake (mg/kg)
Eggs	0.058	0.0028
Muscle	0.0025	0.0001
Liver	0.1687	0.0080
Fat	0.025	0.0012

* calculated feed intake: 0.57 mg/kg

MRL proposal for food of animal origin
(sum of nicobifen and M510F01 including its conjugate):

Milk[1)]	**0.02[2)] mg/kg**
Fat[3)]	**0.10 mg/kg**
Liver[3)]	**0.10 mg/kg**
Kidney[3)]	**0.10 mg/kg**
other products of animal origin	**0.05[2)] mg/kg**

[1)] residues in cream at 0.04 mg/kg
[2)] LOQ
[3)] except for poultry

B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues

No import tolerances have been proposed in the EU or applied for in any EU Member State.

B.7.14 Basis for differences, if any, in conclusion reached having regard to established or proposed Codex MRLs

Not applicable since no Codex MRLs have been established yet.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

The dietary risk assessment is based an **ADI value of 0.04 mg/kg bw/d**. Where no MRL is proposed, the value of the LOQ of 0.05 mg/kg is used in the risk calculation for food of plant and of animal origin. The MRLs proposed for food of animal origin were calculated according to the residue definition as sum of nicobifen and metabolite M510F01 including its conjugates.

Table B.7.15-1: Assessment of the TMDI - German diet

Food	raw[1]	processed[2]	whole	MRL (mg/kg)	Intake (mg/kg) bw
Mean food consumption (g/d) of a 4 to 6 years old girl					
Table grapes	6.1	2.6	8.7	5.00	0.0032222
Beans		3.8	3.8	2.00	0.0005630
Peas	0.1	4.0	4.1	0.30	0.0000911
Rape seed		1.7	1.7	0.05	0.0000063
Other food of plant origin	99.6	374.1	473.7	0.05	0.0017544
Intake whole (mg/kg bw):					**0.0056**
Percent of ADI (%):					**14.1**
Mean food consumption (g/d) of a 36 to 50 years old woman					
Wine grapes (wine)		97.6	97.6	5.00	0.0081333
Tea		1.1	1.1	0.05	0.0000009
Hops		4.9	4.9	0.05	0.0000041
Coffee beans (raw)		26.5	26.5	0.05	0.0000221
Intake whole (mg/kg bw):					**0.0081**
Percent of ADI (%):					**20.4**

[1] raw: without any preparation/processing
[2] processed: e.g. washed, peeled, cooked, baked, preserves

Table B.7.15-2: Assessment of the TMDI - WHO European diet (Mean food consumption in g/d)

Food	Consumption	MRL	Intake
	(g/day)	(mg/kg)	(mg/kg bw)
FOOD OF PLANT ORIGIN			
Table and wine grapes	113.8	5.00	0.0094833
Beans	12.0	2.00	0.0004000
Peas	14.0	0.30	0.0000700
Rape seed	7.3	0.05	0.0000061
Other food of plant origin	1106.3	0.05	0.0009219
FOOD OF ANIMAL ORIGIN			
Milk	342.6	0.02	0.0001142
Fat	10.7	0.10	0.0000178
Edible offals	12.6	0.10	0.0000210
Other food of animal origin	244.1	0.05	0.0002034
Intake whole (mg/kg bw):			**0.0112**
Percent of ADI (%):			**28.1**

Conclusion

The calculations of the TMDI lead to a low utilisation of the ADI as well with the German diet (child 14.1 % + adult woman 20.4 %, Table B.7.15-1) as with the WHO diet (28.1 %, Table B.7.15-2). A chronic 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 of nicobifen was investigated in grapes, lettuce and beans using diphenyl and pyridine labelled test substance.

Unchanged parent compound formed the major part of the residue in these studies. The cleavage products M510F62 (chlorophenylaminobenzene) and M510F47 (chloronicotinic acid) and in addition hydroxy-parent and sugar conjugates were identified in beans. Furthermore a range of small peaks were detected in beans. All metabolites were of minor importance. Therefore parent only is included in the residue definition.

Residue definition plant: Nicobifen

B.7.16.2 Metabolism in livestock

Metabolism studies were carried out in lactating goats and laying hens using diphenyl labelled test substance.

In ruminants and poultry nicobifen is rapidly absorbed, distributed and excreted. In goats the residues in milk, fat, muscle and kidney mainly consisted of unchanged parent and its hydroxy metabolite M510F01 including its conjugates. Metabolites are formed by hydroxylation followed by a glucuronidation to create the glucuronic acid M510F02. The sulfatation of M510F01 leads to the sulfate M510F03. Further hydroxy and thiol substitutions of the biphenyl system occur, followed by methylation. The substitution of the chlorine atom in the pyridine ring system by thiol groups of biomolecules leads to the formation of the cysteine conjugates, as M510F05 and M510F22, detected in urine.

Low extractability could be observed in liver due to a high level of bound residues. By application of a specially developed microwave method, it was possible to characterise these bound residues as M510F53 after solvolytic cleavage of the amide bond. The amide bond of nicobifen was very stable under metabolic conditions in goats.

In hens the residues in eggs, fat, and muscle mainly consisted of unchanged parent. In eggs, beside the parent compound also its hydroxy metabolite M510F01 including conjugates were present. Considerable quantities of nicobifen were also bound in hen liver based on substitution (most likely SH-groups from cysteine containing protein). The amide bond of nicobifen was very stable under metabolic conditions in hens.

The metabolic pathways in rats, goats and hens were qualitatively similar. Thus, the metabolism and the residue situation of nicobifen can be extrapolated from these animal species to pigs.

Based on the results of metabolism studies in goats and hens, unchanged nicobifen and the hydroxylation product M510F01 (including its conjugates) are considered the relevant residue for enforcement purposes. For risk assessment, the bound residues in liver were also considered relevant.

Residues for monitoring:	**Nicobifen, M510F01 (including its conjugates)**
Residues for risk assessment:	**Nicobifen, M510F01 (including its conjugates), M510F53 (for bound residues in liver and minor metabolites in milk)**

B.7.16.3 Residues in treated crops, proposed MRL´s, pre harvest intervals

A sufficient number of trials was presented to support the GAP´s of nicobifen in grapes and rape.
For beans and peas the data are sufficient for North Europe only. For the use in South Europe there are only two trials each supporting the GAP in France. For indoor application to beans, significantly higher residues were found in two out of eight trials where the application was at later growth stages. Further trials are required for glasshouse application.

Proposed MRL's:

Grapes	5	mg/kg
Rape	0.05*	mg/kg
Beans with pods	2	mg/kg
Peas without pods	0.3	mg/kg
Other food of plant origin	0.05*	mg/kg

* limit of quantification

PHI

Grapes: 28 days
Peas: 7 days
Beans: 7 days
Rape: The PHI for is covered by the normal vegetation period between application and harvest.

B.7.16.4 Residues in food of animal origin, proposed MRL's

A residue transfer study with nicobifen was conducted in cows. In the 1 x dose group, no residues could be detected in milk but could be found in cream at 0.04 mg/kg with good dose linear correlation for the higher dosing groups. The 3 x dose group was taken to derive the MRL for other products than milk since calculated dietary burden for beef cattle is 1.5 x the concentration used in the feeding study. In the 3 x dose group residues in fat (0.11 mg/kg), liver (0.06 mg/kg) and kidney (0.07 mg/kg) were quantifiable.

The residue situation in pigs was extrapolated from the cow study and the same MRL are proposed as for ruminants. For hens, it can be expected from the results of the metabolism study and in comparison to potential feed intake that no residues above the LOQ will occur.

Proposed MRL's
(sum of nicobifen and M510F01 including its conjugates)

Milk	0.02[1)] mg/kg
Fat[2)]	0.10 mg/kg
Liver[2)]	0.10 mg/kg
Kidney[2)]	0.10 mg/kg
other products of animal origin	0.05[1)] mg/kg

[1)] LOQ
[2)] except for poultry

B.7.16.5 Stability of residues prior to analysis

Plant matrices

The storage stability of nicobifen in samples of plant origin is proven for a period of 24 months. All samples from residue trials were analysed within this time period.

Animal matrices

The storage stability of nicobifen in samples of animal origin is proven for a period of 5 months. All samples from the livestock feeding study were analysed within this time period.

B.7.16.6 Residues in succeeding crops

In the rotational crop study, diphenyl and pyridine labelled nicobifen was applied at an exaggerated rate to soil. With the exception of wheat grain the major part of the residues were identified as parent. In wheat grain, the concentration of parent was low and most of the radioactive residues were not extractable. For the pyridine label, great parts of the non extractable residues were detected in the starch fraction.

Some of the nicobifen residues found in lettuce leaf and radish root samples were exceeding the LOQ of 0.05 mg/kg of the enforcement method and could be assigned to the exaggerated application rate. But higher levels of nicobifen were found in radish leaves (0.09 – 0.30 mg/kg) and wheat forage (0.19 – 1.47 mg/kg) and very high residues in wheat straw (0.81 – 7.99 mg/kg).

Two field trials were conducted with wheat planted either after two years of several treatments of vegetable or after single treatment of rape. No residues above the LOQ were found in wheat grain. In straw, no residues could be detected in plants grown after rape. Plants grown after the treated vegetables showed residues of nicobifen for wheat forage at 0.10 mg/kg and for wheat straw at 0.75 mg/kg.

From the results of the rotational crop study and the field trial it can not be guaranteed that no residues above the LOQ of 0.05 mg/kg could occur in edible parts of other succeeding crops. Therefore, further trials regarding the residue situation in succeeding crops are necessary.

B.7.16.7 Processing

A processing study was performed with grapes. In none of the consumer products like must or wine a concentration of the nicobifen residues were observed. Nicobifen concentrated in the waste product wet pomace with concentration factors ranging between 1.95 and 3.41.

In peas, it was shown that nicobifen residues were partly washed off and therefore were found in both the washed peas and the washing water.

B.7.16.8 Estimates of dietary exposure to nicobifen

Consumer intake levels were estimated using the proposed MRL values derived from supervised residue trials and from the livestock feeding study. Using the German and European diet and comparing it with an ADI value of 0.04 mg/kg there appears to be no chronic dietary consumer risk. (German diet: child 14.1 % + woman 20.4 %, WHO European diet: 28.1 %)

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[6]
AIIA-6.0	Funk, Horst; Mackenroth, Christiane	2001	Investigation of the Stability of Residues of BAS 510 F in Plant Matrices under Storge Conditions. 2001/1015028 GLP, unpublished RIP2002-192	Y	BAS
AIIA-6.1	Hamm, R.T.	1999	Metabolism of BAS 510 F in Lettuce. BASF DocID: 1999/11240 GLP, unpublished RIP2001-328	Y	BAS
AIIA-6.1	Rabe, U.; Schlüter, H.	2001	Metabolism of BAS 510 F in Grapevine. BASF DocID: 2000/1014860 GLP, unpublished RIP2001-327	Y	BAS
AIIA-6.1	Veit, P.	2001	Metabolism of 14C-BAS 510 F in Beans. BASF DocID: 2000/1014861 GLP, unpublished RIP2001-329	Y	BAS
AIIA-6.2	Fabian, E.; Grosshans, F.	2001	The Metabolism of 14C-BAS 510F in Lactaing Goat. BASF DocID: 2000/1017221 GLP, unpublished RIP2001-331	Y	BAS

[6] 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[6]
AIIA-6.2	Leibold, E.; Hoffmann, H.D.	2000	14C-BAS 510 F- Absorption, Distribution and Excretion after Repeated Oral Administration in Lactating Goats. BASF DocID: 2000/1012353 GLP, unpublished RIP2001-330	Y	BAS
AIIA-6.2	Nietschmann, D.; Lam W.W.	2000	Nature of Residues of 14C-BAS 510 F in Laying Hens. BASF Doc No.: 2000/5154 GLP, unpublished RIP2001-332	Y	BAS
AIIA-6.3	Blaschke, U.	2001	Determination of the Magnitude of the Residue of the Active ingredient of BAS 510 00 F and BAS 510 01 F in/on Grape Raw Agricultural Commodity Specimens from Supervised field Trials in Northern and Southern France in 1999. BASF DocID: 2000/1000251 GLP, unpublished RIP2001-336	Y	BAS
AIIA-6.3	Blaschke, U.	2001	Determination of the magnitude and decline of the residue of BAS 510 KA F in/on grape raw agricultural commodity specimens from supervised field trials in Northern and Southern France in 1998. BASF DocID: 2000/1000250 GLP, unpublished RIP2001-333	Y	BAS
AIIA-6.3	Funk, H.; Mackenroth, C.	2001	Investigation of the Stability of Residues of BAS 510 F in Plant Matrices under normal Storage Conditions. BASF DocID: 2000/1014855 GLP, unpublished RIP2001-351	Y	BAS
AIIA-6.3	Heck, W.; Funk, H.; Mackenroth, C.	2001	Study on the residue behavior of BAS 510 F in peas after treatment with BAS 510 01 F under field conditions in Germay, Denmark and Sweden, 1999. BASF DocID: 2000/1014848 GLP, unpublished RIP2001-347	Y	BAS
AIIA-6.3	Heck, W.; Mackenroth, C.	2001	Study on the residue behavior of BAS 510 F in peas after treatment with BAS 510 01 F under field conditions in Germay, Denmark and Sweden, 2000. BASF DocID: 2000/1014852 GLP, unpublished RIP2001-349	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[6]
AIIA-6.3	Meumann, H.; Funk, H.; Mackenroth, C.	2000	Study on the residue behavior of BAS 510 F in grapes after treatment with BAS 510 00 F and BAS 510 01 F under field conditions in Germany and Spain, 1999. BASF DocID: 2000/1012411 GLP, unpublished RIP2001-337	Y	BAS
AIIA-6.3	Meumann, H.; Funk, H.; Mackenroth, C.	2000	Study on the residue behavior of BAS 510 F in grapes after treatment with BAS 510 KA F under field conditions in Germany and Spain, 1998. BASF DocID: 2000/1012410 GLP, unpublished RIP2001-334	Y	BAS
AIIA-6.3	Perny, A.	2001	Residue study in Beans following treatment with the preparation BAS 510 01 F under Field conditions in France in 2000. BASF DocID: 2000/1014876 GLP, unpublished RIP2001-346	Y	BAS
AIIA-6.3	Perny, A.	2001	Residue study in Green Peas following treatment with the preparation BAS 510 01 F under Field conditions in France in 2000. BASF DocID: 2000/1014878 GLP, unpublished RIP2001-350	Y	BAS
AIIA-6.3	Perny, A.	2001	Residue study in Oil Seed Rape following treatment with the preparation BAS 510 01 F under Field conditions in France in 2000. BASF DocID: 2000/1014877 GLP, unpublished RIP2001-341	Y	BAS
AIIA-6.3	Raunft, E.	2001	Study on the residue behavior of BAS 510 F in winter rape after treatment with BAS 510 01 F under field conditions in Germany, Sweden and Great Britain, 2000. BASF DocID: 2000/1014851 GLP, unpublished RIP2001-340	Y	BAS
AIIA-6.3	Raunft, E.; Funk, H.; Mackenroth, C.	2000	Determination of the residues of BAS 510 F in winter rape following treatment with BAS 510 01 F under field conditions in Denmark, France, Germany and Great Britain, 1999. BASF DocID: 2000/1012409 GLP, unpublished RIP2001-339	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[6]
AIIA-6.3	Schulz, H.	2001	Determination of the Residues of BAS 510 F in Peas following treatment with BAS 510 01 F under field Conditions in France 1999. BASF DocID: 2000/1014879 GLP, unpublished RIP2001-348	Y	BAS
AIIA-6.3	Schulz, H.	2000	Determination of the Residues of BAS 510 F in Grapes Following Treatment with BAS 510 00 F and BAS 510 01 F under field conditions in Italy and France 1999. BASF DocID: 2000/1014880 GLP, unpublished RIP2001-338	Y	BAS
AIIA-6.3	Schulz, H.	2000	Determination of the Residues of Reg. No. 300355 in Grapes following Treatment with bas 510 KA F under Field Conditions in Italy and Northern France 1998. BASF DocID: 2000/1014881 GLP, unpublished RIP2001-335	Y	BAS
AIIA-6.3	Treiber, S.	2001	Study on the residue behavior of BAS 510 F in bush beans after treatment with BAS 510 01 F under field conditions in Denmark, France and Germany, 2000. BASF DocID: 2000/1014850 GLP, unpublished RIP2001-345	Y	BAS
AIIA-6.3	Treiber, S.; Funk, H.; Mackenroth, C.	2001	Study on the residue behavior of BAS 510 F in bush- and climbing beans after treatment with BAS 510 01 F under greenhouse conditions in Spain, 2000. BASF DocID: 2000/1014849 GLP, unpublished RIP2001-344	Y	BAS
AIIA-6.3	Treiber, S.; Funk, H.; Mackenroth, C.	2000	Study on the residue behavior of BAS 510 F in bush-and climbing beans after treatment with BAS 510 01 F under greenhouse condition in Spain, 1999. BASF DocID: 2000/1014847 GLP, unpublished RIP2001-343	Y	BAS
AIIA-6.3	Treiber, S.; Funk, H.; Mackenroth, C.	2000	Study on the residue behavior of BAS 510 F in bush beans after treatment with BAS 510 01 F under field conditions in Germany and Denmark, 1999. BASF DocID: 2000/1014846 GLP, unpublished RIP2001-342	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[6]
AIIA-6.4	Grosshans, F.	2001	Investigation of the Stability of Residues of BAS 510 F and M510F01 in Sample Material of Animal Origin under Usual Storage Conditions. BASF DocID: 2000/1017229 GLP, unpublished RIP2001-354	Y	BAS
AIIA-6.4	Tilting, N.	2001	Residues in Milk and Edible Tissues Following Oral Administration of BAS 510 F to Lactating Dairy Cattle. BASF DocID: 2000/1017228 GLP, unpublished RIP2001-352	Y	BAS
AIIA-6.5.1	Scharf, J.	1998	Hydrolysis of BAS 510 F at 90°C, 100°C, and 120°C. BASF Doc.: 1998/10878 GLP, unpublished RIP2001-355	Y	BAS
AIIA-6.5.2	Meumann, H.; Funk, H.; Mackenroth, C.	2000	Study on the residue behavior of BAS 510 F in grapes in grapes and grape process fractions after treatment with BAS 510 01 F under field conditions in Germany, 1999. BASF DocID: 2000/1012412 GLP, unpublished RIP2001-356	Y	BAS
AIIA-6.5.2	Scharm, M.	2001	Determination of the Residues of Reg. No. 300355 in Peas and Processed Products following treatment with BAS 510 01 F under field Conditions in Germany 2000. BASF DocID.: 2000/1014885 GLP, unpublished RIP2001-372	Y	BAS
AIIA-6.6	Funk, H.; Mackenroth, C.	2001	Determination of the residues of BAS 510 F in wheat obtained from the trial year 2000. BASF DocID.: 2000/1000989 GLP, unpublished RIP2001-375	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[6]
AIIA-6.6	Funk, H.; Mackenroth C.	2000	Determination of the residues of BAS 510 F in wheat obtained from the trial year 2000. BASF DocID.: 2000/1014853 GLP, unpublished RIP2001-374	Y	BAS
AIIA-6.6	Hamm, T.R.; Veit, P.	2001	Confined Rotational Crop Study with 14C-BAS 510 F. BASF DocID.: 2000/1014862 GLP, unpublished RIP2001-373	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

B-8: Environmental fate and behaviour

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

B.8.1.1.1.1 Parent

Stephan A, 1999, BOD2001-283

GLP: yes
Guidelines: BBA-Guideline Part IV, 4-1, SETAC, US EPA

The aerobic soil metabolism of [^{14}C]-BAS 510 F was investigated in a loamy sand soil. The soil characteristics are summarised in Table B.8.1-1. The specific radioactivity was 5.23 MBq/mg with a radiochemical purity > 99% for the diphenyl-label and 5.81 MBq/mg with a radiochemical purity of > 99% for the pyridine-label. The two radiolabels were tested separately.
A concentration of 1.0 mg [^{14}C]-BAS 510 F/kg dry soil was used for both labels. This is equivalent to the maximum single application rate of 700 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. The soil samples were extracted with methanol and methanol/water. The extracts were analysed by HPLC and TLC.

Table B.8.1-1: Soil used to investigate the degradation and metabolism of BAS 510 F

Soil designation	Bruch West (97/060/03)
Textural class (German scheme)	loamy sand
Textural class (USDA scheme)	sandy loam
Origin	Limburgerhof, Germany
Particle size distribution [%] (German scheme): 0.063 – 2 mm 0.002 – 0.063 mm < 0.002 mm (USDA scheme): 0.05 – 2 mm 0.002 – 0.05 mm < 0.002 mm	 76 18 6 77 17 6
Organic C [%]	1.3
Microbial biomass [mg C/100g dry soil]	35.1
CEC [mVal/100g]	12.7
pH[$CaCl_2$]	7.4
MWC [g H_2O/100g dry soil]	43
FC [g H_2O/100g dry soil], 0.33 bar	16.1

CEC cation exchange capacity
MWC maximum water holding capacity
FC field capacity

Results:

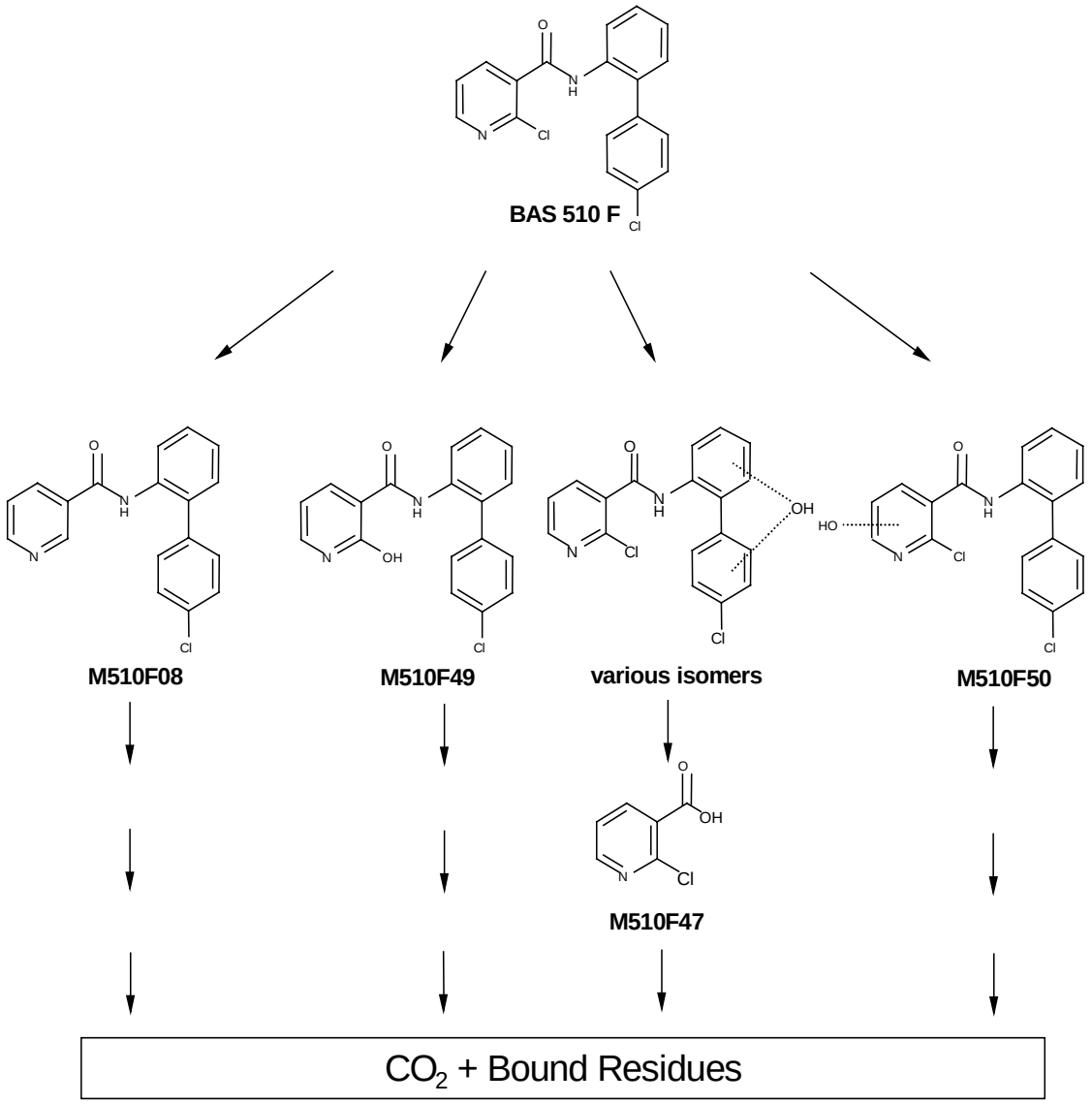
The distribution of radioactivity at different sampling dates is shown inTable B.8.1-2. The average recoveries were 100.3% of the total applied radioactivity (TAR) for the diphenyl-label and 93.0% TAR for the pyridine-label. Substantial mineralization was observed with both radiolabels, which indicates that BAS 510 F is completely degradable in soil. The mineralization rate after 360 days was higher with the pyridine-label (25.4% TAR) than with the diphenyl-label (15.5% TAR).
BAS 510 F was degraded in soil, however, was still detectable in amounts of about 17% TAR after 360 days. No major metabolites were observed during the study. Two transformation products could be identified by MS-analysis, although they never reached more than 0.2% TAR. One of them (M510F49) showed an exchange of the chlorine at the pyridine ring to a hydroxy group, the other one (M510F50) showed a hydroxylation at the pyridine ring. However, the exact position of the hydroxy group could not be determined due to the very low concentration of this compound. The structures of the metabolites are given in the proposed metabolic pathway for BAS 510 F in soil, Figure B.8.1-1. High amounts of bound residues were reached with both labels (50-60% TAR), where most of the radioactivity was associated with the insoluble humins (30-40% TAR). The NaOH-extractable radioactivity was distributed between humic and fulvic acids. Only small amounts of radioactivity (about 2% TAR) could be extracted from the fulvic acids by liquid-liquid partitioning with organic solvents. This indicates that the non-extractable radioactivity is tightly bound to the humic substances in the soil and cannot be released even with harsh extraction methods.

Table B.8.1-2: Recovery of radioactivity in % TAR (total applied radioactivity) and distribution of metabolites after application of [^{14}C]-BAS 510 F to soil and incubation under aerobic conditions

DAT	$^{14}CO_2$	BAS 510 F	M510F49	M510F50	others	bound residues	material balance
diphenyl-label							
0	Ns	99.7	0.0	0.0	0.0	0.3	100.0
7	0.1	95.5	0.0	0.0	0.0	6.1	101.7
14	0.6	93.0	0.0	0.0	0.1	10.8	104.6
29	1.5	81.6	0.0	0.0	0.1	19.7	102.9
57	3.9	61.4	0.2	0.0	0.2	31.4	97.1
93	6.5	51.7	0.2	0.0	0.3	43.1	101.8
119	8.3	44.2	0.2	0.0	0.4	48.6	101.6
182	11.5	32.7	0.1	0.0	0.6	50.8	95.7
266	13.6	26.3	0.2	0.1	1.0	62.7	104.0
364	15.5	16.7	0.2	0.0	0.8	60.0	93.3
pyridine-label							
0	Ns	99.6	0.0	0.0	0.0	0.4	100.0
7	0.6	95.5	0.0	0.0	0.1	5.6	101.8
14	1.8	85.6	0.0	0.0	0.1	8.6	96.1
29	3.9	77.9	0.0	0.0	0.2	14.6	96.6
57	7.7	62.7	0.0	0.0	0.5	22.5	93.4
93	12.5	48.0	0.2	0.1	0.8	28.3	89.9
119	15.0	42.0	0.1	0.1	1.0	32.7	90.8
182	19.1	28.8	0.2	0.0	0.9	35.2	84.1
266	23.2	20.5	0.1	0.1	0.9	38.6	83.4
364	25.4	17.3	0.1	0.1	0.9	50.1	93.9

ns not sampled

Figure B.8.1-1: Proposed route of degradation of BAS 510 F in soil



Ebert D, and Harder D, 2000, BOD2001-284

GLP: yes
Guidelines: BBA-Guideline Part IV, 4-1, SETAC Europe

Four different soils were treated with a concentration of 1.0 mg [diphenyl-U-^{14}C]-BAS 510 F/kg dry soil. This corresponds to a field application rate of 700 g as/ha, assuming an equal distribution in the top 5 cm soil layer and a soil density of 1.5 g/cm^3. Two different batches of the test substance were used during the study. The specific radioactivity of the test substance was either 4.85 MBq/mg or 5.23 MBq/mg, the radiochemical purity was > 99% for both batches. The soil characteristics are given in Table B.8.1-3.
The incubation conditions were: aerobic, in the dark, at 20 °C, 40% maximum water holding capacity. The soil extracts were analysed by radio-HPLC.
Additionally, the rate of degradation at different temperature and moisture was investigated.

Table B.8.1-3: Soils used to investigate the degradation of BAS 510 F

Soil designation	Li 35 b 97/145/04	Lufa 2.2 97/736/04	US soil	Canadian soil
Textural class (German scheme)	loamy sand	loamy sand	sandy loam	n.d.
Textural class (USDA scheme)	loamy sand	loamy sand	loamy sand	loam
Origin	Limburgerhof, RP, Germany	Speyer, RP, Germany	Dinuba, CA, USA	Minto, Manitoba, Canada
Particle size distribution [%] (German scheme): 0.063 – 2 mm 0.002 – 0.063 mm < 0.002 mm (USDA scheme): 0.05 – 2 mm 0.002 – 0.05 mm < 0.002 mm	 79 12 9 80 11 9	 83 11 6 84 10 6	 69 17 14 72 14 14	 n.d. n.d. n.d. 49 36 15
Organic C [%]	1.0	2.5	0.6	3.1
Microbial biomass [mg C/100g dry soil]	30.4	58.1	70.3	49
CEC [mVal/100g]	6.6	11.3	11	33
pH[$CaCl_2$]	6.6	5.6	7.0	7.7
MWC [g H_2O/100g dry soil]	33	39	29	43
FC [g H_2O/100g dry soil], 0.33 bar	9.6	15.8	n.d.	33

n.d. not determined
CEC cation exchange capacity
MWC maximum water holding capacity
FC field capacity

Results:

The findings are summarised in Table B.8.1-4. BAS 510 F was slowly degraded in all cases, but no metabolites of BAS 510 F were detected in all four soils. The main degradate were bound residues. The formation of CO_2 was not measured during the study, however, the material balance calculated as the sum of extractable and bound residues indicates that no significant mineralisation took place.

Table B.8.1-4: Distribution of BAS 510 F and metabolites during soil degradation of [diphenyl-U-^{14}C]-labelled BAS 510 F, concentrations according to HPLC-results (values in % TAR)

	DAT	BAS 510 F	others	bound residues	balance (extractable + bound residues)
Li 35 b	0	96.5	0.0	0.8	97.2
	3	96.8	0.0	2.2	99.0
	7	98.7	0.0	3.2	101.9
	14	82.6	0.0	5.7	88.3
	30	90.6	0.0	7.9	98.5
	60	84.5	0.2	11.6	96.3
	91	85.2	0.3	14.8	100.3
	120	77.7	0.3	16.6	94.6
Lufa 2.2	0	97.6	0.1	0.6	98.3
	3	102.6	0.0	1.8	104.4
	7	100.2	0.0	2.6	102.8
	14	84.8	0.0	4.3	89.1
	30	92.6	0.1	7.0	99.7
	60	93.0	0.7	5.4	99.1
	91	85.2	0.6	11.4	97.2
	120	78.8	0.4	16.8	96.0
US soil	0	96.3	0.0	1.5	97.8
	3	95.6	0.0	2.2	97.8
	7	84.7	0.0	4.7	89.4
	14	99.0	0.0	8.9	107.9
	30	93.8	0.0	15.5	109.3
	60	89.3	0.0	19.7	109.0
	91	83.9	0.0	20.6	104.5
	120	80.9	0.5	21.5	102.9
Canadian soil	0	108.7	0.0	5.4	114.0
	3	99.1	0.0	9.8	108.9
	7	95.5	1.3	12.8	109.6
	14	78.4	0.6	19.4	98.4
	31	78.2	0.0	29.2	107.4
	60	74.4	0.0	33.9	108.3
	90	68.4	0.0	37.0	105.4
	119	53.6	0.0	50.1	103.7

The soil metabolism and degradation studies described so far showed that BAS 510 F is finally degraded in soil to CO_2 and bound residues. However the pathways and intermediary metabolites leading to these final degradates were only partially elucidated. For that reason two small scale supplementary studies with the two subunits of the active substance were set up in order to generate additional data on the degradation pathway under the hypothesis that cleavage of the amide bond should occur at some – however unknown - stage of the degradation cascade.

B.8.1.1.1.2 Metabolites

Kellner O, 1999, BOD2001-285
Ebert D, Harder U, 2000, BOD2001-286

GLP: yes
Guidelines: SETAC

The aerobic soil degradation of the two fragments of BAS 510 F, 2-chloronicotinic acid (= M510 F47, Reg.No. 107 371) and 1-(4-chlorophenyl)-2'-aminobenzene (= M510F62, Reg.No. 363 487) was investigated in a loamy/silty sand soil. The soil characteristics are summarised in Table B.8.1-5. The specific radioactivity was 11.3 MBq/mg with a radiochemical purity > 99% for the chloronicotinic acid and 6.37 MBq/mg with a radiochemical purity of > 97% for the chlorophenyl-aminobenzene. Since the specific radioactivity was very high for the chloronicotinic acid, the radioactive substance was diluted with non-radiolabelled test substance resulting in a specific radioactivity of about 3.4 MBq/mg (206738 dpm/µg).
A concentration of 0.25 mg/kg dry soil was used for both test substances. This is about ¼ of the rate used for the soil metabolism study with the active substance. This concentration was considered as suitable for investigating the fate of the two compounds. However, it must be stated that in all environmental fate studies conducted with BAS 510 F, chloronicotinic acid never exceeded 7% TAR and that chlorophenyl-aminobenzene was never detected in any environmental fate study.
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. The soil samples were extracted with methanol and methanol/water and the extracts were analysed by HPLC. Both incubations were terminated as soon as the test substance had decreased to close to zero.

Table B.8.1-5: Soils used to investigate the degradation rate of the two BAS 510 F molecule fragments (chloronicotinic acid, chlorophenyl-aminobenzene)

Soil designation	Bruch West (99/060/04) (chloronicotinc acid)	Bruch West (98/060/03) (chlorophenyl aminobenzene)
Textural class (German scheme)	silty sand	loamy sand
Textural class (USDA scheme)	sandy loam	sandy loam
Origin	Limburgerhof, RP, Germany	Limburgerhof, RP, Germany
Particle size distribution [%] (German scheme)		
0.063 – 2 mm	59.4	67.3
0.002 – 0.063 mm	33.5	23.2
< 0.002 mm	7.1	9.5
(USDA scheme)		
0.05 – 2 mm	59.3	70.4
0.002 – 0.05 mm	29.8	20.1
< 0.002 mm	10.8	9.5
Organic C [%]	1.9	1.6
Microbial biomass [mg C/100g dry soil]	31.9	44.7
CEC [mVal/100g]	18.7	13
pH[$CaCl_2$]	7.6	7.3
MWC [g H_2O/100g dry soil]	40.0	41.1
FC [g H_2O/100g dry soil], 0.33 bar	n.d.	16.1
Reference	BASF RegDoc# 2000/1013280	BASF RegDoc# 1999/11102

n.d. not determined
CEC cation exchange capacity
MWC maximum water holding capacity
FC field capacity

Results:
This study explains the absence of metabolites in soil extracts.
The results of the two studies are summarized in Table B.8.1-6. Both fragments of BAS 510 F show a fast degradation behavior in soil. The chloronicotinic acid moiety is quickly mineralised (28% within 14 days), but also shows a rapid formation of bound residues. The diphenyl moiety is preferably bound to the soil matrix (65% after 7 days). Degradates of the compounds were only observed in low percentages and were of transient nature.

The very fast binding and mineralisation of both parts of BAS 510 F support the finding that no significant amounts of metabolites could be observed with the parent studies. When assuming that the degradation of BAS 510 F in soil involves a cleavage of the amide bond, both potential degradation products are very rapidly bound to the soil matrix or mineralised under aerobic conditions. Since initial attack on BAS 510 F is obviously very slow and subsequent degradation steps are much faster, no significant amounts of intermediary metabolites can be formed.

