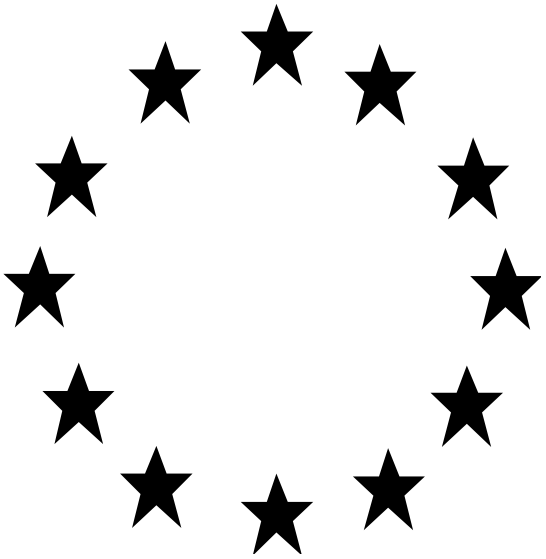


Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State the Netherlands for the existing active substance

PYRIPROXYFEN

of the third stage Part A of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

February 2009

Table of contents

Revisions are highlighted

B.1 Identity

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

All points of Annex IIA, Section I are addressed in Volume 1, Level 1.

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

All points of Annex IIIA, Section 1 are addressed in Volume 1, Level 1.

B.1.3 References relied on

No specific references for this section.

B.2 Physical and chemical properties

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

See Table B.2.1.

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

See Table B.2.2.

Table B.2.1 **Summary of the physical and chemical properties of the active substance** (studies were conducted under GLP and completed to an acceptable standard and results were considered to be valid unless specified otherwise).

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.1 (IIA 2.1)	Melting point	100%	OECD 102, capillary method.	48.0 – 50.0°C		Pesselman, 1993
B.2.1.2 (IIA 2.1)	Boiling point	99.7%	OECD 103, TG/DTA method.	318°C		Isozaki, 2001
B.2.1.3 (IIA 2.1)	Thermal stability	99.7%	OECD 103, TG/DTA method.	Up to the boiling temperature of 318° C no decomposition was observed under N_2 atmosphere.		Isozaki, 2001
B.2.1.4 (IIA 2.2)	Relative density	~~100%~~	~~OECD 109, air comparison pycnometer method.~~	~~1.26 g/cm^3 at 23°C.~~	~~Not GLP, but considered acceptable. The test was not performed at 20°C, therefore, the relative density, D^{20}_4, can not be calculated.~~	~~Kawashima, 2000~~
		100%	Gas comparison method	1.143 at 20 °C	GLP method, acceptable	Bates, 2005

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.5 (IIA 2.3)	Vapour pressure	Pure material	OECD 104, gas saturation method	~~<1.33x10^{-5} Pa~~ <1.0x10^{-7} Pa at 22.81°C	The purity of the test material was not submitted, however as the V.P. could not be determined and therefore the phrase "pure material' can be accepted as the impurities will not have a effect.	Pesselman, 1989
B.2.1.6 (IIA 2.3)	Volatility, Henry's law constant		Calculation	~~<1.16 x 10^{-2}~~ 7.37x10^{-4} Pa.m^3.mol^{-1} at 22 - 25°C	A vapour pressure of ~~<1.33x10^{-5}~~ 1x10 $^{-7}$ Pa ~~and a water solubility of 0.367 mg/L~~ was used to calculate the Henry's law constant.	Takahashi, 1995
B.2.1.7 (IIA 2.4)	Appearance: physical state	100% Tech. a.s. 95.2%	EPA 63-3 Visual assessment	Pure material: granular solid. Technical material: solid at 20°C		Pesselman, 1993 Kimura, 1989
B.2.1.8 (IIA 2.4)	Appearance: colour	100% Tech. a.s. 95.2%	EPA 63-2 ASTM D1535-68	Pure material: white. Munsell colour designation: N9.5/90.0%R Technical material: pale yellowish white Munsell colour designation: 5Y (9/2)		Pesselman, 1993 Kimura, 1989

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.9 (IIA 2.4)	Appearance: odour	100% Tech. a.s. 95.2%	EPA 63-4 Olfactory assessment	Pure material: odourless (0 level of perception). Technical material: faint characteristic odor		Pesselman, 1993 Kimura, 1989
B.2.1.10 (IIA 2.5)	Spectra	100% 100%	Standard UV/Vis, IR, NMR and MS methodology UV/Vis	~~UV/Vis-spectrum: ethanol solution, 0.01 mg/mL. Molar absorption coefficient at 271.6 nm: 6.91 x 10^3 L/(molxcm).~~ ~~IR, ^{1}H-NMR and mass spectra were also determined.~~ UV/Vis-spectrum is determined in water/methanol mixture (10:90 v/v) Acidic: 278 nm (ε = 10354 $L \cdot mol^{-1} \cdot cm^{-1}$) Neutral: 271 nm (ε = 6649 $L \cdot mol^{-1} \cdot cm^{-1}$) Basic: 271 nm (ε = 6749 $L \cdot mol^{-1} \cdot cm^{-1}$)	~~Not GLP.~~ ~~**Spectral data, recorded under GLP conditions, should be provided.**~~	~~Kimura, 2000~~ Pesselman, 1993
		100%	IR, NMR, MS	IR, ^{1}H NMR (chemical shifts: 1.50, 4.15, 5.60, 6.76, 6.85 to 7.00, 7.05, 7.30, 7.58 and 8.17), and mass spectra (major fragment m/z 227) were determined.	GLP, spectral data are considered characteristic for pyriproxifen.	Bates, 2005

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.11 (IIA 2.6)	Solubility in water	~~99.4%~~	~~EPA CG 1500~~	~~0.367 mg/L in double distilled water at 25°C and pH 6.~~	~~Not GLP, performed in 1989 (predated to GLP). Method does not comply with EEC A.6. The test method involved coating of the test substance to the wall of the vessel, adding double distilled water and determining the concentration of the test substance after 18, 19 and 21 days stirring at 25°C. The nominal test substance concentration (0.5 mg/L) is less than two times the quantity of material necessary to saturate the water fraction. However, as the concentrations obtained after 18, 19 and 21 days showed no tendency to increasing values, the test is found to be scientifically acceptable. Effect of the pH on the solubility was not determined or evaluated.~~ **~~Data to be provided: water solubility in the, acidic range (pH 4) and alkaline range~~**	~~Yamada, 1989~~

section (Annex point)	Study	purity	Method	results	comment	reference
		100%	EEC A6/ OECD 105	Purity 100% at 20 °C 0.058 mg/L at pH 5.16 0.101 mg/L at pH 6.53 0.119 mg/L at pH 8.52	GLP method, acceptable	Bates, 2006
B.2.1.12 (IIA 2.7)	Solubility in organic solvents	Tech. a.s. 97.9%	Direct addition (non analytical) technique	At 20°C: n-Heptane: 25 to 29 g/L 1,2-Dichloroethane: >1000 g/L Methanol: 25 to 29 g/L Acetone: >1000 g/L p-Xylene: >1000 g/L Ethyl acetate: >1000 g/L.		Bates, 2002
B.2.1.13 (IIA 2.8)	Partition co-efficient	~~99.4%~~	~~OECD 107, flask-shaking method~~	~~Log P_{ow} (double distilled water/n-octanol) at 25°C (pH 5.6): 5.37.~~	~~Not GLP, performed in 1989 (predated to GLP). **Effect of pH must be determined (pH 4 to 10).**~~	~~Yamada, 1989~~
		100%	EEC method A8; OECD 117	Mean Log Pow=4.85 at pH 5 (95% confidence limits of 4.75 to 4.96) Mean Log Pow=4.86 at pH 7 (95% confidence limits of 4.76 to 4.97) Mean log Pow=4.87 at pH 9 (95% confidence limits of 4.77 to 4.98)	GLP study, acceptable	Bates, 2005

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.15 (IIA 2.9)	Hydrolysis rate	[U-phenoxyphenyl-^{14}C] pyriproxyfen; radiochemical purity 97%; [2,6-pyridyl-^{14}C] pyriproxyfen; radiochemical purity 98%	Guideline not specified.	No radioactivity (<0.1% AR) remained in the aqueous phase after extraction. Extractable radioactivity consisted of mainly pyriproxyfen and ≤2.7% AR (unidentified) "others". No meaningful half-lives could be calculated. Pyriproxyfen is hydrolytically stable at pH 4, 7 and 9.	Study methods agreed with OECD 111. Study acceptable.	Takahashi N., 1989

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.16 (IIA 2.9)	Photochemical degradation	[U-phenoxyphenyl-14C] pyriproxyfen; radiochemical purity >99%; [2,6-pyridyl-14C] pyriproxyfen; radiochemical purity >99%.	EPA N:161-2	Test conditions: Xenon light, buffer pH7, 25°C. Half-lives for pyriproxyfen in the irradiated solutions were 6.36 (phenyl label, r2 0.970) and 3.72 days (pyridyl label, r2 0.966) (calculated by log linear regression using all individual data points). Pyriproxyfen was stable in the dark solution (no half-lives were calculated). The calculated half-lives for the irradiated solution are therefore effective photolytic half-lives. The 14 day irradiation period was equivalent to ~32 days natural sunlight at 43°N, summer. Major degradation products were PYPA (maximum 70% AR at day 14) and assumed (polymerised) phenolic structures (maximum 60% AR at day 14, individual fractions ≤8.2% AR). Remaining radioactive fractions were ≤5.9% AR.	Irradiation was in Pyrex glass. Data were presented which show absorption (25-90%) of light by Pyrex glass in the region 290-330 nm. As these (short) wavelengths are the most energetic and are the most absorbed by the test substance (based on UV spectrum), limited transmission of light of 290-330 nm by Pyrex glass may result in decreased photolysis. In the report it was stated that electronic light measurements were conducted under conditions simulating actual test conditions. It is unclear whether these conditions were identical to those under which the study samples were exposed (i.e. including the passage of light through a water-jacketed beaker, a UV filter and test solution vials).	Fathulla R.N., 1995 Rehani, 2005

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.17 (IIA 2.9)	Quantum yield	[U-phenoxyphenyl-^{14}C] pyriproxyfen; radiochemical purity 99.2%.	Guideline not specified.	Φ = 0.08661	Not GLP. However, the submitted report was a translation of the original Japanese study, completed on July 15, 1988 (predated to GLP).	Takahashi N., 2000
B.2.1.18 (IIA 2.9)	Dissociation constant (pKa)		Titration method, conductometric method, spectrophotometric method	Due to the low water solubility of the test substance (about 0.4 mg/L) none of the three methods were applicable for the determination of the dissociation constant.	RMS: the following pKa was obtained from Pkalc version 5.0 (module in PALLAS version 3.0; estimation performed by RMS): pKa 6.87 (2-subst.-pyridinium). This implies that pH-dependence of certain parameters (e.g. water solubility, UV/Vis spectra, Log Pow) should be investigated.	Shigenaga, 1989
B.2.1.19 (IIA 2.10)	Stability in air, photochemical oxidative degradation		Atkinson Calculation	Half-life in air based on reaction with hydroxyl radicals (9.7x10^5 OH/cm^3; 24-h day time; calculation performed by RMS): 3.8 hrs.	Half-life in air based on reaction with hydroxyl radicals (6x10^5 OH/cm^3; 12-h day time): 6.14 hrs.	Nishiyama, Nambu, Nishioka and Takimoto, 1999

section (Annex point)	Study	purity	Method	results	comment	reference
B.2.1.20 (IIA 2.11)	Flammability and auto-flammability (technical active substance)	Tech. a.s. 97.9%	EEC A.10 EEC A.15	A.10, flammability: not highly flammable. A.15, auto-ignition temperature: no auto-ignition temperature below 400°C		Bates, 2001
B.2.1.20 (IIA 2.11)	Flammability and auto-flammability (technical active substance)	Tech. a.s. 97.9%	EEC A.12	Not hazardous in contact with water.		Bates, 2002
B.2.1.21 (IIA 2.12)	Flash point (technical active substance)				Not applicable since the substance is a solid up to a temperature of about 48°C.	
B.2.1.22 (IIA 2.13)	Explosive properties (technical active substance)	Tech. a.s. 97.9%	EEC A.14	Not explosive.	Evaluated by consideration of the chemical structure and associated thermodynamic properties (DSC).	Bates, 2001
B.2.1.23 (IIA 2.15)	Oxidising properties (technical active substance)	Tech. a.s. 97.9%	EEC A.17	Not oxidizing.	Evaluated by consideration of the chemical structure and associated thermodynamic properties (DSC).	Bates, 2002
B.2.1.24 (IIA 2.14)	Surface tension				Not applicable since the water solubility of pyriproxyfen is <1 mg/L.	

Table B.2.2 Summary of the physical and chemical properties of the plant protection product
Product name: PYRIPROXYFEN 10 EC with 100 g/L Pyriproxyfen (studies were conducted under GLP and completed to an acceptable standard and results were considered to be valid unless specified otherwise).

section (Annex point)	Study	method	results	comment	reference
B.2.2.1 (IIIA 2.1)	Appearance: physical state	EPA 63-3 Visual assessment	Clear, yellowish, free-flowing liquid at room temperature.		Ha, 1995
B.2.2.2 (IIIA 2.1)	Appearance: colour	Visual assessment	Clear, yellowish.		Ha, 1995
B.2.2.3 (IIIA 2.1)	Appearance: odour	Olfactory assessment	Aromatic solvent-based characteristic odour.		Kawashima, 1995
B.2.2.4 (IIIA 2.2)	Explosive properties	EEC A.14 EEC method A 14	Not explosive.		Bernes, 2002
B.2.2.5 (IIIA 2.2)	Oxidising properties	Assessment	Not oxidising. Based on findings under 2.9.1.	Acceptable assessment.	
B.2.2.6 (IIIA 2.3)	Flammability	EEC method A 10, A 11 or A 12	solids and gases	Not required since test substance is a liquid.	

section (Annex point)	Study	method	results	comment	reference
B.2.2.7 (IIIA 2.3)	Auto-flammability	EEC A.15EEC method A 15 or A 16 or UN-Bowes-Cameron-Cage-Test	420°C.		Bernes, 2002
B.2.2.8 (IIIA 2.3)	Flash point	CIPAC MT 12.2	69°C. ~~liquids containing flammable solvents~~	Method equivalent to EEC A.9	Bernes, 2002
B.2.2.8 (IIIA 2.3)	Flash point	EPA 63-15	67°C. ~~liquids containing flammable solvents~~		Ha, 1995
B.2.2.9 (IIIA 2.4)	Acidity/alkalinity	CIPAC Method MT 31 and MT 75		Not required since the pH of a 1% aqueous solution is >4 and <10.	
B.2.2.10 (IIIA 2.4)	PH	CIPAC MT 75CIPAC MT 75	pH of 1% aqueous dispersion: 5.57~~Where relevant, pH of 1% aqueous dilution, emulsion or dispersion~~		Bernes, 2002
B.2.2.10 (IIIA 2.4)	PH	EPA 63-12CIPAC MT 75	pH of 10% aqueous dispersion: 5.7~~Where relevant, pH of 1% aqueous dilution, emulsion or dispersion~~		Ha, 1995
B.2.2.11 (IIIA 2.5)	Surface tension	EEC A.5 OECD 115 EEC method A 5	25.7 mN/m at 20°C 25.2 mN/m at 40°C ~~liquids % suspension = 40.5 mN/m~~		Martel, 2001

section (Annex point)	Study	method	results	comment	reference
B.2.2.12 (IIIA 2.5)	Viscosity	EPA 63-18OECD Test Guideline 114	18.5 mPa.s at 21.2-22.0°~~Cliquids for ULV~~ ~~Non-Newtonian liquids~~		Ha, 1995
B.2.2.12 (IIIA 2.5)	Viscosity	OECD 114 OECD Test Guideline 114	4.3 mPa.s at 20°C 2.7 mPa.s at 40°C~~liquids for ULVliquids for ULVNon-Newtonian liquids~~		Paradis, 2001
B.2.2.13 (IIIA 2.6)	Relative density	EPA 63-7	Density at 19.5°C : 0.9176 g/cm3 ~~Calculated Specific Gravity: 0.92liquids~~		Ha, 1995
B.2.2.14 (IIIA 2.6)	Bulk (tap) density		Not required for an EC formulation		
B.2.2.14 (IIIA 2.7)	Storage stability	CIPAC MT 46	14 days at 54°C in glass ampoule; the content of pyriproxyfen, appearance and pH did not change significantly after storage for 14 days at 54°C. The emulsion stability was good after storage.	Not GLP.	Kawashima, 1994
B.2.2.14 (IIIA 2.7)	Storage stability	CIPAC MT 39	1 month at 0°C and 2 weeks at -5°C; trace amount of separation was observed after storage. These separated materials were dissolved spontaneously at room temperature.	Not GLP.	Kawashima, 1994

section (Annex point)	Study	method	results	comment	reference
B.2.2.15 (IIIA 2.7)	Shelf life	GIFAP Monograph No. 17	Storage in commercial (sealed) containers at 25°C for 2 years. Chemical stability: 10.8% before storage and 10.9% after 24 months; no significant changes. Physical stability, appearance: no significant changes and no significant weight gain or loss in test materials over 24-month storage period. pH and emulsion stability: no significant change in pH (1% emulsion in water) or emulsion stability over the 24-month storage period. ~~If <2 years, shelf life in months at appropriate temps. required~~	The chemical and physical stability was also determined after 6, 12 and 18 months.	Reitz, 2003
B.2.2.16 (IIIA 2.8)	Wettability		Not required for an EC formulation		
B.2.2.17 (IIIA 2.8)	Persistent foaming	CIPAC MT 47.2	0.1% v/v in CIPAC water D at 30°C. After 10 s 8 ml After 1 min 6 ml After 3 min 6 ml After 12 min 6 ml		Bernes, 2002
B.2.2.18 (IIIA 2.8)	Suspensibility		Not required for an EC formulation water dispersible solid products (e.g. WP, WDG or SC)		
B.2.2.19 (IIIA 2.8)	Suspension stability Spontaneity		Not required for an EC formulation		

section (Annex point)	Study	method	results	comment	reference
B.2.2.20 (IIIA 2.8)	Dilution stability		Not required for an EC formulation		
B.2.2.21 (IIIA 2.8)	Dry sieve test	CIPAC Method MT 59.1	Not required for an EC formulationsolids diluted for use		
B.2.2.22 (IIIA 2.8)	Wet sieve test		Not required for an EC formulationsolids diluted for use		
B.2.2.23 (IIIA 2.8)	Particle size distribution	OECD Method 110 CIPAC MT 58.3 or MT 170	Not required for an EC formulationsolids diluted for use		
B.2.2.24 (IIIA 2.8)	Content of dust/fines	CIPAC Method MT 171 or OECD Method 110	Not required for an EC formulationsolids diluted for use	GLP	
B.2.2.25 (IIIA 2.8)	Attrition and friability	CIPAC MT 176	Not required for an EC formulationsolids diluted for use		

section (Annex point)	Study	method	results	comment	reference
B.2.2.26 (IIIA 2.8)	Emulsifiability, re-emulsifiability and emulsion stability	CIPAC MT 36.2 (visual examination) CIPAC Methods MT 36 or MT 173	The emulsions showed satisfactory emulsion characteristics, before and after storage for 2 years at 25°C, in Standard Water D and WHO Soft water at 30°C at concentrations of 0.1% and 1%. Max. 3 mL cream after 24 hours in WHO Soft water at 1% and 30°C. Re-emulsification after standing for 24-hours was possible.		Reitz, 2003
B.2.2.26 (IIIA 2.8)	Emulsion stability	visual examination CIPAC Methods MT 36 or MT 17	The emulsions showed satisfactory emulsion characteristics, before and after storage for 14 days at 54°C, in Standard Water A and C at 20°C at concentrations of 1.0 ml EC/L and 2.86 ml EC/L.	Not GLP. Observations were made after 0.5, 1 and 2 hours. In standard water C no separation was observed. In standard water A traces of separation were observed.	Kawashima, 1994
B.2.2.27 (IIIA 2.8)	Stability of dilute emulsion		Not required for an EC formulationsolids diluted for use		
B.2.2.28 (IIIA 2.8)	Flowability	CIPAC Method MT 172	Not required for an EC formulationsolids diluted for use		
B.2.2.29 (IIIA 2.8)	Pourability (rinsibility)		Not required for an EC formulationsolids diluted for use		
B.2.2.30 (IIIA 2.8)	Dustability	CIPAC Method MT 34	Not required for an EC formulationsolids diluted for use		

section (Annex point)	Study	method	results	comment	reference
B.2.2.31 (IIIA 2.8)	Adherence and distribution to seeds	CIPAC Method MT 175	Not required, not used for seed treatment. distribution and adhesion of seed treatments		
2.9.1	Physical compatibility with other products	EPA 63-14	No apparent reaction with water, (NH4)H2PO4 and granular zinc. Reacted mildly with potassium permanganate.		Ha, 1995
2.9.2	Chemical compatibility with other products		Not required – mixing with other products is not required		

B.2.3 References relied on

References for the active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 2.1.1/01	Pesselman, R.L.	1993a	Melting point determination of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-31-0054 GLP, Unpublished	Y	SUM
IIA, 2.1.2/01	Isozaki, M.	2001	Determination of boiling point of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-0086 GLP, Unpublished	Y	SUM
IIA, 2.2/01	Kawashima, M.	2000	Revision of 'Formulation group monthly report H5-02-PC-100' Sumitomo Chemical Co., Ltd. Report No. NNP-0084 Not GLP, Unpublished	Y	SUM
IIA, 2.2/02	Bates, M.	2005	Pyriproxifen, evaluation of relative density Sumitomo Chemical Co., Ltd. Report No. NNP-0102 GLP, Unpublished	Y	SUM
IIA, 2.3.1/01	Pesselman, R.L.	1989	Vapor pressure determination of Sumilarv Sumitomo Chemical Co., Ltd. Report No. NNP-91-0030 GLP, Unpublished	N	SUM
IIA, 2.3.2/01	Takahashi, M.	1995	Henry's law constant for pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-50-0066 Not GLP, Unpublished	Y	SUM
IIA, 2.4.1/01	Pesselman, R.L.	1993b	Color determination of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-31-0051 GLP, Unpublished	Y	SUM
IIA, 2.4.1/02	Pesselman, R.L.	1993c	Physical state determination of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-31-0053 GLP, Unpublished	Y	SUM
IIA, 2.4.1/03	Kimura, M.	2000a	Revision of 'Color of Sumilarv technical grade' Sumitomo Chemical Co., Ltd. Report No. NNP-0078 Not GLP, Unpublished	N	SUM
IIA, 2.4.1/04	Kimura, M.	2000b	Revision of 'Physical state of Sumilarv technical grade' Sumitomo Chemical Co., Ltd. Report No. NNP-0079 Not GLP, Unpublished	N	SUM
IIA, 2.4.2/01	Pesselman, R.L.	1993d	Odor determination of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-31-0052 GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 2.4.2/02	Kimura, M.	2000c	Revision of 'Odor of Sumilarv technical grade' Sumitomo Chemical Co., Ltd. Report No. NNP-0080 Not GLP, Unpublished	N	SUM
~~IIA, 2.5.1/01~~	~~Kimura, M.~~	~~2000d~~	~~Revision of 'UV, IR, MS and NMR spectral data of pyriproxyfen (pure)'~~ ~~Sumitomo Chemical Co., Ltd. Report No. NNP-0081~~ ~~Not GLP, Unpublished~~	~~Y~~	~~SUM~~
IIA, 2.5.1/02	Pesselman, R.L.	1993e	Ultraviolet-Visible Absorption Spectrum Determination of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-31-0058 GLP, Unpublished	Y	SUM
IIA, 2.5.1/03	Bates, M.	2005	Pyriproxifen:evaluation of spectroscopic properties Sumitomo Chemical Co., Ltd. Report No. NNP-0104 GLP, Unpublished	Y	SUM
IIA, 2.6.1/01	Bates, M.L.	2006	Pyriproxifen: evaluation of the water solubility Sumitomo Chemical Co., Ltd. Report No. NNP-0105 GLP, Unpublished	Y	SUM
IIA, 2.8.1/01	Bates, M.	2005	Pyriproxifen: evaluation of the n-octanol/water partition coefficient Sumitomo Chemical Co., Ltd. Report No. NNP-0103 GLP, Unpublished	Y	SUM
IIA, 2.6/01	Saito, S.	1989a	Water solubility of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-90-0026 Not GLP, Unpublished	N	SUM
IIA, 2.7/01 2.11.1/02 2.15/01	Bates, M.L.	2002	Pyriproxyfen: evaluation of physico-chemical properties (EC Directive 91/414/EEC Annex II, Points 2.15, 2.7 and 2.11.1) Sumitomo Chemical Co., Ltd. Report No. NNP-0094 GLP, Unpublished	Y	SUM
IIA, 2.8/01	Saito, S.	1989b	Partition coefficient (n-octanol/water) of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-70-0025 Not GLP, Unpublished	N	SUM
IIA, 2.9.1/01	Katagi, T., Takahashi, N.	1989	Hydrolysis of S-31183 in buffered aqueous solutions at 50°C Sumitomo Chemical Co., Ltd. Report No. NNM-90-0013 GLP, Unpublished	N	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 2.9.2/01	Fathulla, R. N.	1995	Artificial sunlight photodegradation of pyriproxyfen in aqueous media at pH 7 Sumitomo Chemical Co., Ltd. Report No. NNM-51-0037 GLP, Unpublished	Y	SUM
IIA, 2.9.2/02	Rehani, R.	2005	Covance USA Letter R. Rehani 15 April 2005	Y	SUM
IIA, 2.9.3/01	Takahashi, N.	1988	Quantum yield of direct phototransformation of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-0077 Not GLP, Unpublished	Y	SUM
IIA, 2.9.4/01	Shigenaga, H.	1989	Dissociation constant of Sumilarv Sumitomo Chemical Co., Ltd. Report No. NNP-90-0022 Not GLP, Unpublished	N	SUM
IIA, 2.10/01	Nishiyama, M., Nambu, K., Nishioka, K., Takimoto, Y.	1999	Stability in air of pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNP-0076 Not GLP, Unpublished	Y	SUM
IIA, 2.11.1/01 2.11.2/01 2.13/01	Bates, M.	2001	Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10, A14, A15) Sumitomo Chemical Co., Ltd. Report No. NNP-0091 GLP, Unpublished	Y	SUM

References for the plant protection product

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIIA, 2.1/01 2.6.1/01 2.7.3/01 2.8.7.1/01 2.8.7.2/01	Reitz, G.A.	2003	Shelf-life storage stability of pyriproxyfen 10EC Density of pyriproxyfen 10EC – 24 month interim report Sumitomo Chemical Co., Ltd. Report No. NNF-0053 GLP, Unpublished	Y	SUM
IIIA, 2.1/02	Kawashima, M.	2000a	Odour of pyriproxyfen 10% emulsifiable concentrate Sumitomo Chemical Co., Ltd. Report No. NNF-0042 Not GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIIA, 2.2.1/01 2.3/01 2.4.2/01 2.8.2/01	Bernes, A.	2002	Some physical and chemical properties for pyriproxyfen 10 EC Sumitomo Chemical Co., Ltd. Report No. NNF-0048 GLP, Unpublished	Y	SUM
IIIA, 2.2.2/01	Ha, S.T.	1995	Physical and chemical characteristics of knack insect growth regulator Sumitomo Chemical Co., Ltd. Report No. NNF-0036 GLP, Unpublished	Y	SUM
IIIA, 2.5.2/01	Paradis, B.	2001	Viscosity of pyriproxyfen 10% emulsifiable concentrate Sumitomo Chemical Co., Ltd. Report No. NNF-0049 GLP, Unpublished	Y	SUM
IIIA, 2.5.3/01	Martel, I.	2001	Surface tension of pyriproxyfen 10% emulsifiable concentrate Sumitomo Chemical Co., Ltd. Report No. NNF-0050 GLP, Unpublished	Y	SUM
IIIA, 2.7.1/01	Kawashima, M.	2000b	Stability of pyriproxyfen 10% emulsifiable concentrate after storage for 14 days at 54°C Sumitomo Chemical Co., Ltd. Report No. NNF-0044 Not GLP, Unpublished	Y	SUM
IIIA, 2.7.2/01	Kawashima, M.	2000c	Low temperature stability of pyriproxyfen 10% emulsifiable concentrate Sumitomo Chemical Co., Ltd. Report No. NNF-0043 Not GLP, Unpublished	Y	SUM

B.3 Data on application and further information

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

B.3.1.1 Function (Annex IIA 3.1)

Pyriproxyfen is an insecticide.

B.3.1.2 Effects on harmful organisms (Annex IIA 3.2.1)

Pyriproxyfen is a juvenile hormone agonist: it suppresses embryogenesis and metamorphosis in various insect species. The molecular basis of the mode of action of juvenile hormone (and its agonist pyriproxyfen) has not been clarified.

B.3.1.3 Translocation in plants (Annex IIA 3.2.2)

Dispersed over the foliage, Pyriproxyfen is not translocated in plants, but has translaminar properties. It is not systemic in plants.

B.3.1.4 Fields of use (Annex IIA 3.3)

The field of use is in agriculture (cotton) and on protected crops (tomato- and eggplants).

B.3.1.5 Pests controlled and crops protected (Annex IIA 3.4.1, 3.4.2)

Pyriproxyfen has been found to be effective against the following insects on cotton, tomato- and eggplants: Trialeurodes vaporariorum and Bemisia tabaci.

B.3.1.6 Mode of action (Annex IIA 3.5.1)

Pyriproxyfen is a juvenile hormone agonist: it suppresses embryogenesis and metamorphosis in various insect species. The molecular basis of the mode of action of juvenile hormone (and its agonist pyriproxyfen) has not been clarified.