Table B.8.1-6: Recovery of radioactivity in % TAR (total applied radioactivity) and distribution of metabolites after application of [^{14}C]-chloronicotinic acid and [^{14}C]-chlorophenyl-aminobenzene to soil and incubation under aerobic conditions

DAT	$^{14}CO_2$	test substance	others	bound residues	material balance
chloronicotinic acid					
0	n.a.	98.0	0.0	2.0	100.0
1	1.2	88.6	0.0	7.9	98.0
3	5.6	68.1	0.0	21.7	95.4
7	18.5	13.0	5.1	52.0	88.6
14	27.6	0.4	4.7	55.5	88.2
chlorophenyl-aminobenzene					
0	0.0	95.3	3.5	1.2	100.0
7	4.0	5.1	9.4	65.2	83.7
15	6.8	2.7	7.9	72.3	89.6
52	11.1	1.6	5.0	68.4	86.1

B.8.1.1.2 Supplementary studies

B.8.1.1.2.1 Degradation under anaerobic conditions

Staudenmaier H, Schäfer C, 2000, BOD2001-287
Staudenmaier H, 2000, BOD2001-288

GLP: yes
Guidelines: BBA-Guideline Part IV, 4-1, SETAC

Although anaerobic conditions are not expected to occur following application of BAS 510 F, results from anaerobic soil metabolism studies are submitted as supporting information.
The anaerobic degradation was investigated with the diphenyl- and with the pyridine-labelled test compound. The application rate was 1.0 mg as/kg dry soil. The specific radioactivity was 4.85 MBq/mg with a radiochemical purity > 99% for the diphenyl-label and 5.16 MBq/mg with a radiochemical purity of > 99% for the pyridine-label.

The same soil as for the aerobic soil metabolism was used. The soil characteristics are described in Table B.8.1-7. The soil was incubated in a test apparatus connected to a trapping system for volatiles and was flushed continuously with nitrogen. The incubation conditions were: anaerobic (soil flooded with water), in the dark, temperature 20 °C.

Table B.8.1-7: Soils used to investigate the metabolism and degradation of BAS 510 F under anaerobic conditions

	diphenyl-label	pyridine-label
Soil designation	Bruch West 99/060/01	Bruch West 98/060/02
Textural class (German scheme)	silty sand	sandy loam
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 (USDA scheme): 0.05 – 2 mm 0.002 – 0.05 mm < 0.002 mm	 79 13 8 65 25 10	 64 21 15 68 17 15
Organic C [%]	1.6	1.7
Microbial biomass [mg C/100g dry soil]	27.9	63.9
CEC [mVal/100g]	12.7	16
pH [$CaCl_2$]	7.2	7.5
MWC [g H_2O/100g dry soil]	40.7	39
FC [g H_2O/100g dry soil], 0.33 bar	n.d.	20.4
Reference	BASF RegDoc# 2000/1014986	BASF RegDoc# 2000/1014990

n.d. not determined
CEC cation exchange capacity
MWC maximum water holding capacity
FC field capacity

Results:
The metabolic profile of BAS 510 F in anaerobic soil is shown in Table B.8.1-8. Under these conditions, only very low amounts of CO_2 were detected. Bound residues were formed only in moderate amounts. In contrast to the aerobic studies one significant metabolite was detected in the study with the pyridine label which reached a maximum of 6.7% TAR. This metabolite was identified by MS analysis as M510 F47 (= 2-chloronicotinic acid).

In the study with the diphenyl label no significant metabolite corresponding to M510F47 was present. That means that no derivatives that may result from cleavage of the amide bond of the active substance were detected. Instead, only trace amounts of metabolites could be detected. Nevertheless a variety of these metabolites could be identified by HPLC-MS/MS analysis using an extract of a sample treated at an exaggerated rate. Identified metabolites from this sample included M510F49 and M510 F50 which were already known from the aerobic soil metabolism. Additionally, M510F08 was detected, in which the pyridine-Cl is replaced by H. Furthermore, a variety of derivatives of BAS 510 F mono-hydroxylated in the diphenyl moiety was identified. Although the exact position of the hydroxy group at the diphenyl ring could not be determined in all cases, it can be concluded from the number of different hydroxylated isomers that virtually every free position of the diphenyl ring system was attacked by hydroxylation.

Table B.8.1-8: Recovery of radioactivity in %TAR and distribution of metabolites after application of [^{14}C]-BAS 510 F to soil and incubation under anaerobic conditions. Concentrations of the active substance and metabolites according to HPLC-results

DAT	CO_2	BAS 510 F	M510F47	others	bound residues	material balance
diphenyl-label						
0	0.0	100.9			0.5	101.4
3	0.0	97.4			2.2	99.6
7	0.0	94.7			2.5	97.2
14	0.0	92.8			5.4	98.2
30	0.1	85.9			8.9	94.9
58	0.1	81.1		0.3	8.7	90.2
90	0.1	75.8		0.6	12.7	89.2
120	0.1	73.6		0.3	15.8	89.8
pyridine-label						
0	0.0	96.4		0.8	1.5	98.8
3	0.0	96.2	2.6	0.8	3.2	102.8
7	0.0	95.1	1.7	0.1	4.6	101.5
14	0.0	88.6	4.0	0.6	5.8	98.9
30	0.1	86.2	3.9	0.5	9.5	100.2
62	0.1	81.9	6.0	0.1	12.2	100.2
90	0.2	78.0	5.9	0.2	12.5	96.7
120	0.4	77.0	6.7	0.5	14.4	99.0

B.8.1.1.2.2 Soil photolysis

Götz von N, 2000, BOD2001-289

GLP: yes
Guidelines: SETAC, EPA

For the soil photolysis study soil of the same origin was used as for the aerobic soil metabolism. The soil characteristics are summarized in Table B.8.1-9.
The soil photolytic degradation of BAS 510 F was studied using [pyridine-3-^{14}C]-labelled test substance. The specific radioactivity of the test substance was 5.17 MBq/mg with a radiochemical purity of 100%. The treated soil was incubated at 40% MWC.

Portions of soil corresponding to 30 g dry soil were filled into small dishes (86 mm x 40 mm x 10 mm). Ten dishes with treated soil were arranged in a thermostated bowl adjusted to 22 ± 1 °C. The bowl had an air inlet and an air outlet, was closed airtight with a quartz glass covering 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 4.6 µg [^{14}C]-BAS 510 F/g dry soil (= 140.7 µg as/dish). This corresponds to a field application rate of 700 g as/ha.
The bowl was placed under a xenon lamp in a 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. Samples were taken at 0, 2, 6, 9, 12, and 15 DAT.

Table B.8.1-9: Soil used to investigate the soil photolysis of BAS 510 F

Soil designation batch no.	Bruch West 99/060/03
Textural class (USDA scheme)	loamy sand
Origin	Limburgerhof Germany
Particle size distribution [%] (USDA scheme): 0.050 – 2 mm 0.002 – 0.050 mm < 0.002 mm	 63 27 10
Organic C [%]	1.9
CEC [mval/100g dry soil]	9.8
pH[$CaCl_2$]	7.3
MWC [g H_2O/100g dry soil]	36

Results:

The results of the soil photolysis and the dark control are shown in Table B.8.1-10. No major metabolite appeared during soil photolysis or in the dark control. Two unknown minor metabolites reached intermediary maxima around 1% TAR during the study, both of them being slightly higher in the irradiated samples. Several other minor metabolites were detected at percentages ≤ 0.5% TAR. They are summarised in the column "others".The degradation of BAS 510 F proceeded very slowly under irradiated conditions whereas in the dark control practically no degradation could be observed within 15 days. The amount of bound residues was very low but was slightly higher in the irradiated samples than in the dark controls at the end of the study. Mineralisation was negligible under both photolysis and dark control conditions. In total, a slight difference was observed between photolysis and dark control, indicating that light might gradually enhance the degradation of BAS 510 F on soil.

Table B.8.1-10: Recovery and distribution of radioactivity during soil photolysis of [^{14}C]-BAS 510 F [% TAR]

DAT	CO_2	BAS 510 F	unknown 6.6 min	unknown 30.5 min	others	bound residues	material balance
irradiated							
0	0.0	99.4	0.0	0.6	0.0	0.1	100.1
2	0.0	98.7	0.1	0.8	0.5	2.1	102.1
6	0.1	96.7	0.6	0.8	0.6	3.9	102.6
9	0.1	97.1	0.6	0.9	0.6	4.5	103.7
12	0.1	95.2	1.2	0.9	1.2	4.5	103.0
15	0.2	90.6	0.6	0.5	0.9	5.5	98.2
dark control							
0	0.0	99.4	0.0	0.6	0.0	0.1	100.1
2	0.0	98.1	0.0	0.6	0.3	1.4	100.4
6	0.0	98.6	0.0	0.5	0.3	2.3	101.7
9	0.0	100.1	0.0	0.4	0.1	2.3	102.9
12	0.1	99.4	0.1	0.2	0.6	3.2	103.6
15	0.1	99.2	0.0	0.3	0.3	2.9	102.9

Summary (route):

The behaviour of BAS 510 F after application to aerobic soil is characterised by rather slow but substantial mineralization and the formation of moderate to high amounts of bound residues. By far the major portion of the bound radioactivity was associated with the insoluble humin fraction.

Initial attack of BAS 510 F is via hydroxylation at various positions of all three rings and/or replacement of the pyridine-Cl by OH or H.

Further degradation involves the cleavage of the amide bond. The mineralization rate indicates that ring cleavage and further metabolism to CO_2 occurs from both the pyridine and the diphenyl rings although almost no intermediary breakdown products could be identified.

The initial steps in the degradation are slow and rate limiting. The subsequent steps are faster resulting in extremely low levels of intermediary metabolites. However, certain metabolites e.g. M510F47 become detectable under anaerobic conditions, in which degradation is slowed.

Under the influence of light, degradation of BAS 510 F on soil may be gradually enhanced.

B.8.1.2 Rate of degradation

B.8.1.2.1 Laboratory conditions

Studies considered:

BOD2001-283
BOD2001-284
BOD2001-287
BOD2001-288
BOD2001-289
And additional study on influence of a pretreatment with BAS 510 F on the degradation of BAS 510 F in soil:

Hain W, 2001, BOD2001-290

GLP: yes

The study on the influence of pretreatment was initiated in order to determine whether BAS 510 F-pretreatments of the soil under outdoor conditions will influence the degradation rate of BAS 510 F in soil. Therefore, soil samples taken from two different field sites (treated with BAS 510 F over several vegetation periods and at different application rates) were incubated with ^{14}C-BAS 510 F for 120 days under laboratory conditions (aerobic, in the dark, 20° C, 50% MWC). Origin of soils and pretreatments are listed in Table B.8.1-11. The soil characteristics are shown in Table B.8.1-12. The soils Limburgerhof No. I – IV were from the same field but from different small plots. The same applies for the soils Edesheim No. V – VII.

Table B.8.1-11: Field soils pretreated with BAS 510 F used for investigations on the degradation behaviour of ^{14}C-BAS 510 F under laboratory conditions

Date of treatment	Limburgerhof No. I (RP, Germany) strawberry	Limburgerhof No. II (RP, Germany) strawberry	Limburgerhof No. III (RP, Germany) strawberry	Limburgerhof No. IV (RP, Germany) strawberry
	as/ha	as/ha	as/ha	as/ha
04.05.1995	1000 g	-	-	-
10.05.1995	1000 g			(control)
20.05.1995	1000 g			
26.05.1995	1000 g			
05.05.1996	500 g	500 g		
14.05.1996	500 g	500 g	-	-
22.05.1996	500 g	500 g		
02.05.1997	700 g	500 g	700 g	-
07.05.1997	700 g	500 g	700 g	
15.05.1997	700 g	500 g	700 g	
26.05.1997	700 g	500 g	700 g	
Date of treatment	Edesheim No. V (RP, Germany) vine	Edesheim No. VI (RP, Germany) vine	Edesheim No. VII (RP, Germany) vine	
	as/ha	as/ha	as/ha	
27.06.1996	525 g	225 g	-	
25.07.1996	700 g	300 g	(control)	
20.08.1996	700 g	300 g		

Table B.8.1-12: Characteristics of soils used for BAS 510 F-pretreatment investigations

Soil designation batch no.	Limburgerhof No. IV (strawberry)	Edesheim No. VII (vine)
Textural class (German scheme)	loamy sand	sandy loam
Textural class (USDA scheme)	loamy sand	loam
Origin	Limburgerhof RP, Germany	Edesheim RP, Germany
Particle size distribution [%] (German scheme):		
0.063 – 2 mm	84	46
0.002 – 0.063 mm	12	37
< 0.002 mm	4	17
Organic C [%]	1.4	1.6
Microbial biomass [mg C/100g dry soil]	103	110
pH[$CaCl_2$]	5.9	6.9

Summary (rate):

The degradation rates for BAS 510 F in various soils and under different incubation conditions in the laboratory are shown in Table B.8.1-13.

The half lives for BAS 510 F under standard aerobic incubation conditions (20 °C, 40% MWC) were in the range of 108 – 384 days in the various soils used in the studies. Variation of the incubation conditions (low temperature, lower soil moisture, use of sterilised soil) mostly prolonged the half live to an extent where significant degradation could no longer be

observed within the incubation time of 120 days. Rise of the temperature in contrast only caused an insignificant increase of the degradation rate. However, it should be considered here that the degradation half lives from the "rate study" (BASF RegDoc# 2000/1013279, BOD2001-284) are extrapolations by a factor of about three or more from the actual period of investigation. With this degree of extrapolation the predicted half-lives become increasingly sensitive to variations in the experimental accuracy of individual samplings. The calculated differences in the half-lives due to variation of experimental conditions (temperature, moisture) may also be caused by differences in experimental accuracy, and therefore are not very significant.
In the study which adressed the effect of pretreatment of the soil with the active substance (BASF RegDoc# 1998/10607, BOD2001-290), shorter half-lives were observed with pretreated soil compared to the non-pretreated control from one of the two sites (Edesheim). With soil from the other site (Limburgerhof) this stimulating effect of the pretreatment (which is considered to be an adaptation of the soil microorganisms) could not be observed. However in the latter case, the half-lives were again considerably longer than the period of investigation.
Photolysis does have some effect on the degradation of BAS 510 F which leads to a gradually shorter half-live compared to the dark control. However, half-lives of the dark control and the aerobic soil metabolism study which was performed with the same soil do not match, which is considered to be an effect of the extensive extrapolation from the short experimental period of 15 days in the photolysis study. Therefore only a qualitative but no quantitative assessment of the effect of light should be derived from this study.
Anaerobic soil conditions led to longer half-lives compared to those in the same soil under aerobic conditions, which may be due to reduced microbial activity under anaerobic conditions. But the calculated half-lives were still in the same range that was observed for other soils under aerobic conditions.

Table B.8.1-13: DT_{50}/DT_{90}-values for BAS 510 F in laboratory soil studies

RegDoc#	label	soil	study duration [days]	temp. [° C]	moisture [% MWC]	Half-life (1st order) [days]	DT_{50} (best fit) [days]	DT_{90} [days]
aerobic soil metabolism								
1999/11807 BOD2001-283	diphenyl + pyridine	Bruch West	364	20	40	108		360
aerobic degradation in soil								
2000/1013279 BOD2001-284	diphenyl	Li 35 b	120	20	40	322		n.r.
		Lufa 2.2	120	20	40	384		n.r.
		US soil	120	20	40	376		n.r.
		Minto (Canada)	119	20	40	133		442
		Lufa 2.2	119	5	40	stable		-
		Lufa 2.2	120	30	40	365		n.r.
		Lufa 2.2	120	20	20	stable		-
		Lufa 2.2 sterile	120	20	40	stable		-

RegDoc#	label	soil	study duration [days]	temp. [° C]	moisture [% MWC]	Half-life (1st order) [days]	DT_{50} (best fit) [days]	DT_{90} [days]
aerobic degradation in soil; influence of pretreatment								
1998/10607 BOD2001-290	diphenyl	Limburgerhof I (pretreated)	120	20	50		> 240 d	n.r.
		Limburgerhof II (pretreated)	120	20	50		> 240 d	n.r.
		Limburgerhof III (pretreated)	120	20	50		> 240 d	n.r.
		Limburgerhof IV (control)	120	20	50		> 240 d	n.r.
		Edesheim V (pretreated)	120	20	50		141	n.r.
		Edesheim VI (pretreated)	120	20	50		155	n.r.
		Edesheim VII (control)	120	20	50		201	n.r.
soil photolysis								
2000/1014989 BOD2001-289	pyridine	Bruch West irradiated	15	22	40	135 d		n.r.
		Bruch West dark control	15	22	40	stable		-
anaerobic soil metabolism								
2000/1014986 BOD2001-287	diphenyl	Bruch West	120	20	flooded	261		n.r.
2000/1014990 BOD2001-288	pyridine	Bruch West	120	20	flooded	345		n.r.

n.r. = not reported

The half-life of BAS 510 F in aerobic soil at a soil temperature of 10 °C can be extrapolated from the average half-life observed in the laboratory degradation experiments performed at a soil temperature of 20 °C and a soil moisture of 40% MWC as given in Table B.8.1-13. The average half-life in aerobic soil degradation studies of BAS 510 F at a soil temperature of 20 °C and a soil moisture of 40% MWC is 265 d.

The calculation of the half-life of BAS 510 F for a soil temperature of 10 °C is made with the help of a degradation-temperature relationship (based on Arrhenius) as recommended by FOCUS (formula 1).

$$HL_{at\,10\,°C} = HL_{at\,20\,°C} * Q10^{\frac{20\,°C - 10\,°C}{10}} \qquad (1)$$

$HL_{at\ 10\ °C}$ extrapolated half-life [d] corresponding to a soil temperature of 10 °C

$HL_{at\ 20\ °C}$ average half-life [d] observed in the laboratory experiment at 20 °C and at a soil moisture of 40% MWHC

Q10 temperature correction factor = 2.2

Result:
The calculated half-life in soil of BAS 510 F at 10 °C ($HL_{at\ 10\ °C}$) is 583 d.

No degradation rates for metabolites of BAS 510 F in aerobic soil are provided since no major metabolites higher than 10% of the applied radioactivity were observed in any laboratory soil study. The same applies for degradation rates of metabolites of BAS 510 F in anaerobic soil.

Conclusion
Half-lives of BAS 510 F in aerobic soil range from 108 d to 384 d under standard conditions (20 °C, 40% MWC). This triggers the performance of field soil dissipation studies according to Directive 95/36 EC.
Under the influence of light, degradation of BAS 510 F on soil may be gradually enhanced whereas anaerobic conditions lead to longer half-lives, probably due to reduced microbial activity. Adaption of soils towards a faster degradation may occur at least in certain soils.

B.8.1.2.2 Field conditions

Kellner O, Kellner W, 2000, BOD2001-291
Bayer H, Grote Ch, 2001, BOD2001-292

GLP: yes
Guidelines:BBA-Guideline Part IV, 4-1, SETAC, IVA-Leitlinie

Two field soil dissipation studies have been performed to investigate the degradation and dissipation of BAS 510 F in soil. In total, 6 trials were conducted: 2 trials at two locations in Germany with three different application rates each, two trials in Spain, one trial in Sweden and another trial in Germany. The geographical distribution of the trial locations and the soil parameters of the fields are given in Table B.8.1-14:

Table B.8.1-14: Characterisation of fields in field soil dissipation studies with BAS 510 F

Reference BASF RegDoc# BBA Doc.No.	trial no.	location (postal code)	soil type	soil properties % organic C	 pH ($CaCl_2$)
2000/1000123 BOD2001-291	DU2/15/97	Germany Stetten (74193)	silty loam	0.8	7.5
2000/1000123 BOD2001-291	DU3/06/97	Germany Schifferstadt (67105)	silty sand	0.7	5.4
2000/1013295 BOD2001-292	ALO/05/98	Spain Manzanilla (21890)	Sandy loam	0.6	7.4
2000/1013295 BOD2001-292	ALO/06/98	Spain Alcala del Rio (41200)	Sandy loam	0.9	7.7
2000/1013295 BOD2001-292	D05/03/98	Germany Grossharrie (24625)	Loamy sand	1.2	6.1
2000/1013295 BOD2001-292	HUS/10/98	Sweden Bjärred (23791)	Loamy sand	1.0	5.9

The six locations were distributed over Northern, Central and Southern Europe. A range of soils with organic carbon contents from 0.6 to 1.2% with a pH range from 5.4 to 7.7 was covered. Nevertheless, hydrolytical degradation of BAS 510 F within this pH range is of no importance.

The trials were performed using the formulated products BAS 510 KA F or BAS 510 KB F. The nominal concentration of the active substance in these formulations was 500 g as/l for BAS 510 KA F and 250 g as/l for BAS 510 KB F. The maximum single application rate of BAS 510 F originally was 700 g as/ha for SC type formulations but could later on be reduced to 600 g as/ha for the more efficient WG type formulation. The applied product, the nominal application rates and the verified recoveries with an application verification method are given in Table B.8.1-15.

Table B.8.1-15: Application rate verification for BAS 510 F in field soil dissipation studies

Reference BASF Reg.Doc.#	trial no.	Formulation	target rate [g as/ha]	recovery [% of applied amount]
2000/1000123 BOD2001-291	DU2/15/97	BAS 510 KB F	300	86
2000/1000123 BOD2001-291	DU2/15/97	BAS 510 KB F	600	84
2000/1000123 BOD2001-291	DU2/15/97	BAS 510 KB F	1200	83
2000/1000123 BOD2001-291	DU3/06/97	BAS 510 KB F	300	78
2000/1000123 BOD2001-291	DU3/06/97	BAS 510 KB F	600	76
2000/1000123 BOD2001-291	DU3/06/97	BAS 510 KB F	1200	76
2000/1013295 BOD2001-292	ALO/05/98	BAS 510 KA F	750	92
2000/1013295 BOD2001-292	ALO/06/98	BAS 510 KA F	750	92
2000/1013295 BOD2001-292	D05/03/98	BAS 510 KA F	750	90
2000/1013295 BOD2001-292	HUS/10/98	BAS 510 KA F	750	96

The recovery rate in the field was determined using glass dishes filled with soil. The dishes were spread randomly on the field. They were opened just before application and closed and sealed immediately after application. The average recovery was close to 85%, although the values were 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 1.5 years after application with 9 sampling times (BASF RegDoc# 2000/1000123) or up to 1 year with 7 or 8 sampling times (BASF RegDoc# 2000/1013295).
The soil cores were analysed with BASF method no. 408/1 which determines soil residues of the active substance BAS 510 F down to a limit of quantification of 0.01 mg/kg dry soil. No

corrections, neither for recoveries nor blanks, have been made, but all results were corrected for moisture of the soil.

Results:
One detailed residue table (Table B.8.1-16) for trial DU3/06/97 with an application rate of 300 g as/ha in Germany is presented. The general characteristics of the degradation of BAS 510 F in field soils can be demonstrated with these results as an example. In this trial the degradation of BAS 510 F was followed over a period of ca. 1.5 years.

Table B.8.1-16: Field soil dissipation results from one trial in Germany; Study DE/FA/047/97, trial DU3/06/97, 300 as/ha: summary results

soil depth [cm]	days after application	BAS 510 F [mg/kg]
0 – 10	0	0.187
10 – 25	0	0.011
0 – 10	12	0.175
10 – 25	12	0
0 – 10	28	0.151
10 – 25	28	0
0 – 10	61	0.162
10 – 25	61	0
0 – 10	97	0.115
10 – 25	97	0
25 – 50	97	0
0 – 10	179	0.097
10 – 25	179	0
25 – 50	179	0
0 – 10	367	0.086
10 – 25	367	0
25 – 50	367	0
0 – 10	452	0.067
10 – 25	452	0
25 – 50	452	0
0 – 10	545	0.055
10 – 25	545	0
25 – 50	545	0

0 means below limit of quantification

The results for the other trials are similar. All trials showed that BAS 510 F degraded with an initial degradation rate that decreased later in the year and the concentration in the soils leveled off. In the second year, the degradation continued at a lower rate.

Furthermore, BAS 510 F did not show a tendency to move into deeper layers of soil. It was mostly detected in the top 10 cm soil layer.
The transformation parameters of BAS 510 F 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.8.1-17.

For the trials DU2 and DU3 that were sampled for ca 1.5 years with a slow degradation in winter and a faster degradation the following summer, a fit according to simple first order was not possible. The DT_{50} values were determined graphically. For both trials it could be shown, that the degradation was slowest with the lowest application rate an fastest with the highest application rate.

Table B.8.1-17: Degradation rates of BAS 510 F in field soil dissipation studies

Reference BASF Reg.Doc.#	trial no.	location	as/ha	DT_{50} [days]	r^2
2000/1000123 BOD2001-291	DU2/15/97	Germany Stetten (74193)	300	90	0.952
2000/1000123 BOD2001-291	DU2/15/97	Germany Stetten (74193)	600	49	0.968
2000/1000123 BOD2001-291	DU2/15/97	Germany Stetten (74193)	1200	28	0.988
2000/1000123 BOD2001-291	DU3/06/97	Germany Schifferstadt (67105)	300	208	0.956
2000/1000123 BOD2001-291	DU3/06/97	Germany Schifferstadt (67105)	600	175	0.943
2000/1000123 BOD2001-291	DU3/06/97	Germany Schifferstadt (67105)	1200	147	0.875
2000/1013295 BOD2001-292	ALO/05/98	Spain Manzanilla (21890)	750	27	0.88
2000/1013295 BOD2001-292	ALO/06/98	Spain Alcala del Rio (41200)	750	78	0.81
2000/1013295 BOD2001-292	D05/03/98	Germany Grossharrie (24625)	750	144	0.87
2000/1013295 BOD2001-292	HUS/10/98	Sweden Bjärred (23791)	750	*	0.09

* = could no be evaluated due to inconclusive results
r^2 = coefficient of determination

In general, the DT_{50} values of BAS 510 F in the soil dissipation studies ranged from 27 days in Spain to 208 days in Germany. In all trials a DT_{90} could not be reached within one year after application to bare soil. Therefore and because repeated application in succeeding growing seasons is envisaged, Directive 95/36/EC requires the determination of the level of a possible plateau concentration of the active substance. For this purpose soil accumulation studies were started at two relevant field sites with repeated applications.

Soil residue studies were not performed, because soil residue levels at specified intervals after the use of BAS 510 F (e.g. at harvest of treated crop or at time of sowing of next crop) can be derived from the results of the field soil dissipation trials.

Conclusion

DT_{50} (Best fit) values in the field soil dissipation studies ranged from 27 days in Spain to 208 days in Germany. In all trials, a DT_{90} was not reached within one year after application. According to Directive 95/36 EC, this triggers the performance of soil accumulation studies.

Platz K, 2001, BOD2001-293

GLP: No. Calculation.
Guidelines: none

Nine field dissipation studies with BAS 510 F at five different sites in different European regions (Germany -> Northern Central Europe and in Spain -> Southern Europe) are evaluated according to the Dutch Guideline. The criteria intend to ensure that a field study is adequately performed, sampled and analysed and that the results are critically evaluated whether other processes than microbial or chemical transformation can be neglected or not.

The weather conditions in field soil dissipation studies with BAS 510 F as relevant for the evaluation are listed inTable B.8.1-18.

Table B.8.1-18: Weather conditions in field soil dissipation studies with BAS 510 F

Trial Site	Country/Location/ Study Code	Accumulated Rainfall after Application		Average Temperature after Application	
		3 month [mm]	study period [mm]	3 month [° C]	study period [° C]
1	Germany/ Stetten (74193)/ DU2/15/97	282 (0-90 DAT)	1177 (0-544 DAT)	16 (0-90 DAT)	12 (0-544 DAT)
2	Germany/ Schifferstadt (67105)/ DU3/06/97	194 (0-90 DAT)	712 (0-545 DAT)	16 (0-90 DAT)	12 (0-545 DAT)
3	Spain/ Manzanilla (21890)/ ALO/05/98	33 (0-90 DAT)	455 (0-349 DAT)	25 (0-90 DAT)	18 (0-349 DAT)
4	Spain/ Alcala del Rio (41200)/ ALO/06/98	14 (0-90 DAT)	198 (0-356 DAT)	26 (0-90 DAT)	18 (0-356 DAT)
5	Germany/ Grossharrie (24625)/ D05/03/98	203 (0-90 DAT)	870 (0-357 DAT)	15 (0-90 DAT)	9 (0-357 DAT)

DAT = days after teatment

The studies cover a wide range of experimental weather conditions over Europe. In the South European studies (Spain), very dry periods have to be noted in the first 3 months after the application (precipitation 33 and 14 mm/3 months) with high temperatures (appr. 26 °C). In the studies at the other sites, the conditions were more moderate with rainfall between 200 and 280 mm and average air temperatures of 15 – 16 °C in the first 3 months after application.

Calculation

Seven out of the total of nine field dissipation studies were found to fulfil the guideline criteria, which are located at 3 sites in Northern and Central Europe.
The two field dissipation studies in Spain were rejected within the evaluation process (here: for estimation of biological/chemical degradation rates), because a long drying period of > 3 months was observed after the second sampling. A temperature correction alone was found to be not suitable to estimate standardised degradation parameters.

Seven field studies at 3 locations in Northern and Central Europe were considered to be adequate to estimate field degradation rates of BAS 510 F. Rainfall after application led to an infiltration of the compound, minimising the effect of surface loss processes.

At two of the remaining 3 sites different application rates (3 rates of 0.3, 0.6 and 1.2 kg/ha) have been tested. The 3 application rates are considered as replicates for each test site in the further evaluation. Therefore the results of the 3 replicates are averaged per test site and the degradation rates at the different locations are compiled.

From the suitable studies in Northern Europe, the transformation rates of BAS 510 F standardised at a reference temperature of 20 °C were estimated. This was made with the help of a program that allows to correct the degradation rate for differences between the actual daily temperature and the reference temperature (here 20 °C) with an Arrhenius function. The procedure is described in the EU Guidance Document on Persistence in Soil [9188/VI/97 rev. 8; 12.07.2000]. For the temperature correction a Q10-factor of 2.2 (equivalent to an Arrhenius activation energy of 54 kJ/mol) was used according to the recommendations of FOCUS.

The best fit DT_{50}-values calculated for the residue curves of BAS 510 F in the field dissipation studies together with the standardised half-lives are listed in Table B.8.1-19.

Table B.8.1-19: DT_{50} (best fit) values of BAS 510 F in the field dissipation studies and half-lives standardised to a reference temperature of 20 °C

Trial code/location	DT_{50} best fit [d]	half-life standardised to 20 °C [d]
Germany Stetten (3 replicates) DU2/15/97	55.7	106 d
Germany Schifferstadt (3 replicates) DU3/06/97	176.7	212 d
Spain Manzanilla ALO/05/98	144	-*
Spain Alcala del Rio ALO/06/98	78	-*
Germany Grossharrie D05/03/98	25	98 d
Arithmetic mean	95.9	139

* because of the high standard deviations of the degradation rate a reasonable calculation of the half-life is not possible

The best fit DT_{50}-values of BAS 510 F at the 5 field study sites range from 25 – 177 days, with an average of 95.9 days.
The half-lives of BAS 510 F standardised to 20 °C at the 3 suitable field study sites range from 98 - 212 days with a mean value of 139 days.

The half–lives standardised to 20 °C are higher than the best-fit DT_{50}-values obtained by curve fitting from the field studies without standardisation. This can be attributed to the conservative evaluation and standardisation process.

The average standardised field half-life for BAS 510 F of 139 days is considerably lower than the mean laboratory half-life at 20 °C and 40% of the maximum water holding capacity (ranging from 108 days (minimum) to > 365 days). This may be attributed to the stimulation of the microbial degradation processes by the fluctuating soil temperature and moisture in the dynamic systems of field studies compared to static conditions in the laboratory.

Conclusion
With the chosen evaluation method, the dissipation of BAS 510 F in the 7 suitable out of 5 field dissipation studies at 3 locations can be attributed to transformation in the soil compartment.
A mean value of the half-lives of **139 days** at 20 °C is a reasonable conservative estimate for the prediction of the degradation of BAS 510 F in the field.
The calculated half-lives can therefore be used in simulation models to assess the degradation in soil and potential leaching to groundwater of BAS 510 F under different climatic conditions.

B.8.1.3 Accumulation in soil

Major uses of BAS 510 F will be in vegetable crops and in grapes. Therefore it was decided to perform 2 soil accumulation studies with BAS 510 F according to the targeted crops. For both studies an interim report after 3 years of study duration was written.

Kellner O, Grote C, 2001, BOD2001-294
GLP: yes
Guidelines: SETAC, IVA-Leitlinie

The accumulation behaviour of BAS 510 F under field conditions on grapes was investigated over a three year period from 1998 to 2000 after application onto grapes in a vineyard. The trial was conducted at a site in Germany in Rheinland-Pfalz. The soil was a loamy sand/sandy loam with a pH value of 7.5, an organic carbon content of 1.2%, a cation exchange capacity of 15 meq/100 g dry soil and a maximum water holding capacity of 40 g water/100 g dry soil.
The nominal application rates were 3 times 700 g active substance/ha sprayed onto grapes at BBCH growth stages 67, 77 and 81. The amounts of products actually applied were determined by measuring the volumes in the tank before and after application. The rates were always between 680 and 735 g as/ha and therefore very near to the nominal rates.
BAS 510 KA F (1998) or BAS 510 01 F were always applied onto grapes with a gasoline powered mistblower with nominal amounts of spray mixture of 600, 700 and 800 l/ha at the respective growth stages (Table B.8.1-20).
A summary of the application parameters including dates of application, formulation, growth stage and product and spray mixture applied is given in Table B.8.1-20.

Table B.8.1-20: Application parameters of BAS 510 F in grapes

Application No.	date	DAFT	Formulation	Growth Stage (BBCH)	spray mixture l/ha	product l/ha or kg/ha	ai nominal g/ha
1	19.06.98	0	BAS 510 KA F	67	598	1.39	700
2	28.07.98	39	BAS 510 KA F	77	694	1.39	695
3	18.08.98	60	BAS 510 KA F	81	777	1.36	680
4	17.06.99	363	BAS 510 01 F	67	584	1.36	680
5	27.07.99	403	BAS 510 01 F	77	693	1.39	695
6	18.08.99	425	BAS 510 01 F	81	841	1.47	735
7	16.06.00	728	BAS 510 01 F	67	605	1.41	705
8	24.07.00	766	BAS 510 01 F	77	723	1.45	725
9	16.08.00	789	BAS 510 01 F	81	835	1.46	730

DAFT = days after first treatment

The precipitation and distribution of the spray broth on the plots at the time of application was determined at the first application with a method using Petri dishes filled with soiI. It can be concluded from the results, that the spray broth reaching the soil via application is uniformly distributed throughout the plots. Additionally, the volume of the spray broth was kept small to avoid the formation of droplets rinsing off the leaves. Therefore it was decided, to take the soil cores from 1998 to 2000 as 3 replicates within a subplot at random, but for practical reasons not closer than 45 cm to the vine plants. Soil core samples were taken three times a year, once before the first application in each year, once after the last application (usually in August) and once in October.
Replicate samples were analysed for BAS 510 F by BASF method 408/1. No corrections, neither for recoveries nor blanks, have been made, but all results were corrected for moisture content of soil. Control samples from untreated plots were also analysed. They were free of residues (1 exception). These data demonstrate, that no interferences of the sample material with the analytical procedure occurred and that the control plots were free of residues of BAS 510 F.
The results of the first 3 years are given in. By far the highest amounts of residues of BAS 510 F were detected in the 0 – 10 cm soil layer. Up to sampling no. 4, only very minor quantities above the LOQ were found in the 10 – 25 cm soil layer. At later samplings, the residue level in the 10 – 25 cm layer increased slightly due to agricultural engineering of the plots. Therefore, it was decided to separate the soil cores into increments of 0 – 10, 10 – 20 and 20 – 25 cm starting with the season 2001 to get a clearer picture of the distribution of BAS 510 F with respect to soil depth.