B.3.1.7 Information relative to the formation of active metabolites or degradation products (Annex IIA 3.5.2, 3.5.3)

There are no known active metabolites or degradation products to which the active substance must be converted to exert its intended effect.

B.3.1.8 Information on the possible occurrence of the development of resistance or cross-resistance (Annex IIA 3.6)

Up till now, there is no evidence that pyriproxyfen has induced resistance. Furthermore, pyriproxyfen has not shown in-cross resistance with other active substances. In order to reduce the risk of resistance development by whitefly to pyriproxyfen, only two applications per season will be recommended.

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

Product name: Admiral 10 EC, Admiral, Juvinal 10 EC, Atominal 10 EC, Lano 10 EC, Knack.
(For the sake of simplicity these product names are referred to as Pyriproxyfen 10 EC)

B.3.2.1 Field of use (Annex IIIA 3.1)

The field of use is in agriculture (cotton) and on protected crops (tomato- and eggplants).

B.3.2.2 Nature of the effects on harmful organisms (Annex IIIA 3.2)

The effect of Pyriproxyfen 10 EC is by contact action.

B.3.2.3 Pests controlled and crops protected (Annex IIIA 3.3)

Pyriproxyfen 10 EC is effective against greenhouse whitefly and cotton whitefly on cotton and tomato- and eggplants.

B.3.2.4 Rate of application (Annex IIIA 3.4)

Pyriproxyfen 10 EC is intended to be used as an insecticide on cotton and will be applied as 0.75 L/ha in Southern Europe (max 1 application). Pyriproxyfen 10 EC is intended to be used as an insecticide on tomato- and eggplants at a rate of 0.2-0.3 L/ha (Northern Europe) and 0.5-1.125 L/ha (Southern Europe) per application (max. 2 applications).

B.3.2.5 Concentration of active substance in material used (Annex IIIA 3.5)

Pyriproxyfen 10 EC is an EC formulation containing 100 g/L of pure pyriproxyfen.

B.3.2.6 Description of the method of application, type of equipment used and type and volume of diluent per unit of area or volume (Annex IIIA 3.6)

Pyriproxyfen 10 EC will be applied as a foliar spray (high volume spraying) after dilution in water. The type of equipment used is a knapsack sprayer, automatic green house spraying equipment (greenhouse application) or a tractor mounted field crop sprayer (field application).

B.3.2.7 Number and timing of applications and duration of protection afforded (Annex IIIA 3.7)

Pyriproxyfen 10 EC will be applied as a foliar spray (high volume spraying) maximum twice for tomato and eggplant (greenhouse application, growth stage BBCH 89) and maximum once for cotton (field application, growth stage: before boll opening) as soon as adults are observed. The second application should be performed approximately 10 days after the first one.
The duration of protection afforded by each application is 14 days for tomato and eggplant (greenhouse application) and 21-28 days for cotton. After two applications, the duration of protection is 21 days for tomato and eggplant.

Remark: In document M-III a period of 7 - 15 days is mentioned between the two applications. The GAP, however, mentions a period of 10 days between the two applications. The notifier should explain why there is a deviation from the GAP.

B.3.2.8 Minimum waiting periods or other precautions between last application and sowing or planting of succeeding crops. Limitations on choice of succeeding crops (Annex IIIA 3.8)

It is not necessary to define waiting periods, since pyriproxyfen has no phytotoxic effects on succeeding crops.

B.3.2.9 Proposed instructions for use as printed, or to be printed, on labels (based on document E)

Labels have been submitted.

B.3.3 Summary of data on application

See Table 3.3.1

Table 3.3.1 List of representative uses evaluated (pyriproxyfen) (Annex IIA 3.4; Annex IIIA 3.3 - 3.7, 3.9)

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha min max		
Tomato (greenhouse)	South Europe	Pyriproxyfen 10 EC	G	Greenhouse and cotton whitefly	EC	100 g/L	Foliar spray (High Volume Spraying)	BBCH 89	1-2	10 days	0.005-0.0075	1000-1500	0.05-0.1125	3	First applica-tion: as soon as adults are observed
Tomato (greenhouse)	North Europe	Pyriproxyfen 10 EC	G	Greenhouse and cotton whitefly	EC	100g/L	Foliar spray (High Volume Spraying)	BBCH 89	1-2	10 days	0.002-0.003	800-1200	0.02-0.03	3	
Eggplant (greenhouse)	South Europe	Pyriproxyfen 10 EC	G	Greenhouse and cotton whitefly	EC	100g/L	Foliar spray (High Volume Spraying)	BBCH 89	1-2	10 days	0.005-0.0075	1000-1500	0.05-0.1125	3	
Eggplant (greenhouse)	North Europe	Pyriproxyfen 10 EC	G	Greenhouse and cotton whitefly	EC	100g/L	Foliar spray (High Volume Spraying)	BBCH 89	1-2	10 days	0.002-0.003	800-1200	0.02-0.03	3	
Cotton	South Europe	Pyriproxyfen 10 EC	F	Cotton whitefly	EC	100g/L	Foliar spray (High Volume Spraying)	BBCH 78-79	1	n.a.	0.009-0.015	500-800	0.075	n.a.	

Remarks:

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated

(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) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(g) All abbreviations used must be explained
(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) The minimum and maximum number of application possible under practical conditions of use must be provided
(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)

For details see Material Safety Data Sheet (Reference 3.7/01).
This information is presented in the form of the safety data sheet according to Council Directive 2001/58/EC.

B.3.4.1.1 Handling

The usual precautions for handling chemicals should be observed. Provide adequate ventilation. In case of dust formation, use dust mask. Wear protective gloves. Wear safety goggles or face shield. Wear suitable protective clothing. Launder clothes before re-use.

B.3.4.1.2 Storage

Store in a dry and cool place. Keep container in a well-ventilated place. Keep away from heat. Keep out of the reach of children. Keep away from food, drink, and animal feeding stuffs. Keep only in the original container.

B.3.4.1.3 Transport

The following information was submitted by the notifier:
Land transport:
ADR/RID: Class 9
Warning sign, Hazard No. 90, Substance No. 3077
UN No.: 3077
Packing group: III
Proper shipping name: Environmentally hazardous substance, solid, n.o.s. (Pyriproxyfen)

Sea transport:
IMO/IMDG: Class None
UN No: None
Packing group: NA
EMS: None
Marine pollutant: No
Proper shipping name: None

Air transport:
ICAO/IATA: Class 9
Un No.: 3077
Packing group: III

Proper shipping name: Environmentally hazardous substance, solid, n.o.s. (Pyriproxyfen)

B.3.4.1.4 Fire

Extinguish media: dry chemical powder, carbon dioxide, foam.

Protective equipment: Wear self contained breathing apparatus, Wear suitable protective clothing and eye/face protection.

B.3.4.2 Procedures for destruction or decontamination of the active substance, contaminated packaging and contaminated material

B.3.4.2.1 Controlled incineration

Pyriproxyfen does not have a halogen content greater than 60%, hence pyrolytic behaviour under controlled conditions is not required. The recommended means of safe disposal is by controlled incineration at an approved chemical waste facility (combustion temperature > 800°C).

B.3.4.2.2 Others

No other methods of safe disposal than controlled incineration are recommended.

B.3.4.3 Emergency measures in the case of an accident

There is no readily available method for decontamination of water. Precaution must be taken to avoid contamination. Do not allow spills to escape into sewage system or water courses. Spill contaminated water is to be contained and decontaminated in a suitable sewage plant or incinerated.

Containment of spillages: Clean up spills immediately. Sweep up and place into sealable containers. Dig up heavily contaminated soil and place into drums. Use a damp cloth to clean floors and other objects after removal of spills and place into sealable container. Do not wash residues into drains or other waterways. Dispose of all waste and contaminated clothing in the same manner as waste chemicals (i.e. via an authorized disposal facility).

Fist aid:

General information:	In the case of doubt, seek medical attention.
After inhalation:	Move person to the fresh air. If symptoms persist, seek medical advice. Wash off with plenty of soap and water.
After eye contact:	Rinse eye thoroughly with water. Eyelids should be held away from the eyeball to ensure thorough rinsing. Seek medical advice if irritation develops.
After skin contact:	Remove contaminated clothing. Wash skin with plenty of water. Launder clothes before re-use.
After ingestion:	Rinse mouth. Never induce vomiting in unconscious or confused ersons. Seek medical advice.

B.3.4.4 Summary of further information and assessment

No further information.

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

Product name: Pyriproxyfen 10 EC

B.3.5.1.1 Packaging, compatibility on the preparation with proposed packaging materials (Annex IIIA 4.1)

It is proposed that Pyriproxyfen 10 EC is packed in 0.25 L or 1 L extruded co-ex polyethene bottles (0.25 L - Coex: PE Lupolen 5021 D + Overac 18302 + Grillon A 28 NZ + 2.5% of PB-PE 589; 1 L - Coex: co-extruded polyethylene with EVOH).

Bottle 0.25L:

Closure : DIN cap aluminium sealed (external Φ 50 mm). Additional induction seal stuck by thermo-induction on the bottle opening

Filling quantity: 0.25 L

Material: co-ex polyethene (Coex: PE Lupolen 5021 D + Overac 18302 + Grillon A 28 NZ + 2.5% of PB-PE 589).

Dimensions: Round bottle, Φ 0.063 m, height 0.134 m, thickness 0.5 mm, empty weight 0.030 kg, opening Φ 41 mm, useful capacity 0.25 L.

External packaging:

Square box made of corrugated carton DF (minimum thickness of 4 mm) with internal dimensions (lxdxh) of 0.315x0.252x0.138 m and external dimensions (lxdxh) of 0.323x0.260x0.150 m, and a capacity of 20 bottles of 0.25L), closed using adhesive band 3M 3705.

Bottle 1.0L:

Closure: Thermo-weldable safety seal of PE with disc of polyhexane (external Φ 50 mm). Additional induction seal stuck by thermo-induction on the bottle opening.

Filling quantity: 1.0 L

Material: co-extruded polyethylene with EVOH.

Dimensions: Round bottle, Φ 0.089 m, height 0.241 m (without the stopper), thickness 0.5 mm, empty weight 0.120 kg + 0.012 kg stopper, totalling 0.132 kg, opening Φ 40.8 mm, useful capacity 1.153 L.

External packaging:

Square box made of corrugated carton (weight of 912 g/m^2, composition K B140P P160/B 120/F 160/K 175) with dividers (6 pieces of 363x242 mm) and internal dimensions (lxdxh) of

0.374x0.374x0.263 m and external dimensions (lxdxh) of 0.468x0.374x0.263 m, and a capacity of 16 bottles of 1.0L), closed using adhesive band 3M 3705.

B.3.5.1.2 Packaging suitability

According to the test results (drop tests, stacking tests, complementary drop tests), the internal and external packaging are suitable in terms of strength.

Drop test: No leak of the inner packaging. No deterioration of the outer packaging compromising the safety of the transport.

Complementary drop test: No leaks.

Stacking test: No leak of the inner packaging. No deterioration of the outer packaging compromising the safety of the transport.

B.3.5.1.3 Packaging resistance

After 2 years of storage at ambient temperature (25 °C), the packaging showed no sign of deformation and the closure was intact.

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

Normal procedures should be followed for the cleaning of application equipment. Immediately after spraying, drain tank completely. Any contamination on the outside of the spraying equipment should be removed by rinsing with clean water. Rinse tank inside with clean water and flush through booms and hoses. Drain tank completely. Wash with detergent (sprayer cleaner) and rinse with water. Tank-washings should be disposed of safely and by approved means. Protective clothing should be machine-washed with detergent and rinsed with water.

B.3.5.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIA 4.3)

Pre-harvest interval (in days)

When the formulation is applied according to the good agricultural practices and to the label recommendations a PHI of 3 days is proposed for tomato and eggplant (greenhouse application). For cotton a PHI is not applicable.

Re-entry period (in days) for livestock, to areas to be grazed.

Not relevant since tomatoes, eggplants and cotton are not intended to be grazed.

Re-entry period (in hours or days) for man to crops, buildings or spaces treated

No bystanders should be allowed in greenhouses during the application of pyriproxyfen 10EC on tomatoes and eggplants. No respiratory risk is identified for workers in cotton, tomatoes and eggplants.

Withholding period (in days) for animal foodstuffs

No withholding period is defined (use on protected tomatoes, eggplants and in cotton, which are normally not fed to livestock).

Waiting period (in days) between application and handling treated products

No re-entry periods are proposed. A safe use was identified for re-entry activities without PPE in tomato and eggplant in Northern Europe. A safe use was identified for re-entry activities with PPE in cotton, tomato and eggplant in Southern Europe.

Waiting period (in days) between last application and sowing or planting of succeeding crops

In all aerobic soil degradation studies performed in the laboratory, the DT90 was below 100 days, indicating that residues of pyriproxyfen degrade to less than 10% of the initial within 100 days. There are no major (>10%) metabolites observed in these degradation studies. Additionally, there is sufficient data indicating that pyriproxyfen and residues are not taken up from soil into plants in significant amounts (in practice, <0.01 mg/kg levels are expected in edible rotational crops). Hence, the setting of a waiting period between the last application and sowing or planting of succeeding crops is not required. No waiting period is defined due to the rapid degradation of Pyriproxyfen in soil.

Conditions under which the product should not be used

No restrictions should be imposed on the use of the product in relation to specific agricultural, plant health or environmental conditions.

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

Handling

The usual precautions for handling chemicals should be observed. Provide adequate ventilation. Wear protective gloves. Wear safety goggles or face shield. Wear suitable protective clothing. Launder clothes before re-use.

Storage

Store in a dry and cool place. Keep container in a well-ventilated place. Keep away from heat. Prevent electrostatic discharges. Above the flash point an explosive mixture can be formed. Keep out of the reach of children. Keep away from food, drink and animal feeding stuffs. Keep only in original container.

Transport

Land transport:

ADR/RID: Class 9

Warning sign, Hazard No. 90, Substance No. 3082

UN No.: 3082

Packing group: III

Proper shipping name: Environmentally hazardous substance, liquid, n.o.s. (contains 10% Pyriproxyfen)

Sea transport:

IMO/IMDG: Class None

UN No: None

Packing group: NA

EMS: None

Marine pollutant: No

Proper shipping name: None

Air transport:

ICAO/IATA: Class 9

Un No.: 3082

Packing group: III

Proper shipping name: Environmentally hazardous substance, liquid, n.o.s. (contains 10% Pyriproxyfen)

Fire

Extinguish media: cry chemical powder, carbon dioxide, foam.
Protective equipment: Wear self contained breathing apparatus. Wear suitable protective clothing and eye/face protection. May emit toxic and corrosive fumes under fire conditions (CO and NO_x).

Waste

It is recommended to purchase and store quantities of product required in the short term. Do not open more containers than is necessary for immediate requirements. Do not mix a volume of spray solution greater than is required for immediate use.

B.3.5.5 Emergency measures in the case of an accident (Annex IIIA 4.5)

There is no readily available method for decontamination of water. Precaution must be taken to avoid contamination. Do not allow spills to escape into sewage system or water courses. Contaminated water is to be contained and decontaminated in a suitable sewage plant or incinerated.

Containment of spillages: Use adsorbent material to collect liquid spillage (e.g. sawdust, peat, chemical binder). Sweep up split granules or contaminated absorbent and place into sealable containers. Dig up heavily contaminated soil and place into drums. Use a damp cloth to clean floors and other objects after removal of granules or contaminated absorbent and place into

sealable container. Dispose of all waste and in the same manner as waste chemicals (i.e. via an authorized disposal facility).

Decontamination of areas, vehicles and buildings: In case of spill (liquid), soak it up immediately with suitable absorbent such as sawdust or granular absorbent clay. Sweep up and dispose as waste following local regulations. Do not wash residues into drains or other waterways. Dispose in the same manner as waste chemicals according to local regulations (i.e. via authorized disposal facility).

Fist aid:

General advice:	In all cases of doubt, or when symptoms persist, seek medical attention.
After inhalation:	Bring victim out to fresh air. If symptoms persist, seek medical advice.
After skin contact:	Remove all contaminated clothing immediately. Wash skin with plenty of soap and water. Seek medical advice if irritation develops. Launder clothes before re-use.
After eye contact:	Rinse thoroughly with plenty of water. Eye lids should be held away from the eyeball to ensure thorough rinsing. Seek medical advice if irritation persists.
After ingestion:	Rinse mouth. Do NOT induce vomiting. Seek medical advice.

B.3.5.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6)

B.3.5.6.1 Possibility of neutralisation

A neutralisation procedure cannot be proposed.

B.3.5.6.2 Controlled incineration

The recommended means of safe disposal is by controlled incineration at an approved chemical waste facility. In order to continue perfect combustion, it is desirable that the combustion temperature is > 800°C (standard process).

B.3.5.6.3 Others

No other method than controlled incineration for disposal of the plant protection product, contaminated packaging and contaminated materials is available.

B.3.5.7 Summary of further information and assessment

No further information.

B.3.6 References relied on

Annex point / reference no.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA, 3.6/01	Dennehy, T.J., Williams, L.	1997	Management of resistance in Bemesia in Arizona cotton Pestic. Sci., 51, 398-406 Not GLP, Published	N	-
IIA, 3.6/02	Horowitz, A.R., Forer, G., Ishaaya, I.	1994	Managing resistance in Bemisia tabaci in Israel with emphasis on cotton Pestic. Sci., 42, 113-122 Not GLP, Published	N	-
IIA, 3.6/03	Rufingier, C., Schon, L., Martin, C.	1999	Evaluation de la resistance de l'aleurode des serres Trialeurodes vaporariorum West a la buprofezine, la deltamethrine, au methomyl et au pyriproxyfene ANPP - 5eme Conference Internationale sur les ravageurs en agriculture, Montpellier 7, 8, 9 Decembre 1999, 107-114 Not GLP, Published	N	-
IIA 3.7/01	Mercier, O.	2003	Safety data sheet according to EC Directive 2001/58/EC: Admiral TGMSDS pyriproxyfen TG Sumitomo Chemical Agro Europe S.A. Report No. ALTG/F/101gb Not GLP, Unpublished	Y	SUM
IIIA 4.1.2/01	Meritet, M.	1998	Rapport d'epreuve no 198308 Epreuves mecanique sur un type d'emballage combine Bureau des Verifications Techniques, France CMPA, unpublished Report No. Not allocated Report No: 198308 GLP unknown, Unpublished	N	CMPA
IIIA, 4.2/01	Rzepka, S.	2003a	Testing of the effectiveness of a machine washing procedure of protective clothes stained with Admiral (pyriproxyfen 100 g/l EC) spray solution Sumitomo Chemical Co., Ltd. Report No. NNF-0051 GLP, Unpublished	Y	SUM
IIIA, 4.2/02	Rzepka, S.	2003b	Validation of a method for the determination of residues of pyriproxyfen in protective clothes stained with pyriproxyfen TG Sumitomo Chemical Co., Ltd. Report No. NNA-0096 GLP, Unpublished	Y	SUM

B.4 Proposals for classification and labelling

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

Justified proposals for classification and labelling of pyriproxyfen, relating to physical and chemical properties, human health and ecotoxicological effects, according to Directive 67/548/EEC are listed below:

Physical chemical properties

Based on the results no classification or labelling is proposed.

Toxicology

Based on the results no classification or labelling is proposed.

Environment

In acute toxicity tests with technical pyriproxyfen in fish, *Daphnia magna* and algae, the lowest LC/EC50 values were >0.27, 0.40 and 0.094 mg/L, respectively. Pyriproxyfen has a log Pow value of >3.0, and the experimentally determined BCF is >100 (B.9.2.2.3). It is proposed therefore, that on the basis of its acute toxicity pyriproxyfen should be categorised as “Dangerous for the environment” (N), “Very toxic to aquatic organisms” (R50) and "May cause long-term adverse effects in the aquatic environment" (R53).

It is recommended that the active substance also carries the following ‘S’ safety phrases:

S60	This material and its container must be disposed of as hazardous waste
S61	Avoid release to the environment. Refer to special instructions/Safety data sheet

Justification for the proposal:

N, R50/53	Based on the acute toxicity and the BCF.
S60, S61	Recommended for preparations labelled with R50/R53.

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

Justified proposals for classification and labelling of the preparation PYRIPROXYFEN 10 EC, relating to physical and chemical properties, human health and ecotoxicological effects, according to Directive 1999/45/EC are listed below:

Physical chemical properties

Based on the results no classification or labelling is proposed.

Toxicology

Hazard symbol	:	Xi
Indications of danger	:	Irritant
Risk phrases	:	R38; Irritating to skin

		R65; May cause lung damage if swallowed.
Safety phrases	:	S37; Wear suitable gloves.

Justification for the proposal:

R38 Based on the results of the skin irritation study.

R65 One of the formulants is labelled with R65. Pyriproxyfen 10EC contains approximately 50% of this formulant. However, considering the low viscosity of Pyriproxyfen 10EC and the surface tension of 25.7 mN/m at 20°C, classification of Pyriproxyfen 10EC with R65 is considered necessary.

S37 Recommended for substances irritating the skin.

Environment

In acute toxicity tests with the Pyriproxyfen 10EC formulation in fish, *Daphnia magna* and algae, the lowest LC/EC50 values were 2.1, 1.8 and 0.7 mg product/L, respectively. Pyriproxyfen has a log Pow value of >3.0, and the experimentally determined BCF is >100 (B.9.2.2.3). It is proposed therefore, that on the basis of its acute toxicity the Pyriproxyfen 10EC formulation should be categorised as "Dangerous for the environment" (N), "Very toxic to aquatic organisms" (R50) and "May cause long-term adverse effects in the aquatic environment" (R53).

It is recommended that the Pyriproxyfen 10EC formulation also carries the following 'S' safety phrases:

S60 This material and its container must be disposed of as hazardous waste

S61 Avoid release to the environment. Refer to special instructions/Safety data sheet

B.4.3 References relied on

No specific references for this section.

The proposed classification and labelling is based on the toxicological evaluation as presented in section B.6 and B.9.

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 Technical active substance (IIA 4.1.1)

STUDY 1

Characteristics

Reference	:	Kimura, M., 2000 (IIA 4.1/01)	GLP statement	:	No
Type of study	:	Analytical methods for technical active substance and plant protection product	Guideline	:	Not indicated
Year of execution	:	1998	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Technical, Lot no. 80302, purity not reported, Analytical, Lot no. 971030, purity not reported)	Method reference	:	Not indicated

Description of the method

Pyriproxyfen Technical (0.05 g) was dissolved in 10.0 mL internal standard solution (0.2 g *p*-benzyldiphenyl in 300 mL methanol). Pyriproxyfen was analyzed by HPLC-UV (LiChrosorb RP-18, 10 µm, 4 mm i.d. x 30 cm) using 2/1 (v/v) acetonitrile/water as mobile phase with UV detection at 254 nm.

Results

See Table B.5.1-1.

Conclusion

The method is acceptable for the determination of pyriproxyfen in technical material.

Remarks

None

STUDY 2

Characteristics

Reference	:	Mukumoto, M, 2002 (IIA 4.1/02)	GLP statement	:	No
Type of study	:	Analytical methods for technical active substance and plant protection product	Guideline	:	SANCO/3030/99 rev. 4
Year of execution	:	Not reported	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Technical, Lot no. 80301, purity not reported, Analytical, Lot no. 00427, purity not reported)	Method reference	:	Not indicated

Description of the method

Pyriproxyfen Technical (0.05 g) was dissolved in 10.0 mL methanol. Pyriproxyfen was analyzed by HPLC-MS (LiChrosorb RP-18, 10 µm, 4 mm i.d. x 30 cm, m/z 322 and 227) using 2/1 (v/v) acetonitrile/water as mobile phase.

Results

The identity of pyriproxyfen in the technical material was confirmed by the presence of ions with m/z 322 and 227.

Conclusion

The method is acceptable as confirmatory method (for method B.5.1.1 STUDY 1).

Remarks

None.

B.5.1.2 Plant protection product (IIIA 5.1.1)

STUDY 1

Characteristics

Reference	:	Reitz, G.A., 2001 (IIIA 5.1.1/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for technical active substance and plant protection product	Guideline	:	Directive 96/46/EC SANCO/3030/99
Year of execution	:	2001	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen 10 EC (Product, Lot no. M 73.01, purity 10.9%, Analytical, Lot no. AS 1723b, purity 99.8%)	Method reference	:	Not indicated

Description of the method

Pyriproxyfen 10 EC (0.25 g) was dissolved in 25.0 mL internal standard solution (3 mg/L 4-benzyldiphenyl in acetonitrile). Pyriproxyfen was analyzed by HPLC-UV (Ace C18, 150 mm x 4.6 mm i.d., 5 µm) using 65/35 (v/v) acetonitrile/water as mobile phase (1mL/min) with UV detection at 295 nm.

Results

See Table B.5.1-1.

Conclusion

The method is acceptable for the determination of pyriproxyfen in the plant protection product Pyriproxyfen 10 EC.

Remarks

None.

Table B.5.1-1 Summary of method validation (pyriproxyfen in technical active material and formulations)

	Linearity (between)	Precision – Repeatability	Precision – Reproducibility	Accuracy (%)	Interference/Specificity	Ref.
Company Method Reference Pyriproxyfen in technical material	4020 – 6010 mg/L n=5 (r=0.99996)	n=3, each at 3 concentrations, (RSD 0.38%(A))	Not determined.	Not determined.	No interference.	Kimura, 2000, (5.1.1/01)
Company Method Reference Pyriproxyfen in plant protection product	400 – 1670 mg/L n=5 (r=1.000)	n=5, duplo injections, 10.9% (RSD 0.11%(B))	Not determined	n=3, duplo injections 100%(C) (RSD 0.38%(C))	No interference.	Reitz, 2001, (5.1.2/01)

(A) Analytical standard was used instead of technical active substance.
(B) Recalculated by RMS (Reported as 0.00%; author used rounded figures in calculations).
(C) Recalculated by RMS (Reported as 101% and RSD 0.57; author used rounded figures in calculations).

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

The following residue definitions were determined (section B.7):

Residue definition for plant products
The residue definition for post-registration monitoring and for risk assessment is proposed as pyriproxyfen for tomato, eggplant and cotton.

Residue definition for animal products
No definition of residues in animal products was required, as tomato and eggplant are not fed to animals and as no significant residues are expected in livestock feed after application of pyriproxyfen to cotton.

STUDY 1

Characteristics

Reference	:	Weber, H., Pelz, S., 2000 (IIA 4.2.1/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for plants, animal tissues, milk and eggs	Guidelines	:	Directive 96/46/EC 1996 Draft working document 8064/VI/97 rev. 4 of 15 Dec '98
Year of execution	:	1999-2000	Acceptability	:	Acceptable for pre-registration
Test substance	:	Pyriproxyfen (Lot no. 981021G, purity 99.8%)	Method reference	:	DFG method S19 (extended revision, Modular Multi Method L00.00-34)

Description of the method

The method is based on multi-method S19 (DFG). Samples (cucumber fruit) were homogenized in acetone/water 2:1 (v/v, taking into account the water content of the sample). After addition of sodium chloride the mixture was partitioned using ethylacetate/cyclohexane 1:1 (v/v). The organic phase was partially evaporated and redissolved in ethyl acetate, after which sodium sulphate, sodium chloride and cyclohexane were added. The organic phase was subjected to gel permeation chromatography. The eluate was evaporated and redissolved in ethyl acetate before analysis by GC (Fused silica capillary column DB-5MS (J&W), methyl silicone with 5% phenyl groups, 30m x 0.25 mm i.d., film thickness 0.25 µm) with MS detection (m/z 136 (quantification) and 78, 226 (verification)). Reference standards prepared in solution were used as linearity standards (detector) and used for quantification in recovery samples (one point calibration at appropriate levels).

Results

See Table B.5.2-1.

Conclusions

DFG Method S19 can be considered suitable in pre- and post registration studies for the analysis of pyriproxyfen in cucumber fruit with an LOQ of 0.01 mg/kg.

Remarks

The ILV is provided under STUDY 2.

STUDY 2

Characteristics

Reference	:	Kretschmer, S., 2002a (IIA 4.2.1/02)	GLP statement	:	Yes
Type of study	:	Analytical methods for plants, animal tissues, milk and eggs	Guidelines	:	Directive 91/414/EEC Annex II, 4.2 SANCO/825/00 rev. 6 of 20 June 2000
Year of execution	:	2000	Acceptability	:	Acceptable (together with 5.2-01)
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	DFG method S19 (extended revision, Modular Multi Method L00.00-34)

Description of the method

The method is based on a multi-method S19 (DFG). Samples (cucumber fruit) were homogenized in acetone/water 2:1 (v/v, taking into account the water content of the sample). After addition of sodium chloride the mixture was partitioned using ethylacetate/cyclohexane 1:1 (v/v). The organic phase was partially evaporated and redissolved in ethyl acetate, after which sodium sulphate, sodium chloride and cyclohexane were added. The organic phase was subjected to gel permeation chromatography. The eluate was evaporated and redissolved in ethyl acetate before analysis by GC (Zebron ZB-5 Capillary column: 30 m x 0.32 mm i.d. x 0.25 µm film thickness) with MS detection (m/z 136 (quantification) and 78, 226 (verification). Reference standards prepared in solution were used as linearity standards (detector) and used for quantification in recovery samples (one point calibration at appropriate levels).

Results

See Table B.5.2-1.

Conclusions

DFG Method S19 can be considered suitable in pre- and post registration studies for the analysis of pyriproxyfen in cucumber fruit with an LOQ of 0.01 mg/kg.