Table B.8.1-21: Analytical results: BAS 510 F in soil after application in grapes

Sample ID					Analytical data			
Sample No.	Sampling No.	Soil depth [cm]	Treatment	Sampling date	Date of GC-measure-ment	Water content of the soil [%]	BAS 510 F [mg/kg]	BAS 510 F [kg/ha]*
9801761	1	0 – 10	2	29.05.1998	20.07.1999	14.2	< 0.01	0
9801763	1	0 – 10	3	29.05.1998	20.07.1999	15.7	0.048	0.072
9801765	1	0 – 10	4	29.05.1998	20.07.1999	14.7	< 0.01	0
9801762	1	10 – 25	2	29.05.1998	20.07.1999	14.2	< 0.01	0

Sample ID					Analytical data			
Sample No.	Sampling No.	Soil depth [cm]	Treatment	Sampling date	Date of GC-measure-ment	Water content of the soil [%]	BAS 510 F [mg/kg]	BAS 510 F [kg/ha]*
9801764	1	10 – 25	3	29.05.1998	20.07.1999	15.7	0.236	0.531
9801766	1	10 – 25	4	29.05.1998	20.07.1999	15.3	< 0.01	0
9805877	2	0 – 10	2	20.08.1998	22.07.1999	5.2	0.320	0.480
9805879	2	0 – 10	3	20.08.1998	22.07.1999	6.8	0.295	0.443
9805881	2	0 – 10	4	20.08.1998	22.07.1999	5.8	0.368	0.552
9805878	2	10 – 25	2	20.08.1998	27.07.1999	8.9	< 0.01	0
9805880	2	10 – 25	3	20.08.1998	27.07.1999	11.4	< 0.01	0
9805882	2	10 – 25	4	20.08.1998	27.07.1999	10.6	< 0.01	0
9805965	3	0 – 10	2	26.10.1998	22.07.1999	14.1	0.239	0.359
9805967	3	0 – 10	3	26.10.1998	22.07.1999	16.3	0.256	0.384
9805969	3	0 – 10	4	26.10.1998	22.07.1999	15.8	0.261	0.392
9805966	3	10 – 25	2	26.10.1998	27.07.1999	13.0	0.012	0.028
9805968	3	10 – 25	3	26.10.1998	27.07.1999	13.2	0.051	0.114
9805970	3	10 – 25	4	26.10.1998	27.07.1999	14.8	0.047	0.106
9902491	4	0 – 10	2	09.06.1999	22.07.1999	15.6	0.382	0.573
9902493	4	0 – 10	3	09.06.1999	22.07.1999	17.5	0.530	0.795
9902495	4	0 – 10	4	09.06.1999	22.07.1999	16.8	0.419	0.629
9902492	4	10 – 25	2	09.06.1999	27.07.1999	14.3	0.018	0.039
9902494	4	10 – 25	3	09.06.1999	27.07.1999	14.4	0.013	0.029
9902496	4	10 – 25	4	09.06.1999	27.07.1999	15.4	0.014	0.032
9907638	5	0 – 10	2	19.08.1999	26.11.1999	12.8	0.905	1.36
9907642	5	0 – 10	3	19.08.1999	26.11.1999	12.7	1.26	1.89
9907646	5	0 – 10	4	19.08.1999	26.11.1999	12.6	1.14	1.71
9907639	5	10 – 25	2	19.08.1999	30.11.1999	10.5	0.234	0.527
9907643	5	10 – 25	3	19.08.1999	30.11.1999	11.0	0.046	0.103
9907647	5	10 – 25	4	19.08.1999	30.11.1999	11.7	0.039	0.089
9907640	6	0 – 10	2	27.10.1999	26.11.1999	13.2	0.751	1.13
9907644	6	0 – 10	3	27.10.1999	26.11.1999	14.0	1.24	1.86
9907648	6	0 – 10	4	27.10.1999	26.11.1999	14.4	0.576	0.864
9907641	6	10 – 25	2	27.10.1999	30.11.1999	10.8	0.082	0.185
9907645	6	10 – 25	3	27.10.1999	30.11.1999	11.1	0.026	0.058
9907649	6	10 – 25	4	27.10.1999	30.11.1999	11.9	0.014	0.031
5126	7	0 – 10	2	15.06.2000	04.08.2000	11.7	0.914	1.37
5128	7	0 – 10	3	15.06.2000	04.08.2000	12.2	1.23	1.85
5130	7	0 – 10	4	15.06.2000	04.08.2000	10.7	0.597	0.96
5127	7	10 – 25	2	15.06.2000	14.08.2000	15.3	0.038	0.086
5129	7	10 – 25	3	15.06.2000	14.08.2000	15.2	0.041	0.092
5131	7	10 – 25	4	15.06.2000	14.08.2000	15.5	0.036	0.081
8875	8	0 – 10	2	29.08.2000	10.10.2000	14.9	1.38	2.07
8877	8	0 – 10	3	29.08.2000	10.10.2000	15.2	1.22	1.83
8879	8	0 – 10	4	29.08.2000	10.10.2000	13.2	1.20	1.80
8876	8	10 – 25	2	29.08.2000	10.10.2000	13.1	0.046	0.104
8878	8	10 – 25	3	29.08.2000	10.10.2000	14.0	0.022	0.050
8880	8	10 – 25	4	29.08.2000	10.10.2000	12.0	0.058	0.131
15310	9	0 – 10	2	25.10.2000	08.01.2001	18.3	1.42	2.13
15312	9	0 – 10	3	25.10.2000	08.01.2001	19.5	1.55	2.33
15314	9	0 – 10	4	25.10.2000	08.01.2001	18.6	0.977	1.47
15311	9	10 – 25	2	25.10.2000	08.01.2001	17.8	0.042	0.095
15313	9	10 – 25	3	25.10.2000	08.01.2001	16.8	0.046	0.104
15315	9	10 – 25	4	25.10.2000	08.01.2001	17.3	0.024	0.054

* calculated by soil density of 1.5 g/cm³ and the volume of the soil layer

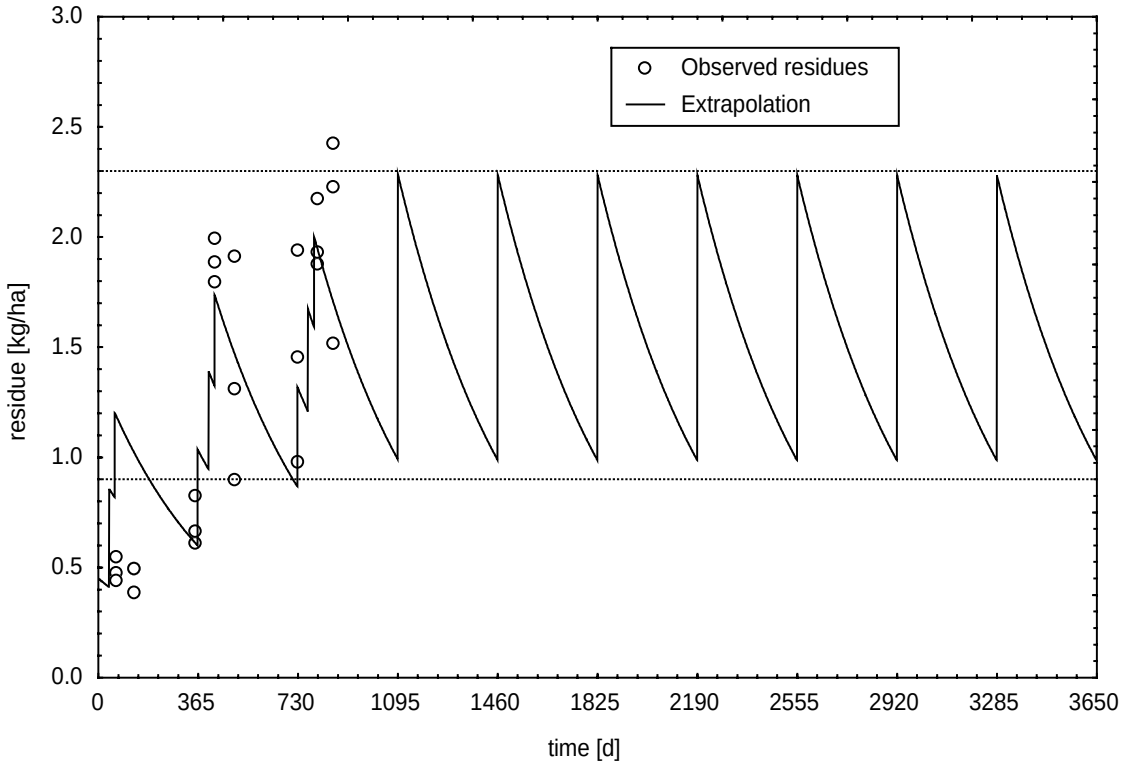
In April 2000 the distribution of the soil residues within the subplots was determined after 3 years of BAS 510 F application and cultivation according to good agricultural practice. It became clear that after 3 years of application and farming practice the residues are not uniformly distributed within the subplots. Right in the middle between the rows of vine the residues are lower than near the plants. Therefore, from the season 2001 on the sampling pattern within the subplots will be modified. The core area between the rows of ca. 70 cm will not be sampled. All samples will be taken at a maximum distance of 60 cm from the trunk of the vine.

Platz K, 2001, BOD2001-295

GLP: no subject to GLP

The prediction of a plateau based on soil residues found during this 3-year application is complex. Therefore, in a separate report the expected minimum and maximum residue levels in soil after repeated application are calculated under consideration of the application rate, interception by the crop, application interval and first order dissipation kinetics of the active substance. In a first step, the degradation rate was standardised to an average annual temperature of 10 °C, which is a suitable temperature for the field trials. This was done by the Arrhenius equation formula. The calculated half-life at 10 °C in the field studies is 306 days. Average crop interception ranging from 70% to 85% (in accordance with the actual growth stages as given by FOCUS) was considered in a first evaluation step. To achieve a better agreement between observed and simulated residues the effective interception had to be reduced by a factor of 0.49.
Considering the application pattern of the soil accumulation study in grapes the maximum and minimum plateau residue level of BAS 510 F is about 2.3 kg/ha and 0.9 kg/ha (see Figure B.8.1-2). The observed data up to 3 years are in good agreement with this predicted concentration range. The accumulation study will be continued until a final plateau is reached.

Figure B.8.1-2: Simulated and observed residues of BAS 510 F after repeated application onto grapevine



Kellner O, Grote Ch, 2001, BOD2001-296

GLP: yes
Guidelines: SETAC, IVA-Leitlinie

The accumulation behaviour of BAS 510 F in vegetables was conducted at a site in Germany in Rheinland-Pfalz (BASF Reg.Doc.# 2000/1017040, BOD2001-296). The soil was a loamy sand with an organic carbon content of 1%, a pH value of 7.7, cation exchange capacity of 13 mVal/100 g dry soil and a maximum water holding capacity of 43 g water/100 g dry soil.
BAS 510 F was applied in 1998 onto lettuce (nominal 2 x 300 g as/ha) and green beans (nominal 3 x 500 g as/ha) and in 1999 onto carrots (nominal 3 x 300 g as/ha) and cauliflower (nominal 2 x 400 g as/ha) (Table B.8.1-22). The total nominal amounts of BAS 510 F applied were 2100 g in 1998 and 1700 g in 1999. The actual values as determined by spray broth calculation differ only slightly. In 2000 spring wheat was grown on the plots and no product containing BAS 510 F was applied to the plots. Growth of vegetables in 2 succeeding years followed by cultivation of cereals during the third year is typical agricultural practice in Germany. It represents a reasonable worst case for the application of BAS 510 F in a crop rotation.

Table B.8.1-22: Application of BAS 510 F in vegetables

Application No.	date	DAFT	Formulation	crop	Growth stage (BBCH)	spray mixture l/ha	product l/ha or kg/ha	ai nominal g/ha
1	14.05.98	0	BAS 510 KA F	Lettuce	17	595	0.595	298
2	03.06.98	20	BAS 510 KA F	Lettuce	43	811	0.608	304
3	25.08.98	103	BAS 510 KA F	Green bean	61	589	0.982	491
4	07.09.98	116	BAS 510 KA F	Grean bean	65	799	0.999	500
5	17.09.98	126	BAS 510 KA F	Green bean	67	823	1.029	515
6	20.05.99	371	BAS 510 01 F	Carrot	14	395	0.593	297
7	07.06.99	389	BAS 510 01 F	Carrot	41	575	0.575	288
8	22.06.99	404	BAS 510 01 F	Carrot	47	756	0.567	284
9	02.09.99	476	BAS 510 01 F	Cauliflower	19	617	0.822	411
10	17.09.99	491	BAS 510 01 F	Cauliflower	41	781	0.781	391

DAFT = days after first treatment

Soil samples were taken twice a year in 3 replicates, once before application and once after harvest . Results up to sampling after harvest in August 2000 are reported.
Replicate samples were analysed for BAS 510 F by BASF method 408/1. No correction, neither for recoveries nor blanks, have been made, but all results were corrected for moisture content of soil. Control samples from untreated plots were analysed from sampling before application. They were free of residues. These data demonstrate, that no interferences of the sample material with the analytical procedure occured and that the control plots were free of residues of BAS 510 F.

Results:
The results of the first 3 years of the soil accumulation study are given in Table B.8.1-23 and confirm the results, that were found after application of BAS 510 F onto bare soil in the field soil dissipation studies. After application in the growth season, significant residues of BAS 510 F can be detected in soil in the following spring. In contrast to the field soil dissipation studies, BAS 510 F was found in this study also in deeper layers of soil. This was caused by the tillage of the soil including ploughing once a year down to 35 cm depth. But the highest amounts of residues were detected from 0 to 25 cm depth.

Table B.8.1-23: Analytical results for BAS 510 F in soil after application in vegetables

Sample ID					Analysis Data			
Sample no.	sampling no.	soil depth [cm]	Treatment	sampling date	Date of GC-measurement	water content of the soil [%]	BAS 510 F [mg/kg]	BAS 510 F [kg/ha]*
9801728	1	0 - 10	2	30.04.98	21.05.1999	13.79	< 0.01	0
9801731	1	0 - 10	3	30.04.98	21.05.1999	11.07	< 0.01	0
9801734	1	0 - 10	4	30.04.98	21.05.1999	15.05	< 0.01	0
9801729	1	10 - 25	2	30.04.98	28.05.1999	16.81	< 0.01	0
9801732	1	10 - 25	3	30.04.98	28.05.1999	16.18	< 0.01	0
9801735	1	10 - 25	4	30.04.98	28.05.1999	17.10	< 0.01	0
9801730	1	25 - 50	2	30.04.98	28.05.1999	16.65	< 0.01	0
9801733	1	25 - 50	3	30.04.98	29.05.1999	17.71	< 0.01	0
9801736	1	25 - 50	4	30.04.98	29.05.1999	17.11	< 0.01	0
9805854	2	0 - 10	2	12.10.98	21.05.1999	16.89	0.386	0.579
9805857	2	0 - 10	3	12.10.98	21.05.1999	17.41	0.619	0.929

Sample ID					Analysis Data			
Sample no.	samp-ling no.	soil depth [cm]	Treat-ment	samp-ling date	Date of GC-measure-ment	water content of the soil [%]	BAS 510 F [mg/kg]	BAS 510 F [kg/ha]*
9805860	2	0 - 10	4	12.10.98	21.05.1999	17.95	0.904	1.356
9805855	2	10 - 25	2	12.10.98	28.05.1999	16.74	< 0.01	0
9805858	2	10 - 25	3	12.10.98	28.05.1999	17.38	< 0.01	0
9805861	2	10 - 25	4	12.10.98	28.05.1999	15.83	0.0789	0.178
9805856	2	25 - 50	2	12.10.98	29.05.1999	17.34	0.0106	0.040
9805859	2	25 - 50	3	12.10.98	29.05.1999	17.78	0.0204	0.077
9805862	2	25 - 50	4	12.10.98	01.06.1999	17.88	0.253	0.949
9900965	3	0 - 10	2	08.03.99	21.05.1999	16.49	0.128	0.192
9900968	3	0 - 10	3	08.03.99	21.05.1999	17.11	0.132	0.198
9900971	3	0 - 10	4	08.03.99	21.05.1999	18.63	0.0810	0.122
9900966	3	10 - 25	2	08.03.99	28.05.1999	17.27	0.183	0.412
9900969	3	10 - 25	3	08.03.99	01.06.1999	19.75	0.361	0.812
9900972	3	10 - 25	4	08.03.99	28.05.1999	18.28	0.115	0.259
9900967	3	25 - 50	2	08.03.99	29.05.1999	17.60	< 0.01	0
9900970	3	25 - 50	3	08.03.99	29.05.1999	18.95	< 0.01	0
9900973	3	25 - 50	4	08.03.99	29.05.1999	18.79	0.0293	0.110
9908546	4	0 - 10	2	03.11.99	06.12.1999	16.66	0.774	1.161
9908549	4	0 - 10	3	03.11.99	06.12.1999	19.22	0.874	1.311
9908552	4	0 - 10	4	03.11.99	03.12.1999	16.82	< 0.01	0
9908547	4	10 - 25	2	03.11.99	03.12.1999	15.66	0.196	0.441
9908550	4	10 - 25	3	03.11.99	03.12.1999	16.67	0.206	0.464
9908553	4	10 - 25	4	03.11.99	06.12.1999	15.36	0.401	0.902
9908548	4	25 - 50	2	03.11.99	03.12.1999	16.50	< 0.01	0
9908551	4	25 - 50	3	03.11.99	03.12.1999	15.93	0.0255	0.096
9908554	4	25 - 50	4	03.11.99	03.12.1999	14.09	0.0466	0.175
3208	5	0 - 10	2	13.03.00	17.08.2000	13.98	0.222	0.333
3211	5	0 - 10	3	13.03.00	17.08.2000	13.25	0.221	0.332
3214	5	0 - 10	4	13.03.00	17.08.2000	13.45	0.2390	0.359
3209	5	10 - 25	2	13.03.00	18.08.2000	17.48	0.305	0.686
3212	5	10 - 25	3	13.03.00	17.08.2000	17.72	0.246	0.554
3215	5	10 - 25	4	13.03.00	17.08.2000	18.32	0.197	0.443
3210	5	25 - 50	2	13.03.00	17.08.2000	18.47	0.0183	0.069
3213	5	25 - 50	3	13.03.00	17.08.2000	18.69	0.0118	0.044
3216	5	25 - 50	4	13.03.00	17.08.2000	19.36	< 0.01	0
8892	6	0 - 10	2	21.08.00	13.10.2000	17.36	0.132	0.198
8895	6	0 - 10	3	21.08.00	13.10.2000	18.46	0.145	0.218
8898	6	0 - 10	4	21.08.00	13.10.2000	15.67	0.139	0.209
8893	6	10 - 25	2	21.08.00	19.10.2000	17.15	0.363	0.817
8896	6	10 - 25	3	21.08.00	20.10.2000	15.43	0.28	0.630
8899	6	10 - 25	4	21.08.00	20.10.2000	16.35	0.238	0.536
8894	6	25 - 50	2	21.08.00	19.10.2000	16.38	0.0544	0.204
8897	6	25 - 50	3	21.08.00	19.10.2000	15.79	0.0628	0.236
8900	6	25 – 50	4	21.08.00	19.10.2000	14.28	0.0523	0.196

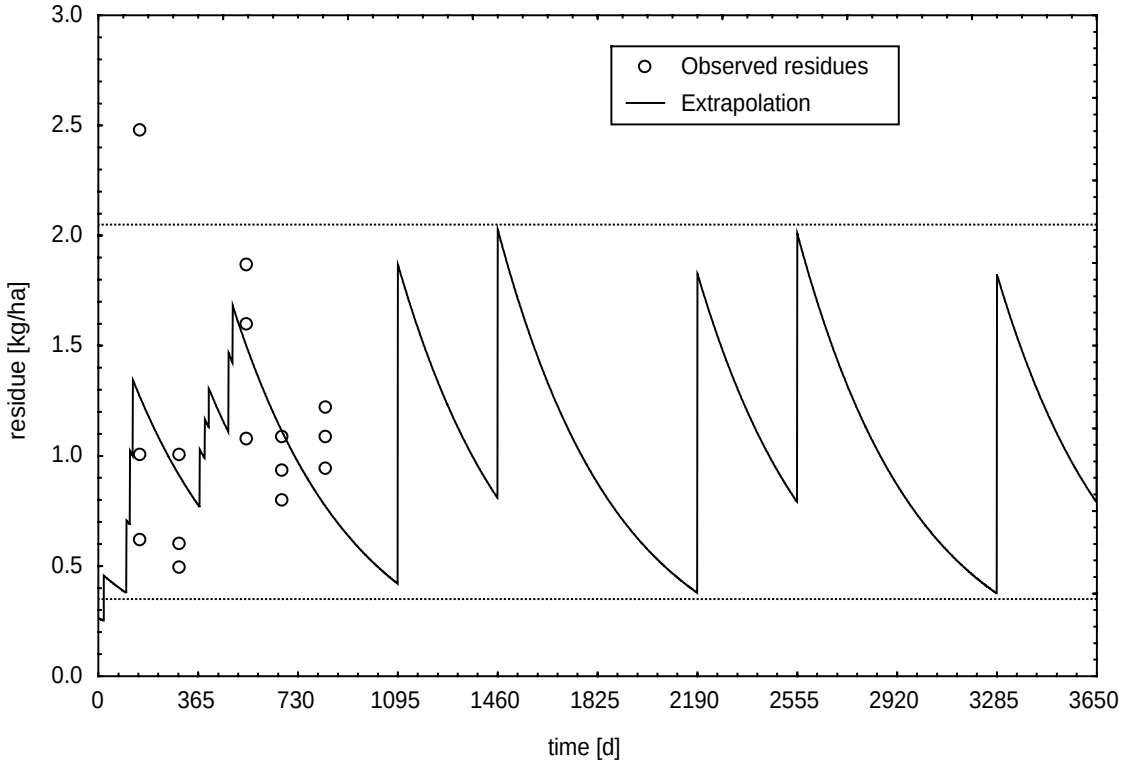
* calculated by soil density of 1.5 g/cm³ and the volume of the soil layer

Platz K, 2001, BOD2001-298

GLP: no subject to GLP (calculation)

The prediction of a plateau based on soil residues found during this 3-year application is complex. Therefore, in a separate report (BASF Reg.Doc.# 2000/1017046, BOD2001-298) the expected minimum and maximum residue levels in soil after repeated application are calculated under consideration of the application rate, interception by the crop, application interval and first order dissipation kinetics of the active substance. In a first step, the degradation rate was standardised to an average annual temperature of 10 °C, which is a suitable temperature for the field trials. The calculated half- life at 10 °C in the field studies is 306 days. Average crop interception ranging from 25% to 80% (in accordance with the actual growth stages as given by FOCUS) was considered in a first evaluation step. To achive a better agreement between observed and simulated residues the effective interception had to be reduced by a factor of 0.53.
Considering the application pattern of the soil accumulation study in vegetables the maximum and minimum plateau residue level of BAS 510 F is about 2.1 kg/ha and 0.3 kg/ha (see Figure B.8.1-3). The observed data up to 3 years are in good agreement with this predicted concentration range. The accumulation study will be continued until a final plateau is reached.

Figure B.8.1-3: Simulated and observed residues of BAS 510 F after repeated application to vegetables



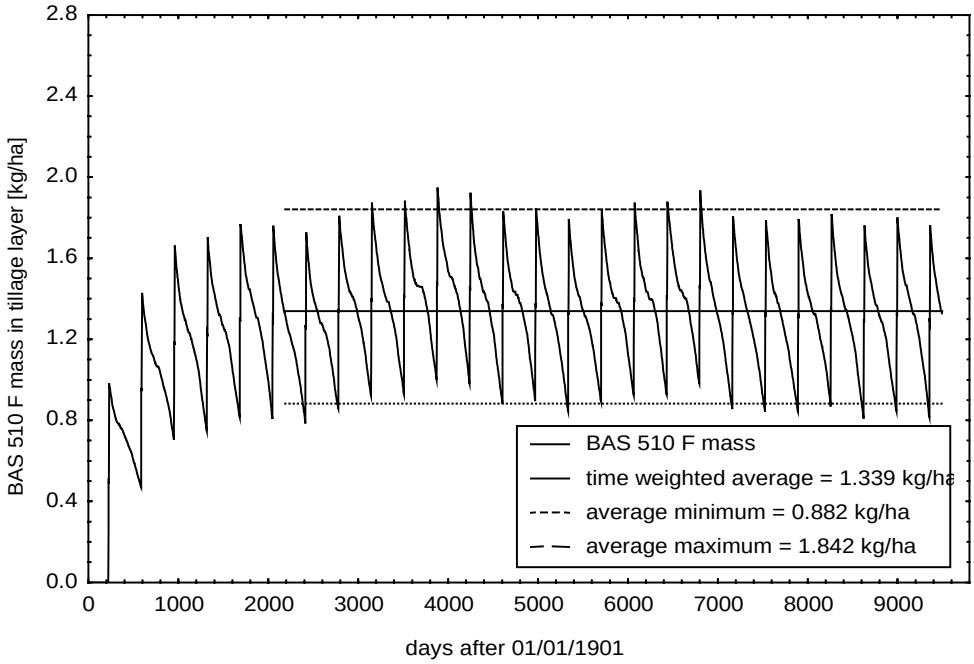
Hauck T, 2001, BOD2001-301

GLP: No subject to GLP (calculation)

Although plateau levels of BAS 510 F in soil could be derived from the two accumulation studies as described above, these plateau levels can not be used for risk assessment since the application regime in these studies is no longer in accordance with the intended agricultural practice for BAS 510 F. During the development of the compound, the number of applications has been significantly reduced for many crops as well as the application rate has partially been

reduced. E.g. in grapes only one application per year at 600 g as/ha is attempted instead of 3 times 700 g as/ha, which has been used in the accumulation study. Therefore, a final assessment of the accumulation potential and predicted environmental concentrations of BAS 510 F in soil was made by using the FOCUS groundwater scenarios.
The calculations were conducted with the simulation model FOCUS PEARL v 1.1.1 for three grapevine scenarios (Hamburg, Piacenza and Sevilla) and for three beans scenarios (Jokioinen, Hamburg and Sevilla). For the vine scenarios, one application per year of 600 g as/ha in autumn was used. For the beans scenarios, two applications of each 500 g as/ha in August was assumed. All scenarios were calculated without interception and with an interception of 80% for beans and 85% for vines as recommended by FOCUS.
To describe the degradation in soil, the average field half-life of BAS 510 F, after standardisation to a reference temperature of 20 °C, was used (139 d). During the simulation the half-life is adjusted automatically by the model subroutines from the reference temperature to actual daily temperature for the different scenarios.
The formation of a plateau of areic mass of BAS 510 F in the tillage layer was observed within the first years. An example is shown in Figure B.8.1-4.

Figure B.8.1-4: Areic mass of BAS 510 F in the tillage layer of the Hamburg (North Central) soil following annual application onto vegetables (no interception)



Thus, as an estimate of the $PEC_{soilaccu}$ the minimum, the mean and the maximum areic mass of BAS 510 F in the tillage layer of the years 1907 to 1926 of the FOCUS scenarios was calculated from the daily values that the model put out.
The calculations for the different FOCUS scenarios representing agroclimatic conditions of southern, central and northern Europe for two crops predicted an annual areic mass of BAS 510 F in the tillage layer of the soil (Table B.8.1-24):

Table B.8.1-24: Predicted plateau residue levels in the tillage layer ($RES_{Plateau}$)

Scenario	crop type	interception	Minimum [kg/ha]	Maximum [kg/ha]	Mean [kg/ha]
Without interception					
Jokioinen	Beans	0%	1.53	2.48	2.02
Hamburg	Beans	0%	0.88	1.84	1.34
Sevilla	Beans	0%	0.26	1.21	0.66
Hamburg	Vines	0%	0.51	1.10	0.83
Piacenza	Vines	0%	0.21	0.81	0.52
Sevilla	Vines	0%	0.12	0.72	0.39
Interception according to FOCUS					
Jokioinen	Beans	80%	0.31	0.50	0.42
Hamburg	Beans	80%	0.18	0.37	0.28
Sevilla	Beans	80%	0.05	0.25	0.14
Hamburg	Vines	85%	0.08	0.17	0.13
Piacenza	Vines	85%	0.03	0.12	0.08
Sevilla	Vines	85%	0.02	0.11	0.06

Conclusion

The accumulation plateau of BAS 510 F was investigated in two field soil accumulation studies accompanied by modelling. The residue data from the first three years of these studies which were available for evaluation were in good agreement with modelling results based on half-lives from field soil dissipation studies.

Since the intended agricultural practice for BAS 510 F has changed significantly since the start of the accumulation studies, the actual prediction of the accumulation plateau is based on a separate modelling approach. In this way a plateau can be reasonably predicted for a variety of conditions based on the FOCUS scenarios.

This modelling approach shows that the accumulation plateau is reached within the first years, depending on the actual scenario. Predicted maximum plateau residue levels of BAS 510 F range from 2.48 kg as/ha for the worst case scenario (beans at Jokioinen, Finland without any interception) down to 0.11 kg as/ha for the vines scenario at Sevilla, Spain under consideration of the crop interception.

B.8.1.4 Storage stability

Stephan A, 2000, BOD2001-302

GLP: yes
Guidelines: EPA, IVA Guideline residue Chemistry

A loamy sand soil was treated with [diphenyl-U-^{14}C]-labelled BAS 510 F at a rate of 0.930 mg as/kg dry soil at 40% maximum water holding capacity. The soil was stored for about two years at –18 °C to –22 °C. Samples were taken at different time periods up to two years after treatment.

Results:

Over the period of 2 years BAS 510 F residues in soil were stable.

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

Seher A, 1998, BOD2001-303

GLP: yes
Guideline: OECD 106, EPA 163-1

Adsorption and desorption of BAS 510 F were measured using a batch equilibrium procedure to determine the K_F- and K_{oc}-values of [diphenyl-U-^{14}C]-labelled BAS 510 F 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 the adsorption/desorption of BAS 510 F

Soil designation	LUFA 2.2 97/736/02	Bruch West 97/060/02	Li 35b 97/145/02	USA 538-30-5 97/995	USA 538-31-2 97/996	Canada 95024 97/994
Origin	Speyer, RP, Germany	Limburgerhof RP, Germany	Limburgerhof RP, Germany	Holly Springs NC, USA	Greenville MS, USA	Minto MA, CAN
Textural class (German scheme)	sand / loamy sand	loamy sand	loamy sand	loamy sand	silty loamy sand	sandy loam
Textural class (USDA scheme)	sand / loamy sand	sandy loam	loamy sand	loamy sand	loam	sandy clay loam
Particle size distribution [%] (German scheme):						
0.063 – 2 mm	85	72	81	81	40	46
0.002 – 0.063 mm	10	18	12	11	47	31
< 0.002 mm	5	10	7	8	13	23
Particle size distribution [%] (USDA scheme):						
0.050 – 2 mm	86	73	83	83	44	49
0.002 – 0.050 mm	9	17	10	9	43	28
< 0.002 mm	5	10	7	8	13	23
Organic C [%]	2.5	1.5	1.1	0.4	0.5	3.4
CEC [mVal/100g]	11.2	12.1	7.2	4.0	10.0	26.0
pH[$CaCl_2$]	5.8	7.5	6.5	5.8	5.2	7.5

Results:
A summary of the results obtained can be found in Table B.8.2-2. The adsorption process for BAS 510 F in the investigated concentration range of 0.02 – 2.5 µg as/ml can be described with a high degree of accuracy using the Freundlich equation. The adsorption constants K_F calculated from the Freundlich isotherms for the six test soils range from 3.3 to 27.8. K_{oc}-values of 507 – 1110 were obtained.

A subsequent determination of the desorption in two steps with 0.01 M $CaCl_2$-solution resulted for the six soils in K_{FdesI}-values from 9.4 to 112.9 and in K_{FdesII} values from 8.9 to 53.8.

Table B.8.2-2: Adsorption and desorption of [diphenyl-U-^{14}C] BAS 510 F in different soil types

Soil designation	Textural class	Adsorption constant K_F [ml/g]	Adsorption exponent 1/n	Adsorption constant K_{oc} [ml/g]	Desorption constant K_{Fdes} [ml/g]	
LUFA 2.2	sand / loamy sand	27.8	0.875	1110	35.2*	37.3**
Bruch West	sandy loam	7.6	0.870	507	15.4*	18.6**
Li 35 b	loamy sand	6.5	0.839	594	14.2*	17.8**
USA 538-30-5	loamy sand	3.9	0.887	987	9.4*	8.9**
USA 538-31-2	loam	3.3	0.860	655	11.6*	14.9**
CAN-95024	sandy clay loam	26.4	0.851	776	112.9*	53.8**

* desorption step 1
** desorption step 2

Adsorption and desorption data for metabolites of BAS 510 F are not provided since no major metabolites higher than 10 % of the applied radioactivity were observed in any laboratory environmental fate study.

Conclusion:
On the basis of the findings of the adsorption/desorption study, BAS 510 F can be classified as non-mobile in soil.

B.8.2.2 Mobility in soil

B.8.2.2.1 Column leaching studies

No column leaching studies were performed since reliable adsorption coefficient values were obtained for the active substance in the adsorption/desorption study. Nevertheless column leaching in one soil without ageing was performed along with the "aged column leaching" study.
No column leaching studies with metabolites of BAS 510 F were performed since no major metabolites higher than 10% of the applied radioactivity were observed in any laboratory environmental fate study.

B.8.2.2.2 Aged residue column leaching

Richter T, 2001, BOD2001-305
Richter T, 2001, BOD2001-320 (Amendment)

GLP: yes
Guidelines: BBA IV, 4-2, SETAC

The leaching characteristics of aged and non-aged BAS 510 F soil residues were studied in a German soil using [diphenyl-U-^{14}C]-labelled BAS 510 F. Details of the soil used are provided

in Table B.8.2-3. Soil samples containing labelled BAS 510 F (at rates equivalent to about 750 g as/ha, resp. 150 µg per column) were incubated in the dark for 28 days at 20 °C at 40% water holding capacity. After ageing or right after the application, respectively, the soil was transferred 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 leachates were collected in four fractions of about 100 ml and analysed for radioactive residues.

Table B.8.2-3: Soil used to investigate aged soil leaching of BAS 510 F

soil designation	LUFA 2.1 Speyer, Germany
textural class (German scheme)	sand
particle size distribution [%] (German scheme): 0.063 – 2 mm 0.002 – 0.063 mm < 0.002 mm	 92 4 4
organic C [%]	0.6
microbial biomass [mg C/100 g dry soil]	6.4
CEC [meq/100 g]	5.0
pH value (0.01 M $CaCl_2$)	6.0
MWC [g H_2O/100 g dry soil]	25
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.8.2-4.

Table B.8.2-4: Leaching behaviour of aged and non-aged soil residues of [diphenyl-U-^{14}C]-labelled BAS 510 F (values in % of applied radioactivity)

amount applied	150 µg (750 g as/ha)		150 µg (750 g as/ha)	
ageing [days]	0		28	
individual tests	A	B	A	B
1. volatile compounds ^{14}C-CO_2	 0.32	 0.32	 0.32	 0.32
2. soil (total)	104.31	110.33	101.49	104.95
segment I	38.73	41.01	60.99	60.68
segment II	65.41	68.68	40.37	43.31
segment III	0.13	0.17	0.12	0.23
segment IV	0.05	0.06	0.00	0.18
segment V	0.00	0.21	0.00	0.14
quartz sand	0.00	0.20	0.00	0.42
3. leachate (total)	0.04	0.04	0.04	0.04
fraction a	0.00	0.00	0.00	0.00
fraction b	**0.00 0.00**		**0.00 0.00**	
fraction c	0.02	0.02	0.02	0.02
fraction d	0.02	0.02	0.02	0.02
(1 – 3) total	104.67	110.69	101.85	105.31

In both experiments, very low amounts of radioactivity were found in the leachates. The upper two soil segments (0 – 6 and 6 - 12 cm) of the columns together contained almost the whole radioactive residues (TRR). The amounts below were very low.
Analysis of methanol and methanol/water extracts of the upper soil segments (radio-HPLC) showed that the radioactivity consisted of unchanged BAS 510 F. There is no significant difference in the results obtained from the experiments with and without ageing of the residues.

Conclusion
The aged and non-aged soil column leaching experiments show that BAS 510 F is not mobile in soil. There is no risk of displacement of BAS 510 F into deeper soil layers.

B.8.2.2.3 Lysimeter or field leaching studies

The adsorption/desorption studies revealed high adsorption coefficient values (K_{oc} > 500). Under the worst case conditions of laboratory leaching experiments (unaged and aged), no movement of the active substance or any other radioactive compound within the soil columns could be observed.
Initial PELMO-simulations using worst case parameters as well as the FOCUS PEARL, PELMO and MACRO calculations clearly show that there is no risk of displacement of BAS 510 F into deeper soil layers or into the groundwater. Therefore, no lysimeter study or field leaching study was necessary.

B.8.3 Predicted environmental concentrations in soil (Annex IIIA 9.1.3)

Hauck T, 2001, BOD2001-330

GLP: no
Guidelines: none

The PEC_{soil}-values for BAS 510 F after application of BAS 510 01 F are calculated according to first order kinetics for a 5 cm soil layer with a bulk density of 1.5 kg/dm³. The worst case field half-life (212 d at 20 °C) is considered after standardisation from a reference temperature of 20 °C to 15 °C (worst case half-life at 15 °C = 314.5 d).

The maximum accumulated application rate of 1 kg a.i./ha (= 2 x 0.5 kg/ha for beans) is considered. Crop interception is neglected and as a worst case scenario it is assumed that 100 % of the applied amount reaches the soil.

Calculation of the $PEC_{soil,ini}$ (Initial Soil Concentrations) for BAS 510 F

The initial predicted environmental concentrations in soil ($PEC_{s,ini}$) are calculated according to Equation 1.