Remarks

None

STUDY 3

Characteristics

Reference	:	Weeren, R.D., Pelz, S., 1999 (IIA 4.2.1/03)	GLP statement	:	Yes
Type of study	:	Analytical methods for plants, animal tissues, milk and eggs	Guidelines	:	Directive 96/46/EC Draft working document 8064/VI/97 rev. 1 of 09 Apr '98
Year of execution	:	1999	Acceptability	:	Acceptable for pre registration
Test substance	:	Pyriproxyfen (Lot no. 961216G, purity 100%)	Method reference	:	DFG method S19 with DFG Cleanup Method 5

Description of the method

The method is based on multi-method S19 (DFG). Samples (cotton seed) were homogenized in acetonitrile/acetone, calcium silicate and Celite 545. After evaporation until dryness the residue was redissolved in ethylacetate/cyclohexane and subjected to gel permeation chromatography. The eluate was concentrated and redissolved in ethyl acetate before analysis by GC (fused silica capillary column XTI-5: 30 m x 0.25 mm i.d. x 0.25 µm film thickness) with MS detection (m/z 136 (quantification) and 78 (verification)). Reference standards prepared in solution were used for quantitation in recovery samples (one point calibration at appropriate levels) and linearity standards (detector).

Results

See Table B.5.2-1.

Conclusions

DFG Method S19 can be considered suitable in pre- and post registration studies for the analysis of pyriproxyfen in cotton seed with an LOQ of 0.01 mg/kg.

Remarks

1. No verification results (m/z 78) have been reported.
2. The ILV is reported under STUDY 4 (commodities with high fat content)

STUDY 4

Characteristics

Reference	:	Kretschmer, S., 2002b (IIA 4.2.1/04)	GLP statement	:	Yes
Type of study	:	Analytical methods for plants, animal tissues, milk and eggs	Guidelines	:	Directive 91/414/EEC, Annex II, 4.2. SANCO/825/00, rev. 6 of 20 June 2000
Year of execution	:	2000-2002	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	DFG method S19 with DFG Cleanup Method 5

Description of the method

The method is based on multi-method S19 (DFG). Samples (olives) were stoned and homogenized in acetonitrile/acetone, calcium silicate and Celite 545. After evaporation until dryness the residue was redissolved in ethylacetate/cyclohexane and subjected to gel permeation chromatography. The eluate was concentrated and redissolved in ethyl acetate before analysis by GC (Zebron ZB-5 Capillary column: 30 m x 0.32 mm i.d. x 0.25 µm film thickness) with MS detection (m/z 136 (quantification) and 78, 226 (verification)). Reference

standards prepared in solution were used for quantitation in samples and linearity standards (detector).

Results

See Table B.5.2-1.

Conclusions

DFG Method S19 can be considered suitable in pre- and post registration studies for the analysis of pyriproxyfen in olives with an LOQ of 0.01 mg/kg.

Remarks

None.

Table B.5.2-1 Summary of method description and validation (treated plants, plant products, foodstuffs, feedingstuffs, environmental samples)

Substrate	Analyte	Dissolution/ extraction	Partitioning/ clean-up	Quantifi-cation	Limit of quantifica-tion (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)	Linearity demon-strated	Ref.
Cucumber	Pyriproxyfen	Water/ acetone extraction	Ethyl acetate/ cyclohexane partitioning, followed by Gel Permeation Cleanup	GC-MS	0.01	0.01	90-105 (97)	7.2 (5)	Yes	Weber and Pelz, 2000 (5.2/01)
						0.10	82-97 (90)	6.1 (5)		
Cucumber	Pyriproxyfen	Water/ acetone extraction	Ethyl acetate/ cyclohexane partitioning, followed by Gel Permeation Cleanup	GC-MS	0.01	0.01	79-88 (83)	5 (5)	Yes	Kretsch-mer, 2002a (5.2/02)
						0.10	80-99 (90)	9 (5)		
Cotton (seed)	Pyriproxyfen	Acetonitrile/ acetone extraction	Gel Permeation Cleanup	GC-MS	0.01	0.01	76-92 (86)	7.4 (5)	Yes	Weeren and Pelz, 1999 (5.2/03)
						0.10	86-91 (88)	2.2 (5)		
Olive (fruit)	Pyriproxyfen	Acetonitrile/ acetone extraction	Gel Permeation Cleanup	GC-MS	0.01	0.01	75-101 (86)	13 (6)	Yes	Kretsch-mer, 2002b (5.2/04)
						0.10	71-109 (95)	15 (7)		

B.5.3 Analytical methods (residue) soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2)

The major components of the environmental residue are therefore as follows:

Soil:	Pyriproxyfen, 4'-OH-Pyr and PYPAC
Groundwater:	Pyriproxyfen
Surface water:	Pyriproxyfen, 4'-OH-Pyr, DPH-Pyr and PYPAC
Sediment:	Pyriproxyfen, 4'-OH-Pyr, PYPAC
Air:	Pyriproxyfen

The residue definition for monitoring is the parent only for all compartments.

STUDY 1

Characteristics

Reference	:	Wolf, S., 2002a (IIA 4.2.2/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC SANCO/825/00 rev. 6, 20 June 2000 SANCO/3029/99 rev. 4, 11 July 2000 Nachrichtenbl. Deut. Pflanzenschutzd. 52, 2000, p 292
Year of execution	:	2002	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	BBA multi method L 00.00-34 (§ 35 LMBG, Nov. 1999)

Description of the method

The method is a multi method (BBA multi method L 00.00-34). Soil (sandy loam, 10/32/58 % clay/silt/sand, pH 6.3, 50 g dry mass) was extracted with acetone/water 2:1 (v/v, taking into account the water content of the sample) and filtered over Celite. The extract was partitioned using ethylacetate/cyclohexane 1:1 (v/v). The organic phase was evaporated until dryness, redissolved in ethylacetate/cyclohexane and subjected to gel permeation chromatography. The eluate was evaporated until dryness and redissolved in ethyl acetate. After addition of iso-octane, the residue was partially evaporated and passed through a silica gel column using subsequently hexane/toluene 65/35 (v/v) (twice), toluene (twice) and toluene-acetone 95/5 (v/v) (twice). The toluene-acetone eluate was evaporated to dryness and redissolved in toluene before analysis by GC/NPD (quantification, DB-17 column 18 m x 0.18 mm (0.3 µm)) and GC/MSD (verification, DB-17 column 20 m x 0.18 mm (0.3 µm), m/z 226 and (qualification) m/z 186, 136, 96). Reference standards prepared in toluene were used as calibration standards (calibration curve $Y=AX^B$). Although r>0.99 (recalculated by RMS), linear regression yields deviations >20% for concentrations at the lower end of the calibration curve. Regression using $Y=AX^B$ covers the whole calibration range (0.01 – 1.0 mg/L), with r>0.997.

Results

See Table B.5.3-1.

Conclusion

BBA multi method L 00.00-34 is considered suitable for the determination of pyriproxyfen in soil with an LOQ of 0.01 mg/kg.

Remarks

None.

STUDY 2

Characteristics

Reference	:	Wolf, S., 2002b (IIA 4.2.3/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC SANCO/825/00 rev. 6, 20 June 2000 SANCO/3029/99 rev. 4, 11 July 2000 Nachrichtenbl. Deut. Pflanzenschutzd. 52, 2000, p 292
Year of execution	:	2002	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-untersuchung)

Description of the method

The method is a multi-residue method (F12, Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). Subsequently tap water (1L), distilled water, air (for drying) and acetone were passed through a SPE C18 column. The acetone eluate was evaporated to an aqueous residue, to which ethanol was added followed by evaporation until dryness. The residue was reconstituted in toluene and was analyzed by GC/NPD (quantification, DB-17 column 18 m x 0.18 mm (0.3 µm)) and verified by GC/MS (DB-17 column 20 m x 0.18 mm (0.3 µm), m/z 226 and (qualification) m/z 186, 136, 96). Reference standards prepared in solution were used as calibration standards (calibration curve $Y=AX^B$). Although r>0.99 (recalculated by RMS), linear regression yields deviations >20% for concentrations at the lower end of the calibration curve. Regression using $Y=AX^B$ covers the whole calibration range (0.01 – 1.0 mg/L), with r>0.997.

Results

See Table B.5.3-1.

Conclusion

Multi residue method F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung) is considered suitable for the determination of pyriproxyfen in tap water (LOQ 0.1 µg/L).

Remarks

None.

STUDY 3

Characteristics

Reference	:	Wais, A., 2001a (IIA 4.2.3/02)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC EC guidance 8064/VI/97 rev 4, 15 Dec 1998 SANCO/3029/99 rev. 4, 11 July 2000
Year of execution	:	2000	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-untersuchung)

Description of the method

The method is a multi-residue method (F12, Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). Subsequently surface water (1L, river water, TOC 32.8 mg/L, pH 7.85), distilled water, air (for drying) and hexane were passed through a SPE C18 column. The hexane was evaporated to an aqueous residue, to which methanol was added followed by evaporation until dryness. The residue was reconstituted in toluene and analyzed by GC/NPD (quantification, DB-17 column 20 m x 0.18 mm (0.3 µm)) and verified by GC/MS (DB-17 column 20 m x 0.18 mm (0.3 µm), m/z 226 and (qualification) m/z 186, 136, 96). Reference standards prepared in solution were used as calibration standards.

Results

See Table B.5.3-1.

Conclusion

Multi residue method F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung) is considered suitable for the determination of pyriproxyfen in surface water (LOQ 0.01 µg/L) in pre-registration studies and for post-registration monitoring purposes.

Remarks

The LOQ of 0.01 µg/L is below the level of the lowest appropriate toxicity value for aquatic organisms (in this case the NOEC for daphnia (0.015 µg/L) (See B.9.2.3.1.2)). The established LOQ is >NOEC/10 (i.e. including the risk assessment safety factor of 10).

STUDY 4

Characteristics

Reference	:	Wais, A., 2001b (IIA 4.2.4/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC EC guidance 8064/VI/97 rev 4, 15 Dec 1998 SANCO/3029/99 rev. 4, 11 July 2000
Year of execution	:	2000-2001	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	Not indicated

Description of the method

The front sections of Tenax adsorption tubes (100 mg adsorbent in the front and 50 mg in the back section) were fortified with pyriproxyfen (0.36 and 3.6 µg). Air was passed through each tube at a rate of 1L/min for 6 hours (total 360 L, equivalent to 1.0 and 10 µg/m3 of pyriproxyfen, respectively). For both concentrations, air conditions were ~20°C, ~30% rH and ~35°C, ≥ 80% rH. Front and back sections were extracted separately in toluene. Extracts were analyzed by GC/NPD (DB-17 column, 20 m x 0.18 mm, 0.3 µm film thickness). Results were confirmed by GC/MS (DB-17 column, 20 m x 0.18 mm, 0.3 µm film thickness, m/z 226 (and m/z 186, 136, 96 and 77 for qualification)). Reference standards prepared in toluene were used as calibration standards (calibration curve $Y=AX^B$).

Results

See Table B.5.3-1. No interferences in the blanks and no breakthrough were observed.

Conclusion

The method is considered suitable for the analysis of pyriproxyfen in air with an LOQ of 1.0 µg/m3 (sampling at a rate of 1L/min during 6 hours; conditions ~20°C, ~30% rH and ~35°C, ≥80% rH). The LOQ (1 $\mu g/m^3$) is sufficient according to SANCO/825/00 as it is below the limit of 15 $\mu g/m^3$ calculated using: limit = $AOEL_{systemic}$*0.1*60/20. The $AOEL_{systemic}$ (0.05 mg/kg bw/day) is set in B.6.10.5.

Remarks

None.

Table B.5.3-1 Summary of method description and validation (soil, water and air)

Substrate	Analyte	Dissolution/ extraction	Partitioning/ clean-up	Quantifi-cation	Limit of quantifica-tion (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)	Linearity demon-strated	Ref.
Soil	Pyriproxyfen	Acetone/ water extraction	Ethyl acetate/ cyclohexane partitioning, followed by Gel Permeation and Silica Gel cleanup	GC-NPD	0.01	0.01	104-107 (105)	1.2 (5)	No[(A)]	Wolf, 2002a (5.3/01)
						0.10	92-111 (103)	7.5 (5)		
Confirmatory method, soil	Pyriproxyfen	Acetone/ water extraction	Ethyl acetate/ cyclohexane partitioning, followed by Gel Permeation and Silica Gel cleanup	GC-MS	0.01	0.01	106-110 (108)		Yes	
						0.10	102 (102)			
Tap water (pH 7.3, DOC 4.2)	Pyriproxyfen	SPE C18 column	-	GC-NPD	0.1 µg/L	0.1 µg/L	97-111 (104)	5.4 (5)	No[(A)]	Wolf, 2002b (5.3/02)
						1.0 µg/L	75-84 (79)	4.3 (5)		
Surface water (pH 7.85, TOC 32.8 mg/L)	Pyriproxyfen	SPE C18 column	-	GC-NPD	0.01 µg/L	0.01 µg/L	107-109 (108)	0.6 (5)	Yes	Wais, 2001a (5.3/03)
						0.1 µg/L	89-106 (98)	7.1 (5)		
Confirmatory method, surface water	Pyriproxyfen			GC-MS	0.01	0.01			Yes	
						0.10	100.7			

Air ~20°C, ~30% rH, 1 L/min for 6 hours	Pyriproxyfen	Flow of air through Tenax tubes. Extraction with toluene	-	GC-NPD	1.0 µg/m^3	1.0 µg/m^3	89-94[(B)] (91)	2.8 (5)	No[(A)]	Wais, 2001b (5.3/04)
						10 µg/m^3	95-107[(B)] (101)	4.9 (5)		
Air ~35°C, ≥80% rH, 1 L/min for 6 hours	Pyriproxyfen	As above	-	As above	1.0 µg/m^3	1.0 µg/m^3	107-109[(B)] (108)	0.7 (5)		
						10 µg/m^3	107-108[(B)] (108)	0.6 (5)		

(A) Although r>0.99 (linear regression, recalculated by RMS), linear regression yields deviations >20% for concentrations at the lower end of the calibration curve. Regression using $Y=AX^B$ covers the whole calibration range (0.01 – 1.0 mg/L), with r>0.997.

(B) No pyriproxyfen was detected in the back sections of the Tenax tubes (i.e. no breakthrough was observed).

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

Not required, not a toxic or very toxic compound.

B.5.5 Evaluation and assessment

Analytical methods for technical active substance and plant protection product

A HPLC-UV method was submitted for the determination of pyriproxyfen in technical material and in the plant protection product Pyriproxyfen 10 EC. The identity of pyriproxyfen in technical material was confirmed using HPLC-MS. The validation results fulfilled all criteria.

Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs

One analytical method (DFG multi-residue method S19) was submitted for the determination of pyriproxyfen in cucumber fruit (study 1, LOQ 0.01 mg/kg) and in cotton seed (study 3, LOQ 0.01 mg/kg). Both studies contained fully acceptable validation results. An ILV for cucumber was submitted (study 2) and deemed acceptable. An ILV for cotton seed was submitted which was conducted using olives (study 4). This ILV was acceptable since cotton seed and olives belong to the same commodity group (commodities with high fat content). Hence, DFG method S19 can be accepted as pre- and post registration method for the determination of pyriproxyfen in cucumber fruit (LOQ 0.01 mg/kg) and in cotton seed (LOQ 0.01 mg/kg).

Analytical methods for soil, water and air

Analytical methods were submitted for the determination of pyriproxyfen. Validations for soil, water (surface water and tap water) and air were included.

One method (BBA multi method L 00.00-34, GC/NPD) was submitted for the determination of pyriproxyfen in soil (study 1). The method was fully validated and confirmed by GC-MS. BBA multi method L 00.00-34 is considered suitable for the determination of pyriproxyfen in soil with an LOQ of 0.01 mg/kg.

One GC-NPD method (Multi residue method F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung) was submitted for the determination of pyriproxyfen in tap water (study 2) and surface water (study 3). The method was fully validated and confirmed by GC-MS. Method F12 is considered suitable for the determination of pyriproxyfen in tap water and surface water (LOQ 0.1 µg/L). The LOQ of 0.01 µg/L for surface water is below the level of the lowest appropriate toxicity value for aquatic organisms (in this case the NOEC for daphnia (0.015 µg/L) (See B.9.2.3.1.2)). The established LOQ is >NOEC/10 (i.e. including the risk assessment safety factor of 10).

One GC-NPD method was submitted for the determination of pyriproxyfen in air (study 4). The method was fully validated and confirmed by GC-MS. The method is considered suitable for the analysis of pyriproxyfen in air with an LOQ of 1.0 µg/m^3 (~20°C, ~30% rH and

~35°C, ≥80% rH; 1L/min during 6 hours). The method is considered suitable for post-registration monitoring because the LOQ (1 µg/m^3) is below the limit of 15 µg/m^3 calculated using: limit = $AOEL_{systemic}$*0.1*60/20. The $AOEL_{systemic}$ (0.05 mg/kg bw/day) is set in B.6.10.5.

B.5.6 References relied on

References for the active substance

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 4.1/01	Kimura M	2000	Analytical methods for the determination of active substance and impurities in pyriproxyfen technical substance Sumitomo Chemical Co., Ltd. Report No. NNA-0083 Not GLP, Unpublished	Y	SUM
IIA, 4.1/02	Mukumoto, M	2002a	Confirmation of identification of active substance and impurities in pyriproxyfen technical material Sumitomo Chemical Co., Ltd. Report No. NNA-0088 Not GLP, Unpublished	Y	SUM
IIA 4.2.1/01	Weber H Pelz S	2000	Validation of DFG method S19 (extended revision) for the determination of residues of pyriproxyfen in samples of commodities with high water content (cucumber) Sumitomo Chemical Co., Ltd. Report No. NNA-0079 GLP, Unpublished	Y	SUM
IIA 4.2.1/02	Kretschmer S	2002a	Pyriproxyfen: Independent laboratory validation (ILV) of the multi-residue method DFG S19 (extended revision) for the determination of residues of pyriproxyfen in watery crops (cucumber) Sumitomo Chemical Co., Ltd. Report No. NNA-0093 GLP, Unpublished	Y	SUM
IIA 4.2.1/03	Weeren RD Pelz S	1999	Validation of DFG method S19 with DFG cleanup method 5 for the determination of residues of pyriproxyfen in field samples of cottonseed Sumitomo Chemical Co., Ltd., Report No. NNA-0076 GLP, Unpublished	Y	SUM
IIA 4.2.1/04	Kretschmer S	2002b	Pyriproxyfen: Independent laboratory validation (ILV) of the multi-residue method DFG S19 for the determination of residues of pyriproxyfen in oily crops Sumitomo Chemical Co., Ltd., Unpublished Report No. NNA-0094 GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 4.2.2/01	Wolf S	2002a	Validation of the BBA multi method L 00.00-34 (§ 35 LMBG, Nov. 1999) for the determination of pyriproxyfen in soil Sumitomo Chemical Co., Ltd. Report No. NNA-0091 GLP, Unpublished	Y	SUM
IIA 4.2.3/01	Wolf S	2002b	Validation of a multi-residue method for the determination of pyriproxyfen in drinking water Sumitomo Chemical Co., Ltd. Report No. NNA-0092 GLP, Unpublished	Y	SUM
IIA 4.2.3/02	Wais A	2001a	Development and validation of the residue analytical method for pyriproxyfen in surface water Sumitomo Chemical Co., Ltd Unpublished Report No. NNA-0084 GLP, Unpublished	Y	SUM
IIA 4.2.4/01	Wais A	2001b	Development and validation of the residue analytical method for pyriproxyfen in air Sumitomo Chemical Co., Ltd Unpublished Report No. NNA-0085 GLP, Unpublished	Y	SUM

References for the plant protection product

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA 5.1.1/01	Reitz GA	2001	Quantitation of pyriproxyfen in pyriproxyfen 10 EC Sumitomo Chemical Co., Ltd Report No. NNA-0090 GLP, Unpublished	Y	SUM

European Commission

B.5.7 **PYRIPROXYFEN**

ADDENDUM

VOLUME 3 (B8)

ANNEX B

Rapporteur Member State: The Netherlands

DECEMBER 2008 revised

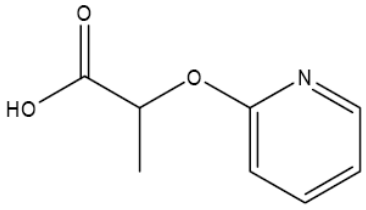
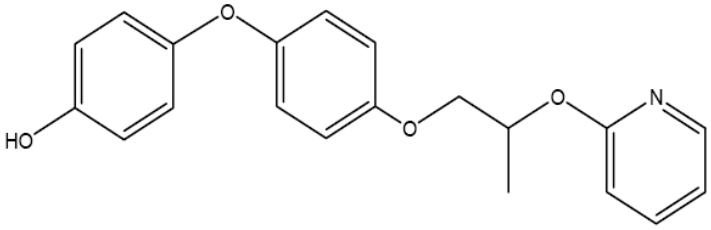
Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of pyriproxyfen in Annex I of Council Directive 91/414/EEC

4.1 Point of clarification for the applicant.

Applicant to provide pKa estimates (QSAR calculations) for the metabolites PYPAC and 4-OH-Pyr together with their argumentation how adsorption of pyriproxyfen PYPAC and 4-OH-Pyr would or would not be significantly affected at the pH range normally associated with agricultural soils.

Applicant provided the following information:
To evaluate the possible pH dependent sorption to soil and sediment of the two main soil metabolites of pyriproxyfen, PYPAC and 4'-OH-Pyr, pKa values for each metabolite were estimated using the ACD/pKa DB Program (version 4.5)[1]. The dissociation constants (pKa) were estimated to be 2.06 and 4.35 for PYPAC and 3.63 and 10.13 for 4'-OH-Pyr. The results are summarised in Table 1 below.

Table 1
Estimated pK_a values for metabolites PYPAC and 4'-OH-Pyr using the ACD/pK_a DB Program

Test compound	Chemical Structure	Dissociation constant (pK_a)	
		Dissociation centre	
		OH	NH
PYPAC		2.06	4.35
4'-OH-Pyr		10.13	3.63

It is not possible to draw any clear conclusions concerning the influence of pH on the adsorption of metabolites PYPAC and 4'-OH-Pyr. No clear influence of pH was observed during the adsorption / desorption studies. Given their chemical properties, it is possible that adsorption of these metabolites may be pH dependent. However, based on the estimated pKa values for the metabolites, it can be assumed that the ionised form of

[1] ACD/pK_a DB Program Version 4.5, Calculating the Acid-Base Ionization Constant, pK_a, Advanced
Chemistry Development (2000)

these metabolites will not be significantly affected at the pH range normally associated with agricultural soils (pH 5.0 – 7.5).
RMS agrees with the applicant that at soil pH relevant for agricultural soils the metabolites will not be present in their ionised form. It is therefore not expected that sorption is pH dependent at relevant soil pH.

B.5.7.1 Predicted concentrations in groundwater

4.2 Point of clarification for the applicant:
Applicant to provide an assessment of the potential for groundwater exposure from pyriproxyfen or its metabolites 4-OH-Pyr and PYPAC as a result of the applied for uses in glasshouses.

Reference	:	Jarvis, T	GLP	:	Not applicable
Type of study	:	PEC groundwater calculations	Guideline	:	Not applicable
Year of execution	:	2007	Acceptability	:	Acceptable
Study titel	:	Predicted Environmental Concentrations of Pyriproxyfen, and its soil metabolites, 4'-OH pyriproxyfen and PYPAC, in Groundwater in the EU using the FOCUS Groundwater Scenarios with the PEARL model			
Test substance	:	Not applicable			

Study design

To provide a wider assessment of pyriproxyfen groundwater contamination potential following glasshouse use, simulations were undertaken for the outdoor tomato scenarios provided by FOCUS (i.e. Chateaudun, Piacenza, Porto, Thiva and Sevilla) but using the worst case application rates and timings supported for indoor tomatoes and eggplants.
Ground water modelling of pyriproxyfen has been undertaken with the FOCUS groundwater scenarios using the FOCUS PEARL 3.3.3 model. The modelling undertaken in this exercise was based on the use of a foliar spray (10 EC formulation).
Simulations were undertaken for outdoor tomatoes with a GAP of 2 x 0.1125 kg a.s./ha (i.e. the maximum rate used for indoor tomatoes and eggplants).
The FOCUS scenarios, Chateaudun, Piacenza, Porto, Sevilla and Thiva include outdoor tomatoes and hence these locations were considered for this modelling exercise. A summary of the chemical specific inputs used are shown below. These are consistent with those used in the DAR.

Compound	Pyriproxyfen (parent)	4'-OH-Pyriproxyfen	PYPAC
Molecular Weight	321.37	337.37	167.16
Vapour Pressure (Pa)	$<1.33 \times 10^{-5}$	1.05×10^{-7}	0.078
Aqueous Solubility (mg/L)	0.367	1.4	65000
Maximum amount (%) in soil	-	6.3% (aerobic)	8.6% (aerobic)
DT50 in aerobic soil (days) at 20ºC	8.6	34.8	15.7
Soil Adsorption Coefficient (Koc) (ml/g)	21175 (Kom =12282)	2598 (Kom =1507)	20.7 (Kom =12)
Freundlich Exponent (1/n)	1.15	0.87	1.10
Crop uptake factor	0	0	0.5

The agronomic parameters used as inputs for these simulations were therefore as follows with interception percentages determined according to FOCUS guidance (2002):

Crop: Tomatoes
Application Rate: 2 x 0.1125 kg a.s./ha (Southern European GAP)
Application Dates: 13 days prior to harvest, 3 days prior to harvest (latest possible application considered as worst case)
Crop Interception: 80% (based on stated BBCH growth stage of 89)
Crop uptake factor: 0 (pyriproxyfen is not expected to be systemic)

Results

FOCUS scenario			80th percentile concentration in groundwater [µg/L] of:		
Crop	**GAP**	**location**	**Pyriproxyfen**	**4'-OH-Pyr**	**PYPAC**
Tomatoes	cGAP Southern Europe	Chateaudun	<0.001	<0.001	0.0149 (0.0195)
		Piacenza	<0.001	<0.001	0.0267 (0.0329)
		Porto	<0.001	<0.001	0.0131 (0.0224)
		Sevilla	<0.001	<0.001	0.0014 (0.0007)
		Thiva	<0.001	<0.001	0.0106 (0.0173)

From the results it can be seen that for pyriproxifen and metabolites 4'OH-pyriproxyfen and PYPAC simulated concentrations are <0.1 µg/L for all scenarios following outdoor applications to tomato and eggplant. It is considered that indoor uses on tomato and eggplant are sufficiently covered by this simulation, and that predicted concentrations in groundwater for indoor uses will not exceed 0.1 µg/L.

Remarks

DT_{50} was not only corrected for temperature as stated in the table, but also for moisture content. Therefore the value is at 20 ºC and pF2.
RMS recalculated using the same input values and came to slightly different values (values in brackets).

Open point 4.5
RMS to add the reference Study Report No. NNP-0067, '4'-OH-Pyriproxyfen - Water solubility'
WATER SOLUBILITY OF METABOLITES

Characteristics

Reference	:	Lorence, P.J., 1996	GLP	:	Yes
Type of study	:	Water solubility	Guideline	:	Pest. Assess. Guideline, subdiv. D, product chemistry, guidline 63-8
Study title	:	4'OH-pyriproxyfen water solubility.			
Year of execution	:	1995	Acceptability	:	Acceptable
Test substance	:	4'-OH-pyriproxyfen; batch 941117; chemical purity 99.1%;			

This study was available in the original dossier. The endpoint has been used in the DAR for calculations. However, the study was not included in Vol.3, nor was it referred to at any point.

Study design

The shake flask method was used to determine the solubility of 4'-OH-pyriproxyfen at 25 ºC. Saturated solutions of the test material in water were prepared in triplicate. This was accomplished by dissolving the test material in an appropriate solvent (acetone), coating it on the interior walls of Erlenmeyer flasks, and than removing the solvent. The sample solutions and appropriate blanks were equilibrated in a temperature-controlled

environment with continuous agitation. At sampling intervals of 3, 5 and 7 days equilibration aliquots were removed and centrifuged to remove suspended particles. The content of 4'-OH-pyriproxyfen in the solutions was quantified by liquid chromatography and UV detection. Stability at test conditions was evaluated based on the recovery ratio using the procedure as for the solubility samples, but extract them from the interior using 25 ml acetonitril.

Results

Upon reaching equilibrium 4'-OH-pyriproxyfen was determined to be soluble at a concentration of 1.4 ppm in water at 25 ºC. Results were statistically evaluated using SAS software.

European Commission

B.5.8 **PYRIPROXYFEN**

B.5.9

ADDENDUM

VOLUME 4 (B9)

ANNEX B

Rapporteur Member State: The Netherlands

DECEMBER 2008

Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of pyriproxyfen in Annex I of Council Directive 91/414/EEC

FRONT PAGE ONLY

European Commission

PYRIPROXYFEN

ADDENDUM TO VOLUME 4

ANNEX C

Rapporteur Member State: The Netherlands

DECEMBER 2008

Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of pyriproxyfen in Annex I of Council Directive 91/414/EEC

FRONT PAGE ONLY

European Commission

B.5.10 **PYRIPROXYFEN**

REVISED ADDENDUM

VOLUME 3 (B6)

ANNEX B

PRAPeR 64

Rapporteur Member State: The Netherlands

DECEMBER 2008

revised JANUARY 2009

Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of pyriproxyfen in Annex I of Council Directive 91/414/EEC

CONTENTS

38

Introduction

Based on the comments from the Member States, a reporting table and evaluation table were composed for pyriproxyfen. There are 21 open points and 1 point for clarification for mammalian toxicology. Additional information with regard to the open points and point of clarification will be presented in this addendum, to be discussed in PRAPeR 64 dd. January 21-23, 2009.

This addendum was discussed at PRAPeR 64 (Jan. 2009). Changes after the PRAPeR meeting are highligted (a few small changes have been made in Table 6.14.1.4-1 (operator exposure) and an additional remark on worker exposure is made).

B.6 TOXICOLOGY AND METABOLISM

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

Open point 2.1: RMS to provide more details on the study of Isobe (1988a) to clarify the calculation of oral absorption (based on reporting table 2(1)).