Equation 1:

$$PEC_{s,ini} = \frac{A * f_s * 10}{d * \rho_b}$$

with:

$PEC_{s,ini}$	=	initial PEC_{soil}	(mg/kg)
A	=	application rate of active ingredient	(kg a.i./ha)
f_s	=	fraction reaching soil	(-)
d	=	depth of soil layer	(cm)
ρ_b	=	bulk density	(kg/dm³)

Calculation of actual and time weighted average predicted environmental concentrations in soil ($PEC_{s,act}$ and $PEC_{s,twa}$) for BAS 510 F

The actual concentrations in soil ($PEC_{s,act}$) for BAS 510 F are calculated according to first order kinetics from the half-lives using Equation 2.

Equation 2:

$$PEC_{s,act} = PEC_{s,ini} * \exp^{-t * \frac{\ln 2}{half-life}}$$

with

$PEC_{s,act}$	=	Actual Predicted Environmental Concentration	(mg/kg)
$PEC_{s,ini}$	=	Initial Concentration in soil	(mg/kg)
half-life	=	Half-life	(d)
t	=	Time Period	(d)

Assuming first order kinetics for the decline of the concentrations the predicted time weighted average concentrations ($PEC_{s,twa}$) can be calculated using Equation 3.

Equation 3:

$$PEC_{S,TWA} = PEC_{s,ini} * \frac{half-life}{t * \ln 2} * \left[1 - \exp^{-t * \frac{\ln 2}{half-life}}\right]$$

with

$PEC_{s,twa}$	=	Time Weighted Average Concentration	(mg/kg)
$PEC_{s,ini}$	=	Initial Concentration in soil	(mg/kg)
half-life	=	Half-life	(d)
t	=	Time Period	(d)

The parameters used for the calculation of the PEC_{soil}-values for BAS 510 F are listed in Table B.8.3-1.

Table B.8.3-1: Parameters used for calculation of PEC_{soil}-values for BAS 510 F

Parameter	Meaning	Value	Unit
A	application rate of active ingredient	1000	[g as/ha]
f_s	fraction reaching soil	1	[-]
d	depth of soil layer	5	[cm]
ρb	bulk density	1.5	[kg/dm³]
half-life at 15 °C		314.5	[d]

The actual ($PEC_{s,act}$) and the time weighted averages ($PEC_{s,twa}$) of the predicted environmental concentrations for BAS 510 F in soil are given in Table B.8.3-2.

Table B.8.3-2: Predicted actual (PECs,act) and time weighted average environmental concentrations (PECs,twa) of BAS 510 F in soil (0 - 5 cm) (mean temperature of 15 °C and extrapolated worst-case field half-life of 314.5 d)

	DAT [d]	PECs,act [mg/kg]	PECs,twa [mg/kg]
Initial	0	1.333	1.333
Short-term	1	1.330	1.332
	2	1.327	1.330
	3	1.325	1.329
	4	1.322	1.327
Long-term	7	1.313	1.323
	14	1.293	1.313
	21	1.273	1.303
	28	1.254	1.293
	50	1.194	1.262
	100	1.070	1.197

DAT: days after treatment

The initial $PEC_{s,act}$ concentrations for BAS 510 F are 1.333 mg/kg. The $PEC_{s,act}$- and the $PEC_{s,twa}$-concentrations for BAS 510 F after 100 days are 1.070 mg/kg and 1.197 mg/kg, respectively.

In the terrestrial studies no metabolite was found in amounts greater than 10% of the applied parent. Therefore no PEC_{soil} calculations are performed for metabolites of BAS 510 F.

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

Goetz von N, 1999, WAS2001-153

GLP: yes
Guidelines: EPA 161-1, EC Method C 7

Hydrolysis of [diphenyl-U-^{14}C]-labelled BAS 510 F was tested in aqueous buffer solutions at 50 °C at pH values 4, 7 and 9, and at 25 °C at pH values 5, 7 and 9. Duplicate samples were taken at each sampling time and analysed by radio-HPLC. Selected samples were additionally analysed by radio-HPTLC. The specific radioactivity of the test substance was 5.23 MBq/mg with a radiochemical purity of 97 - 100%. The concentration of BAS 510 F in the buffer solutions was 3 mg/l. The solutions were incubated in the dark under sterile conditions. Sampling times were 0, 1, 2, 3, 4, and 5 DAT for the 50 °C study and 0, 6, 10,15, 20 and 30 DAT and for the 25 °C study.

Results:
The results of the hydrolysis studies at 50 °C and at 25 °C are shown in Table B.8.4-1. No degradation was observed neither at pH values 4, 7, and 9 in the 50 °C study nor at pH values 5, 7 and 9 in the 25 °C study. BAS 510 F was stable under these conditions. No degradates were detected.
No DT_{50}-values were calculated because they will exceed the period of reliable extrapolation (twice the duration of the studies).

Table B.8.4-1: Percentage of BAS 510 F in % TAR during hydrolysis at 50 °C and 25 °C

DAT	BAS 510 F at			
	pH 4	pH 5	pH 7	pH 9
50 °C study				
0	100.0		100.0	100.0
1	100.5		100.4	100.5
2	102.0		99.8	101.4
3	101.0		100.4	100.5
4	101.6		100.6	100.6
5	101.6		100.8	101.3
25 °C study				
0		100.0	100.0	100.0
6		99.6	99.0	99.5
11		98.9	99.3	99.0
15		98.9	99.3	99.3
20		99.5	99.2	99.3
30		99.5	100.1	99.8

Hydrolysis studies with metabolites of BAS 510 F were not performed since no major metabolites higher than 10% of the applied radioactivity were observed in any laboratory environmental fate study.

Conclusion
BAS 510 F is hydrolytically stable between pH 4 and pH 9. Hydrolysis of metabolites was not investigated since no major metabolites above 10% of the applied radioactivity were observed.

B.8.4.1.2 Photochemical degradation

Goetz von N, 1999, LUF2001-269

GLP: yes
Guidelines: FAO revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993), EPA 161-2

The direct aqueous photolysis was performed because the absorption coefficients of BAS 510 F for wavelengths above 290 nm were > 10 l mol^{-1} cm^{-1} as determined in this study. The absorption coefficients were used for the determination of the quantum yield.
The direct photolysis was performed with [pyridine-3-^{14}C]-labelled BAS 510 F. The study was performed at pH 5 (acetate buffer). BAS 510 F is equally hydrolytically stable at all pH-values tested. The concentration of the active substance in the sterile aqueous buffer solution was about 3 mg/l.

Sterilised glass vessels with a quartz glass covering 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 con-

nected 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 15 days with continuous irradiation. Samples were analysed by radio-HPLC.
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 BAS 510 F, a mixture of p-nitroacetophenon 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). Two vessels with the actinometer solution were irradiated together with the test solutions.

Results:
Except traces of $^{14}CO_2$ all of the radioactivity remained in the water. Table B.8.4-2 shows the overall material balance in the direct photolysis and the dark control. By radio-HPLC all of the radioactivity in the water was shown to be BAS 510 F. No degradation was observed in the direct photolysis and in the dark control. No DT_{50} value is reported since it would exceed the period of reliable extrapolation (twice the study duration).

Table B.8.4-2: Material balance of applied radioactivity for photolysis and dark control (mean values of 2 measurements)

DAT	Photolysis water	CO_2	Balance	Dark Control Water
0	100.0	0.0	100.0	100.0
1	98.3	0.0	98.3	97.7
4	95.2	0.1	95.3	95.0
8	98.9	0.1	99.0	100.2
15	94.4	0.1	94.5	101.0

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 BAS 510 F was estimated to be smaller than $2.45 * 10^{-4}$.

For BAS 510 F no half-life in the top layer of aqueous systems was calculated. In the study on direct aqueous photolysis, in the course of which the quantum yield was estimated, the substance was stable. Therefore, if one extrapolates from this result it has to be assumed that the substance is stable in the top layer of aqueous systems.

However, another study was performed, WAS2001-149, that shows the fate of BAS 510 F in an irradiated water/sediment system, which better reflects realistic outdoor conditions than the direct photolysis performed in sterile buffer.
Photolysis studies with metabolites of BAS 510 F were not performed since no major metabolites higher than 10% of the applied radioactivity were observed in any laboratory environmental fate study.

Conclusion:
BAS 510 F is not significantly degraded under the rather artificial conditions of the direct aqueous photolysis. Photolysis of metabolites was not investigated since no major metabolites above 10% of the applied radioactivity were observed.

B.8.4.2 Biological degradation

B.8.4.2.1 Ready biodegradability

Werner D I, 1999, WAS2001-147

GLP:yes
Guidelines: EEC 92/69 (Method C 4-D), OECD 301 F, ISO 9408

The aerobic biodegradability of BAS 510 F 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 not pre-adapted inoculum were incubated in a respirometer (Sapromat). The inoculum was municipal 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. BAS 510 F was considered as poorly biodegradable and not readily biodegradable in this test.

B.8.4.2.2 Degradation in water/sediment system

B.8.4.2.2.1 Laboratory conditions

Ebert D, 2000, WAS2001-148

GLP: yes
Guidelines: BBA IV 5-1, EPA 162-4, SETAC Europe

The distribution and degradation of BAS 510 F was studied in two natural systems of water and sediment. The water/sediment systems were taken from a pond named "Kellmetsch-weiher" close to Schifferstadt (System A) and a pond-like side arm of the Rhine river close to Speyer (System B), both in Rhineland-Palatinate, Germany.

Both radiolabelled forms of BAS 510 F, [pyridine-3-^{14}C] and [diphenyl-U-^{14}C], were used and applied separately to the test systems. The specific radioactivity of the active substance was 5.81 MBq/mg for the [pyridine-3-^{14}C]-label and 5.23 MBq/mg for the [diphenyl-U-^{14}C]-label, both with a radiochemical purity of > 99%.

Characteristics of the water/sediment systems are given in Table B.8.4-3. BAS 510 F was applied to the water at a rate of 70 µg as per test vessel which corresponds to the maximum rate of 700 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 potential degradation products, some water/sediment systems were additionally treated at an application rate of about 215 µg as per test vessel. The test vessels were incubated in the dark at a temperature of 20 ± 2 °C for up to 100 days. Aeration was achieved by a stream of air over the water surface.

Table B.8.4-3: Characterisation of the water/sediment systems

Designation		System A	System B
Origin		Kellmetschweiher Rhineland-Palatinate, FRG	Berghäuser Altrhein Rhineland-Palatinate, FRG
Sediment	sand [%]	83	48
	silt [%]	7	42
	clay [%]	10	10
	textural class (German scheme)	clayey sand	silty loamy sand
	pH ($CaCl_2$)	6.8	7.5
	organic C [%]	0.8	4.1
	total N [%]	0.08	0.37
	total P [%]	0.01	0.1
	CEC [mVal/100g]	11	18
	ATP [µg/kg]	13	1605
	plate counts [cfu/g]		
	bacteria	3.9×10^7	5.3×10^7
	actinomycetes	2.5×10^6	2.3×10^6
	fungi	7.7×10^4	1.6×10^6
Water	pH	8.5	8.1
	hardness [mmol/l]	1.96	1.31
	TOC [mg/l]	12.0	15.2
	total N [mg/l]	2	2
	total P [mg/l]	< 3	< 3

Results:
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.8.4-4, the corresponding results from system B are presented in Table B.8.4-5.
The radioactivity moved quite fast from the water to the sediment. The radioactivity in the water decreased within 14 days to 42% TAR in system A and 22% TAR in system B. A further decrease to less than 20% TAR in system A and less than 10% TAR in system B after was reached after 100 days. In the sediment a corresponding increase could be observed which accounted for more than 80% / 90% TAR (system A/B) at the end of the incubation period. Mineralization was low in both systems with 0.5% TAR and no other volatile degradates were detected.

Moderate amounts of bound residues were formed in the sediment which accounted for up to 13% TAR in system A and 10% TAR in system B after 100 days. These residues were fractionated into humins, humic acids and fulvic acids. In system A, most of the radioactivity was located in the fulvic acids (maximum 5.2% TAR), whereas in system B most of the NaOH extractable radioactivity was associated with humic acids (maximum 3.9% TAR).

The distribution of radioactivity in the sterilised test systems was very similar to the viable trials at the end of the study.

Table B.8.4-4: Material balance and distribution of radioactivity after application of [^{14}C]-BAS 510 F to water/sediment system A (%TAR)

DAT	water	sediment					CO_2	balance
		extractable residues			bound residues	total		
		ACN/H_2O	ACN	total				
0	96.6	0.4	0.1	0.5	0.1	0.5	n.d.	97.1
1	75.9	16.8	3.1	19.9	0.8	20.7	0.0	96.6
2	70.3	21.0	4.6	25.6	1.5	27.1	0.0	97.4
7	53.5	33.9	6.5	40.4	2.7	43.1	0.0	96.6
14	42.3	41.8	8.9	50.7	4.2	54.9	0.1	97.2
29	31.0	48.9	11.4	60.2	6.5	66.7	0.1	97.8
59	23.4	51.2	13.0	64.2	12.0	76.1	0.2	99.8
100	17.4	55.5	12.2	67.7	12.9	80.6	0.5	98.5
101 s	14.6	55.9	15.6	71.5	8.7	80.2	n.d.	94.8

s = sterilised
n.d. = not determined

Table B.8.4-5: Material balance and distribution of radioactivity after application of [^{14}C]-BAS 510 F to water/sediment system B (%TAR)

DAT	water	sediment					CO_2	balance
		extractable residues			bound residues	total		
		ACN/H_2O	ACN	total				
0	96.4	0.5	0.2	0.7	0.0	0.7	n.d.	97.1
1	63.4	24.4	7.7	32.1	1.4	33.4	0.0	96.8
2	52.2	34.0	9.1	43.1	1.8	44.9	0.0	97.1
7	33.1	47.0	13.9	60.9	3.0	64.0	0.0	97.1
14	22.2	54.7	14.8	69.5	4.6	74.1	0.1	96.4
29	12.1	57.6	21.2	78.8	6.1	84.8	0.3	97.2
59	7.9	57.3	21.0	78.3	8.1	86.5	0.5	94.9
100	6.1	59.8	20.1	79.9	10.4	90.3	0.5	96.9
101 s	7.3	63.3	20.6	83.9	4.5	88.4	n.d.	95.7

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.8.4-6 for system A and system B.

In the water and sediment extracts of both water/sediment systems, the active substance was found to be the only radiolabelled compound. No metabolites were detected.

Table B.8.4-6: HPLC analysis of the water and the sediment extracts after application of [^{14}C]-BAS 510 F (%TAR)

DAT	System A		System B	
	BAS 510 F	total	BAS 510 F	total
water				
0	96.6	96.6	96.4	96.4
1	75.9	75.9	63.4	63.4
2	70.3	70.3	52.2	52.2
7	53.5	53.5	33.1	33.1
14	42.3	42.3	22.2	22.2
29	31.0	31.0	12.1	12.1
59	23.4	23.4	7.9	7.9
100	17.4	17.4	6.1	6.1
101 s	14.6	14.6	7.3	7.3
sediment extract				
0	n.d.	0.5	0.0	0.7
1	19.9	19.9	32.1	32.1
2	25.6	25.6	43.1	43.1
7	40.4	40.4	60.9	60.9
14	50.7	50.7	69.5	69.5
29	60.2	60.2	78.8	78.8
59	64.2	64.2	78.3	78.3
100	67.7	67.7	79.9	79.9
101 s	71.5	71.5	83.9	83.9

s = sterilised

Disappearance times of the active substance were calculated for the water. For this purpose a simple compartment model was established for the two water/sediment systems which were used for parameter estimation by the computer program ModelMaker (version 3.0.4, Cherwell Scientific Publishing Ltd., UK). In the calculations the results of the two radiolabels were treated as replicates.

The coefficient of determination was $r^2 = 0.995$ for both systems. The DT_{50} values for the active substance were determined graphically instead by a 1st order estimation. The DT_{50} for BAS 510 F in the water was 9 days for system A and 3 days for system B. The corresponding DT_{90}-values were 133 and 43 days for system A and B, respectively.

B.8.4.2.2.2 Outdoor conditions

Fent G, 2001, WAS2001-149

GLP: yes
Guidelines: based on BBA IV 5-1

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). The water/sediment characteristics are summarised in Table B.8.4-7. Test vessels were filled with about 2.0 cm sediment (about 400 g) and a water layer of about 20 cm height (1950 ml). The system was allowed to equilibrate for 8 days before treatment. The water surface was treated with [diphenyl-U-^{14}C]-labelled BAS 510 F. The specific radioactivity of the test substance was 6.27 MBq/mg, the radiochemical purity was > 98%. BAS 510 F was applied at a rate of 464 µg per test vessel. This roughly corresponds to an application rate of 700 g active substance/ha, when assuming direct overspray of a 30 cm deep water body.

The water/sediment systems were placed in big isolated plastic tanks, filled to a distinct level with water in order to simulate a bigger water body with respective temperature compensation. The tanks were located outdoors in order to have outdoor temperature and light conditions (treatment date July 5th, 2000). In order to protect the vessels from rainfall they were placed under a special plexiglass cover which allowed UV and visible light transmission. If no rainfall was forecasted the plexiglass cover was removed.

Samples were taken at 0, 1, 2, 7, 14, 30, 58, 103, and 120 DAT. One test vessel was worked up per sampling day. The water was analysed by HPLC without further treatment. The sediment was extracted, and the extracts were analysed by HPLC. Volatiles could not be trapped, however, it can be concluded that the increasing balance deficiencies occuring during this study can be attributed to the formation of CO_2.

Table B.8.4-7: Characterization of the water/sediment system Kellmetschweiher used for the water/sediment study under outdoor conditions

water/sediment designation origin			Kellmetschweiher Schifferstadt, Rhineland Palatinate, Germany
water	pH at site of sampling		8.8
	TOC	[mg/l]	16.8
	total hardness [mmol/l]		1.04
	plate counts [cfu/ml]		
	bacteria		8.14×10^2
	fungi		2
	actinomycetes		0
sediment	textural class (German scheme)		sand / clayey sand
	clay	[%]	12
	silt	[%]	4
	sand [%]		84
	plate counts [cfu/g]		
	bacteria		4.77×10^6
	fungi		4.39×10^4
	actinomycetes		2.52×10^4
	organic C [%]		1.6
	nitrogen total	[%]	0.14
	phosphorus [mg/kg]		150

Results:
The distribution of radioactivity and material balance in the water/sediment system is shown in Table B.8.4-8.

Table B.8.4-8: Distribution of radioactivity and material balance in the water/sediment system after application of ^{14}C-BAS 510 F and incubation under outdoor light and temperature conditions

time after treatment	% TAR					
	water	sediment			material balance (water + sediment)	CO_2 *
		extractable residues	bound residues	total		
0 d	96.2	0.0		0.0	96.2	3.8
1 d	88.5	8.4	0.6	9.0	97.5	2.5
2 d	84.3	12.1	1.1	13.2	97.5	2.5
7 d	70.6	20.0	2.2	22.2	92.8	7.2
14 d	60.9	25.4	6.3	31.7	92.6	7.4
30 d	41.1	24.0	12.4	36.4	77.5	22.5
58 d	28.3	27.1	27.9	55.0	83.3	16.7
103 d	19.8	32.1	48.3	80.4	100.2	-
120 d	22.0	30.7	20.5	51.2	73.2	26.8

* calculated as material balance difference

The results of this study show that BAS 510 F follows two dissipation and degradation pathways in a natural water system. When reaching the water, BAS 510 F undergoes a photolytical

transformation where the diphenyl structure is completely degraded, forming para-Cl-benzoic acid (M510F 64) as metabolite (Table B.8.4-9), and simultaneously, it adsorbs fast to the sediment where it is finally bound to the sediment matrix. The sum of radioactivity in water and sediment declined to about 73% TAR after 120 days which indicates a mineralization of about 27% TAR.

No major metabolites (> 10% TAR) were formed. The metabolite M510 F64 reached 9.4% TAR after 30 days in the water phase, however, it degraded thereafter reaching 1.9% TAR at the end of the study. Almost no other degradation and breakdown products could be detected in the water during the study, and also in the sediment, only trace amounts of various metabolites were visible in the HPLC-chromatograms.

Table B.8.4-9: HPLC analysis of the water samples and sediment extracts after application of ^{14}C-BAS 510 F to a water/sediment system and incubation under outdoor light and temperature conditions

time after treatment	% TAR			
	total	BAS 510 F	M510F64	others
water				
0 d	96.2	96.2		
1 d	88.5	88.5		
2 d	84.3	84.3		
7 d	70.6	63.3	7.3	
14 d	60.9	51.9	9.0	
30 d	41.1	31.7	9.4	
58 d	28.3	25.7	2.6	
103 d	19.8	19.8		
120 d	22.0	19.2	1.9	0.9
sediment				
0 d	0.0	0.0		0.0
1 d	8.4	8.4		
2 d	12.1	11.8		0.2
7 d	20.0	18.8		1.2
14 d	25.4	24.0		1.4
30 d	24.0	22.1		1.9
58 d	27.1	23.6		3.5
103 d	32.1	28.2		3.9
120 d	30.7	26.5		4.2

Half life time calculations were performed in a separate study reported below.

Platz K, 2001, WAS2001-150

GLP: No subject to GLP
Guidelines: none

In this modelling excercise, the half life time calculation for the degradation of BAS 510 F in a water/sediment system under outdoor conditions was performed on the basis of the study described above.

In order to evaluate the degradation and partitioning of BAS 510 F and its metabolite M510 F64 three fit approaches were used to describe the observed residues with simple and more elaborate assumptions. In a first approach, the residues of BAS 510 F in the water were fitted assuming first order kinetics. This approach made use of the observed data up to day 58 since up to this date the temperature was fairly constant around 20 °C.

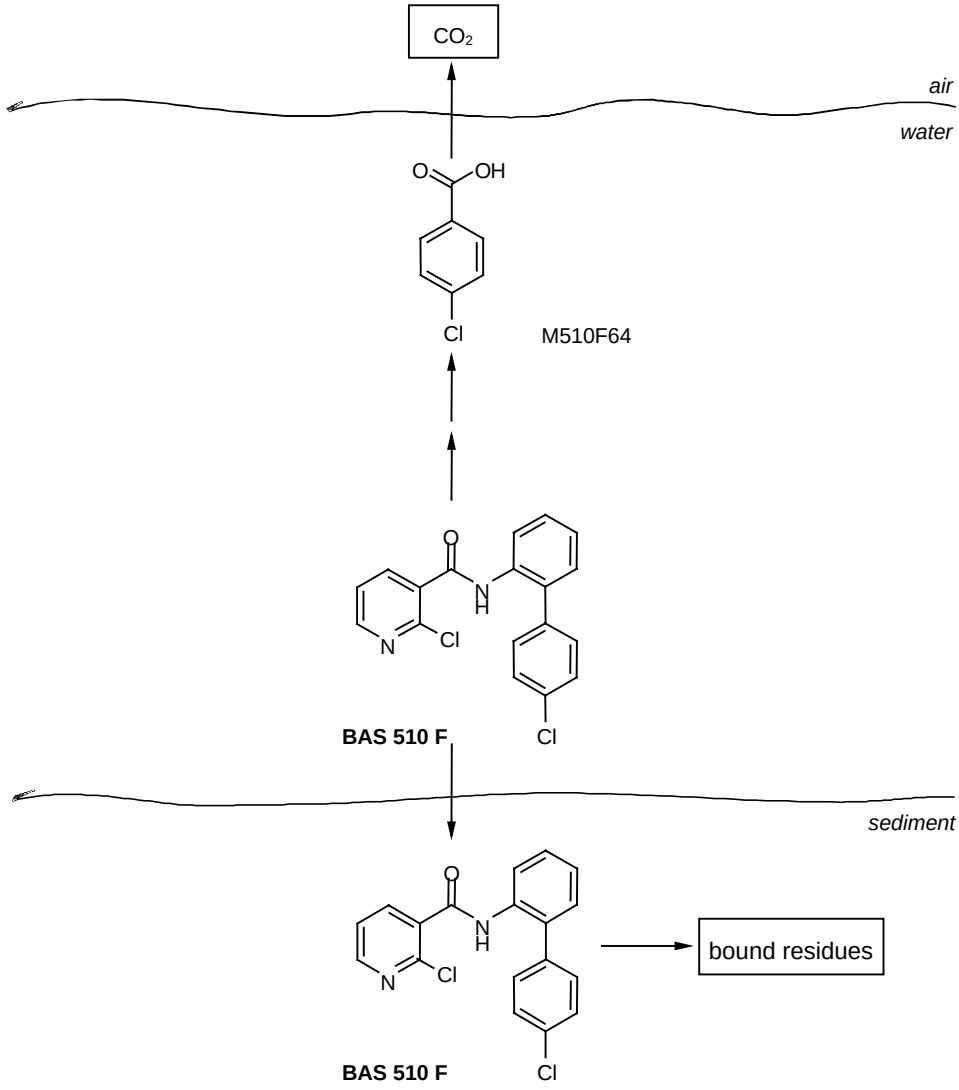
In a second approach, additionally the partitioning to the sediment and the formation and decline of M510 F64 was considered with the help of a compartment model. In the third approach, the daily water temperatures were considered and the observed residues of the whole study period were taken into account.
Estimation of the kinetic parameters (initial concentration, transfer and transformation rates) using compartment models was done with the program ModelMaker version 4 (Cherwell Scientific Publishing, Oxford 1993-97).

Results:
The half-lives and DT_{50} values, respectively resulting from the different modelling approaches are summarized in Table B.8.4-10. High coefficients of determination were achieved for all three models (0.94, 0.99 and 0.98 for approaches 1, 2 and 3, respectively).

Table B.8.4-10: Half-lives and DT_{50}-values of BAS 510 F and its metabolite M510F64 in the water/sediment study under outdoor conditions estimated by different modelling approaches

Compound	Phase	Modelling approach	Half-life (1^{st} order)	DT_{50} (best fit)
BAS 510 F	Water	1^{st} order kinetics	21 d	-
BAS 510 F	Water	approach 2	-	16 d
BAS 510 F	Water	approach 3	-	18 d
M510 F64	Water	approach 2	8 d	-
		approach 3	7 d	-
BAS 510 F	Sediment	approach 2	-	66 d

Figure B.8.4-1: Proposed route of degradation of BAS 510 F in aqueous systems under outdoor conditions



Conclusion:

The degradation of BAS 510 F in natural aqueous systems is insufficiently described by the basic laboratory studies (hydrolysis, aqueous photolysis and water/sediment). BAS 510 F has a low water solubility and a high adsorption coefficient, which leads to a fast movement into the sediment where it is finally bound to the sediment matrix. As can be deduced from the soil photolysis study, the compound is more susceptible to degradation under the influence of light. Therefore, an additional study was designed where all relevant mechanisms (sorption to the sediment, photolysis in natural water and biological degradation) are integrated. This study shows that under natural conditions, where all these mechanisms act together, BAS 510 F will be degraded to CO_2 or will be bound to the sediment. Compared to the standard laboratory studies the degradation under natural conditions is enhanced. This is accompanied by the appearance of an additional metabolite (M510 F64), that was not detected in the other studies. However, this metabolite remained below 10% TAR and was of transient nature. Additionally this compound is of no toxicological and ecotoxicological relevance.

Half-life/DT_{50} of BAS 510 F in the water is 16 – 21 days (depending on the modelling approach), the DT_{50} in the sediment was calculated to be 66 days. The half-life of M510F64 in the water is 7 – 8 days.

B.8.4.3 Degradation in the saturated zone

In the adsorption/desorption studies with BAS 510 F 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. FOCUS PEARL, PELMO and MACRO calculations show that there is no risk of displacement of BAS 510 F 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)

BAS 510 F is not supposed to contaminate the ground water. Therefore an impact on water treatment procedure has not to be addressed.

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 ground water (PECGW)

Predicted environmental concentrations in groundwater (PEC_{gw}) of the fungicidal active ingredient BAS 510 F after application of BAS 510 01 F were calculated for the FOCUS groundwater Scenarios for European registration (*FOCUS (2000) "FOCUS groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC, Document Reference Sanco/321/2000 rev.2, 202pp*).

Veen van de J R, 2001, WAS2001-157

GLP: no
Guidelines: see above

The simulation models FOCUS-PEARL v. 1.1.1, FOCUS-MACRO v. 1.1.1 and PELMO v. 3.0 were used for the calculation of the predicted environmental concentrations in groundwater (PEC_{gw}) for BAS 510 F. The model FOCUS-PEARL v. 1.1.1 was used for all FOCUS groundwater scenarios which grapevine or beans are cultivated, FOCUS-MACRO v. 1.1.1 was used to demonstrate the effect of macropore flow with one scenario (grapevine in Chateaudun) and PELMO v. 3.0 was used with German Standard Scenario (beans in Hamburg, Borstel soil) as a reference scenario for which numerous calculations have been submitted.

Soil and climate scenarios

The soil and climate scenarios defined by FOCUS (2000) were selected to represent an overall vulnerability approximating the 90th percentile of all scenarios with agricultural significance (realistic worst case). The nine locations cover all climatic regions of agricultural relevance in Europe which are briefly characterised in Table B.8.6-1. For all scenarios, daily weather data are available for a period of 20 years.

In this modelling study, calculations were performed for all locations for a period of 26 years and the results of 20 years after a warm up period of 6 years are presented.

Table B.8.6-1: Characteristics of the nine weather- and soil scenarios created by FOCUS

Scenario (code)	Soil type	OM [%]	Annual average air temperature [°C]	Precipitation [mm]
Châteaudun (C)	silty clay loam	2.4	11.3	648 + I
Hamburg (H)	sandy loam	2.6	9.0	786
Jokioinen (J)	loamy sand	7.0	4.1	638
Kremsmünster (K)	loam/silt loam	3.6	8.6	900
Okehampton (N)	loam	3.8	10.2	1038
Piacenza (P)	loam	1.7	13.2	857 + I
Porto (O)	loam	6.6	14.8	1150
Sevilla (S)	silt loam	1.6	17.9	493 + I
Thiva (T)	loam	1.3	16.2	500 + I

I = irrigation
OM = organic matter

Crop scenario

According to the recommendations of FOCUS (2000), a continuous cropping of the same crop (here grapevine and beans) is assumed for the risk assessment, with an application of the product on the same day in every year (for 26 years).

Rates, number of applications and factors contributing to soil residues

The calculations were conducted with the simulation model FOCUS-PEARL v.1.1.1 for seven scenarios with an application of 1 x 0.6 kg/ha of BAS 510 F onto grapevine and for 5 scenarios with 2 x 0.5 kg/ha onto beans. With FOCUS-Macro v.1.1.1 the grapevine scenario in Chateaudun is simulated. With PELMO 3.0 calculations were performed for the German Standard Scenario with beans as crops, the 'Borstel' soil and weather from 'Hamburg'.

Other crops like oilseed rape (2*0.25 kg/ha) and peas (2*0.5 kg/ha) are covered by the beans scenario.

It was assumed that 100 % of the applied amount did reach the soil (worst case assumption) and interception was not considered, which would have reduced the amount on soil by 85 % and 80 % for grapevine and for beans, respectively.

The application dates were chosen to be the same for the same crop (grapevine or beans, respectively) in the different regions. It is assumed that the product is applied on October 1st for the grapevine scenario and on August 11th and 18th for the beans scenario.

Input data for the simulation model related to the crop and application scenario are summarized in Table B.8.6-2.

Table B.8.6-2: Crop-related model input parameters

Input parameter	Unit	Grapevine scenario	Vegetable scenario
Crop	-	Grapevine	Vegetables (beans)
Application dates	-	Châteaudun, Hamburg, Kremsmünster, Piacenza, Porto, Sevilla, Thiva October, 1st each year	Hamburg, Kremsmünster, Okehampton, Porto, Sevilla August, 11th each year August, 18th each year
Timing in cropping period	-	28 ± 2 d before harvest	7, 14 days before harvest
Application rate	kg/ha	1 x 0.6	2 x 0.5
crop interception	%	0	0
amount reaching soil	kg/ha/a	0.6	1.0

Significant differences exist between the degradation of BAS 510 F in the laboratory incubation studies (slower rates with half lives of 133 d to > 1 year) and the field studies (faster rates, DT_{50}bestfit between 25 and 177 d). In a thorough evaluation of all studies those degradation parameters where estimated which can be attributed solely to the transformation in the soil compartment [see Field studies]. The average half-life of BAS 510 F obtained after standardisation from the field studies to a reference temperature of 20 °C is 139 days.

The sorption parameters of BAS 510 F ($K_{F,OM}$-values) range from 294 to 644 L/kg (= $K_{F,OC}$-values 507-1110 L/kg) with an average $K_{F,OM}$ of 447.5 L/kg. The average Freundlich Exponent is 0.864.

The substance-related input parameters for BAS 510 F the specifications which parameter was considered in which of the models (FOCUS_PEARL, FOCUS_MACRO or PELMO 3.0) are listed in Table B.8.6-3.

Table B.8.6-3: Summary of FOCUS Input parameters for BAS 510 F

Input parameter	Unit	Measured value	Default value	Model
PHYSICO-CHEMICAL PARAMETERS				
Molecular weight	g/mol	343.2		Pearl+Pelmo
Water solubility	mg/L	5 at 20 °C		Pearl
Molar enthalpy of dissolution	kJ/mol	-	27	Pearl
Saturated vapor pressure	Pa mPa	0.1×10^{-9} (20 °C) 0.1×10^{-6} (20 °C)		Pearl Macro
Molar enthalpy of vaporisation	kJ/mol	-	95	Pearl
Diffusion coefficient in water	m²/d m²/s	- -	4.3×10^{-5} (20 °C) 5.0×10^{-11} (20 °C)	Pearl Macro
Diffusion coefficient in gas	m²/d	-	0.43 (20 °C)	Pearl
DEGRADATION PARAMETERS				
Half-life at reference conditions (20 °C; matric potential -10 kPa)	d	139		Pearl+Macro+Pelmo
Exponent of temperature function	-	-	0.079	Macro
Arrhenius activation energy	kJ/mol	-	54	Pearl
Exponent of moisture correction function	-	-	0.7	Pearl+Macro+Pelmo
SORPTION PARAMETERS				
$K_{F,OC}$-value	dm³/kg	771		Macro+Pelmo
$K_{F,OM}$-value	dm³/kg	447.5		Pearl
Freundlich exponent 1/n	-	0.864		Pearl+Macro+Pelmo
Method of subroutine description	-	pH independent		Pearl
CROP RELATED PARAMETERS				
TSCF (crop uptake)	-	-	0.5	Pearl+Macro

Calculation procedure of PEC_{gw} for BAS 510 F

The PEC_{gw}-values were calculated with the model FOCUS-PEARL v. 1.1.1 to cover the relevant use areas in the EU of BAS 510 F in grapevine and in beans, with FOCUS-MACRO v. 1.1.1 at one site (Châteaudun) to demonstrate the effect of macropore flow and with PELMO v. 3.0 for the German Standard Scenario (Borstel soil, Hamburg weather).

With FOCUS-MACRO and with FOCUS-PEARL the 80th percentile of the annual average flux concentration leaching below one meter soil depth is calculated for each scenario. For PELMO 3.0 the highest average annual concentration in the leachate is considered.

Results

The PEC_{gw}–values for BAS 510 F are given in Table B.8.6-4 for the grapevine scenario and in Table B.8.6-5 for the beans scenario.

Table B.8.6-4: PEC_{gw}-values for BAS 510 F for the grapevine scenario

Scenario	Model used	BAS 510 F [µg/L]
Châteaudun	Macro	0.005
Châteaudun	Pearl	< 0.001
Hamburg	Pearl	< 0.001
Kremsmünster	Pearl	< 0.001
Piacenza	Pearl	0.042
Porto	Pearl	< 0.001
Sevilla	Pearl	< 0.001
Thiva	Pearl	< 0.001

Table B.8.6-5: PEC_{gw}-values for BAS 510 F for the beans scenario

Scenario	Model used	BAS 510 F [µg/L]
Hamburg	Pearl	< 0.001
Kremsmünster	Pearl	< 0.001
Okehampton	Pearl	< 0.001
Porto	Pearl	< 0.001
Thiva	Pearl	< 0.001
German Standard Scenario	PELMO	< 0.001

For all scenarios the PEC_{gw}-values for BAS 510 F simulated with FOCUS-PEARL v.1.1.1 did not exceed 0.1 µg/L even without consideration of any crop interception. The highest PEC_{gw}-value was 0.04 µg/L for the Piacenza-scenario.

The PEC_{gw}-values calculated with FOCUS-MACRO v.1.1.1 for the Chateâudun-scenario (grapevines) and with PELMO 3.0 for the German Standard Scenario (beans) were 0.005 µg/l and < 0.001 µg/l, respectively.

In the terrestrial studies no metabolite was found in amounts greater than 10% of the applied parent. Therefore no PEC_{gw}-calculations are performed for metabolites of BAS 510 F.

No additional field testing was required since the calculation of the PEC_{gw} could be performed with appropriate accuracy.

Conclusion:
The simulations for BAS 510 F show concentrations below 0.1 µg/l in all FOCUS scenarios indicating a safe use of BAS 510 F with respect to groundwater.

In the terrestrial studies no metabolite was found in amounts greater than 10% of the applied parent. Therefore no PEC_{gw}-calculations are performed for metabolites of BAS 510 F.

B.8.6.2 Predicted environmental concentration in surface water (PECSW)

Gottesbueren B, 2001, WAS2001-158

The PEC_{sw} were calculated for BAS 510 F for a static water body of 30 cm water depth.
In the terrestrial and the aquatic studies no metabolite was found in amounts greater than 10% of the applied parent. Therefore no PEC_{sw}-calculations are performed for metabolites of BAS 510 F.

The static water body is assumed to be a worst case scenario for a slow moving water body. Therefore no PEC_{sw}-calculations are performed for a slow moving water body.

Pesticide parameters
The dissipation behaviour (degradation and partitioning to sediment) of BAS 510 F in surface water is predicted on the basis of the results of a water-sediment study under outdoor conditions.
Different approaches were used to determine the dissipation and metabolism behaviour of BAS 510 F in a water sediment study under outdoor conditions.

Approach 1: Estimation of the half life of BAS 510 F in the water phase assuming first order kinetics without considering the water temperature. Only the observed residues in water up to day 58 (with a fairly constant water temperature after application were taken into account

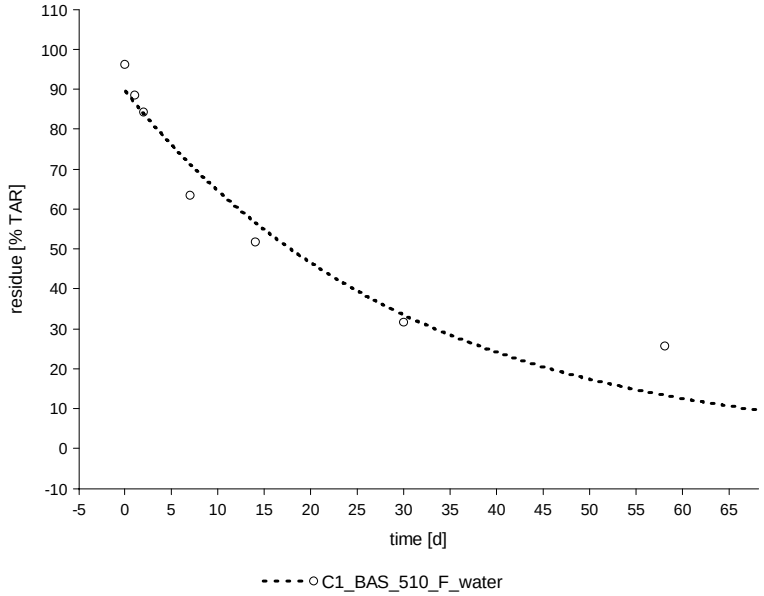
Approach 2: Estimation of the parameters of BAS 510 F without considering the water temperature from observed residues in water and sediment up to day 58 (with a fairly constant water temperature) after application

Approach 3: Estimation of the kinetic parameters of BAS 510 F in water and sediment considering the actual water temperature and the observed residues over the whole study period

The water sediment study that provide the basis for the parameters to calculate the PEC_{sw}-values for BAS 510 F was conducted under variable outdoor conditions (radiation, temperatures) with water temperatures that show a distinct decline after a study period of 58 days.
Not only the temperature but also the global radiation was considerably decreasing in the later phase (> 58 d) of the water sediment study under outdoor conditions.
It was therefore decided to consider the half-life of BAS 510 F in the water phase obtained with approach 1 for the period of 0-58 days for the calculation of the PEC_{sw}-values of BAS 510 F. A half life of 21 days was obtained with a good fit that is represented also in a coefficient of determination of 0.94
The dissipation of BAS 510 F from the water phase of the water-sediment study (day 0 - 58) is a result of (i) degradation in the water and (ii) partitioning onto the sediment. Both processes are accounted for the PEC_{sw}-calcuations if the half life in the water phase is used.

The observed residues of BAS 510 F and the fitted curve in the water sediment study under outdoor conditions are shown in Figure B.8.6-1.

Figure B.8.6-1: Comparison of the observed residues of BAS 510 F and the fitted curve investigated in water sediment study under outdoor conditions



Entry routes
Different entry routes were assessed, and entries into the water body via drift, via runoff and via drainage were calculated.

The assessments are made for two crop scenarios which either have the highest application rate and drift values (grapevine with 1 x 0.6 kg a.i./ha and 7.5 % drift in 3 m distance) or the highest accumulated application rate (beans with 2 x 500 g a.i./ha in an interval of 7 days).

BAS 510 F is applied with the product BAS 510 01 F with a maximum single rate of 0.6 kg a.i./ha onto grapevine or with a maximum accumulated application rate onto beans with 2 x 0.5 kg a.i./ha in a minimum interval of 7 days.

The applications are made in the late growth stages of grapevine (September - October) and of beans (June - August).

Estimation of drift entry into surface water
Entry into surface water by drift is calculated using (case 1) the 95th and (case 2) the overall 90th percentile drift values of *Ganzelmeier*. The 95th and the 90th percentile drift values are listed in the EU Guidance Document on Aquatic Ecotoxicology *[EU Guidance document on Aquatic Ecotoxicology (8075/VI/97 rev 7 from 08.07.2000]* and in Kohsiek (2000) [*Kohsiek, H. (2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden.- Vom 8. Mai. Bundesanzeiger Nr. 100/26.05.2000, 9878-9880.*], respectively.

Different drift scenarios were considered with 3 m, 5 m and 10 m buffer for grapevine and with 1 m and 5 m buffer for beans.

Case (1): Using 95th percentile drift factors it is assumed that 7.5 %, 5.0 % and 1.5 % of the applied amount reach the water body via drift in 3 m, 5 m and 10 m buffer distance for grape-

vine and 4.0 % and 0.6 % of the applied amount reach the water body via drift in 1 m and 5 m buffer distance for beans.

Case (2): Using the 90th percentile drift factors it is assumed that 8.02 %, 3.62 % and 1.23 % of the applied amount reach the water body via drift in 3 m, 5 m and 10 m buffer distance for grapevine.
To achieve an overall 90th percentile drift scenario for the double application in the beans scenario the 82nd percentile drift factors for arable crops given by Kohsiek (2000) are used and 2.38 % and 0.47 % of the applied amount are assumed to reach the water body via drift in 1 m and 5 m buffer distance for the double application in the beans scenario.

The amount of pesticide loaded to the surface water body via drift is calculated with Equation 4.

Equation 4: Loading by drift

$$LOAD_{DRIFT} = A * f_{drift}$$

with

$LOAD_{DRIFT}$	=	amount of substance loaded to water body via drift	[kg/ha]
A	=	application rate	[kg/ha]
f_{drift}	=	drift factor	[-]

The drift factors and the calculated loadings by drift are listed in Table B.8.6-1.

Table B.8.6-6: Drift factors (95th, 90th and 82nd percentiles) and calculated loadings by drift for the grapevine and for the beans scenario

Distance [m]	Parameter	95th percentile drift factors for Grapevine (late)	95th percentile drift factors for field crops (early + late)	90th percentile drift factors for Grapevine (late)	82nd percentile drift factors for field crops (early + late)	Unit
1	f_{DRIFT}	-	4.0		2.38	% of applied
3	f_{DRIFT}	7.5	-	8.02	-	% of applied
5	f_{DRIFT}	5.0	0.6	3.62	0.47	% of applied
10	f_{DRIFT}	1.5	-	1.23	-	% of applied
	A (Application rate)	**0.6**	per application **0.5**	**0.6**	per application **0.5**	**kg/ha**
1	$LOAD_{DRIFT}$	-	0.020	-	0.0119	kg/ha
3	$LOAD_{DRIFT}$	0.045	-	0.04810	-	kg/ha
5	$LOAD_{DRIFT}$	0.030	0.003	0.00217	0.0240	kg/ha
10	$LOAD_{DRIFT}$	0.009	-	0.00740	-	kg/ha

Estimation of run-off entry into surface water

The entry of BAS 510 F into the water body via the runoff pathway is calculated according to proposals made by Kloskowski et al. (1999) (*Kloskowski, R.; Fischer, R.; Binner, R.; Winkler, R. (1999) Draft guidance on the calculation of predicted environmental concentration values of plant protection products for soil, ground water, surface water and sediment.- in: Human and Environmental Exposure to Xenobiotics. eds. Del Re, A.A.M.; Brown, C.; Capri, E.; Errera, G.; Evans, S.P.; Trevisan, M.; Proceedings of the XI Symposium Pesticide Chemistry, September 11-15 1999, Cremona, Italia.- 835-850*) and an announcement by BBA/UBA (2001) [*BBA/UBA (2001) Bekanntmachung über die Durchführung der Berechnung von Einträgen in Oberflächengewässer durch Run-off im Nationalen und EU Verfahren.- Notiz einer Sitzung zwischen Vertretern des Industrieverbandes Agrar e., FA Ökchemie, des Umeltbundesamtes und der Biologischen Bundesanstalt – Fachgruppe Chemische Mittelprüfung- am 7. Februar 2001 in Berlin Dahlem*].

For 1st step calculations a simple approach is applied which assumes that the residues of the plant protection product on soil can potentially reach the surface water via runoff. In case of no buffer zones a fraction of 0.5 % of the soil residue on day 3 after last application is transported to the surface water body via runoff. Interception by crops and degradation in soil is accounted for in the calculation of the residue on soil that can be transported by runoff.

In case of a planted buffer zone of 5 m between the edge of the field and the surface water body the amount of runoff water and substance in the runoff water is reduced by a factor of 2. Therefore, he amount of the pesticide on soil that can reach the surface water is reduced to 0.25 %.

It is assumed that the residue reaches the water body in the runoff water only and not absorbed onto eroded soil particles. In this first step a partitioning of the compound between runoff water and eroded soil particles is therefore not considered.

The interception by grapevine and by beans in the development stage 'ripening' according to the recommendations of the FOCUSgroundwater scenario group (FOCUS 2000) is 85 % and 80 %, respectively ($f_{INTERCEPT}$ for grapevine = 0.85, $f_{INTERCEPT}$ for beans = 0.80). Only 15 % and 20 % of the applied amount reaches the soil at each application of BAS 510 F onto grapevine and onto beans, respectively. The degradation of BAS 510 F in soil from day of application to day of runoff event (day 3 after last application) is simulated with an average field half-life of 139 d.

A field of 100 m x 100 m and an adjacent surface water basin of 30 cm depth, 100 m length and 1 m width is further assumed. The water volume in the water course is 30000 l. The simple scenario implies 20 mm precipitation (rain event 3 days after last application) and 50 % (10 mm) run-off, which results in 100 000 l of run-off water that enters the water course.

By a planted buffer zone of 5 m width a reduction of 50 % of the run-off water is achieved (from 100000 l down to 50000 l). The same reduction applies also to the amount of the plant protection product transported with the run-off water. The final water volume in the basin can reach 130 000 l (without buffer zone) or 80 000 l (with buffer zone).

Equation 5 is used to calculate the load of substance via runoff.

Equation 5: Loading by Runoff

$$LOAD_{RUNOFF} = PEC_{soil\ at\ t\_RUNOFF} * f_{RUNOFF} * AREA$$

with

$LOAD_{RUNOFF}$	=	amount of substance loaded to water body via runoff	[kg]
$PEC_{soil\ at\ t\_RUNOFF}$	=	Soil residue at day of runoff	[kg/ha]
f_{RUNOFF}	=	runoff factor as fraction of soil residue transported via runoff to surface water body at time of runoff (0.5% or 0.25%)	[-]
AREA	=	surface area of the field from which the runoff occurs (1 ha)	[ha]

The PEC in soil at the day of the runoff event is calculated with:

$$PEC_{soil\ at\ t\_RUNOFF} = PEC_{soil\_ini} * e^{-k*t_{RUNOFF}} \quad \textbf{2a)}$$

with

PEC_{soil_ini}	=	Initial PEC in soil at day of (last) application	[kg/ha]
k	=	degradation rate in soil	[1/d]
t_{RUNOFF}	=	time of runoff event after last application	[d]

The initial concentration in soil at day of a single application is calculated by:

$$PEC_{soil\ ini,1} = A * (1 - f_{INTERCEPT}) \quad \textbf{2b)}$$

with:

$PEC_{soil\ ini,1}$	=	Initial PEC in soil at day of application	[kg/ha]
A	=	application rate	[kg/ha]
$f_{INTERCEPT}$	=	interception factor	[-]

In case of multiple application the concentration at day of last application is calculated by:

$$PEC_{soil\_ini,n} = \frac{PEC_{soil\_ini,1} \cdot (1 - e^{-nki})}{(1 - e^{-ki})} \quad \textbf{2c)}$$

where

$PEC_{soil\_ini,n}$	=	PEC_{soil} at day of last application	[kg/ha]
$PEC_{soil\_ini,1}$	=	initial PEC_{soil} after one application	[kg/ha]
n	=	number of applications	[-]
i	=	application interval	[d]
k	=	degradation rate in soil	[1/d]

The parameters required to calculate the load of the surface water body by drainage for the grapevine and for the beans scenario are listed in Table B.8.6-7.

Table B.8.6-7: Parameters used for the calculation of the runoff entry of BAS 510 F for the scenarios grapevine and beans

Parameter	Values for grape-vine	Values for beans	Unit	Used in equation
A application rate	0.6	0.5	kg/ha	2a
$f_{INTERCEPT}$	0.85	0.80	-	2b
n number of applications	1	2	-	2c
i application interval	-	7	d	2c
k degradation rate in soil equal to a half life	0.00499 139	0.00499 139	1/d d	2a
t_{RUNOFF}	3	3	d	2a
f_{RUNOFF} (no buffer)	0.5	0.5	%	2
f_{RUNOFF} (5 m buffer)	0.25	0.25	%	2
AREA	1	1	ha	2

The calculated runoff entries for BAS 510 F are listed in Table B.8.6-8.

Table B.8.6-8: Runoff entry of BAS 510 F calculated for the scenarios grapevine and beans

Parameter	Values for grape-vine	Values for beans	Unit	Calculated with equation
$PEC_{soil,ini}$	0.09	0.1965673	kg/ha	2b, 2c
$PEC_{soil\ at\ T\text{-}RUNOFF}$	0.08866273	0.1936466	kg/ha	2a
$LOAD_{RUNOFF}$ (no buffer)	0.00044331	0.00096823	kg	2
$LOAD_{RUNOFF}$ (5 m buffer)	0.00022166	0.000484117	kg	2

A reduction factor of 0.5 (= 50 %) is to be applied for the calculation of the $PEC_{sw,ini}$-values by runoff to account for the dilution by the moving water (induced by the runoff event).

Estimation of Drainage Entry into Surface Water

The entry of BAS 510 F into surface water via drainage is assessed according to a recommendation of PSD (2000) [*PSD (2000) Method for the calculation of the predicted environmental concentrations in surface water (PEC_{sw}) for exposure via drainflow.- Letter of PSD to MAFF approval holders dated on 10 January 2000., Ref. AAHL/1/2000.*].

Equation 6 is used to calculate the entry via drainage.

Equation 6: Loading by drainage

$$LOAD_{DRAINAGE} = PEC_{soil\ at\ t\_DRAINAGE} * f_{DRAINAGE} * AREA$$

with

$LOAD_{DRAINAGE}$ = amount of substance loaded to the water body via drainage [kg]

$f_{DRAINAGE}$ = drainage factor as fraction of soil residue at time of drainage transported via drainage to the surface water body [-]

AREA = surface area of the field from which the drainage occurs (1 ha) [ha]

and

$$PEC_{soil\ at\ t\_DRAINAGE} = PEC_{soil\ ini} * e^{-k*t_{DRAINAGE}} \quad \textbf{3a)}$$

with

$PEC_{soil\ at\ t\_DRAINAGE}$	=	Soil residue at day of drainage	[kg/ha]
PEC_{soil_ini}	=	Initial PEC in soil	[kg/ha]
k	=	degradation rate in soil	[1/d]
$t_{DRAINAGE}$	=	time of drainage event after application	[d]

The initial concentration in soil is calculated at day of single application by:

$$PEC_{soil\ ini,1} = A * (1 - f_{INTERCEPT}) \quad \textbf{3b)}$$

with

$PEC_{soil\ ini,1}$	=	Initial PEC in soil at day of application	[kg/ha]
A	=	application rate	[kg/ha]
$f_{INTERCEPT}$	=	interception factor	[-]

In case of multiple application the concentration at day of last application is calculated by:

$$PEC_{soil\_ini,n} = \frac{PEC_{soil\_ini,1} \cdot (1 - e^{-nki})}{(1 - e^{-ki})} \quad \textbf{3c)}$$

where

$PEC_{soil\_ini,n}$	=	PEC_{soil} at day of last application	[kg/ha]
$PEC_{soil\_ini,1}$	=	initial PEC_{soil} after one application	[kg/ha]
n	=	number of applications	[-]
i	=	application interval	[d]
k	=	degradation rate in soil	[1/d]

After application of BAS 510 F onto grapevine and onto beans a drainage event is not to be expected in the application periods (September - October for grapes and June – August for beans). Therefore, the dissipation of BAS 510 F in the field between the time of application and the time of drainage (not before November) has to be taken into account. The time of drainage event after application ($t_{DRAINAGE}$) is assumed to be at 30 days and at 60 days after last application onto grapes and onto beans, respectively.

The interception by grapevine and by beans in the development stage 'ripening' according to the recommendations of the FOCUSgroundwater scenario group is 85 % and 80 %, respectively ($f_{INTERCEPT}$ for grapevine = 0.85, $f_{INTERCEPT}$ for beans = 0.80). Therefore only 15 % and 20 % of the applied amount reaches the soil at each application of BAS 510 F onto grapevine and onto beans, respectively. The degradation of BAS 510 F in soil from day of application to day of the drainage event is simulated with an average field half-life of 139 d.

According to the SSLRC Mobility Classification given in PSD (2000) BAS 510 F is in the slightly mobile class ($K_{F,OC}$ = 771 dm³/kg). It is assumed that 0.5 % of the residue in soil at the time of drainage is transported in 10 mm of drainflow (equivalent to 100.000 l water) to the surface water body (30000 l) and in the stream the total dilution is then in 130 000 l.

The parameters required to calculate the load of the surface water body by drainage and the loadings by drainage of BAS 510 F for the grapevine and for the beans scenario are listed in Table B.8.6-9.

Table B.8.6-9: Parameters for the calculation of the drainage entry of BAS 510 F for the scenarios grapevine and beans

Parameter	Values for grapevine	Values for beans	Unit	Used in equation
A application rate	0.6	0.5	kg/ha	3a
$f_{INTERCEPT}$	0.85	0.80	-	3b
N number of applications	1	2	-	3c
i application interval	-	7	d	3c
k degradation rate in soil equal to a half life of	0.00499 139	0.00499 139	1/d d	3a
$t_{DRAINAGE}$	30	60	d	3a
$f_{DRAINAGE}$	0.5	0.5	%	3
AREA	1	1	ha	3

The loadings of BAS 510 F by drainage for the grapevine and for the beans scenario are listed in Table B.8.6-10.

Table B.8.6-10: Drainage entry of BAS 510 F for the scenarios grapevine and beans

Parameter	Values for grapevine	Values for beans	Unit	Calculated with equation
$PEC_{soil,ini}$	0.09	0.196567301	kg/ha	3b, 3c
$PEC_{soil\ at\ tDRAINAGE}$	0.07748696	0.145708037	kg/ha	3a
$LOAD_{Drainage}$	0.000387435	0.00072854	kg	3

The loadings by drainage for the beans scenario are higher than the loadings for the grapevine scenario.

Calculation of the PEC_{ini} (Initial Concentrations) in surface water

The initial PEC_{sw}-values for BAS 510 F in surface water are determined considering the loads by spraydrift ($LOAD_{DRIFT}$), runoff ($LOAD_{RUNOFF}$) and drainage ($LOAD_{DRAINAGE}$) as calculated with
Equation 4, Equation 5 and Equation 6 and the specified water layer depths and dilution factors (for runoff).

In case of single loading the $PEC_{sw,ini}$-values after loading by spraydrift are calculated with Equation 7.

Equation 7: Calculation of $PEC_{sw,ini}$ by drift

$$PEC_{ini,DRIFT} = \frac{Load_{DRIFT} * 10^5}{Vsw}$$

with

$PEC_{INI,DRIFT}$	=	initial predicted environmental concentration by drift	[µg/l]
$LOAD_{DRIFT}$	=	loading by drift	[kg/ha]
Vsw	=	water volume per m^2 [300 l/m²]	[l/m^2]

(note that a factor of 100000 has to be applied to convert kg/ha into µg/m²)

In case of the multiple loading by drift in the beans scenario the $PEC_{sw,ini}$-values by drift is the maximum concentration after the last application which is calculated using a ModelMaker program. The residues in the water phase (in % of total applied) are simulated with a half life of 21 d in the water phase and 2 applications in an application interval of 7 days and converted into concentrations using the total application rate and the water volume per m² using Equation 8.

Equation 8: Calculation of $PEC_{sw,ini}$-values after repeated application

$$PEC_{sw,act} = \frac{A * \%applied\_max * 10^5}{V_{SW} * 100}$$

with

$PEC_{sw,act}$	=	actual predicted environmental concentration	[µg/l]
A	=	yearly application rate (= 1.0 kg/ha for beans)	[kg/ha]
%applied_max	=	maximum residue in water (considering the drift factors)	[% of total yearly applied]
V_{SW}	=	water volume per m^2 [300 l/m²]	[l/m^2]

(note that a factor of 100000 has to be applied to convert kg/ha into µg/m²)

For the multiple application scenario (beans) the maximum residue in the water phase (% applied_max) are 3.59 % and 0.54 % (95^{th} drift percentile) and 2.31 % and 0.42 % (90^{th} drift percentile) of the total yearly application rate for the 1 m and the 5 m buffer zone (with 4 % and 0.6 % drift (95^{th} percentile) and 2.38 an d 0.47 % drift (90^{th} percentile) of each individual of the 2 applications, which is equal to 2 % and 0.3 % and 1.19 % and 0.24 %drift of the annual application rate).

The $PEC_{sw,ini}$-values after loading by runoff are calculated using Equation 9.

Equation 9: Calculation of $PEC_{sw,ini}$ considering runoff

$$PEC_{ini,RUNOFF} = \frac{Load_{RUNOFF} * 10^9}{VOLwater} * f_{DILUTE}$$

with

$PEC_{INI,RUNOFF}$	=	initial predicted environmental concentration by runoff	[µg/l]
$LOAD_{RUNOFF}$	=	loading by runoff	[kg]
VOL_{WATER}	=	Volume of Water (130000 l with no buffer, 80000 l with 5 m buffer)	[l]
f_{DILUTE}	=	dilution factor by moving water	[-]

The $PEC_{sw,ini}$-values after loading by drainage are calculated using Equation 10.

Equation 10: Calculation of $PEC_{sw,ini}$ considering drainage

$$PEC_{ini,DRAINAGE} = \frac{Load_{DRAINAGE} * 10^9}{VOLwater}$$

$PEC_{INI,DRAINAGE}$	=	initial predicted environmental concentration by drainage	[µg/l]
$LOAD_{DRAINAGE}$	=	loading by drainage	[kg]
VOL_{WATER}	=	Volume of Water (= 130000 l)	[l]

It has to be noted that for the loading by drift events the depth of the static water body is assumed to be 30 cm. For the runoff the loading is diluted in a water body of 130000 l (no buffer) or 80000 l (5m buffer) and subsequently a reduction factor of 0.5 (= 50 %) is to be applied for the calculation of the $PEC_{sw,ini}$-values by runoff to account for the dilution by the moving water (induced by the runoff event).

For the drainage the loading is diluted in a water body of 130000 l.

The loadings and the parameters to calculate the $PEC_{sw,ini}$-values of BAS 510 F are summarised in Table B.8.6-11 and in Table B.8.6-12 respectively.

Table B.8.6-11: Summary of loadings of BAS 510 F into the surface water body

Entry route	Scenario Grapevine	Scenario Beans
$LOAD_{DRIFT}$ 95th percentile drift factors 1m buffer 3 m buffer 5 m buffer 10 m buffer	 - 0.045 kg/ha 0.030 kg/ha 0.009 kg/ha	 3.59 % of total yearly rate 0.54 % of total yearly rate
$LOAD_{DRIFT}$ 90th percentile drift factors 1m buffer 3 m buffer 5 m buffer 10 m buffer	 - 0.0481 kg/ha 0.0217 kg/ha 0.0074 kg/ha	 2.31 % of total yearly rate – 0.42 % of total yearly rate -
$LOAD_{RUNOFF}$ (no buffer; 0.5 %)	0.00044331 kg	0.00096823 kg
$LOAD_{RUNOFF}$ (5 m buffer; 0.25 %)	0.00022166 kg	0.000484117 kg
$LOAD_{DRAINAGE}$	0.000387435 kg	0.00072854 kg

Table B.8.6-12: Summary of parameters to calculate PECsw,ini for BAS 510 F

Parameter	Drift	Runoff	Drainage
A total yearly application rate	0.6 kg/ha (grapevine) 1.0 kg/ha (beans)	-	-
Vsw Volume of water per m²	300 l/m²	-	-
VOL_{WATER} Volume of water in the water course	-	130000 l (no buffer) 80000 l (5m buffer)	130000 l
f_{DILUTE} dilution factor by moving water	-	0.5	-

Calculation of the $PEC_{sw,act}$-values

The PEC_{act} is the concentration of the compound at days after treatment (DAT) assuming single first order exponential decline of the concentrations in the water phase.

For the single application (vine scenario) the $PEC_{sw,act}$-values are calculated using Equation 8.

Equation 11: Calculation of $PEC_{sw,act}$-values after single application

$$PEC(t) = PEC_{ini} \cdot e^{-k\_water * t} = PEC_{ini} \cdot e^{-\frac{t \cdot \ln 2}{DT50\_water}}$$

with

PEC_{ini}	=	initial PEC_{sw}	[µg/l]
k_water	=	first order dissipation rate in water (=0.033)	[1/d]
DT50_water	=	Half Life in water (21 d)	[d]
t	=	time	[d]

For the repeated application (beans scenario) the $PEC_{sw,act}$-values are calculated using a ModelMaker Programm.

A half life of 21 days of BAS 510 F in the water phase is used to simulate the PEC_{act}-values after loading by drift. Two drift events with an interval of 7 days are considered. The simulated residues [in % of yearly total applied] are transferred into an Excel-spreadsheet and converted to concentrations [in µg/L] using Equation 9.

Equation 9: Calculation of $PEC_{sw,act}$-values after repeated application

$$PEC_{sw,act} = \frac{A * \%applied * 100}{bd * depth}$$

with

$PEC_{sw,act}$	=	actual predicted environmental concentration	[µg/l]
A	=	yearly application rate (= 1.0 kg/ha for beans)	[kg/ha]
%applied	=	residue in water	[% of total applied a.i. per year]
depth	=	depth of top water layer (=30 cm)	[cm]
bd	=	bulk density (= 1 kg/dm³)	[kg/dm³]

<u>Calculation of the PECtime weighted average ($PEC_{sw,twa}$)</u>

The $PEC_{sw,twa}$-values are defined as the average concentrations of the compound at different time steps (days).

For the single application scenario (application onto grapevine) the $PEC_{sw,twa}$ concentrations are calculated with Equation 12.

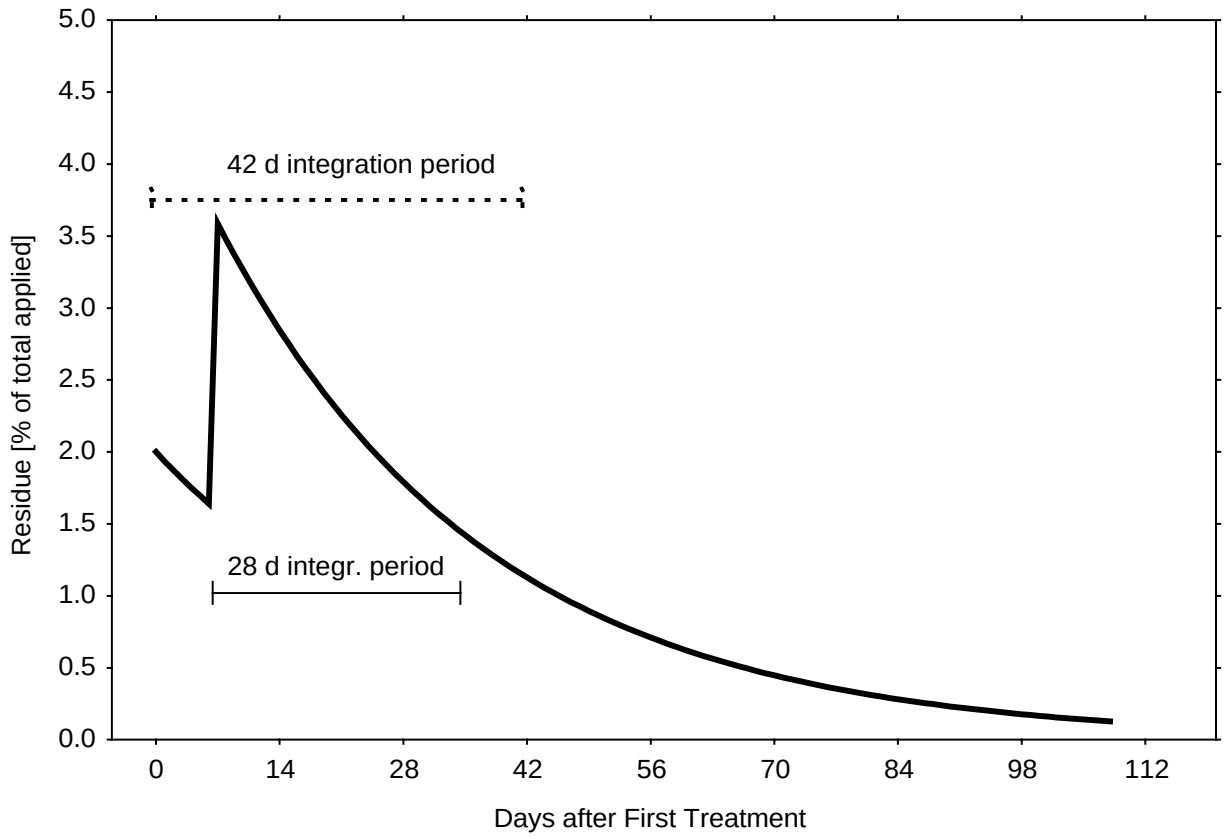
Equation 12: Calculation of $PEC_{sw,twa}$-values after single application

$$PEC_{sw,twa}(t) = PEC_{ini} \cdot \frac{DT50water}{t \cdot \ln 2} \cdot (1 - e^{-\frac{t \cdot \ln 2}{DT50water}})$$

For the repeated application scenario (beans) the $PEC_{sw,act}$-concentrations as simulated with ModelMaker are transferred to an Excel-spreadsheet. Then the maximum average concentrations for integration periods of 1, 2, 3, 4, 7, 14, 21, 48 and 100 days are calculated with a moving timeframe. This means that all time periods are checked for the highest average concentration. The integration period is not necessarily the time after the last application and in case of multiple applications it could also include the peaks of different loading events.

This is illustrated in an example given in Figure B.8.6-2 with the multiple application scenario (beans). In this example the period with the highest average concentration for 28 d starts after the second application (= day 7) whereas the $PEC_{sw,twa}$-values for 42 days are calculated from the day of first application onwards including the first and the second peak.

Figure B.8.6-2: PECsw,act for BAS 510 F [% of total applied] and integration periods for the calculation of time weighted average concentrations (repeated application scenario 'beans', 95th percentile drift factor)



Results

$PEC_{sw,ini}$

The initial PEC_{sw}-values for BAS 510 F as calculated Equation 8, Equation 9 and Equation 10 for the different entry routes are given in Table B.8.6-13.

Table B.8.6-13: Initial PECsw-values for BAS 510 F for different entry

	Grapevine Scenario [µg/L]	Beans Scenario [µg/L]
DRIFT 95th percentile drift factors		
1 m buffer	-	12.0
3 m buffer	15.0	-
5 m buffer	10.0	1.8
10 m buffer	3.0	-
DRIFT 90th percentile drift factors		
1 m buffer	-	7.1
3 m buffer	16.0	-
5 m buffer	7.2	1.4
10 m buffer	2.5	-
$PEC_{INI,RUNOFF}$ (no buffer) (0.5 % runoff VOL_{WATER} = 130000 l + reduction factor of 0.5)	1.7	3.7
$PEC_{INI,RUNOFF}$ (5 m buffer) (0.25 % runoff VOL_{WATER} = 80000 l + reduction factor of 0.5)	1.4	3.0
$PEC_{INI,DRAINAGE}$ (VOL_{WATER} = 130000 l)	1.5	2.8

The $PEC_{sw,ini}$-values by drift for the grapevine scenario are 15.0, 10.0 and 3.0 µg/l (95th percentile drift factors) and 16.0, 7.2 and 2.5 µg/l (90th percentile drift factors) for the 3 m, 5 m and 10 m buffer zone, respectively. The $PEC_{sw,ini}$-values by runoff and drainage are lower (1.7 and 1.5 µg/l) than the $PEC_{sw,ini}$ by drift. They are therefore not considered in the calculation of actual and time weighted average PEC-values in the surface water.

The $PEC_{sw,ini}$-values by drift for the beans scenario are 12.0 and 1.8 µg/l (95th percentile drift) and 7.1 and 1.4 µg/l (90th percentile drift) for the 1 m and for the 5 m buffer zone, respectively.

The $PEC_{sw,ini}$-values for the beans scenario by runoff and drainage are higher than the $PEC_{sw,ini}$-value by drift for the 5 m buffer zone (3.7 and 2.8 µg/l). The actual and time weighted average PEC-values for the beans scenario are therefore calculated also after the runoff event, assuming two cases (1) 'no buffer' and (2) '5 m buffer'.

$PEC_{sw,act}$ and $PEC_{sw,twa}$ for the grapevine scenario

The actual and the time weighted average concentrations of BAS 510 F in surface water ($PEC_{sw,act}$ and $PEC_{sw,twa)}$ are provided for the grapevine scenario after loading by drift in Table B.8.6-14 for the 95th percentile drift value and in Table B.8.6-15 for the 90th percentile drift factors.

Table B.8.6-14: PECsw,act- and PECsw,twa-values for BAS 510 F at the grapevine scenario (95th percentile drift factors)

		3 m buffer		5 m buffer		10 m buffer	
	Time / Integration Period	PECsw,act	PECsw,twa	PECsw,act	PECsw,twa	PECsw,act	PECsw,twa
	[d]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
Initial	0	15.0	15.0	10.0	10.0	3.0	3.0
Short-term	1	14.5	14.8	9.7	9.8	2.9	3.0
	2	14.0	14.5	9.4	9.7	2.8	2.9
	3	13.6	14.3	9.1	9.5	2.7	2.9
	4	13.1	14.1	8.8	9.4	2.6	2.8
Long-term	7	11.9	13.4	7.9	8.9	2.4	2.7
	14	9.5	12.0	6.3	8.0	1.9	2.4
	21	7.5	10.8	5.0	7.2	1.5	2.2
	28	6.0	9.8	4.0	6.5	1.2	2.0
	42	3.8	8.1	2.5	5.4	0.8	1.6
	100	0.6	4.4	0.4	2.9	0.1	0.9

Table B.8.6-15: PECsw,act- and PECsw,twa-values for BAS 510 at the grapevine scenario (90th percentile drift factors)

		3 m buffer		5 m buffer		10 m buffer	
	Time / Integration Period	PECsw,act	PECsw,twa	PECsw,act	PECsw,twa	PECsw,act	PECsw,twa
	[d]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]	[µg/L]
Initial	0	16.0	16.0	7.2	7.2	2.5	2.5
Short-term	1	15.5	15.8	7.0	7.1	2.4	2.4
	2	15.0	15.5	6.8	7.0	2.3	2.4
	3	14.5	15.3	6.6	6.9	2.2	2.3
	4	14.1	15.0	6.3	6.8	2.2	2.3
Long-term	7	12.7	14.3	5.8	6.5	2.0	2.2
	14	10.1	12.8	4.6	5.8	1.6	2.0
	21	8.0	11.6	3.6	5.2	1.2	1.8
	28	6.4	10.5	2.9	4.7	1.0	1.6
	42	4.0	8.7	1.8	3.9	0.6	1.3
	100	0.6	4.7	0.3	2.1	0.1	0.7

The short-term and long-term actual concentrations ($PEC_{sw,act}$) for BAS 510 F decrease to 0.6 µg/l, 0.4 µg/l and 0.1 µg/l and to 0.6 µg/l, 0.3 µg/l and 0.1 µg/l after 100 days for the 3 m,. 5 m and 10 m buffers at the 95th and at the 90th drift scenario with grapevine, respectively.