To facilitate the discussion on the oral absorption in the expert meeting, the summary of the study of Isobe as presented in the DAR is copied, and the calculation of oral absorption is explained and amended.

STUDY 1

Characteristics

reference	:	Isobe N., 1988a	exposure	:	Single or repeated by gavage
type of study	:	Absorption, distribution, metabolism, excretion	doses	:	2 and 1000 mg/kg bw
year of execution	:	1987-1988	vehicle	:	Corn oil
test substances	:	S-31183 (Pyriproxyfen), lot no PTG-86012, chemical purity 99.0% [Phenoxyphenyl-^{14}C] S-31183, lot no C-86-92, radiochemical purity >99%,chemical purity >99%, specific activity 58.2 mCi/mmol	GLP statement	:	yes
route	:	oral	guideline	:	EPA 85-1, Directive 88/302/EEC
species	:	Rat, Sprague-Dawley CD, 5-7 weeks (at administration)	acceptability	:	acceptable
group size	:	See table 6.1.1.1			

Study design

The study investigated the absorption, distribution, metabolism and excretion of 14C-pyriproxyfen after a single oral dose of 2 and 1000 mg/kg bw, and a single oral dose of 2 mg/kg bw after 14 daily pre-treatments at the same dose with unlabelled pyriproxyfen or corn oil. Absorption was investigated in bile duct cannulated rats after a single oral dose of 2 mg/kg bw (study E). Exposure and sampling of urine, faeces, bile, cage wash and tissues was as described in table 6.1.1.1. No samples of expired air were collected (based on the outcome of a preliminary study, showing <0.01% of the radioactivity administered trapped in alkaline solution).

Table 6.1.1.1 Experimental groups for each dose level

Study	No. of animals/ sex	Treatment	Sampling times (days after dosing)	Sacrifice time (h after last dose)
A (low dose)	5	Single oral dose at 2 mg/kg bw [Phenoxyphenyl-^{14}C] S-31183	1, 2, 3, 5 and 7: urine and faeces; 7 cage wash	Day 7[1]
B (high dose)	5	Single oral dose at 1000 mg/kg bw [Phenoxyphenyl-^{14}C] S-31183	1, 2, 3, 5 and 7: urine and faeces; 7 cage wash	Day 7[1]
C (repeated dose)	5	14 daily pretreatments of unlabelled pyriproxyfen (2 mg/kg bw) followed by one oral dose at 2 mg/kg bw [Phenoxyphenyl-^{14}C] S-31183	1, 2, 3, 5 and 7: urine and faeces; 7 cage wash	Day 7[1]
D (repeated vehicle)	3 males	14 daily pretreatments with corn oil (5 ml/kg bw) followed by one oral dose at 2 mg/kg bw [Phenoxyphenyl-^{14}C] S-31183	1, 2, 3, 5 and 7: urine and faeces	Day 7[1]
E (bile study)	3[2]	Single oral dose at 2 mg/kg bw [Phenoxyphenyl-^{14}C] S-31183	24 and 48 hour; bile, urine and faeces	48 hour[3]

1. At sacrifice blood was collected and the following tissues and organs were taken from all animals: bone, brain, fat, heart, liver, kidney, lung, muscle, spleen, stomach, small intestines, caecum, large intestines, testis/uterus, ovaries and the remaining carcass (sciatic nerve and spinal cord only in study A)
2. Bile duct cannulated rats
3. At sacrifice the intestinal contents was collected. No organs or tissues were taken

At termination blood and selected tissues and organs (see footnote 1 of table 6.1.1.1) were collected from the animals of study A-C. Clinical signs were observed daily and body weights were determined just before daily dosing.

Tissues (2 samples of ca. 200 mg), carcass and homogenised faeces were combusted and analysed by LSC. Radioactivity in urine (cage washings were combined with urine over the last sampling period) and bile (study E only) was quantified by LSC.

Acetone extracts of 0-2 day faeces homogenate were separated by TLC using toluene/diethyl ether (3/2). Metabolites were identified on TLC using cochromatography. Polar metabolites were subjected to enzymatic hydrolysis. Composite samples of 0-2 day urine were separated by TLC. Quantification of the metabolites was carried out by scraping the silica gel from the TLC plates followed by LSC analysis. In study E no metabolites were identified in faeces and urine. Bile was subjected to TLC analysis as described above.

Results

In high dose animals loose stool/diarrhoea was observed 10 hours after dosing in all males and 4 out of 5 females. No other treatment related symptoms were observed.

Data on excretion are presented in tables 6.1.1.2. At the end of the study (7 days post-dosing), overall excretion for all groups was found to be 92-98% AR.

After a single oral dose of 2 and 1000 mg/kg bw, or an oral dose of 2 mg/kg bw after 14 oral daily pre-treatments at 2 mg/kg bw (unlabelled pyriproxyfen), radioactivity recovered after 7 days (range for males and females) represented 5-12% AR (urine and cage wash) and 81-92% AR (faeces). Excretion within the first 24 hours after

dose administration was essentially 63-83% AR. The amount of RA retained in tissues and residual carcass of male and female rats was low (0.1-0.3% AR).

From the study in bile cannulated rats after a single oral dose of 2 mg/kg bw biliary excretion was 34-37% of RA after 48 hours. There were no remarkable differences between patterns of absorption and excretion of sexes and oral dosing regimes.

Table 6.1.1.2 Excretion of radioactivity (% AR) in rats after single or repeated oral exposure to ^{14}C-Pyriproxyfen

		single oral, 2 mg/kg bw		single oral, 1000 mg/kg bw		Repeated oral 2 mg/kg bw			single oral (bile duct cann.) 2 mg/kg bw	
sample	period (d)	M	F	M	F	M	F	period (h)	M	F
urine	0-1	7	4	5	3	10 (12)	7	0-24	2	1
	0-7[1]	8	5	7	5	12 (13)	9	0-48	3	2
faeces	0-1	76	74	72	68	60 (58)	57	0-24	24	48
	0-7	89	92	90	92	81 (78)	83	0-48	38	51
total tissues	7	0.1	0.1	0.3	0.1	0.3	0.2			
bile								0-24	28	34
								0-48	34	37
Intestinal contents								0-48	5	1
Total	0-7	98	97	97	97	93 (91*)	92	0-48	80	90

1. RA recovered from urine including cage wash.
Values in brackets are from the repeated dose study with corn oil (D)

* Total without tissues

The distribution of radioactivity in tissues and carcass for all groups 7 days post-dose is presented in table 6.1.1.3. Tissue concentrations in male and female rats of the same treatment group were comparable.

There were no remarkable differences between tissue concentrations of rats after treatment with a single or repeated oral dose of 2 mg/kg bw or after a single dose at 1000 mg/kg bw. Tissue concentrations were in general very low (0.1% AR).

Table 6.1.1.3 Residual radioactivity in tissues and organs in % AR and ng pyriproxyfen equivalents/gram tissue taken at 7 days post-radio labelled-dose.

	single oral, 2 mg/kg bw				single oral, 1000 mg/kg bw				repeated oral, 2 mg/kg bw			
	M		F		M		F		M		F	
tissue	**conc**	**% AR**	**conc**	**% AR**	**conc**	**% AR**	**conc**	**% AR**	**conc**	**% AR**	**conc**	**% AR**
blood	<1	0	<1	0	<300	0	<300	0	2 (2)	0	2	0
bone	1	0	<1	0	<200	0	<200	0	1 (1)	0	1	0
brain	<1	0	<1	0	200	0.2	<300	0.1	1(1)	0.1	<1	0.1
caecum	1	0	1	0	500	0	500	0	2 (2)	0	2	0
fat (abdom.)	10	0	13	0	8000	0	9500	0	48 (32)	0	35	0
heart	<1	0	<1	0	<200	0	<200	0	1 (1)	0	1	0
Intestine small	1	0	1	0	600	0	500	0	2 (2)	0	3	0
Intestine large	1	0	1	0	400	0	500	0	1 (2)	0	1	0
kidney	1	0	1	0	400	0	400	0	2 (1)	0	2	0
liver	3	0	4	0	1700	0	1500	0	5 (6)	0	6	0
lungs	<1	0	<1	0	<200	0	<200	0	1 (1)	0	1	0
muscle	<1	0	<1	0	300	0	<200	0	1 (1)	0	<1	0
ovaries			2	0			900	0			4	0
sciatic nerve	<2	0	<3	0								
spinal cord	<1	0	<1	0								
spleen	1	0	1	0	200	0	200	0	1 (1)	0	1	0
stomach	1	0	<1	0	300	0	<300	0	1 (2)	0	1	0
testes	<1	0			<200	0			1 (1)	0		
uterus			<1	0			300	0			1	0
carcass	1	0.1	1	0	2600	0.1	2300	0.1	3 (2)	0.1	2	0.1
contents												
stomach	<1		<1		600		300		1 (1)		1	
intestines	7		6		7000		4600		15 (13)		10	

total	0.1	0.1	0.3	0.1	0.3	0.2

Values in brackets are from the repeated dose study with corn oil (D)

Results of the metabolite identification in urine and faeces are presented in Table 6.1.1.4. Only the fraction sampled two days after administration of the labelled compound was investigated.

In faeces 10 metabolites were identified (17 detected) and in urine 2 (out of 11 detected). 4'-OH-pyriproxyfen was the major metabolite in faeces, representing on average 25-35% and 43-54% of the total administered radioactivity in males and females, respectively. In the studies with a single oral dose in faeces the parent compound accounted for 25-37% of the total radioactivity administered in both sexes. After repeated dosing 11.4% (males) and 6.5% (females) of the parent was found in the faeces. In the repeated dose study with corn oil 5.5% of the administered radioactivity found in the faeces was identified as the parent. The author of the report therefore concludes that the vehicle is facilitating the uptake of the parent (leading to less parent in the faeces).

In low dosed males 5",4'-OH-pyriproxyfen and 4'-OH-POPA were identified in small amounts (3.0-8.5% of administered radioactivity) and in females in even smaller amounts (0.8-2.7%). Sulfate-conjugates of 4'-oxydiphenol (0.2-0.5% AR), 5",4'-OH-pyriproxyfen (0.4-1.3% AR), 4'-OH-POPA (1.1-2.6% AR) and 4'-OH-pyriproxyfen (2.1-3.7% AR) were found in small amounts in faeces of high dosed animals.

In urine the sulfate-conjugates of 4'-oxydiphenol (0.3-3.8% AR) and 4'-OH-pyriproxyfen (0.4-1.4%) were identified in minor amounts. In general in urine 3-7% of radioactivity could not be identified. In faeces up to 20.6% of the administered radioactivity could not be identified (or was not extractable).

In bile the following metabolites were identified (not quantified): 4'-OH-pyriproxyfen sulfate, 4'-oxidiphenol sulfate, 4'-OH-POPA sulfate and 5",4'-OH-pyriproxyfen sulfate. The parent compound was not detected in bile.

Table 6.1.1.4 Urinary and faecal metabolite identification (% AR; mean for males and females)

	single oral, 2 mg/kg				single oral, 1000 mg/kg				Repeated oral, 2 mg/kg				Corn oil
	urine		faeces		urine		faeces		urine		faeces		faeces
Metabolites	M	F	M	F	M	F	M	F	M	F	M	F	M
Pyriproxyfen			37.2	31.1			31.1	25.1			11.4	6.5	5.5
2'-OH-pyriproxyfen			0.2	0.2			0.2	0.2			0.2	0.2	0.2
4'-OH-pyriproxyfen			24.5	43.3			35.2	48.3			34.5	54.4	39.9
4'-OH-pyriproxyfen sulfate	0.4	1.0			0.5	1.0	3.7	2.1	0.6	1.4			
POPA[1]			0.2	0.2			0.2	0.2			0.1	0.4	0.2
4'-oxydiphenol			0.5	0.4			0.2	0.3			0.6	0.4	0.5
4'-oxydiphenol sulfate	3.1	0.5			1.6	0.3	0.5	0.2	3.8	0.8			
5",4'-OH-pyriproxyfen			8.5	2.0			1.5	1.0			3.0	0.8	2.7
5",4'-OH-pyriproxyfen sulfate							1.3	0.4					
4'-OH-POPA			3.3	1.3			1.4	0.8			8.3	2.7	9.1
4'-OH-POPA sulfate							2.6	1.1					
unidentified	4.4	3.5	7.1	6.1	4.3	3.1	5.0	5.1	6.8	6.2	11.7	7.6	10.1
not extractable			6.4	5.3			4.4	3.9			8.9	6.5	6.5
total RA chromatographed	7.9	5	88	90	6.4	4.4	87	89	11	8.4	79	80	75
total RA identified	3.5	1.5	74	79	2.1	1.3	78	80	4.4	2.2	58	65	58

1. POPA = 4-phenoxyphenyl(RS)-2-(2-hydroxy)propyl ether

Acceptability

The totals of the metabolites were recalculated by the reviewer, because the totals in the report were not in agreement with values for individual metabolites.
The study is considered acceptable.

Conclusion

Based on the absence of the parent compound in bile, the author concludes that the pyriproxyfen found in faeces was not absorbed. According to the author of the report the low amounts of parent compound found in faeces after repeated dosing of pyriproxyfen are caused by facilitation of absorption of pyriproxyfen by the vehicle corn oil, as a similar effect was seen after repeated dosing with corn oil followed by pyriproxyfen. Although this effect was substantiated for corn oil, it cannot be accepted as prove that in general absorption of pyriproxyfen is not increased at longer exposure periods.
Metabolites formed after first pass metabolism were shown to contribute substantially to the amount of radioactivity excreted via the faeces. In the DAR it was concluded that absorption can be assumed to be ca. 39-49% of the applied dose, based on radiolabel recovered from urine, bile and tissues, and 40% absorption was used for the risk assessment.

In the DAR it was not clearly explained how absorption was calculated. The values of 39-49% were based on the overall values from the study. The values were comparable for all groups, single low and high dose, repeated dose, males and females. Therefore, the values were combined:
Urine and cage wash: 5-12%
Tissues and carcass: 0.1-0.3%
Bile: 34-37%

However, it is not correct to combine the results from bile cannulated animals (radioactivity in bile) with results from non-cannulated animals (radioactivity in urine, cage wash, tissues and carcass). Therefore, absorption should be based on radioactivity in bile and urine from the bile-cannulated rats. In the bile study, radioactivity was not determined in tissues and carcass, but the results from the non-cannulated animals show that this is very low. This results in an absorption of 37-39%. Since this is a worst-case approach, it is still proposed to use 40% oral absorption for the risk assessment.

The notifier considers the amount of unchanged pyriproxyfen in the faeces as the minimum unabsorbed dose. However, the metabolic profiles of the bile and faeces are not comparable, e.g. one of the major metabolites found in faeces (4'-OH-pyriproxyfen) was not found in bile. Therefore, it cannot be excluded that metabolism of pyriproxyfen occurs in the intestines. In absence of further data, which exclude the possibility of metabolism in the intestines, a worst-case assumption for oral absorption should be made. Based on these considerations it is concluded that for risk assessment purposes, 40% oral absorption is taken as a worst-case estimate.