The short-term and long-term time weighted average concentrations ($PEC_{sw,twa}$) for BAS 510 F decrease to 4.4 µg/l, 2.9 µg/l and 0.9 µg/l and to 4.7 µg/l, 2.1 µg/l and 0.7 µg/l after 100 days for the 3 m, 5 m and 10 m buffers at the 95th and at the 90th drift scenario with grapevine, respectively.

$PEC_{sw,act}$ and $PEC_{sw,twa}$ for the beans scenario

The actual and the time weighted average concentrations for BAS 510 F in surface water ($PEC_{sw,act}$ and $PEC_{sw,twa}$) are provided for the beans scenario after loading by drift in Table B.8.6-16 for the 95th percentile drift value, in Table B.8.6-17 for the 90th percentile drift factors. In Table B.8.6-18 the PEC_{sw}-values are given after loading by runoff.

Table B.8.6-16: PECsw,act- and PECsw,twa-values for BAS 510 F at the beans scenario (95th percentile drift factors)

		1 m buffer		5m buffer	
	Time / Integration Period [d]	PECsw,act [µg/L]	PECsw,twa [µg/L]	PECsw,act [µg/L]	PECsw,twa [µg/L]
Initial	0	12.0		1.8	
Short-term	1	11.6	12.0	1.7	1.8
	2	11.2	11.8	1.7	1.8
	3	10.8	11.6	1.6	1.7
	4	10.5	11.4	1.6	1.7
Long-term	7	9.5	10.9	1.4	1.6
	14	7.5	9.7	1.1	1.5
	21	6.0	8.8	0.9	1.3
	28	4.7	8.1	0.7	1.2
	42	3.0	7.0	0.4	1.1
	100	0.4	3.9	0.1	0.6

Table B.8.6-17: PECsw,act- and PECsw,twa-values for BAS 510 at the beans scenario (90th percentile drift factor)

	Time / Integration Period [d]	1 m buffer PECsw,act [µg/L]	1 m buffer PECsw,twa [µg/L]	5m buffer PECsw,act [µg/L]	5m buffer PECsw,twa [µg/L]
Initial	0	7.1		1.4	
Short-term	1	6.9	7.1	1.4	1.4
	2	6.7	7.0	1.3	1.4
	3	6.4	6.9	1.3	1.4
	4	6.2	6.8	1.2	1.3
Long-term	7	5.6	6.5	1.1	1.3
	14	4.5	5.8	0.9	1.1
	21	3.6	5.2	0.7	1.0
	28	2.8	4.8	0.6	1.0
	42	1.8	4.2	0.4	0.8
	100	0.3	2.3	0.1	0.5

Table B.8.6-18: PECsw,act- and PECsw,twa-values for BAS 510 at the beans scenario (loading by runoff) for cases (1) 'no buffer' and (2) '5m

	Time / Integration Period [d]	no buffer PECsw,act [µg/L]	no buffer PECsw,twa [µg/L]	5 m buffer PECsw,act [µg/L]	5 m buffer PECsw,twa [µg/L]
Initial	0	3.7	3.7	3.0	3.0
Short-term	1	3.6	3.7	2.9	3.0
	2	3.5	3.6	2.8	2.9
	3	3.4	3.6	2.7	2.9
	4	3.3	3.5	2.7	2.8
Long-term	7	3.0	3.3	2.4	2.7
	14	2.4	3.0	1.9	2.4
	21	1.9	2.7	1.5	2.2
	28	1.5	2.4	1.2	2.0
	42	0.9	2.0	0.8	1.6
	100	0.1	1.1	0.1	0.9

The short-term and long-term actual concentrations ($PEC_{sw,act}$) for BAS 510 F decrease to 0.4 µg/l and 0.1 µg/l and to 0.3 µg/l and 0.1 µg/l after 100 days for the 1 m and 5 m buffers at the 95th and at the 90th drift scenario with beans, respectively.

The short-term and long-term time weighted average concentrations ($PEC_{sw,twa}$) for BAS 510 F decrease to 3.9 µg/l and 0.6 µg/l and to 2.3 µg/l and 0.5 µg/l after 100 days for the 1 m and 5 m buffers at the 95th and at the 90th drift scenario with beans, respectively.

The short-term and long-term actual concentrations ($PEC_{sw,act}$) for BAS 510 F decrease to 0.1 µg/l and 0.1 µg/l after 100 days for the no buffer and 5 m buffer at the runoff scenario with beans, respectively.

The short-term and long-term time weighted average concentrations ($PEC_{sw,twa}$) for BAS 510 F decrease to 1.1 µg/l and 0.9 µg/l after 100 days for the no buffer and 5 m buffer at the runoff scenario with beans, respectively.

No additional field testing was required since the calculation of the PEC_{sw} could be performed with appropriate accuracy.

Conclusion:
Drift is considered to be the main entry route of BAS 510 F into surface water compared to runoff or drainage. After loading of the surface water body the PEC_{sw}-values of BAS 510 F decrease moderately fast.

In the terrestrial and the aquatic studies no metabolite was found in amounts greater than 10% of the applied parent. Therefore no PEC_{sw} calculations are performed for metabolites of BAS 510 F.

The static water body is assumed to be a worst case scenario for a slow moving water body. Therefore no PEC_{sw}-calculations are performed for a slow moving water body.

B.8.6.3 Predicted environmental concentration in sediment (PECSED)

Gottesbueren B, 2001, WAS2001-159

Predicted initial, short-term and long-term concentrations for BAS 510 F in sediment (PEC_{sed}) after application of BAS 510 01 F are calculated based on the results of a water sediment study under outdoor conditions.

The conditions of the water/sediment study under outdoor conditions represent a more realistic environmental scenario, than the classic water/sediment study in the dark in the laboratory.

The assessments are made for two crop scenarios which either have the highest application rate and drift values (grapevine with 1 x 0.6 kg a.i./ha) or the highest accumulated application rate (beans with 2 x 0.5 kg a.i./ha in an interval of 7 days).

In the terrestrial and the aquatic studies no metabolite was found in amounts greater than 10% of the applied parent. Therefore no PEC_{sed}-calculations are performed for metabolites of BAS 510 F.

Pesticide parameters

The dissipation behaviour (degradation and partitioning to sediment) of BAS 510 F in sediment is predicted on the basis of the results of a water-sediment study under outdoor conditions. The observed residues are listed in Table B.8.6-19.

Table B.8.6-19: BAS 510 F residues in the water sediment study

T [d]	BAS 510 F in water [% TAR]	BAS 510 F in sediment [% TAR]
0	96.15	0.02
1	88.50	8.38
2	84.33	11.82
7	63.32	18.79
14	51.86	24.04
30	31.68	22.06
58	25.70	23.56
103	**19.84**	**28.21**
120	19.17	26.53

On day 103 the highest concentration in the sediment was observed with 28.21 % of applied amount.

Loadings via drift

Application details

BAS 510 F is applied with the product BAS 510 01 F with a maximum single rate of 0.6 kg a.i./ha onto grapevine or with a maximum accumulated application rate onto beans with 2 x 0.5 kg a.i./ha in a minimum interval of 7 days.

Estimation of drift entry into surface water

Partitioning of BAS 510 F onto sediment is calculated after loading of the surface water body by drift.

Entry into surface water by drift is calculated using (case 1) the 95th and (case 2) the overall 90th percentile drift values of *Ganzelmeier*. Different drift scenarios were considered with 3 m, 5 m and 10 m buffers for grapevine and with 1 m and 5 m buffers for beans.

The amount of pesticide loaded to the surface water body via drift is calculated and described in 1.1.2.
The $PEC_{ini,sw}$-concentrations are listed in Table B.8.6-20.

Table B.8.6-20: Initial PECsw-values for BAS 510 F for drift

	Grapevine scenario [µg/L]	Beans scenario [µg/L]
DRIFT 95th percentile drift factors (depth of water body 30 cm)		
1 m buffer	-	12.0
3 m buffer	15.0	-
5 m buffer	10.0	1.8
10 m buffer	3.0	-
DRIFT 90th percentile drift factors (depth of water body 30 cm)		
1 m buffer	-	7.1
3 m buffer	16.0	-
5 m buffer	7.2	1.4
10 m buffer	2.5	-

Calculation of $PEC_{sediment}$

The concentrations in sediment (PEC_{sed}) are calculated according to recommendations of Kloskowski et al. (1999) (*Kloskowski, R.; Fischer, R.; Binner, R.; Winkler, R. (1999) Draft guidance on the calculation of predicted environmental concentration values of plant protection products for soil, ground water, surface water and sediment.- in: Human and Environmental Exposure to Xenobiotics. eds. Del Re, A.A.M.; Brown, C.; Capri, E.; Errera, G.; Evans, S.P.; Trevisan, M.; Proceedings of the XI Symposium Pesticide Chemistry, September 11-15 1999, Cremona, Italia.- 835-850*) using the residues of the compound in the sediment in the water/sediment study under outdoor conditions (% a. s. in sediment at day t). The PEC_{sw}-values as calculated in M III 9.2.3 for the loadings by drift are considered using Equation 13 for the simplified calculation of the actual concentration in sediment (PEC_{sed}):

Equation 13: Calculation of PEC_{sed}

$$PEC_{sed}(t) = \frac{PEC_{ini,sw} \cdot V_{sw} \cdot P_{sed}(t)}{V_{sed} \cdot bd_{sed} \cdot 100}$$

where

$PEC_{sed(t)}$ = PEC_{sed} at time t [µg/g] or [mg/kg]

$PEC_{ini,sw}$ = 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 proposed depth of the sediment layer
- of 2.0 cm [20000 cm³] if $K_{oc} < 500$
- of 1.0 cm [10000 cm³] if $K_{oc} > 500$

bd_{sed} = bulk density of the wet sediment consisting of 80 vol. % water and 20 vol. % of dry sediment [1.3 g/cm³]

As the K_{OC}-value of BAS 510 F is > 500 kg/dm³ a depth of the sediment of 1.0 cm is considered for the PEC_{sed}-calculations.

Results

The PEC_{sed}-values for BAS 510 F as calculated with Equation 13 are listed for the grapevine and for the beans scenarios in Table B.8.6-21 and in Table B.8.6-22, respectively.

Table B.8.6-21: PEC_{sed}-values for BAS 510 F for the grapevine scenario (loading by drift using 95th and 90th percentile drift factors)

Time	95th percentile drift factors			90th percentile drift factors		
[d]	3 m buffer [mg/kg]	5 m buffer [mg/kg]	10 m buffer [mg/kg]	3 m buffer [mg/kg]	5 m buffer [mg/kg]	10 m buffer [mg/kg]
0	0.000	0.000	0.000	0.000	0.000	0.000
1	0.029	0.019	0.006	0.031	0.014	0.005
2	0.041	0.027	0.008	0.044	0.020	0.007
7	0.065	0.043	0.013	0.070	0.031	0.011
14	0.083	0.055	0.017	0.089	0.040	0.014
30	0.076	0.051	0.015	0.082	0.037	0.013
58	0.082	0.054	0.016	0.087	0.039	0.013
103	**0.098**	**0.065**	**0.020**	**0.104**	**0.047**	**0.016**
120	0.092	0.061	0.018	0.098	0.044	0.015

Table B.8.6-22: PEC_{sed}-values for BAS 510 F for the beans scenario (loading by drift using 95th and 82nd percentile drift factors)

Time	95th percentile drift factors		82nd percentile drift factors to achieve a 90th percentile total drift	
[d]	1 m buffer [mg/kg]	5 m buffer [mg/kg]	1 m buffer [mg/kg]	5 m buffer [mg/kg]
0	0.000	0.000	0.000	0.000
1	0.023	0.003	0.014	0.003
2	0.033	0.005	0.019	0.004
7	0.052	0.008	0.031	0.006
14	0.067	0.010	0.039	0.008
30	0.061	0.009	0.036	0.007
58	0.065	0.010	0.039	0.008
103	**0.078**	**0.012**	**0.046**	**0.009**
120	0.073	0.011	0.043	0.009

The highest PEC_{sed}-values are calculated for day 103.

For the grapevine scenario the highest PEC_{sed}-values range from 0.016 to 0.104 [mg/kg wet sediment], depending on the buffer zone (1 m, 5 m or 10 m buffer) and the spray drift percentile (95th or 90th percentile) that is applied.

For the beans scenario the highest PEC_{sed}-values range from 0.009 to 0.078 [mg/kg wet sediment], depending on the buffer zone (1 m or 5 m buffer) and the spray drift percentile (95^{th} or 90^{th} percentile) that is applied.

No additional field testing was required since the calculation of the PEC_{sed} could be performed with appropriate accuracy.

Conclusions:
The maximum PEC_{sed}-values of BAS 510 F are low and range from 0.009 to 0.106 [mg/kg wet sediment] depending on the buffer zone and the crops.

In the terrestrial and the aquatic studies no metabolite was found in amounts greater than 10% of the applied parent. Therefore no PEC_{sed}-calculations are performed for metabolites of BAS 510 F.

B.8.7 Fate and behaviour in air (Annex IIA 7.2.2; Annex IIIA 9.3)

Ohnsorge U, 2000, LUF2001-147

GLP: no (calculation)
Guidance: none

The volatilization from water was determined by calculating the Henry's law constant according to the equation:

$H = p * MW/c$ (kPa m^3/mol).

p:	vapor pressure	($7.0 \cdot 10^{-10}$ kPa at 20 °C)
MW:	molecular weight	(343.2 g/mol)
c:	water solubility	(4.64 g/m^3 at 20 °C)

The resulting Henry's law constant of the active substance BAS 510 F is
$H = 5.178 \times 10^{-8}$ kPa m^3/mol.

Goetz von N, 2000, LUF2001-134

GLP: yes
Guidelines: BBA IV, 6-1

The volatilization study was performed with the formulation BAS 510 01 F (containing nominal 500 g active substance/kg) based on a field application rate of 600 g active substance/ha. The formulation was spiked with about 1.6% diphenyl-U-[^{14}C]-labelled active substance to enable a total balance. The specific radioactivity of the labelled BAS 510 F was 6.27 MBq/mg, the radiochemical purity was 100%. Soil and plant were treated in a special glass container. The formulation was applied via a nozzle (1.2 bar) to a Petri dish filled with soil, and to a glass tray with a plant (bush bean, soil covered). The soil characteristics were: 86%

sand, 13% silt, 1% clay, organic C 0.5%, pH 6.0, MWC 25.8 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 (19 – 20 °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 103% for the plant experiment and 94% for the soil experiment. The volatilization rates were about 1% from the plant surface and about 0.5% from the soil surface.

Goetz von N, 2000, LUF2001-149

GLP: No, no subject to GLP
Guidelines: EEC 94/37

The rate constant for reactions of BAS 510 F with OH radicals in the atmosphere was calculated using the AOPWIN Program based on the chemical structure of the molecule. (Atmospheric Oxidation Program for Microsoft Windows 3.1, Version 1.88, Syracuse Research Corp., 1997) This program was developed on the basis of 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). In the case that the AOPWIN program provides no increments for a group in question, approximations are made by the program. These approximations have been checked and modified if considered necessary.

The resulting value for the rate constant k_{OH} of the active substance was

$k_{OH} > 8.8053\ 10^{-12}\ cm^3/(molecule \cdot s)$.

By averaging the OH-radical concentration in the troposphere diurnally and seasonally, the OH-radical concentration in the troposphere can be assumed as being constant. Accordingly, the degradation of the active substance can be assumed to follow pseudo-first order kinetics with the rate constant $k' = k_{OH} \cdot [OH\ radicals]$:

$-d[BAS\ 510\ F]/dt = k' \cdot [BAS\ 510\ F]$

The half-life of this process was calculated by the following equation:

$t_{1/2} = \ln 2/k' = \ln 2/k_{OH} \cdot [OH\ radicals]$

The diurnally and seasonally averaged tropospheric OH radical concentration ([OH radicals]) for the northern hemisphere is $8 \cdot 10^5\ cm^{-3}$ (Prinn, R. et al. (1992) Global average concentration and trend for hydroxyl radicals deduced from ALE/GAGE trichlorethane (methyl chloroform) data for 1978-1990, J. Geophys. Res. 97, D2, 2445-2461).

The half life of BAS 510 F was calculated to be

$t_{1/2} < 27.3$ h (1.1 d, 24h day).

Additionally, the degradation of BAS 510 in the atmosphere by ozone was estimated taken into account the attack to the Cl-substituted ring as lowest possible value. The resulting degradation rate was:
$k_{O3} > 3.3 \cdot 10^{-18}$ $cm^3 molecule^{-1} s^{-1}$.
With an ozone concentration of $7 \cdot 10^{11}$ cm^{-3} for unpolluted areas, the half life of BAS 510 F for photochemical degradation by ozone was estimated to be $t_{1/2} < 3.5$ d (24 h day).

Conclusion:
BAS 510 F has low volatilisation potential and, if reaching the troposphere, is degraded by photochemical processes.

B.8.8 Predicted environmental concentrations in air (Annex IIIA 9.3)

The volatilisation behavior of BAS 510 F from plant and soil surfaces after application according to good agricultural practice is discussed in detail in section 8.07 (Fate and behavior in air). Since only about 1% of the as from plant surfaces and only about 0.5% of the as from soil surfaces volatilise within 24 hours after application, the active substance has no tendency to enter the air.

Furthermore, the DT_{50} of BAS 510 F in air due to photochemical oxidative degradation is less than 1.1 days.

Conclusion:
Because of the low volatility and a fast photochemical oxidative degradation, no detailed assessment of atmospheric concentrations of BAS 510 F is necessary.

B.8.9 Definition of the residue (Annex IIA 7.3)

According to the results presented, the parent compound is the only relevant residue for quantification in soil, water and air. A characteristic feature of BAS 510 F are the low amounts of intermediary metabolites that are formed in the environment. Only one environmental metabolite approached, but did not exceed 10% TAR. This compound is of transient nature and in addition is of no toxicological or ecotoxicological relevance.

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[7]
AIIA-7.1.1.1.1; AIIA-7.1.1.2.1	Stephan, A.	1999	Metabolism of BAS 510 F (14C-Diphenyl and 14C-Pyridin) in soil under aerobic conditions. BASF Reg.Doc.#1999/11807 GLP, unpublished BOD2001-283	Y	BAS
AIIA-7.1.1.1.2	Staudenmaier, H.	2000	Anaerobic metabolism of BAS 510 F in soil (14C-pyridine-label). BASF DocID 2000/1014990 GLP, unpublished BOD2001-288	Y	BAS
AIIA-7.1.1.1.2	Staudenmaier, H. u. Schäfer, C.	2000	Anaerobic metabolism of BAS 510 F in soil (diphenyl-14C-label). BASF DocID 2000/1014986 GLP, unpublished BOD2001-287	Y	BAS
AIIA-7.1.1.1.2	von Götz, N.	2000	Soil Photolysis of BAS 510 F. BASF DocID 2000/1014989 GLP, unpublished BOD2001-289	Y	BAS
AIIA-7.1.1.2.1	Ebert, D. u. Harder, U.	2000	Degradation of 14C-chloronicotinic acid (Reg.No. 107371) in soil under aerobic conditions. BASF Doc ID 2000/1013280 GLP, unpublished BOD2001-286	Y	BAS
AIIA-7.1.1.2.1	Ebert, D. u. Harder, U.	2000	The Degradation Behaviour of 14C-BAS 510 F in Different Soils (DT50 / DT90). BASF Doc ID 2000/1013279 GLP, unpublished BOD2001-284	Y	BAS
AIIA-7.1.1.2.1	Hein, W.	1998	Influence of a Pretreatment with BAS 510 F on the Degradation of BAS 510 F in Soil (Einfluss der Vorbehandlung mit BAS 510 F auf das Abbauverhalten von BAS 510 F im Boden). BASF98/ 10607 GLP, unpublished BOD2001-290	Y	BAS

[7] 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[7]
AIIA-7.1.1.2.1	Kellner, O.	1999	Degradation of 14C-Reg. No. 363 487 in Aerobic Soil. BASF Reg.Doc.#1999/11102 GLP, unpublished BOD2001-285	Y	BAS
AIIA-7.1.1.2.1; AIIIA-9.1.3	Stephan, A.	2000	Storage stability of BAS 510 F in soil. BASF DocID 2000/1000136 GLP, unpublished BOD2001-302	Y	BAS
AIIA-7.1.1.2.2	Bayer, H. u. Grote, Ch.	2001	Field soil dissipation of BAS 510 F (300355) in formulation BAS 510 KA F. BASF Reg.Doc.#2000/10 13295 GLP, unpublished BOD2001-292	Y	BAS
AIIA-7.1.1.2.2; AIIIA-9.1.3	Hauck, T.	2001	Calculation of the Accumulation Potential and the Predicted Environmental Concentrations for BAS 510 F in Soil (PECsoil) after Repeated Application. BASF DocID 2000/1017050 not GLP, unpublished BOD2001-301	Y	BAS
AIIA-7.1.1.2.2	Kellner, O. u. Grote, Ch.	2001	Accumulation behaviour of BAS 510 F under field conditions over a 3-year-period (1998 - 2000) after application onto vegetables. BASF DocID 2000/1017040 GLP, unpublished BOD2001-296	Y	BAS
AIIA-7.1.1.2.2	Kellner, O. u. Grote. Ch.	2001	Accumulation behaviour of BAS 510 F under field conditions over a 3-year-period (1998-2000) after application onto grapes in a vineyard. BASF DocID 2000/1017039 GLP, unpublished BOD2001-294	Y	BAS
AIIA-7.1.1.2.2	Kellner, O. u. Keller, W.	2000	Field soil dissipation of BAS 510 F (300 355) in formulation BAS 510 KB F (1997 - 1998). BASF DocID 2000/1000123 GLP, unpublished BOD2001-291	Y	BAS
AIIA-7.1.1.2.2; AIIIA-9.1.3	Platz, K.	2001	Comparison of actual residues in soil after repeated application of BAS 510 F (accumulation study) with expected residues for repeated applications onto vegetable crops. BASF DocID 2000/1017046 not GLP, unpublished BOD2001-298	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[7]
AIIA-7.1.1.2.2; AIIIA-9.1.3	Platz, K.	2001	Comparison of actual residues in soil after repeated application of BAS 510 (accumulation study) with expected residues for repeated applications onto a vineyard . BASF DocID 2000/1017045 not GLP, unpublished BOD2001-295	Y	BAS
AIIA-7.1.1.2.2; AIIIA-9.1.3	Platz, K.	2001	Assessment whether field dissipation studies with BAS 510 F can be used to estimate transformation rates in soil and standardisation of half-lives to reference conditions. BASF Doc ID 2000/1017044 not GLP, unpublished BOD2001-293	Y	BAS
AIIA-7.1.2	Seher, A	1998	Soil Adsorption / Desorption Study of 300 355 (Bas 510 F). Reg. Doc.# BASF 98/10513 GLP, unpublished BOD2001-303	Y	BAS
AIIA-7.1.3.1; AIIA-7.1.3.2	Richter, T.	2001	Investigation of the leaching behaviour of aged and non-aged BAS 510 F residues in soil. BASF DocID 2000/1000965 not GLP, unpublished BOD2001-320	Y	BAS
AIIA-7.1.3.1; AIIA-7.1.3.2	Richter, T.	2001	Investigation of the leaching behaviour of aged and non-aged BAS 510 F residues in soil. BASF DocID 2000/1017037 GLP, unpublished BOD2001-305	Y	BAS
AIIA-7.2.1.3.1	Werner, D.I.	1999	Determination of the Biodegradabiltity of BAS 510 F in the Manometric Respirometry Test according to GLP, EN 45001 and ISO 9002. Reg.Doc.#BASF 99/10290 GLP, unpublished WAS2001-147	Y	BAS
AIIA-7.2.1.3.2	Ebert, D.	2000	Degradation of BAS 510 F in Aerobic Aquatic Environment. BASF DocID 2000/1000135 GLP, unpublished WAS2001-148	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[7]
AIIA-7.2.1.3.2	Kellner, O.	2001	Degradation and distribution of BAS 510 F in a water-sediment system under outdoor conditions. BASF DocID 2000/1017038 GLP, unpublished WAS2001-149	Y	BAS
AIIA-7.2.1.3.2	Platz, K.	2001	Estimation of the kinetic parameters of the degradation of BAS 510 F in an aerobic water sediment system under outdoor conditions. BASF DocID 2000/1017047 not GLP, unpublished WAS2001-150	Y	BAS
AIIA-7.2.2	Götz, N.	2000	Volatilization of BAS 510 F after Application of BAS 510 01 F on Soil and on Plant Surface. BASF DocID 2000/1014979 GLP, unpublished LUF2001-134	Y	BAS
AIIIA-9.1.3; AIIIA-11	anonym	2001	Fate and behaviour in the environment. Document M-III not GLP, unpublished BOD2001-329	Y	BAS
AIIIA-9.1.3; AIIIA-11	Anonym	2001	Fate and behaviour in the environment. Document M-III not GLP, unpublished LUF2001-145	Y	BAS
AIIIA-9.1.3	Hauck, T.	2001	Calculation of Predicted Environmental Concentrations for BAS 510 F in Soil (PECsoil). BASF DocID 2000/1017049 not GLP, unpublished BOD2001-330	Y	BAS
AIIIA-9.2.1; AIIIA-9.2.3; AIIIA-11	anonym	2001	Fate and behaviour in the environment. Document M-III not GLP, unpublished WAS2001-152	Y	BAS
AIIIA-9.2.1; AIIIA-9.2.3	Platz, K.	2001	Assessment whether field dissipation studies with BAS 510 F can be used to estimate transformation rates in soil and standardisation of half-lives to reference conditions. BASF Doc ID 2000/1017044 not GLP, unpublished WAS2001-133	Y	BAS
AIIIA-9.2.1	van de Veen, J.R.	2001	Calculation of Predicted Environmental Concentrations (PECgw) for BAS 510 F in Groundwater on an European Level. BASF DocID 2000/1017048 not GLP, unpublished WAS2001-157	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[7]
AIIIA-9.2.3	Gottesbüren, B.	2001	Calculation of Predicted Environmental Concentrations (PECsed) for BAS 510 F in Sediment. BASF DocID 2000/1017053 not GLP, unpublished WAS2001-159	Y	BAS
AIIIA-9.2.3	Gottesbüren, B.	2001	Calculation of Predicted Environmental Concentrations (PECsw) for BAS 510 F in Static Surface Water. BASF DocID 2000/1017051 not GLP, unpublished WAS2001-158	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Annex B

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

B-9: Ecotoxicology

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 510 F - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	AVS2001-119
Test substance:	Technical nicobifen
Purity:	95.3 %
Guideline:	EPA 71-1
Test species:	Bobwhite quail (Colinus virginianus)
Age:	ca. 4.5 months
Birds per treatment:	5 M + 5 F
Administration:	Intubation
Solvent / vehicle:	Carboxymethl-cellulose
Dose levels:	0/500/1000/2000 mg/kg
Findings:	No signs of intoxication neither effects on body weight and feed consumption in any treatment group.
LD50:	>2000 mg/kg bw
Lowest lethal dose:	>2000 mg/kg bw
NOED:	2000 mg/kg bw
valid:	yes
GLP compliance:	yes

B.9.1.2 Dietary toxicity (Annex IIA 8.1.2)

Title:	**Test Report: BAS 510 F - Avian dietary LC50 test in chicks of the bobwhite quail (Colinus virginianus)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	AVS2001-120
Test substance:	Technical nicobifen
Purity	95.3 %
Guideline:	EPA 71-2 / OECD 205
Test species:	Bobwhite quail (Colinus virginianus)
Age:	14 d
Birds per treatment:	10 unsexed
Solvent / vehicle:	none

Exposure period:	5 d
Conc. levels (nom.):	0/313/625/1250/2500/5000 ppm
Conc. levels (meas.):	0/360/651/1281/2714/5427 ppm
Findings:	No signs of intoxication neither effects on body weight and feed consumption in any treatment group.
LC50:	>5000 ppm
Lowest lethal conc.:	>5000 ppm
NOEC:	5000 ppm
valid:	yes
GLP compliance:	yes

Title:	**Test Report: BAS 510 F - Avian dietary LC50 test in chicks of the mallard duck (Anas platyrhynchos)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	AVS2001-121
Test substance:	Technical nicobifen
Purity	95.3 %
Guideline:	EPA 71-2 / OECD 205
Test species:	Mallard duck (Anas platyrhynchos)
Age:	7 d
Birds per treatment:	10 unsexed
Solvent / vehicle:	none
Exposure period:	5 d
Conc. levels (nom.):	0/328/656/1312/2623/5247 ppm
Conc. levels (meas.):	0/312/629/1197/2562/5175 ppm
Findings:	No signs of intoxication were observed in any treatment group. There appeared to be a reduction in feed consumption during the 5-day-exposure period in the 3 highest test groups (1250 ppm and above), however the response was not clearly dose related.
LC50:	>5000 ppm
Lowest lethal conc.:	>5000 ppm
NOEC:	625 ppm
valid:	yes
GLP compliance:	yes

B.9.1.3 Effects on reproduction (Annex IIA 8.1.3)

Title: **Report: BAS 510 F - 1-generation reproduction study on the bobwhite quail (Colinus virginianus) by administration in the diet + Amendment No. 1 to the report**

Author: Zok, S. (2000)
BBA-Ref.-No.: AVS2001-122

Test substance: Technical nicobifen
Purity: 96.3 %

Guideline: EPA 71-4 / OECD 206
Test species: Bobwhite quail (Colinus virginianus)
Age: 6 months
Birds per treatment: 16 pairs
Solvent / vehicle: none
Exposure period: 22 w
Conc. levels (nom.): 0/100/300/1000 ppm
Conc. levels (meas.): 0/96/99/99 %

Findings:

Treatment (ppm)	**0**	**100**	**300**	**1000**
Number of eggs laid per hen and week	4.8	4.6	4.8	4.2
% viable embryos of eggs set	98.2	94.8	96.4	92.4
% live 11-day embryos of eggs set	97.3	93.3	95.4	85.6
% live 18-day embryos of eggs set at day 11	98.8	99.9	99.5	96.9
% normal hatchlings of live 18-day embryos	85.0	82.3	83.9	76.9
% surviving of normal hatchlings	81.9	78.9	84.7	74.3
Number of 14-day survivors per hen and week	2.9	2.5	3.0	1.8
Egg shell thickness (mm)	0.21	0.21	0.21	0.20

Conclusion: At 1000 ppm substance-related effects were seen on the following parameters: Number of eggs laid, embryo survival, and chick survival, resulting in a clear decrease of overall reproductive success at this treatment level.
NOEC: 300 ppm

valid: yes
GLP compliance: yes

Title: **Report: BAS 510 F - 1-generation reproduction study on the mallard duck (Anas platyrhynchos) by administration in the diet**

Author: Zok, S. (2000)
BBA-Ref.-No.: AVS2001-123

Test substance: Technical nicobifen
Purity: 96.3 %

Guideline: EPA 71-4 / OECD 206
Test species: Mallard duck (Anas platyrhynchos)
Age: 6-7 months
Birds per treatment: 16 pairs
Solvent / vehicle: none
Exposure period: 22 w
Conc. levels (nom.): 0/100/300/1000 ppm
Conc. levels (meas.): 0/98/94/95 %

Findings:

Treatment (ppm)	**0**	**100**	**300**	**1000**
Number of eggs laid per hen and week	4.7	4.9	5.3	4.6
% viable embryos of eggs set	89.2	94.3	86.3	92.1
% live 14-day embryos of eggs set	85.9	89.3	84.8	87.2
% live 21-day embryos of eggs set at day 14	98.9	99.3	99.5	99.2
% normal hatchlings of live 21-day embryos	75.7	68.0	85.5	76.5
% surviving of normal hatchlings	96.0	95.1	98.3	98.0
Number of 14-day survivors per hen and week	2.8	2.6	3.5	2.7
Egg shell thickness (mm)	0.39	0.40	0.39	0.39

Conclusion: No compound-related effects were observed on parent, eggs and chicks at any treatment level.
NOEC: 1000 ppm

valid: yes
GLP compliance: yes

B.9.1.4 Summary of avian toxicity data

Test material	Species	Test	NOEL	LD50/LC50	Unit
Nicobifen	Bobwhite quail	Acute	2000	>2000	mg/kg bw
Nicobifen	Bobwhite quail	5-day-dietary	5000	>5000	ppm
Nicobifen	Mallard duck	5-day-dietary	625	>5000	ppm
Nicobifen	Bobwhite quail	Reproduction	300		ppm
Nicobifen	Mallard duck	Reproduction	1000		ppm

B.9.1.5 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 nicobifen formulations are to be applied exclusively as sprays.

B.9.1.6 Risk assessment for birds

Birds may be exposed to nicobifen mainly by the consumption of contaminated feed. Depending on species this may be insects, green plant material or fruits. The risk assessment will be based on a maximum single rate of 0.5 kg as/ha in field crops and 0.6 kg as/ha in grapes.

Relevant food items and residue estimates

- Grape, insects:
  Residues estimates are based on generic data according to Fischer and Bowers assuming 21 mg/kg for 1 kg/ha (upper 95 percentile of the distribution); with 0.6 kg/ha the estimate is 12.6 mg/kg which will be used for short-term and long-term assessment.
- Grapes, earthworms
  With a PEC_{soil} of 1.33 mg/kg and assuming a bioaccumulation factor from soil to earthworm of 5 the residues in earthworms would be about 7 mg/kg and lower than residues in insects; therefore this food item is covered by the consideration of insects.
- Grape, fruit
  According to residue trials the maximum day-0 residue was 5.76 mg/kg.
- Grape, vegetation on the ground
  It is unlikely that birds feed exclusively on ground vegetation in vineyards; therefore this scenario is not considered.
- Field crops, insects
  As insects in grapes; with 0.5 kg/ha the estimate is 10.5 mg/kg which will be used for short-term and long-term assessment.

- Field crops, earthworms:
  As in grapes this food item is covered by the consideration of insects.
- Field crops, vegetation
  Residue estimates are based on generic data for the „leafy crop“ category of Hoerger and Kenaga (31 mg/kg for 1 kg/ha); with 0.5 kg as/ha the estimate is 15.5 mg/kg which is in general agreement with measured residues (day-0 residues for bean plants were 10.6-30.1 mg/kg, for peas 4.4-13.3 mg/kg, for rape 0.7-3.2 mg/kg). For the long-term assessment a time-weighted average for a period of 21 days based on a DT50 of 10 days is used which is 0.53 of the initial residue = 8.2 mg/kg.

Food consumption

- Insectivorous birds
  Body weight 10 g; food consumption (dry) 2.8 g (according to Nagy); assuming a moisture content of 70 % the consumption of fresh insects would be 9.4 g equivalent to 94 % of the body weight.
- Frugivorous birds
  Body weight 80 g; food consumption (fresh) equivalent to 87 % of body weight (Skorupa et al.: Consumption of commercially-grown grapes by American robins. J Field Ornithol 56, 369-378, 1985).
- Herbivorous birds
  Body weight 300 g; food consumption (dry) 27 g (according to Nagy); assuming a moisture content of 80 % the consumption of fresh vegetation would be 132 g equivalent to 44 % of the body weight.

Table B.9.1-1 : Exposure assessment for birds

Use	Maximum application rate (kg/ha)	Feed	Initial residue (mg/kg)	twa residue (mg/kg)	Relative feed demand (%)	Maximum initial daily intake (mg/kg bw)
Grape	0.6	Insects	12.6	12.6	94	11.8
		Fruit	5.8	5.8	87	5.0
Field crops	0.5	Insects	10.5	10.5	94	9.8
		Vegetation	15.5	8.2	44	6.8

Toxicity/exposure ratios

Acute TER: the LD_{50} is related to the maximum initial daily intake
Short-term TER: the LC_{50} is related to the initial residue
Long-term TER: the NOEC from the reproduction test is related to the twa residue.

Table B.9.1-2 : Toxicity/exposure ratios for birds

Use	Feed	Time-scale	Toxicity/Exposure ratio
Grapes	Insects	acute	TERa = >2000/11.8 = >170
		short-term	TERst = >5000/12.6 = >390
		long-term	TERlt = 300/12.6 = 23
	Fruits	acute	TERa = >2000/5.0 = >400
		short-term	TERst = >5000/5.8 = >860
		long-term	TERlt = 300/5.8 = 52
Field crops	Insects	acute	TERa = >2000/9.8 = >200
		short-term	TERst = >5000/10.5 = >470
		long-term	TERlt = 300/10.5 = 28
	Vegetation	acute	TERa = >2000/6.8 = >294
		short-term	TERst = >5000/15.5 = >322
		long-term	TERlt = 300/8.2 = 37

All TER-values are above the Annex-VI-triggers; so the risk to birds is considered acceptable.

Bioaccumulation

According to toxicokinetic studies with rats there is no evidence for potential for bioaccumulation in mammals (see chapter 5). Also the logPow of 2.96 doesn't raise concerns with regard to bioaccumulation and food-chain effects.

Metabolites

All metabolites in environmental media are of minor importance (see chapter B.8). The same is true for metabolites in plants (chapter B.7).

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 unless stated otherwise. 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 510 F - Acute toxicity study on the bluegill (*Lepomis macrochirus* Raf.) in a static system (96 hours)**
Author: Zok, S. (2001)
BBA-Ref.-No.: WAT2001-364

Test substance: Technical nicobifen
Purity: 95.3 %
Guideline: OECD 203
Test species: *Lepomis macrochirus*
Exposure mode: Static
Conc. levels (nom.): 0/0.6/1.0/1.6/2.5/4.0 mg/L
Conc. levels (meas.): 90.0 - 95.8 %

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
96 h	Mortality	> 4.0			> 4.0

Remarks: From 24 h on precipitations at the water surface at all concentrations. Limit of solubility of the test substance was 4.0 mg/L.

valid: yes
GLP compliance: yes

Title: **BAS 510 F - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) in a static system (96 hours)**
Author: Zok, S. (2001)
BBA-Ref.-No.: WAT2001-363

Test substance: Technical nicobifen
Purity: 95.3 %
Guideline: OECD 203
Test species: *Oncorhynchus mykiss*
Exposure mode: Static
Conc. levels (nom.): 0/0.5/0.7/1.2/1.9/3.0 mg/L
Conc. levels (meas.): 88.0 - 109.3 %

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
96 h	Mortality	1.9			2.7

Remarks: Results based on mean analytically determined concentrations.

valid: yes
GLP compliance: yes

B.9.2.1.2 Formulated product (BAS 510 01 F)

Title: **BAS 510 01 F - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) in a static system (96 hours)**
Author: Zok, S. (2000)
BBA-Ref.-No.: WAT2001-383

Test substance: Formulation BAS 510 01 F (nicobifen 51.3 %)
Guideline: OECD 203
Test species: *Oncorhynchus mykiss*
Exposure mode: Static
Conc. levels (nom.): 0/1.0/2.2/5.0/10.0/22.0/50.0/100.0 mg/L
Conc. levels (meas.): 96 h: 70.1 - 90.8 %

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
96 h	Mortality	5.0	10.0		100.0

Remarks: Turbidity was observed at 5.0 mg/L and higher concentrations.

valid: yes
GLP compliance: 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 510 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.:	WAT2001-365
Test substance:	Technical nicobifen
Purity:	95.3 %
Guideline:	OECD 204
Test species:	*Oncorhynchus mykiss*
Exposure mode:	Flow-through
Conc. levels (nom.):	0/0.100/0.464/1.000/2.150 mg/L
Conc. levels (meas.):	d 1 at 1.0 mg/L only 53.3 %

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
28 d	Mortality	1.0	>1.0 <2.15		
28 d	Growth	1.0	>1.0 <2.15		
28 d	Behaviour	1.0	>1.0 <2.15		

valid:	no. The study has several shortcomings and was not conducted according to the recommendations of the relevant guideline. Body length of test animals varied between 4.0 and 7.0 cm, with a mean of 5.6 cm (guideline: 5 cm max.). In addition, due to technical problems fish were exposed to only 10 % of the nominal concentration of the test compound during the first week. Therefore the test was prolonged for an additional period of 1 week. However, nominal concentrations were not achieved until day 4 in the 1.0 mg/L-group. Only 4 concentration levels have been tested (guideline: 5). The water volume in the test aquaria was only exchanged twice a day due to the reduction of the dilution water flow rates in order to achieve nominal concentration levels (guideline recommends 4 times).
GLP compliance:	yes

B.9.2.2.1.2 Fish early life stage toxicity test (Annex IIa 8.2.2.2)

Title:	**BAS 510 F - Early life-stage toxicity test on the rainbow trout (*Oncorhynchus mykiss* Walbaum 1792)**
Author:	Zok, S. (1999)
BBA-Ref.-No.:	WAT2001-366
Test substance:	Technical nicobifen
Purity:	95.3 %
Guideline:	OECD 210
Test species:	*Oncorhynchus mykiss*
Exposure mode:	Flow-through
Conc. levels (nom.):	0/0.125/0.25/0.50/1.0/2.0 mg/L
Conc. levels (meas.):	83.4 - 96.4 %

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
97 d	Mortality	0.125	0.25		
97 d	Hatch rate	0.25	0.5		
97 d	Growth	0.25	0.5		
97 d	Behaviour	0.125	0.25		

valid:	yes
GLP compliance:	yes

B.9.2.3 Bioconcentration in fish (Annex IIA 8.2.3)

Title:	**Bioaccumulation and metabolism of [14C]-BAS 510F in rainbow trout**
Author:	Chapleo, S., Caley, C.Y. (2000)
BBA-Ref.-No.:	WAT2001-367
Test substance:	Technical nicobifen
Purity:	>97 %
Guideline:	OECD 305
Test species:	*Oncorhynchus mykiss*
Exposure mode:	Flow-through
Conc. levels (nom.):	0.020/0.200 mg/L
Conc. levels (meas.):	0.0162-0.0225; 0.175-0.228 mg/L
Duration exposure phase:	35 d
Duration depuration phase:	15 d

Maximum BCF:	89-125 after 28 d (steady-state achieved)
Depuration:	90 % elimination after 3.3 d
ct50/ct90:	1.0 / 3.3 d
valid:	yes
GLP compliance:	yes

B.9.2.4 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4, Annex IIIA 10.2.1)

B.9.2.4.1 Active substance

Title:	**Effect of BAS 510 F on the immobility of *Daphnia magna* Straus in a 48 hour static, acute toxicity test**
Author:	Dohmen, P. (2001)
BBA-Ref.-No.:	WAT2001-378
Test substance:	Technical nicobifen
Purity:	94.4 %
Guideline:	OECD 202 I
Test species:	*Daphnia magna*
Exposure mode:	Static
Conc. levels (nom.):	0/0.5/0.8/1.5/2.5/4.3/7.5 mg/L
Conc. levels (meas.):	0 h: 94.5 - 109.4 %; 48h: 62.8 % (4,3 mg/L) 42.3 % (7.5 mg/L)

Results (mg/L) related to measured concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
48 h	Mortality	0.49			5.33

valid:	yes
GLP compliance:	yes

B.9.2.4.2 Formulated product (BAS 510 01 F)

Title:	**BAS 510 01 F - Determination of the acute effect on the swimming ability of the water flea *Daphnia magna* Straus**
Author:	Jatzek (2001)
BBA-Ref.-No.:	WAT2001-384
Test substance:	Formulation BAS 510 01 F Nicobifen 51.3 %
Guideline:	OECD 202 I
Test species:	*Daphnia magna*
Exposure mode:	Static
Conc. levels (nom.):	0/6.25/12.5/25.0/50.0/100.0 mg/L
Conc. levels (meas.):	

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
48 h	Mortality	12.5			50.0

valid:	yes
GLP compliance:	yes

B.9.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

B.9.2.5.1 Active substance

Title:	**BAS 510 F - Determination of the chronic effect on the reproduction of the water flea *Daphnia magna* Straus**
Author:	Hisgen (2001)
BBA-Ref.-No.:	WAT2001-379
Test substance:	Technical nicobifen
Purity:	96.3 %
Guideline:	OECD 202 II
Test species:	*Daphnia magna*
Exposure mode:	Semi-static
Conc. levels (nom.):	0.05/0.1/0.2/0.4/0.8/1.6/3.2 mg/L
Conc. levels (meas.):	75.5 - 82.3 % (mean values)

Results (mg/L) related to measured concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
21 h	Mortality	> 2.63			
21 h	Reproduction	1.31	2.63		

valid: yes
GLP compliance: yes

B.9.2.6 Effects on algal growth (Annex IIA 8.2.6, Annex IIIA 10.2.1)

B.9.2.6.1 Active substance

Title: **Effect of BAS 510 F on the growth of the green alga *Pseudokirchneriella subcapitata***
Author: Kubitza, J. (2001)
BBA-Ref.-No.: WAT2001-380

Test substance: Technical nicobifen
Purity: 94.4 %
Guideline: OECD 201 (Grünalge)
Test species: *Pseudokirchneriella subcapitata*
Exposure mode: Static
Conc. levels (nom.): 0/0.1/0.5/1.0/1.5/2.0/2.5/3.0 mg/L
Conc. levels (meas.): 0 h: 82.5 - 96.7 % %

Results (mg/L) related to measured concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
96 h	Biomass				1.34
96 h	Growth				3.75

Remarks: Two preliminary invalid tests showed approximately similar results.

valid: yes
GLP compliance: yes

B.9.2.6.2 Formulated product (BAS 510 01 F)

Title:	**Effect of BAS 510 01 F on the growth of the green algae *Pseudokirchneriella subcapitata***
Author:	Kubitza, J. (2001)
BBA-Ref.-No.:	WAT2001-385
Test substance:	Formulation BAS 510 01 F (nicobifen 51.3 %)
Guideline:	OECD 201 (green algae)
Test species:	*Pseudokirchneriella subcapitata*
Exposure mode:	Static
Conc. levels (nom.):	0/2.5/2.8/3.1/3.5/4.0/4.5/5.0 mg/L
Conc. levels (meas.):	0 h: 96.6 - 103.9 %

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
72 h	Biomass				3.37
72 h	Growth				4.50

valid:	yes
GLP compliance:	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 510 F on the development of sediment dwelling larvae of *Chironomus riparius* in a water-sediment system**
Author:	Dohmen, P. (2001)
BBA-Ref.-No.:	WAT2001-381
Test substance:	Technical nicobifen
Purity:	94.4 %
Guideline:	BBA
Test species:	*Chironomus riparius*
Exposure mode:	static, spiked water
Conc. levels (nom.):	0.25/0.5/1.0/2.0/4.0 mg/L
Conc. levels (meas.):	0 h: 106.1 - 115.0 %

Results (mg/L) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
28 d	Development	2.0	4.0		
28 d	Emergence	2.0	4.0		

valid: yes
GLP compliance: yes

The effects of the active substance nicobifen and the formulated product BAS 510 01 F on aquatic organisms are summarised in Table B.9.2-1. The results of the bioconcentration study are summarised in Table B.9.2-2.

Table B.9.2-1: Summary of aquatic toxicity data

Test material	**Species**	**Duration**		**NOEC (mg/L)**	**EC50 (mg/L)**	
Nicobifen	*L. macrochirus*	96 h (st)	Mortality	> 4.0	> 4.0	Nom
Nicobifen	*O. mykiss*	96 h (st)	Mortality	1.9	2.7	Nom
Nicobifen	*O. mykiss*	97 d (fl)	Mortality	0.125		Nom
			Hatch rate	0.25		Nom
			Growth	0.25		Nom
			Behaviour	0.125		Nom
Nicobifen	*D. magna*	48 h (st)	Mortality	0.49	5.33	Meas
Nicobifen	*D. magna*	21 d (ss)	Mortality	> 2.63		Meas
			Reproduction	1.31		Meas
Nicobifen	*C. riparius*	28 d (st)	Development	2.0		Nom
			Emergence	2.0		Nom
Nicobifen	*P. subcapitata*	96 h (st)	Biomass		1.34	Meas
			Growth		3.75	Meas
Nicobifen	Activated sludge	0.5 h (st)	Respiration rate		>1000	Nom
BAS 510 01 F	*O. mykiss*	96 h (st)	Mortality	5.0	100.0	Nom
BAS 510 01 F	*D. magna*	48 h (st)	Mortality	12.5	50.0	Nom
BAS 510 01 F	*P. subcapitata*	72 h (st)	Biomass		3.37	Nom
			Growth		4.50	Nom

fl = flow-through; st = static; ss = semi-static; sm = special method;

Nom = nominal concentration; Meas = measured concentration.

Table B.9.2-2: Summary of aquatic bioconcentration studies

Test material	Species	Duration Expos. + Elim.	BCF	Elimination
Nicobifen	*O. mykiss*	35 + 15 d	89-125	90 % after 3.3 d

B.9.2.8 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 and the formulated product has been submitted.

The formulated product is less toxic than could be predicted from the active substance. From the data submitted for fish, daphnia, algae and sediment dwelling insects the NOEC of 125 µg as/L from a 97 d-ELS-study with *Oncorhynchus mykiss* is considered most relevant for the overall risk assessment. As this toxicity value is derived from a flow-through test, time-weighted average PEC-values should be used for calculation of TER-values. Lethal and behavioural effects observed in this study were obvious from about day 40 onwards.

The respective TER-values are summarised in

Table B.9.2-3: TER values for the most sensitive test organism (*Oncorhynchus mykiss;* NOEC: 125 µg as/L, 97 d ELS-study)

Scenario: Grapevines, late growth stage, 90th percentile drift factors Application rate: 1 x 0.600 kg as/ha (DT_{50} water: 21 d; $PEC_{twa;\ 42\ d}$)				
Distance	Drift rate [%]	$PEC_{sw;\ twa}$ (µg as/L)	TER	Annex VI Trigger
3 m	8.02	8.7	14.4	10
5 m	3.62	3.9	32.0	10
Scenario: Field crops (beans), 90th percentile drift factors Application rate: 2 x 0.500 kg as/ha (DT_{50} water: 21 d; $PEC_{twa;\ 42\ d}$) PEC_{sw} for 5 m buffer zone calculated also after run off event (for explanation see chapter B.8.6)				
Distance	Drift rate [%]	$PEC_{sw;\ twa}$ (µg as/L)	TER	Annex VI Trigger
1 m (drift)	2.77	4.2	29.8	10
5 m (drift)	0.57	0.8	156.3	10
5 m (runoff)	-	1.6	78.1	10

All relevant trigger values according to Annex VI of Directive 91/414/EEC are met. The overall risk to aquatic organisms arising from the uses described above is considered acceptable.

Bioconcentration
Nicobifen has a log Pow of 2.96 indicating a medium liability to bioaccumulation. Results from a bioconcentration study with *Oncorhynchus mykiss* showed a maximum BCF (whole fish) of 125 after 35 days of exposure. However, the substance was eliminated with a half-life of 1.0 day. After 3.3 days 90 % of the accumulated net total radioactivity had been eliminated. Therefore the overall risk of bioaccumulation is considered to be acceptable.

Metabolites
No major metabolites were identified in the water/sediment study. Only one metabolite, 4-Cl-benzoic acid (M510F64) reached levels just below the trigger value (9.4 % TAR after 30 days in the water phase). However, data available from the open literature indicated, that this metabolite is by orders of magnitude less toxic than the active substance and therefore of no ecotoxicological relevance.

Classification and labelling
The active substance nicobifen is not readily biodegradable. The $E_bC_{50\ (96\ h)}$ *for P. subcapitata* is 1.34 mg. as/L. According to Directive 67/548/EEC nicobifen should be labelled with N, R51 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 nicobifen for rats is >5000 mg/kg body weight. Regarding the long-term risk the assessment will be based on 100 ppm that was the NOAEL for reproductive effects in a multi-generation study with rats. This is a rather conservative value as in the next higher dose group of the multi-generation study (1000 ppm) effects were confined to hepatocellular hypertrophy in a few parental animals and slight decrease in body weight of second generation male pups (see section B.06, Toxicology).

B.9.3.2 Risk assessment for mammals

Mammals may be exposed to nicobifen mainly by the consumption of contaminated feed. Depending on species this may be insects, earthworms and green plant material. The risk assessment will be based on a maximum single rate of 0.5 kg as/ha in field crops and 0.6 kg as/ha in grapes.

Relevant food items and residue estimates

- Grape, insects
  Residues estimates are based on generic data according to Fischer and Bowers assuming 21 mg/kg for 1 kg/ha (upper 95 percentile of the distribution); with 0.6 kg/ha the estimate is 12.6 mg/kg which will be used for short-term and long-term assessment.

- Grapes, earthworms
  With a PEC_{soil} of 1.33 mg/kg and assuming a bioaccumulation factor from soil to earthworm of 5 the residues in earthworms would be about 7 mg/kg and lower than residues in insects; therefore this food item is covered by the consideration of insects.
- Grape, vegetation on the ground
  It is assumed that half of the applied amount reaches the ground; residue estimates are based on generic data for the „short grass“ category of Hoerger and Kenaga (112 mg/kg for 1 kg/ha); with 0.6 kg/ha the estimate is 34 mg/kg. For the long-term risk assessment a time-weighted average for a period of 21 days based on a DT50 of 10 days is used which is 0.53 of the initial residue = 18 mg/kg.
- Field crops, insects
  As insects in grapes; with 0.5 kg/ha the estimate is 10.5 mg/kg which will be used for short-term and long-term assessment.
- Field crops, earthworms
  As in grapes this food item is covered by the consideration of insects.
- Field crops, vegetation
  Residue estimates are based on generic data for the „leafy crop“ category of Hoerger and Kenaga (31 mg/kg for 1 kg/ha); with 0.5 kg as/ha the estimate is 15.5 mg/kg which is in general agreement with measured residues (day-0 residues for bean plants were 10.6-30.1 mg/kg, for peas 4.4-13.3 mg/kg, for rape 0.7-3.2 mg/kg). For the long-term assessment a time-weighted average for a period of 21 days based on a DT50 of 10 days is used which is 0.53 of the initial residue = 8.2 mg/kg.

Food consumption

- Insectivorous mammal
  Body weight 10 g; food consumption (fresh) equivalent to 100 % of body weight (Churchfield S: Food availability and the diet of the common shrew, Sorex araneus, in Britain, J Anim Ecol 51, 15-28, 1982).
- Herbivorous mammal
  Body weight 3000 g, food consumption (dry) 170 g (according to Nagy); assuming a moisture content of 80 % the consumption of fresh vegetation would be 850 g equivalent to 28 % of the body weight.

Table B.9.3-1: Exposure assessment for mammals

Use	Maximum application rate (kg/ha)	Feed	Initial residue (mg/kg)	twa residue (mg/kg)	Relative feed demand (%)	Maximum initial daily intake (mg/kg bw)
Grape	0.6	Insects	12.6	12.6	100	12.6
		Ground vegetation	34	18	28	9.5
Field crops	0.5	Insects	10.5	10.5	100	10.5
		Vegetation	15.5	8.2	28	4.3

Toxicity/exposure ratios

Acute TER: the LD_{50} is related to the maximum initial daily intake
Long-term TER: the NOEC from the reproduction test is related to the twa residue.

Table B.9.3-2 : Toxicity/exposure ratios for mammals

Use	Feed	Time-scale	Toxicity/Exposure ratio
Grapes	Insects	acute	TERa = >5000/12.6 = >390
		long-term	TERlt = 100/12.6 = 8
	Ground vegetation	acute	TERa = >5000/9.5 = >520
		long-term	TERlt = 100/18 = 6
Field crops	Insects	acute	TERa = >5000/10.5 = >470
		long-term	TERlt = 100/10.5 = 9
	Vegetation	acute	TERa = >5000/4.3 = >1160
		long-term	TERlt = 100/8.2 = 12

The long-term TER-values for all scenarios are only marginally above the Annex-VI-trigger of 5. However, the corresponding toxicity figure is conservative as explained above; furthermore, it is assumed that animals feed exclusively on contaminated material and spend the entire time in the treated area. Therefore, the risk to mammals is considered acceptable.

Bioaccumulation

According to toxicokinetic studies with rats there is no evidence for potential for bioaccumulation in mammals (See chapter 5). Also the logPow of 2.96 doesn't raise concerns with regard to bioaccumulation and food-chain effects.

Metabolites

All metabolites in environmental media are of minor importance (see chapter B.8). The same is true for metabolites in plants (chapter B.7).

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 Acute oral toxicity of nicobifen (technical)

Reference:	King, A.: Assessment of side-effects of BAS 510 01 F to the honey bee, Apis mellifera L. in the laboratory (study code 20001059/01-BLEU, 17.07.2000)
Testguideline:	OECD guideline No. 213
valid:	yes
GLP compliance:	yes
Test design:	test substance - nicobifen toxic standard – Perfektion, active substance dimethoat untreated control
Method:	The test was performed as a limit-test. The test substance was offered only in one high dosage of 100 µg as/bee. The toxic standard was tested in the nominal concentration of 0.08 µg as/bee, 0.11µg as/bee, 0.16 µg as/bee and 0.22 µg as/bee. Each concentration comprised 5 replicates with 10 bees each.
Results:	test substance – LD_{50} oral > 100 µg as/bee toxic standard – LD_{50} oral 0.12 µg as/bee control: no mortality

B.9.4.1.2 Acute contact toxicity of nicobifen (technical)

Reference:	King, A.: Assessment of side-effects of BAS 510 01 F to the honey bee, Apis mellifera L. in the laboratory (study code 20001059/01-BLEU, 17.07.2000)
Testguideline:	OECD guideline No. 214
valid:	yes
GLP compliance:	yes
Test design:	test substance - nicobifen toxic standard – Perfektion, active substance dimethoat untreated control
Method:	The test was performed as a limit-test. The test substance was applied only in one high dosage of 100 µg as/bee. The toxic standard was applied in dosages of 0.09 µg as/bee, 0.13 µg as/bee, 0.19 µg as/bee and 0.25 µg as/bee. Each concentration comprised 5 replicates with 10 bees each.
Results:	test substance – LD_{50} oral > 100 µg as/bee toxic standard – LD_{50} oral 0.12 µg as/bee control: no mortality

B.9.4.1.3 Acute oral and contact toxicity of formulated nicobifen to honeybees

Reference:	Sack, D.:Effect of Reg. No. 300355 on the honeybee (Apis mellifera L.) in laboratory trials.
Testguideline:	EPPO guideline No. 170
valid:	yes
GLP compliance:	yes
Test design:	test substance – Reg. No. 30035 reference substance – Perfektion control
Method:	The test was performed as a limit-test. The test substance was applied only in one high dosage of 200µg as/bee for both the oral and contact toxicity test. The reference substance was applied with 0.5µg/bee, 0.25 µg/bee, 0.2 µg/bee, 0.15 µg/bee, 0.125 µg/bee and 0.1 µg/bee in the oral toxicity test and with 0.5 µg/bee, 0.4 µg/bee, 0.35 µg/bee, 0.3 µg/bee, 0.25 µg/bee and 0.1 µg/bee in the contact toxicity test. The test substance comprised 5 replicate with 10 bees each. The reference substance comprised 3 replicates per concentration with 10 bees each.
Results:	test substance: oral – LD_{50} ~ 166 µg/bee (since the bees fed less than estimated total sugar solution) contact test – LD_{50} > 200 µg/bee reference substance: oral – 0.38 µg/bee contact – 0.36 µg/bee control: no mortality

B.9.4.2 Bee brood feeding test (Annex IIA 8.3.1.2)

Tests are not required as the test substance is not an IGR.

B.9.4.3 Residue test (Annex IIIA 10.4.2)

Tests are not required, as the oral and contact toxicity are very low.

B.9.4.4 Cage test (Annex IIIA 10.4.3)

Tests are not required, as the oral and contact toxicity are very low.

B.9.4.5 Field test (Annex IIIA 10.4.4)

Tests are not required, as the oral and contact toxicity are very low.

B.9.4.6 Tunnel test (Annex IIIA 10.5.5)

Tests are not required, as the oral and contact toxicity are very low.

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 the highest amount of 600 g nicobifen/ha.

active substance nicobifen

HQ oral – 6
HQ contact – 6

formulation (Reg. No. 300355)

HQ oral – 3.61
HQ contact - 3

All values are clearly below the trigger of 50. This indicates a low risk for honeybees by the practical use of nicobifen-containing 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 use and nominal field rates outlined in this monograph. Investigations into the toxicity of nicobifen 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 of the acute toxicity of formulated nicobifen in laboratory tests:

Predatory mites

Title:	**Effects of BAS 510 01 F on the Predatory Mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the Laboratory - Dose Response Design -**
Author:	Goßmann, A. (2000)
BBA-Ref.-No.:	ANA2001-425
Test substance:	formulation BAS 510 01 F nicobifen 50 %
Guideline:	Typhlodromus (Blümel et al. 2000)
Test species:	*Typhlodromus pyri*
Developmental stage:	protonymphs
Substrate:	glass plates
Exposure route:	deposit
Exposure duration:	14 d
valid:	yes
GLP compliance:	yes

Results:

Appl. rate	Mortality	Sublethal effects
92 g/ha	0 %	
230 g/ha	0 %	
576 g/ha	0 %	+14 % (Fertility)
1400 g/ha	0 %	3 % (Fertility)
3600 g/ha	0 %	+3 % (Fertility)

Parasitoids

Title:	**Effects of BAS 510 01 F on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) in the Laboratory - Dose Response Test -**
Author:	Moll, M. and Groer, M. (2000)
BBA-Ref.-No.:	ANA2001-424
Test substance:	formulation BAS 510 01 F nicobifen 50 %
Guideline:	Aphidius, Lab (Polgar 1998)
Test species:	*Aphidius rhopalosiphi*
Developmental stage:	Imagines
Substrate:	glass plates
Exposure route:	deposit
Exposure duration:	48 h
valid:	yes
GLP compliance:	yes

Results:

Appl. rate	Mortality	Sublethal effects
711 g/ha	0 %	
1067 g/ha	3 %	
1600 g/ha	5 %	
2400 g/ha	11 %	25 % (parasitation capacity)
3600 g/ha	11 %	34 % (parasitation capacity)

Plant dwelling species

Title:	**Effect of BAS 510 01 F on the Green Lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) in a Laboratory Trial**
Author:	Ufer, A. (2000)
BBA-Ref.-No.:	ANA2001-426
Test substance:	formulation BAS 510 01 F nicobifen 50 %
Guideline:	Chrysopa (Vogt et al. 2000)
Test species:	*Chrysopa carnea*
Developmental stage:	larvae
Substrate:	glass plates
Exposure route:	deposit
Exposure duration:	8 d
valid:	yes
GLP compliance:	yes

Results:

Appl. rate	Mortality	Sublethal effects
2400 g/ha	2 %	11 % (Fertility)

Soil dwelling species

Title:	**BAS 510 01 F:Acute Toxicity Test With Spiders, *Pardosa* sp. (Araneae: Lycosidae)**
Author:	Nienstedt, K. (2001)
BBA-Ref.-No.:	ANA2001-428
Test substance:	formulation BAS 510 01 F nicobifen 50 %
Guideline:	Pardosa (BBA 23-2.1.9, Heimbach et al. 1999)
Test species:	*Pardosa spp.*
Developmental stage:	adults
Substrate:	quartz sand
Exposure route:	overspray + deposit
Exposure duration:	14 d
valid:	yes
GLP compliance:	yes

Results:

Appl. rate	Mortality	Sublethal effects
2400 g/ha	0 %	5 % (Food uptake)

Title:	**Effect of BAS 510 01 F on the ground dwelling predator *Poecilus cupreus* (Coleoptera, Carabidae) in a laboratory trial**
Author:	Bühler, A. (2000)
BBA-Ref.-No.:	ANA2001-427
Test substance:	formulation BAS 510 01 F nicobifen 50 %
Guideline:	Poecilus (BBA 23-2.1.8, Heimbach et al. 1999)
Test species:	*Poecilus cupreus*
Developmental stage:	imagines
Substrate:	quartz sand
Exposure route:	overspray + deposit
Exposure duration:	14 d
valid:	yes
GLP compliance:	yes

Results:

Appl. rate	Mortality	Sublethal effects
2400 g/ha	0 %	5 % (Food uptake)

Table B.9.5-1: Summary of arthropod toxicity data with the formulation effects of BAS 510 01 F (WG), (nicobifen 50 %)

Test material	Species	Developmental stage	Substrate	Dosage g/ha	Effects (%) lethal	sublethal
	Predatory mites					
BAS 510 01 F	*T. pyri*	Protonymphs	I	92	0	
BAS 510 01 F	*T. pyri*	Protonymphs	I	230	0	
BAS 510 01 F	*T. pyri*	Protonymphs	I	576	0	+14
BAS 510 01 F	*T. pyri*	Protonymphs	I	1400	0	3
BAS 510 01 F	*T. pyri*	Protonymphs	I	3600	0	+3
	Parasitoids					
BAS 510 01 F	*A. rhopalosiphi*	Imagines	I	711	0	
BAS 510 01 F	*A. rhopalosiphi*	Imagines	I	1067	3	
BAS 510 01 F	*A. rhopalosiphi*	Imagines	I	1600	5	
BAS 510 01 F	*A. rhopalosiphi*	Imagines	I	2400	11	25
BAS 510 01 F	*A. rhopalosiphi*	Imagines	I	3600	11	34
	Plant dwelling species					
BAS 510 01 F	*C. carnea*	Larvae	I	2400	2	11
	Soil dwelling species					
BAS 510 01 F	*Pardosa spp.*	Adults	I	2400	0	5
BAS 510 01 F	*P. cupreus*	Imagines	I	2400	0	5

I = Inert substrate, N = Natural substrate

B.9.5.2 Field tests (Annex III 10.5.2)

Predatory mites

Title:	**Effect of BAS 510 01 F on Populations of the Predatory Mite *Typhlodromus Pyri*, Scheuten in a Field Study (Vineyard)**
Author:	Ufer, A. (2001)
BBA-Ref.-No.:	ANA2001-431
Test substance:	formulation BAS 510 01 F nicobifen 50 %
Guideline:	BBA VI 23-2.3.4 (Heimann-Detlefsen, 1991)
Species:	*Typhlodromus pyri*
Developmental stage:	lifecycle(s)
Substrate:	natural substrate (grapes, southern Germany)

Test design:	four variants: 0.06 kg BAS 510 01 F/ha, 1.2 kg BAS 510 01 F/ha, reference substance (Antracol WG 3.2 kg/ha), water treated control, 5 replicates, 3 application per season in an interval of 14 days +/- 1 day (3rd application: 16 days) between mid June and the mid-July. method: number of live mites / 25 leaves were counted at 7 days before 1. treatment, 7-8 days after 1st, 2 nd and 3rd application and 27 days after 3rd application.
Exposure route:	overspray + deposit + oral
Calculation of effects:	Henderson and Tilton
valid:	yes
GLP compliance:	yes

Results:

	Effects in comparison to control % (Henderson & Tilton)			
Assessment	8 days after 1st application	8 days after 2nd application	7 days after 3rd application	27 days after 3rd application
BAS 510 01 F 0.06 kg/ha	25.2	15.6	0	6
BAS 510 01 F 1.2 kg/ha	0	0	0	0

Title:	**A Field Study to Evaluate the Effects of BAS 510 01 F Against the Predatory Mite *Typhlodromus pyri* Scheuten in Vines**
Author:	Müther, J. (2001)
BBA-Ref.-No.:	ANA2001-430
Test substance:	formulation BAS 510 01 F nicobifen 50 %
Guideline:	BBA VI 23-2.3.4, Bluemel et al. 2000
Species:	*Typhlodromus pyri*
Developmental stage:	lifecycle(s)
Substrate:	natural substrate (grapes, south-west Germany)
Test design:	four variants: 0.06 kg BAS 510 01 F/ha, 1.2 kg BAS 510 01 F/ha, reference substance (Antracol WG 3.2 kg/ha), water treated control, 5 replicates, 3 application per season in an interval of 13 to 14 days between mid June and the mid-July. method: number of live mites / 25 leaves were counted at 3 days before 1. treatment, 6 days after 1st, 7 days after 2 nd and 3rd application and at 27 days after 3rd application.
Exposure route:	overspray + deposit + oral
Calculation of effects:	Henderson and Tilton
valid:	yes
GLP compliance:	yes

Results:

	Effects in comparison to control % (Henderson & Tilton)			
Assessment	6 days after 1st application	7 days after 2nd application	7 days after 3rd application	27 days after 3rd application
BAS 510 01 F 0.06 kg/ha	25.9	19.9	33.6	5.0
BAS 510 01 F 1.2 kg/ha	31.6	13.6	39.7	2.8

Title:	**Effects of "BAS 510 01 F" on predatory mites (*Typhlodromus pyri*) under typical vine culture conditions on grape vines, Germany 2000**
Author:	Ipach, R. (2000)
BBA-Ref.-No.:	ANA2001-429
Test substance:	Formulation BAS 510 01 F Nicobifen 50 %
Guideline:	BBA VI 23-2.3.4
Species:	*Typhlodromus pyri*
Developmental stage:	lifecycle(s)
Substrate:	natural substrate (grapes, south-west Germany)
Test design:	four variants: 0.06 kg BAS 510 01 F/ha, 1.2 kg BAS 510 01 F/ha, reference substance (Antracol WG 3.2 kg/ha), water treated control, 4 replicates, 3 application per season (09 June, 03 July and 02. Aug.). method: number of live mites / 25 leaves were counted at 1 day before 1. treatment, 7 days after 1st, 2 nd and 3rd application and at 29 days after 3rd application.
Exposure route:	overspray + deposit + oral
Calculation of effects:	Henderson and Tilton
valid:	yes
GLP compliance:	yes

Results:

	Effects in comparison to control % (Henderson & Tilton)			
Assessment	7 days after 1st application	7 days after 2nd application	7 days after 3rd application	29 days after 3rd application
BAS 510 01 F 0.06 kg/ha	7	2	12	9
BAS 510 01 F 1.2 kg/ha	26	9	21	9

Table B.9.5-2: **Summary of arthropod field and semi-field testing**

Test material	Species	Test	No. of appl.	Dosage per appl. (g/ha)	Effect (%) Final bonitur *
	Predatory mites				
BAS 510 01 F	*T. pyri*	Field	3	1200	0 / 0
BAS 510 01 F	*T. pyri*	Field	3	60	0 / 6
BAS 510 01 F	*T. pyri*	Field	3	1200	39.7 / 2.8
BAS 510 01 F	*T. pyri*	Field	3	60	33.6 / 5.0
BAS 510 01 F	*T. pyri*	Field	3	1200	21 / 9
BAS 510 01 F	*T. pyri*	Field	3	60	12 / 9

* 7 / 27 days resp. 29 days after last application

B.9.5.3 Risk assessment for non-target terrestrial arthropods

Details of use patterns
The fungicide BAS 510 01 F (50 % nicobifen) is a water dispersible granule formulation (WG) containing 500 g/kg nicobifen and is used in agriculture (production of field crops and grapevines). The maximum recommended field rate of BAS 510 01 F in vineyards is 1.2 kg/ha and in field crops 2 x 1 kg/ha.

Risk assessment
Non-target arthropods are likely to be exposed to formulated nicobifen 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.

The field rates tested given in Table B.9.5-1 and Table B.9.5-2 correspond to the intended uses given above. According to the data submitted a low toxicity was demonstrated in basic laboratory tests on a number of species (i.e. *T. pyri* , *A. rhopalosiphi, C. carnea, Pardosa spp., P. cupreus)* and in field tests with *T. pyri*.

It is therefore concluded, that use of nicobifen 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:	**Acute Toxicity (14 Days) of BAS 510 to the Earthworm *Eisenia fetida* (Savigny 1826) in Artificial Soil**
Author:	Lührs, U. (1999)
BBA-Ref.-No.:	ARW2001-84
Test substance:	Technical nicobifen
Purity:	99.7 %
Guideline:	OECD 207
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	0/ 198/ 296/ 444/ 667/ 1000 mg/kg
Findings:	
LC50:	>1000 mg as/kg
Lowest lethal conc.:	>1000 mg as/kg
NOEC:	1000 mg as/kg
valid:	yes
GLP compliance:	yes

Title:	**Acute toxicity (14 days) of BAS 510 01 F to the earthworm *Eisenia fetida* (Savigny 1826) in artificial soil**
Author:	Lührs, U. (2000)
BBA-Ref.-No.:	ARW2001-76
Test substance:	Formulation BAS 510 01 F (nicobifen 500 g/kg)
Guideline:	OECD 207
Test species:	*Eisenia fetida*
Exposure duration:	14 d
Worms per treatment:	4 x 10
Conc. levels (nom):	0, 198, 296, 444, 667, 1000 mg/kg

Findings:
LC50: >1000 mg/kg
Lowest lethal conc.: >1000 mg/kg
NOEC: 667 mg/kg

valid: yes
GLP compliance: yes

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: **Effects of BAS 510 01 F on growth and reproduction of the earthworm *Eisenia foetida***
Author: Krieg, W. (2000)
BBA-Ref.-No.: ARW2001-77

Test substance: Formulation BAS 51001 F (nicobifen 500 g/kg)

Guideline: BBA 2-2
Test species: *Eisenia foetida*
Exposure duration: 8 w
Worms per treatment: 4 x 10

Findings:
NOEC: 3.6 kg/ha (equivalent 2,39 mg as/kg)
valid: yes
GLP compliance: yes

Table B.9.6-1: Effects of BAS 51001 F on reproduction and biomass of *Eisenia foetida*

Dose level	Adult mortality [%]	Adult weight (% of initial)	Number of juveniles
control	0	160.93	130.25
1.8 kg/ha	0	171.78	97.75
3.6 kg/ha	0	165.30	98.25
9.0 kg/ha	0	176.51*	52.50*
18.0 kg/ha	0	170.03	29.50*

* sign. $\alpha = 0.05$, Dunett-test

B.9.6.2.2 Effects in the field

B.9.6.2.2.1 Field study to evaluate the effects of BAS 510 01 F on earthworms (season 2000), Krieg, W., 2001

Interim Report: **BASF DocID 2001/1000101 (BBA Ref-No. ARW 2001-78)**
Final Report: **BASFDocID 2001/1014661 (BBA Ref-No. ARW 2001-168)**

Year: 2000/2001
Guideline: BBA guideline part VI, 2-3 (1994), ISO/CD 11268-3 (1999)
GLP: yes
valid: yes

Test substance: BAS 510 01 F
(nicobifen 500 g/kg)

Test design: a randomised block design with four replicates and 4 treatments: untreated control, reference substance benomyl with 4 kg as/ha and two rates of BAS 510 01 F
Application: 3 applications with 1.2 kg BAS 510 01 F/ha (corresponding to 0.6 kg as/ha) and 3 applications with 0.6 kg BAS 510 01 F/ha (corresponding to 0.3 kg as/ha) with intervals of approx. 12 days
Study site: Rheinland-Pfalz/ Germany
Grassland, 4 mulching dates /year
Soil: silty sand
Irrigation: 1. irrigation 5 days before the last application and 4 times after the last application
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.9.6-2: Abundance of earthworms (number of individuals; % of untreated control)

Sampling date	**27. 04.2000 prior to application**		**22.05.2000 12 days after 2nd application**		**22.08.2000 ≈ 3 month after last application**		**18.10.2000 ≈ 5 month after last application**		**14.05.2001 ≈ 1 year after last application**	
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 510 01 F 3 x 1.2 kg/ha	259.8	98.4	221.8	79.5	357.8	77.7	246.5	65.0*	187.5	68.4
BAS 510 01 F 3 x 0.6 kg/ha	251.3	95.2	219.0	78.5	399.5	86.8	294.0	77.5	238.0	86.8
Benomyl 4 kg as/ha	262.0	99.2	172.8	61.9*	279.0	60.6*	230.8	60.8*	172.3	62.8
Control	264.0	100.0	279.0	100.0	460.3	100.0	379.3	100.0	274.3	100.0

* sign. $\alpha = 0.05$, Dunett-test

Table B.9.6-3: Biomass of earthworms (biomass in g/m², and % of untreated control)

Sampling date	27. 04.2000 prior to application		22.05.2000 12 days after 2nd application		22.08.2000 ≈ 3 month after last application		18.10.2000 ≈ 5 month after last application		14.05.2001 ≈ 1 year after last application	
Treatment	g/m²	% of contr.	g/m²	% of contr.	g/m²	% of contr.	g/m²	% of contr.	g/m²	% of contr.
BAS 510 01 F 3 x 1.2 kg/ha	71.76	106.4	69.20	81.9	106.47	77.3*	70.59	65.8*	45.4	72.5
BAS 510 01 F 3 x 0.6 kg/ha	59.98	88.9	70.42	83.3	124.28	90.3	76.06	70.9*	54.56	87.1
Benomyl 4 kg as/ha	71.28	105.7	34.07	40.3*	91.10	66.2*	87.49	81.5	47.78	76.3
Control	67.46	100.0	84.50	100.0	137.65	100.0	107.33	100.0	62.66	100.0

*sign. $\alpha = 0.05$, Dunett-test

B.9.6.2.2.2 Field study to evaluate the effects of BAS 510 01 F on earthworms (Season 2000), Ehlers, H. A. 2001

Interim Report: BASF DocID 2001/1000102 (BBA Ref-No. ARW 2001-79)
Final Report: BASF DocID 2001/1014681 (BBA Ref-No. ARW 2001-169)

Year: 2000/2001
Guideline: ISO/CD11268-3 (draft 1997), BBA guideline part VI, 2-3(1994)
GLP: yes
valid: yes

Test substance: BAS 510 01 F
(nicobifen 500 g/kg)

Application: 3 applications with 1.2 kg BAS 510 01 F/ha (corresponding to 0.6 kg as/ha) and 3 applications with 0.6 kg BAS 510 01 F/ha (corresponding to 0.3 kg as/ha) with intervals of approx. 14 days
Site: Darmstadt/Germany
grassland, 2 mulching dates/year
Soil: silty loam to silty clavey loam
Irrigation: none
Test design: 4 replicates with 10 x 10 m plot size;
4 treatments: untreated control, reference substance benomyl with 4 kg as/ha and two rates of BAS 510 01 F
Earthworm sampling: electrical octett method, 0.25 m² sample with 4 samples /sampling date and plot (= 16 samples/treatment and date)

Findings:

Table B.9.6-4: Abundance of earthworms (individuals/m^2 and % of untreated control)

Sampling date	27. 04.2000 prior to application		19.06.2000 13 days after 2nd application		28.08.2000 ≈ 9 weeks after last application		25.10.2000 ≈ 18 weeks after last application		21.05.2001 11 month after last application	
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 510 01 F 3 x 1.2 kg/ha	152.0	89.8	37.5	81.9	283.5	89.9	253.5	80.2	98.0	71.6
BAS 510 01 F 3 x 0.6 kg/ha	150.3	88.8	47.5	103.7	273.5	86.7	265.8	84.1	205.0	149.9*
Benomyl 4 kg as/ha	168.3	99.4	17.8	38.9*	194.8	61.8*	239.0	75.6	131.0	95.8
Control	169.3	100.0	45.8	100.0	315.3	100.0	316.0	100.0	136.8	100.0

* sign. α = 0.05 (Bonferroni U-test for data which were not normally distributed and homogenous)

Table B.9.6-5: Biomass of earthworms (g/m^2 and % of untreated control)

Sampling date	27. 04.2000 prior to application		19.06.2000 13 days after 2nd application		28.08.2000 ≈ 9 weeks after last application		25.10.2000 ≈ 18 weeks after last application		21.05.2001 11 month after last application	
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 510 01 F 3 x 1.2 kg/ha	98.3	112	22.6	90.4	167.2	95.2	147.4	113.3	38.9	71
BAS 510 01 F 3 x 0.6 kg/ha	88.8	102	24.3	97.2	149.5	85.1	132.0	101.5	70.8	129.2
Benomyl 4 kg as/ha	103.5	118	10.4	41.6*	147.7	84.1*	139.2	107.0	55.6	101.5
Control	87.4	100	25.0	100	175.6	100	130.1	100	54.8	100

* sign. α = 0.05 (Bonferroni U-test for data which were not normally distributed and homogenous)

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). Initial PECs, TER_a and TER_{lt} are summarised in Table B.9.6-6.

Table B.9.6-6: TER_a and TER_{lt} for earthworms

Test material	Toxicity data (corrected) mg as/kg substrate	$PEC_{initial}$ mg as/kg*	Time scale	TER
nicobifen	>500	1.333	Acute	>375
BAS 510 01 F	>250	1.333	Acute	>187
BAS 510 01 F	1.197	1.333	Longterm	0.9

* see chapter B.08.03

Laboratory tests

The acute TER values for the active substance and the product (calculated for the as content) are far above the relevant Annex VI trigger of 10 (see Table B.9.6-6). Therefore it is concluded that the active substance and the product do not pose an acute risk to earthworms.

The long term risk is assessed using a NOEC of 3.6 kg product/ha, corrected to 1.8 kg product/ha, since Log Pow is > 2. The NOEC converted into a soil concentration corresponds to 1.197 mg as/kg soil. For long term risk, concern is raised due to the TER_{lt} value for reproduction of 0.9 (Bean scenario, 2 x 0.5 kg as/ha, no interception, worst case) being below the trigger value of 5.

Field tests

In the first field study (BBA Ref-No. ARW 2001-78) there was a significant reduction in abundance of about 35 % compared to control five month after the third application of 1.2 kg BAS 510 01 F/ha. In the case of biomass of earthworms the reduction compared to control five month after the last application was significant for both concentrations 0.6 and 1.2 kg BAS 510 01 F/ha. One year after the last application the effects on abundance and biomass decreased in comparison to the control in case of abundance of about 32 % for the higher application rate respectively 13 % for the lower concentration and are no longer significant. The abundance and the biomass of epilobous juveniles and of *Aporrectodea caliginosa* were reduced in both treatments after one year.

In the second field study (BBA Ref-No. ARW 2001-79) there are no significant reductions in the abundance or biomass of earthworms. However the effects have the same tendency in the first half year as in the first study. The reduction of abundance compared to control increased after the last application. In the first study in both concentrations a reduction of the effects were observed whereas in the second field study the not significant effects increase in case of the higher application rate. Also in this study effects on single species about 30 % in comparison to control were detected. The biomass of *Aporrectodea caliginosa* were reduced about 56 % eleven month after the last application.

In an additional statistical evaluation of the first earthworm field study (BASFDocID 2001/1014661) the community level was analysed using diversity indices, similarity analysis and Principal Response Curves. In addition, each taxon was analysed separately with Williams`and Dunnett`s tests to detect significant differences to controls. The lower treatment had only short-term effects (observed on sampling 2, 25 days after the 1st treatment). One year after the applications, no effects were found anymore.
The laboratory data submitted have shown that a longterm risk to earthworms can not be excluded. However, on the basis of the additional field data submitted a potential risk to

earthworms can be excluded only for the lower treatment (3 x 0.6 kg BAS 510 01 F/ha respectively 0.9 kg as/ha).
As the highest application rate according to the intended uses is 2 x 0.5 kg as/ha in beans which is only slightly higher than 0.9 kg as/ha, this is considered acceptable too. Higher application rates than 1 kg as/ha and year should not be applied on the basis of the data submitted.

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

B.9.7.1 Collembola *Folsomia candida*

1. Laboratory test

Title:	**Effects of BAS 510 01 F on reproduction of the Collembola *Folsomia candida* in artificial soil**
Author:	Meister, A. (2001)
BBA-Ref.-No.:	ARW 2001-80
Test substance:	formulation BAS 510 01 F nicobifen 500 g/kg
Guideline:	ISO 11267 (1999)
Test species:	*Folsomia candida*
Test design:	28-days exposure in treated artificial soil; 5 different concentrations of the test substance (62.5, 125, 250, 500, and 1000 mg BAS 510 01 F/kg soil), untreated water control and toxic standard (200 mg Betosip/kg soil equivalent to 30.3 mg Phenmedipham/kg soil); 5 replicates/concentration with 10 Collembola.
GLP:	yes
valid:	The test is not valid with respect to reproduction. The validity criteria concerning the coefficient of variation for the reproduction assessment is 64.8 % and so far above the trigger of 30 %. Therefore only the data for the mortality are considered.
Findings:	NOEC (mortality): 250 mg BAS 510 01 F/kg soil

2. Laboratory test

Title:	**Effects of BAS 510 01 F on reproduction of the Collembola *Folsomia candida* in artificial soil**
Author:	Meister, A. (2001)
BBA-Ref.-No.:	ARW 2002-15
Test substance:	formulation BAS 510 01 F nicobifen 500 g/kg
Guideline:	ISO 11267 (1999)
Test species:	*Folsomia candida*
Test design:	28-days exposure in treated artificial soil; 5 different concentrations of the test substance (62.5, 125, 250, 500, and 1000 mg BAS 510 01 F/kg soil), untreated water control and toxic standard (200 mg Betosip/kg soil equivalent to 30.3 mg Phenmedipham/kg soil); 5 replicates/concentration with 10 Collembola.
GLP:	yes
valid:	yes
Findings:	NOEC (mortality): 125 mg BAS 510 01 F/kg soil NOEC (reproduction) >1000 mg BAS 510 01 F /kg soil

Title:	Monitoring of collembola populations following an exposure to BAS 510 01 F in the field (grassland)

A field monitoring to evaluate potential effects on collembola populations was conducted together with the earthworm field study (BBA Ref.-No. ARW 2001-78), see chapter B.09.06.

Author:	Krieg, W., Schick, H. (2001)
BBA-Ref.-No.:	ARW 2001-81
Test substance:	formulation BAS 510 01 F (WG) nicobifen 500 g/kg
Guideline:	ISO 11268-3 (1999), BBA guideline part VI, 2-3 (1994)
Test design:	a randomised block design with four replicates and 4 treatments: untreated control, reference substance Benomyl with 4 kg as/ha and two rates of BAS 510 01 F
Application:	3 applications with 1.2 kg BAS 510 01 F/ha (recommended application rate, corresponding to 0.6 kg as/ha) and 3 applications with 0.6 kg BAS 510 01 F/ha (half of the application rate, taking into account vegetation cover during application, corresponding to 0.3 kg as/ha) with intervals of approx. 12 days
Study site:	Rheinland-Pfalz/ Germany Grassland, 4 mulching dates /year
Soil:	silty sand
Irrigation:	1. irrigation 5 days before the last application and 4 times after the last application
Sampling:	In October 2000 four month after the last application 5 soil cores (8 cm length and 6.4 cm diameter) per plot (i.e. 20 cores / variant) were taken.

Evaluation:	extraction with McFayden within 7 days, abundance [Ind/m2] and identi fication up to the family level
GLP:	yes

Findings: *Isotomidae* generally dominated by about 40 % to 60 %, followed by *Onychiuridae* with about 20 % to 50 %. *Sminthuridae* were found in all plots (about < 5 % - 15 %). *Poduridae* were determined in the majority of plots at lower numbers (in average < 10 %). *Entomobryidae* could be found only sporadically (0 % - about 5 %).
The Collembola population density showed high fluctuations of total abundance between the different samples of a test plot. Significantly different results were calculated for the treatment with the higher application rate (3 x 1.2 kg BAS 510 01 F) not for mean values of abundance but on dominances of *Onychiuridae* and *Isotomidae*. *Onychiuridae* showed a decline whereas *Isotomidae* an increase corresponding to the control. In the family of *Isotomidae* a significant reduction of the number of species could be detected in this treatment. The lower concentration did not show any significant deviations from the control.

B.9.7.2 Effects on organic matter breakdown

Title:	**Effects of BAS 510 01 F on the organic matter degradation under field conditions (litter bag method) study 1**
Author:	Krieg, W. (2001)
BBA-Ref.-No.:	ARW 2001-82
Test substance:	formulation BAS 510 01 F nicobifen 500 g/kg
Guideline:	draft method (BBA, 2000)
GLP:	yes
valid:	yes
Test species:	naturally occurring soil organisms
Test design:	Main study on earthworms (BBA Ref.-No. ARW 2001-78) of randomised block design with four replicates with 10 x 10 m plot size. Litterbag study with 3 treatments: untreated control, reference substance Benomyl with 4 kg as/ha and one rate of BAS 510 01 F Litterbags (12 x 20 cm, mesh size 8 x 8 mm) filled with about 4.0 g (dry weight) wheat straw; horizontally buried at a depth of about 5 cm, shortly before the first treatment in April 2000
Application:	3 applications with 1.2 kg BAS 510 01 F/ha (recommended application rate, corresponding to 0.6 kg as/ha) with intervals of approx. 12 days
Study site:	Rheinland-Pfalz/ Germany Grassland, 4 mulching dates /year
Soil:	silty sand
Irrigation:	1. irrigation 5 days before the last application and 4 times after the last application

Sampling: Four sampling dates were scheduled over a period of a half year. At each sampling date 32 bags per treatment (with four plots as replicates) were taken from the soil.

Evaluation: The cleaned bag content was oven dried at 50 °C for 24 hours, weight and finally ashed at 600 °C for 2 hours

Findings: Breakdown of leaf material in the BAS 510 01 F treated plots was similar to that in the water treated control plots and the Benomyl treated plots over the whole sampling time. Statistical analysis indicated significant difference at the second evaluation date for the Benomyl variant (-11.4 % deviation from control) and at the last evaluation date for the BAS 510 01 F treated plots variant (-6.5 % deviation from control) only.

Table B.9.7-1: Weight loss [%] of ash-free wheat straw – exposed to BAS 510 01 F – and deviation from control

	Weight loss [%] of ash-free wheat straw at each sampling date			
	05.06.2000 13 d after last application	**11.07.2000 49 d after last application**	**25.08.2000 3 month after last application**	**06.11.00 ≈ 6 month after last application**
Control	32.5	43.1	58.5	75.5
BAS 510 01 F 3 x 1.2 kg ha	30.0 (-7.7)	41.7 (-3.2)	55.2 (-5.6)	70.6* (-6.5)
Benomyl 4 kg as / ha	31.7 (-2.5)	38.2* (-11.4)	57.7 (-1.4)	71.6 (-5.2)

* sign. A = 0.05 (Wilcoxon`s rank sum test and Bonferroni t-Test)

Title: **Effects of BAS 510 01 F on the organic matter degradation under field conditions (litter bag method) study 2**

Author: Krieg, W. (2001)

BBA-Ref.-No.: ARW 2001-83

Test substance: formulation BAS 510 01 F
nicobifen 500 g/kg

Guideline: draft method (BBA, 2000)

GLP: yes

valid: yes

Test species: naturally occurring soil organisms

Test design: The litterbag study was of randomised block design with four replicates (10 x 2 m plot size) and 3 treatments: untreated control, reference sub stance Benomyl and one rate of BAS 510 01 F.
Litterbags (12 x 20 cm, mesh size 8 x 8 mm) filled with about 4.0 g (dry weight) wheat straw; horizontally buried at a depth of about 5 cm, shortly after the treatment in April 2000.
Four sampling dates were scheduled over a period of a half year. At each sampling date 32 bags per treatment (with four plots as replicates) were taken from the soil.

Application:	1 application with 3.6 kg BAS 510 01 F/ha (corresponding to 1.8 kg as/ha) and one application with 4 kg Benomyl/ha
Study site:	Rheinland-Pfalz/ Germany Fallow land, 3 mulching dates /year
Soil:	sandy loam
Irrigation:	none
Sampling:	Four sampling dates were scheduled over a period of a half year. At each sampling date 32 bags per treatment (with four plots as replicates) were taken from the soil.
Evaluation:	The cleaned bag content was oven dried at 50 °C for 24 hours, weight and finally ashed at 600 °C for 2 hours.
Findings:	Breakdown of leaf material in the BAS 510 01 F treated plots was significantly reduced over the whole sampling time. The deviations from the control were at the first sampling date –17.8 % and at the last application date –10.7 %. The Benomyl treatment did not result in clear effects on straw weight loss.

Table B.9.7-2: Weight loss [%] of ash-free wheat straw – exposed to BAS 510 01 F – and deviation from control

	Weight loss [%] of ash-free wheat straw at each sampling date			
	19.05.2000 1 month after application	**28.06.2000 ≈ 2 month after application**	**08.08.2000 ≈ 4 month after last application**	**23.10.00 ≈ 6 month after last application**
Control	24.7	37.5	53.5	68.3
BAS 510 01 F 3.6 kg ha	20.3* (-17.8)	32.5* (-13.3)	45.2* (-15.5)	61.0* (-10.7)
Benomyl 4 kg as / ha	24.1 (-2.4)	36.1 (-3.7)	54.4 (+1.7)	72.8 (+6.6)

* sign. A = 0.05 (Wilcoxon`s rank sum test and Bonferroni t-Test)

B.9.7.3 Risk assessment for other soil non-target macro-organisms

Collembola *Folsomia candida*

The field study is not valid as no pre-treatment check was done and because only one evaluation after 6 month was reported, therefore no assessment of the seasonal population development is possible.

Organic matter breakdown

The application of 3 x 1.2 kg BAS 510 01 F/ha (equivalent to 2.4 mg as / kg soil) on the buried bags showed no unacceptable effects to the organic matter break down (ARW 2001-82).

After one application of 3.6 kg BAS 510 01 F/ha on the bags before they were buried showed significant reduced organic matter breakdown in comparison to the untreated control (ARW 2001-83). However the effects were less than 20 % and at the last sampling date the deviation

between treatment and control were less than at the beginning which could indicate recovery. It has to be taken into consideration that the application rate of 3.6 kg /ha BAS 510 01 F directly applied onto the ground of the test site is a highly conservative scenario.
Therefore no unacceptable risk to the organic matter breakdown is given.

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 nicobifen on microbial activities in soil. The tests were carried out with the formulation BAS 510 01 F.

B.9.8.1 Carbon conversion (Annex IIA 8.5; Annex IIIA 10.7)

Title:	**Assessment of the Side Effects of BAS 510 01 F on the Activity of the Soil Microflora, Short-Term Respiration**
Author:	Wachter, S. (2001)
BBA-Ref.-No.:	BMF2001-65
Test substance:	Formulation BAS 510-01 F (nicobifen 500 g/kg)
Guideline:	BBA 1-1 (C)
Type of test:	C-mineralisation
Activity:	Short term respiration
Amendment:	Glucose
valid:	yes
GLP compliance:	yes

Findings:

Table B.9.8-1: Effects of BAS 510 01 F on CO_2-respiration rates

type of soil	test substance application rate [kg /ha]	effect compared to untreated control [%]	test duration [d]	influence tolerable
loamy sand	BAS 510-01 F 1.2 kg/ha	-7.4	28	yes
	BAS 510-01 F 12 kg/ha	-8.4	28	yes
loamy silt	BAS 510-01 F 1.2 kg/ha	-4.9	28	yes
	BAS 510-01 F 12 kg/ha	-11.2	28	yes

B.9.8.2 Nitrogen conversion

Title:	**Assessment of the Side Effects of BAS 510 01 F on the Activity of the Soil Microflora, Nitrogen Turnover**
Author:	Wachter, S. (2001)
BBA-Ref.-No.:	BMF2001-66
Test substance:	Formulation BAS 510 01 F (nicobifen 500 g/kg)
Guideline:	BBA 1-1 (N)
Type of test:	N-Mineralisation
Amendment:	Lucerne meal (0.5 %)
valid:	yes
GLP compliance:	yes

Findings:

Table . 9.8-2: Effects of BAS 510 01 F on nitrate formation

type of soil	test substance application rate [kg /ha]	effect compared to untreated control [%]	test duration [d]	influence tolerable
loamy sand	BAS 510-01 F 1.2 kg/ha	-10.9	28	yes
	BAS 510-01 F 12 kg/ha	+3.1	28	yes
loamy silt	BAS 510-01 F 1.2 kg/ha	-2.4	28	yes
	BAS 510-01 F 12 kg/ha	0.0	28	yes

B.9.8.3 Risk assessment

The influence of the formulation BAS 510 01 F (500 g nicobifen/kg) on the soil respiration and the nitrogen turnover was evaluated in a loamy sand soil and a loamy silt soil. The presented results (<25 % effect in comparison to untreated control) show that when applying nicobifen containing plant protection products no lasting effects on microbial activities are to be expected at application rates up to 6 kg as/ha.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

Title: **BAS 510 01 F: Effects on non-target plants in the greenhouse – A limit test**
Author: Oberwalder, C.; Schmidt, O. (2000)
BBA-Ref.-No.: PFL 2001-63

Test substance: Formulation BAS 510 01 F
(nicobifen 500 g/kg)
Guideline: OECD guideline no. 208 (draft 1999)
Test species: *Avena sativa, Allium cepa, Brassica oleracea, Pisum sativum, Daucus carota, Zea mays*
Test design: Limit test (vegetative vigour study) in a greenhouse:
3 variants: two treatment variants with application (post emergence) of 1.2 kg and 3.6 kg BAS 510 01 F/ha and one water treated control
4 replicates/variant; 1pot/replicate, 3-4 plants per pot
Evaluation: Phytotoxicity was evaluated after 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.

Findings:

Table B.9.9-1: Effect of BAS 510 01 F on plant biomass and plant conditions (visible damage) after 14 days of exposure

Treatment	***Daucus carota***	***Brassica oleracea***	***Pisum sativum***	***Zea mays***	***Avena sativa***	***Allium cepa***
	Mean plant weight [% of control]					
Control	100.0	100.0	100.0	100.0	100.0	100.0
BAS 510 01 F 1.2 kg/ha	109.4	100.2	91.2	101.9	105.7	108.2
BAS 510 01 F 3.6 kg/ha	110.6	98.9	97.5	103.5	99.5	113.6
	Mean visible damage [% damage compared to control]					
Control	0.0	0.0	0.0	0.0	0.0	0.0
BAS 510 01 F 1.2 kg/ha	2.5	0.0	0.0	0.0	0.0	0.0
BAS 510 01 F 3.6 kg/ha	5.0	0.0	0.0	0.0	0.0	0.0

No significant effects on weight were observed (Dunett test, $p<0.05$). The maximum observed reduction in weight was 8.8 % in the single application rate. There were no effects on phytotoxicity of more than 5 %.

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)

B.9.10.1 Toxicity data

Title:	**Determination of the inhibition of oxygen consumption by activated sludge by BAS 510 F in the activated sludge respiration inhibition test according to GLP, EN 45001 and ISO 9002**
Author:	Werner, D.I. (1999)
BBA-Ref.-No.:	WAT2001-382
Test substance:	Technical nicobifen
Purity:	94.4 %
Guideline:	OECD 209
Test species:	Activated sludge
Exposure mode:	
Conc. levels (nom.):	500/1000 mg/l

Results (mg/l) related to nominal concentrations

Time	End point	NOEC	LOEC	Effect (%) at LOEC	EC50
0.5 h	respiration rate				>1000

valid:	yes
GLP compliance:	yes

B.9.10.2 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 sludge 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[8]
AIIA-8.1.1	Zok, S.	1999	Report BAS 510 F - Avian single-dose oral LD50 on the bobwhite quail (Colinus virginianus). 11W0179/97043 GLP, unpublished AVS2001-119	Y	BAS
AIIA-8.1.2	Zok, S.	1999	Test Report: BAS 510 F - Avian dietary LC50 test in chicks of the mallard duck (Anas platyrhynchos). 32W0179/97045 GLP, unpublished AVS2001-121	Y	BAS
AIIA-8.1.2	Zok, S.	1999	Test Report: BAS 510 F - Avian dietary LC50 test in chicks of the bobwhite quail (Colinus virginianus). 31W0179/97042 GLP, unpublished AVS2001-120	Y	BAS
AIIA-8.1.3	Zok, S.	2000	Report: BAS 510 F - 1-generation reproduction study on the mallard duck (Anas platyrhynchos) by administration in the diet. 72W0179/97122 GLP, unpublished AVS2001-123	Y	BAS
AIIA-8.1.3	Zok, S.	2000	Report: BAS 510 F - 1-generation reproduction study on the bobwhite quail (Colinus virginianus) by administration in the diet + Amendment No. 1 to the report. 71W0179/97044 GLP, unpublished AVS2001-122	Y	BAS
AIIA-8.2.1	Zok, S.	2001	BAS 510 F Acute toxicity study on the bluegill (Lepomis macrochirus Raf.) in a static system (96 hours). 2001/1001727 ! 14F0179/975132 GLP, unpublished WAT2001-364	Y	BAS
AIIA-8.2.1	Zok, S.	2001	BAS 510 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 2001/1001726 ! 12F0179/975131 GLP, unpublished WAT2001-363	Y	BAS

[8] 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[8]
AIIA-8.2.2.1	Zok, S.	1999	BAS 510 F - Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss Walbaum 1792). 1999/11847 ! 52F0179/975051 GLP, unpublished WAT2001-366	Y	BAS
AIIA-8.2.3	Chapleo, S., Caley, C.Y.	2000	Bioaccumulation and metabolism of [14C]-BAS 510F in rainbow trout. 2000/1017222 ! 394178 ! 18219 GLP, unpublished WAT2001-367	Y	BAS
AIIA-8.2.4	Dohmen, P.	2001	Effect of BAS 510 F on the immobility of Daphnia magna Straus in a 48 hour static, acute toxicity test. 2000/1018537 ! 41898 GLP, unpublished WAT2001-378	Y	BAS
AIIA-8.2.5	Hisgen	2001	BAS 510 F - Determination of the chronic effect on the reproduction of the water flea Daphnia magna Straus. 2000/1018539 ! 00/0618/51/2 GLP, unpublished WAT2001-379	Y	BAS
AIIA-8.2.6	Kubitza, J.	2001	Effect of BAS 510 F on the growth of the green alga Pseudokirchneriella subcapitata. 2000/1018524 ! 41893 GLP, unpublished WAT2001-380	Y	BAS
AIIA-8.2.7	Dohmen, P.	2001	Effects of BAS 510 F on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system. 2000/1018538 ! 44152 GLP, unpublished WAT2001-381	Y	BAS
AIIA-8.3	Sack, D.	1999	Effect of Reg. No. 300 355 on the Honeybee (Apis mellifera L.) in Laboratory Trials. BASF 1999/10823 GLP, unpublished BIE2001-27	Y	BAS
AIIA-8.4.1	Lührs, U.	1999	Acute Toxicity (14 Days) of BAS 510 to the Earthworm Eisenia fetida (Savigny 1826) in Artificial Soil. 1999/10816 GLP, unpublished ARW2001-84	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[8]
AIIA-8.6; AIIIA-10.8	Oberwalder, Ch. and Schmidt, O.	2000	BAS 510 01 F: Effects on non-target plants in the greenhouse - A limit test. 2000/1018515 GLP, unpublished PFL2001-63	Y	BAS
AIIA-8.7	Werner, D.I.	1999	Determination of the inhibition of oxygen consumption by activated sludge by BAS 510 F in the activated sludge respiration inhibition test according to GLP, EN 45001 and ISO 9002. 99/10289 ! 98/0715/08/1 GLP, unpublished WAT2001-382	Y	BAS
AIIIA-10.2.1	Jatzek, H.-J.	2001	BAS 510 01 F - Determination of the acute effect on the swimming ability of the water flea Daphnia magna Straus. 2000/1018540 ! 00/0295/50/1 GLP, unpublished WAT2001-384	Y	BAS
AIIIA-10.2.1	Kubitza, J.	2001	Effect of BAS 510 01 F on the growth of the green alga Pseudokirchneriella subcapitata. 2000/1018525 ! 59064 GLP, unpublished WAT2001-385	Y	BAS
AIIIA-10.2.1	Zok, S.	2000	BAS 510 01 F Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours). 2000/1018528 ! 12F0295/005010 GLP, unpublished WAT2001-383	Y	BAS
AIIIA-10.4	Kling, A.	2000	Assessment of the Side Effects of BAS 510 01 F to the Honey Bee, Apis mellifera L. in the Laboratory. 20001059/01-BLEU GLP, unpublished BIE2001-25	Y	BAS
AIIIA-10.5.1	Bühler, A.	2000	Effect of BAS 510 01 F on the ground dwelling predator Poecilus cupreus (Coleoptera, Carabidae) in a laboratory trial. 2000/1012479 GLP, unpublished ANA2001-427	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[8]
AIIIA-10.5.1	Goßmann, A.	2000	Effects of BAS 510 01 F on the Predatory Mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the Laboratory - Dose Response Design -. 2000/1018520 GLP, unpublished ANA2001-425	Y	BAS
AIIIA-10.5.1	Ipach, R.	2000	Effects of "BAS 510 01 F" on predatory mites (Typhlodromus pyri) under typical vine culture conditions on grape vines, Germany 2000. 2000/1014931 GLP, unpublished ANA2001-429	Y	BAS
AIIIA-10.5.1	Moll, M. and Groer, M.	2000	Effects of BAS 510 01 F on the Parasitoid Aphidius rhopalosiphi (Hymenoptera, Braconidae) in the Laboratory - Dose Response Test -. 2000/1018519 GLP, unpublished ANA2001-424	Y	BAS
AIIIA-10.5.1	Müther, J.	2001	A Field Study to Evaluate the Effects of BAS 510 01 F Against the Predatory Mite Typhlodromus pyri Scheuten in Vines. 2000/1014938 GLP, unpublished ANA2001-430	Y	BAS
AIIIA-10.5.1	Nienstedt, K.	2001	BAS 510 01 F: Acute Toxicity Test With Spiders, Pardosa Sp: (Araneae: Lycosidae). 2000/1018518 GLP, unpublished ANA2001-428	Y	BAS
AIIIA-10.5.1	Ufer, A.	2001	Effect of BAS 510 01 F on Populations of the Predatory Mite Typhlodromus Pyri, Scheuten in a Field Study (Vineyard). 2000/1014939 GLP, unpublished ANA2001-431	Y	BAS
AIIIA-10.5.1	Ufer, A.	2000	Effect of BAS 510 01 F on the Green Lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in a Laboratory Trial. 2000/1014932 GLP, unpublished ANA2001-426	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[8]
AIIIA-10.6.1	Ehlers, E.	2002	Addendum No. 1 to Final Report: 7584023 Field Studie to Evaluate the Effects of BAS 510 01 F on Earthworms. 2002/1000252 GLP, unpublished ARW2002-14	Y	BAS
AIIIA-10.6.1	Ehlers, H.A.	2001	Interim Report: Field Study to Evaluate the Effects of BAS 510 01 F on Earthworms (Season 2000). 2001/1000102 GLP, unpublished ARW2001-79	Y	BAS
AIIIA-10.6.1	Krieg, W.	2001	Interim Report: Field study to evaluate the effects of BAS 510 01 F earthworms (season 2000). 2001/1000101 GLP, unpublished ARW2001-78	Y	BAS
AIIIA-10.6.1	Krieg, W.	2000	Effects of BAS 510 01 F on Growth and Reproduction of the Earthworm Eisenia foetida. 2000/1011482 GLP, unpublished ARW2001-77	Y	BAS
AIIIA-10.6.1	Lührs, U.	2000	Acute Toxicity (14 Days) of BAS 510 01 F to the Earthworm Eisenia fetida (Savigny 1826) in Artificial Soil. 2000/1014952 GLP, unpublished ARW2001-76	Y	BAS
AIIIA-10.6.1.3	Ehlers,H.A.	2001	Final Report: Field Study to Evaluate the Effects of BAS 510 01 F on Earthworms. 2001/1014681 GLP, unpublished ARW2001-169	Y	BAS
AIIIA-10.6.1.3	Hommen, U.	2002	Final Report: Statistical evaluation of the earthworm field study with the test substance BAS 510 01 F (study code: 61000; BASF Doc ID 2001/1014661). BASF DocID 2002/1004385 not GLP, unpublished ARW2002-44	Y	BAS
AIIIA-10.6.1.3	Krieg, W.	2001	Final Report: Field study to evaluate the effects of BAS 510 01 F on earthworms. 2001/1014661 GLP, unpublished ARW2001-168	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[8]
AIIIA-10.6.2	Krieg, W.	2001	Effects of BAS 510 01 F on the organic matter degradation under field conditions (litter bag method) study 2. 2001/1000107 GLP, unpublished ARW2001-83	Y	BAS
AIIIA-10.6.2	Krieg, W.	2001	Effects of BAS 510 01 F on the organic matter degradation under field conditions (litter bag method) study 1. 2001/1000106 GLP, unpublished ARW2001-82	Y	BAS
AIIIA-10.6.2	Krieg, W. and Schick, H.	2001	Monitoring of Collembola populations following an exposure to BAS 510 01 F in the field (grassland). 2001/1000105 GLP, unpublished ARW2001-81	Y	BAS
AIIIA-10.6.2	Meister, A.	2001	Effects of BAS 510 01 F on Reproduction of the Collembola Folsomia candida in Artificial soil. 2001/1015075 GLP, unpublished ARW2002-15	Y	BAS
AIIIA-10.6.2	Meister, A.	2001	Effects of BAS 510 01 F on Reproduction of the Collembola Folsomia candida in Artificial Soil. 2001/1000104 GLP, unpublished ARW2001-80	Y	BAS
AIIIA-10.7.1	Wachter, S.	2001	Assessment of the Side Effects of BAS 510 01 F on the Activity of the Soil Microflora, Nitrogen Turnover. 2000/1018517 GLP, unpublished BMF2001-66	Y	BAS
AIIIA-10.7.1	Wachter, S.	2001	Assessment of the Side Effects of BAS 510 01 F on the Activity of the Soil Microflora, Short-Term Respiration. 2000/1018516 GLP, unpublished BMF2001-65	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Appendix 1

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

Standard Terms and Abbreviations

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 inter-change 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

Nicobifen
Alternative common name proposed to ISO in August 2002: Boscalid

Specific Terms and Abbreviations

B.10.2 Appendix II: Specific terms and abbreviations

PAS	pure active substance
TAS	technical active substance
eq.	equivalents
TRR	Total radioactive residue
ERR	Extractable radioactive residue
RRR	Residual radioactive residue
DMSO	Dimethylsulfoxide
DAT	Days after treatment
DAP	Days after planting
DALT	Days after last treatment
DALA	Days after last application
GS	Growth stage
TAR	Total applied radioactivity