B.6.3.3 Subacute inhalation studies

Open point 2.3 and point of clarification 2.1: NOAEL in the subacute inhalation study to be discussed by the experts. RMS could provide a revised table 6.3.3.1 with additional figures for the discussion. Point of clarification for the applicant: historical control data for changes in clinical chemistry have to be provided (based on reporting table 2(3)).

To facilitate the discussion on the subacute inhalation study in the expert meeting, the summary of the study as presented in the DAR is copied, and additional figures are presented. The RMS however disagrees with the point of clarification (provide historical control data for changes in clinical chemistry), because for these kind of parameters the results should be compared to the concurrent control group and **not** to historical control data.

STUDY 1

Characteristics

Reference	:	Kawaguchi, 1988	exposure	:	28 days, 4 hours/day, whole body
type of study	:	Subacute inhalation toxicity study	dose	:	0, 269, 482 and 1000 mg/m^3, MMAD 0.71-0.88 µm with GSD 1.29-1.41µm
year of execution	:	1987	vehicle	:	Corn oil
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.0%	GLP statement	:	yes
Route	:	Inhalation	guideline	:	OECD guideline 412
Species	:	Rat, Sprague-Dawley	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	482 mg/m^3

Study design

The study was performed in accordance with OECD 412.

Results

The results are summarised in tables 6.3.3.1 – 6.3.3.4.

Table 6.3.3.1

Dose (mg/m^3)	**0**		**269**		**482**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No treatment-related mortality								
Clinical signs - salivation							+	+	
Body weight							d		
Food consumption	No treatment-related findings								

Dose (mg/m^3)	0		269		482		1000		dr
	m	f	m	f	m	f	m	f	
Water consumption	No treatment-related findings								
Haematology	No treatment-related findings								
Clinical chemistry									
- albumin							ic		
- LDH							ic		
Urinalysis	No treatment-related findings								
Ophthalmoscopy	No treatment-related findings								
Organ weights									
- lung							dc[a]		
- spleen							dc[a]		
- liver							ic[r]		
Pathology									
Macroscopy	No treatment-related findings								
Microscopy	No treatment-related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight

Table 6.3.3.2 Results of clinical chemistry: albumin (g/dL) and LDH (lactic dehydrogenase) (U/L)

	Solvent control		269 mg/m^3/day		482 mg/m^3/day		1000 mg/m^3/day	
	Male	Female	Male	Female	Male	Female	Male	Female
Alb	3.2	3.5	3.3	3.5	3.2	3.6	3.3*	3.6
LDH	72	71	59	66	88	83	104*	79

* Statistically different from solvent control ($p<0.05$)
** Statistically different from solvent control ($p<0.01$)

The notifier submitted historical control data for LDH. The data is derived from three studies, conducted at the same laboratory and in the same time period as the subacute inhalation study.

Table 6.3.3.3 Historical control data on LDH (lactic dehydrogenase) (U/L)

	Year of execution	Species	No. of animals	LDH (U/L) ± SD
Study 1	1987-1988	Rat, Sprague-Dawley, male	10	96 ± 26
Study 2	1989	Rat, Sprague-Dawley, male	5	66 ± 30
Study 3	1990-1991	Rat, Sprague-Dawley, male	10	82 ± 25

Table 6.3.3.4 Organ weights

	Solvent control		269 mg/m^3/day		482 mg/m^3/day		1000 mg/m^3/day	
	Male	Female	Male	Female	Male	Female	Male	Female
Absolute organ weight (g)								
Lung	1.53	1.10	1.46	1.16	1.45	1.09	1.36**	1.08
Spleen	0.86	0.52	0.78	0.58	0.80	0.51	0.75*	0.51
Relative organ weight (/100 g body weight)								
Liver	2.90	3.06	2.98	3.11	3.04	3.09	3.15**	3.23

* Statistically different from solvent control ($p<0.05$)
** Statistically different from solvent control ($p<0.01$)

Acceptability

The study is considered acceptable for evaluation.

Conclusions

After subacute inhalation exposure one female of the vehicle control and one female of the negative control (not exposed) were found dead. No treatment-related mortality was noted. Salivation was noted in one to three animals per sex of the high dose group, during the first 5 days of the study. Similar salivation was seen in the acute inhalation toxicity study, therefore, this finding was attributed to the test substance. Slightly reduced body weights (93-94% of control values) were noted among males in the high dose group, reaching statistical significance on days 10 and 24. No treatment-related effect on food or water consumption was observed. There were no significant treatment-related changes in haematology parameters.

At clinical biochemistry, a statistically significant increase in albumin (103% of control) and in LDH (144%) were observed in males at 1000 mg/m^3. Further statistically significant changes were considered incidental due to the absence of a dose relationship. The slight increase in albumin, although statistically significant, is considered not toxicologically relevant. The notifier submitted historical control data on LDH, because this was requested in the reporting table, but as the RMS already stated above, the RMS is of the opinion that for these kind of parameters the results should be compared to the concurrent control group and not to historical control data.

In males at 1000 mg/m^3, relative liver weight was increased (109% of control) and absolute spleen and lung weights were decreased (87 and 89% of controls, respectively).

Macroscopic and microscopic examinations revealed no treatment-related findings.

The NOAEL was established at 482 mg/m^3 (equivalent to 87 mg/kg bw/day), based on salivation, increased LDH and changes in organ weights.

B.6.3.4 Semichronic oral studies

Open point 2.4: NOAEL in the 52-week dog study to be confirmed by the experts. RMS could provide a revised version of table 6.3.4.4 with additional figures in order to ease the discussion (based on reporting table 2(4)).

To facilitate the discussion on the 52-week dog study in the expert meeting, the summary of the study as presented in the DAR is copied, and additional figures on cholesterol and liver weight are presented.

STUDY 4

Characteristics

Reference	:	Chapman, 1991	exposure	:	52 weeks, gelatin capsule
Type of study	:	52-week oral toxicity study	dose	:	0, 30, 100, 300 and 1000 mg/kg bw/ day
year of execution	:	1988/89	vehicle	:	gelatin capsule
test substance	:	S-31183 tech. (pyriproxyfen), Lot no. PYG-87074, purity 95.3%)	GLP statement	:	yes
route	:	oral	guideline	:	predominantly in accordance with OECD 452 (1981)
species	:	dog, Beagle	acceptability	:	acceptable
group size	:	4/sex/dose	**NOAEL**	:	<30 mg/kg bw/day for males and 30 mg/kg bw/day for females

Study design

The study was generally in compliance with OECD 452 (1981). Electrocardiography was not performed.

Samples for haematology and clinical biochemistry were taken at the start of the exposure and after 12, 24, 37 and 50 weeks of treatment. Urinalysis was performed at the start of the exposure and after 11, 23, 35 and 49 weeks of treatment.

Results

The results are summarised in table 6.3.4.4 – 6.3.4.6.

Table 6.3.4.4 Results

Dose (mg/kg bw)	**0**		**30**		**100**		**300**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4	0/4	
Clinical signs											
- diarrhea									i[1]	i[1]	
- salivation									i	i	
- emesis									i	i	
Body weight gain							dc	dc	d	dc	
Food consumption									d		
Ophthalmoscopy	No treatment-related findings										
Haematology											
- RBC						dc[8]	dc[9]	dc[8]	dc[2]	dc[8]	
- HB						dc[8]	dc[9]	dc[8]	d[3]	dc[4]	
- HCT						dc[8]	d[3]	dc[8]	d[3]	dc[4]	
- MCV						ic[7]	ic[3]	ic[9]	ic[3]	ic[7]	
- platelet count							ic[5]		ic[7]	ic[7]	
- prothrombin time							ic[10]		ic[7]	ic[2]	
Clin. Chemistry											
- ALP							ic[5]	ic[8]	ic[7]	ic[9]	

Dose (mg/kg bw)	0		30		100		300		1000		dr
	m	f	m	f	m	f	m	f	m	f	
- ALT									ic[7]	ic[7]	
- AST									ic[9]		
- total bilirubin									ic[12]	ic[4]	
- cholesterol			ic[10]		ic[7]	ic[12]	ic[7]	ic[8]	ic[3]	ic[11]	m/f
- triglycerides							ic[6]	ic[9]	ic[9]	ic[9]	m/f
- glucose									dc[13]		
- chloride									dc[10]	dc[3]	
Urinalysis											
- volume									i		
Organ weights											
- liver			i[a], ic[r]		ic[a,r]	i[a,r]	ic[a,r]	ic[a,r]	ic[a,r]	ic[a,r]	m/f
- thyroid						ic[r]		ic[a,r]		ic[a,r]	
Pathology											
macroscopy											
- liver, enlarged	0/4	0/4	0/4	0/4	0/4	0/4	0/4	04	2/2	0/4	
- liver, irregular surface	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/2	2/4	
- liver, firm irregular masses	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/2	0/4	
- kidney, firm dark mass replacing lymph nodes	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/2	0/4	
microscopy											
liver:											
- bile duct hyperplasia	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	2/2	3/4	
- nodular hyperplasia	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/2	0/4	
- active chronic inflammation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/2	2/4	
- cyst. degen.	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/2	1/4	
- centriacinar fibr.	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/2	3/4	
gall bladder:											
- submuc. fibrosis	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	2/2	3/4	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
1 after week 1
2 week 12
3 week 12 and 24
4 week 37
5 week 24,37 and 50
6 week 37 and 50
7 whole exposure period
8 week 12 and 37
9 week 12,24 and 37
10 week 24 and 37
11 at start of exposure
12 week 12, 37 and 50
13 week 24 and 50

Table 6.3.4.5 Cholesterol (mg/dL)

	0 mg/kg bw/day		30 mg/kg bw/day		100 mg/kg bw/day		300 mg/kg bw/day		1000 mg/kg bw/day	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Week 12	128	155	172	157	210**	244**	262***	283***	225**	207
Week 24	109	199	172*	156	223***	197	265***	213	221**	181
Week 37	129	162	195*	189	247***	298**	294***	306**	142	211
Week 50	103	222	154	168*	265***	172*	251**	179	103	173*

* Statistically different from control ($p<0.05$)
** Statistically different from control ($p<0.01$)
*** Statistically different from control ($p<0.001$)

Table 6.3.4.6 Absolute and relative liver weight

	0 mg/kg bw/day		30 mg/kg bw/day		100 mg/kg bw/day		300 mg/kg bw/day		1000 mg/kg bw/day	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Absolute weight (g)										
Liver	365	389	476	416	538*	486	613**	561*	697*	542*
Organ weight relative to bodyweight										
Liver	2.4	2.8	3.1*	2.9	3.8**	3.8	4.6**	4.4**	4.8**	4.3**

* Statistically different from control ($p<0.05$)
** Statistically different from control ($p<0.01$)

Acceptability

The study is considered acceptable.

Conclusions

Dogs were exposed to 0, 30, 100, 300 or 1000 mg/kg bw/day of pyriproxyfen technical via capsules for 52 weeks. Two males at 1000 mg/kg bw were killed in extremis in week 17 and 31, respectively; these animals had shown marked weight loss and a general deterioration in condition prior to sacrifice. The examination of ante mortem blood samples revealed in both dogs low haematocrit, haemoglobin, and erythrocyte counts, high platelet counts, high alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activities, high cholesterol and triglyceride concentrations, and low calcium and phosphorus concentrations; microscopic examinations revealed significant liver damage in both dogs.
Salivation associated with dosing, emesis, and an increased incidence of diarrhea was noted at 1000 mg/kg bw in both sexes. Body weight gains were significantly reduced in males at 300 mg/kg bw and in females at 300 and 1000 mg/kg bw after 13 weeks of treatment. At termination, there was a significant weight gain deficit in males and females at 300 mg/kg bw and in females at 1000 mg/kg bw. A small reduction of food consumption was observed in males at 1000 mg/kg bw during the whole exposure period. Ophthalmoscopy revealed no treatment-related findings.

Slightly low haematocrit, haemoglobin and erythrocyte counts were apparent in males at ≥300 mg/kg bw (87-89% of control) after 12 and 24 weeks of treatment and in females at ≥100 mg/kg bw (78-90% of control) after 12 and 37 weeks of treatment, pointing to mild anaemia. Mean corpuscular volume was significantly increased

in males at ≥300 mg/kg bw (105-106% of control) in the first six months of treatment and in females at ≥100 mg/kg bw (103-107% of control) throughout treatment, suggesting that the anaemia was macrocytic. Platelet count was significantly increased at ≥300 mg/kg bw in males (138-221% of control) and at 1000 mg/kg bw in females (123-147% of control). Prothrombin time was significantly increased at ≥300 mg/kg bw in males (106-164% of control) and at 1000 mg/kg bw in females (109-116% of control), possibly a result of diminished synthesis of prothrombin and other clotting factors by the liver. Bone marrow examinations showed no treatment-related findings.

Alanine aminotransferase activity was significantly and markedly increased during the whole exposure period at 1000 mg/kg bw in males (504-983% of control) and females (432-831% of control), consistent with significant hepatocellular injury. Aspartate aminotransferase activity was also significantly and moderately increased at 1000 mg/kg bw in males (135-247% of control) after 12, 24 and 37 weeks. Alkaline phosphatase activity was markedly increased at ≥300 mg/kg bw in males (227-496% of control) and females (221-356% of control) throughout treatment. Total bilirubin was significantly increased at 1000 mg/kg bw in males (250-271% of control) after 12, 37 and 50 weeks and females (175% of control) after week 37 only. The elevated ALP and bilirubin levels point to cholestasis. Cholesterol was increased in all groups of treated males (151-243% of control) and in females at 100 mg/kg bw/day and higher (157-189% of control); these changes were dose-related, except at the highest exposure level. Triglycerides were significantly increased in males and females at 300 and 1000 mg/kg bw throughout treatment (176-983% of control for males and 156-335% of control for females). Glucose was significantly decreased in males at 1000 mg/kg bw after 24 and 50 weeks (76-80% of control), possibly secondary to significant liver damage. Plasma chloride levels were significantly lower in males and females at 1000 mg/kg bw, probably a result of frequent vomiting.

Urinalysis showed a higher urine volume (140-252% of control) in males at 1000 mg/kg bw.

Absolute liver weights were significantly increased in males at ≥ 100 mg/kg bw (147-191% of control) and in females at ≥ 300 mg/kg bw (135-139% of control); relative liver weights were significantly increased in all groups of treated males (129-200% of control) and in females at ≥ 300 mg/kg bw (157-148% of control). Absolute thyroid weights were significantly increased in females at ≥ 300 mg/kg bw (163-182% of control) and relative thyroid weights were significantly increased in females at ≥ 100 mg/kg bw (175-200% of control). There was no microscopic correlate for the thyroid weight changes.

Treatment-related macroscopic changes in animals killed after 52 weeks were large livers in both males at 1000 mg/kg bw, an irregular surface of the liver of three animals at 1000 mg/kg bw, numerous firm irregular masses in the liver and a firm dark mass replacing the renal lymph nodes in one male at 1000 mg/kg bw. Histopathological examination revealed significant liver damage in all but one of the animals at 1000 mg/kg bw, characterised by centriacinar fibrosis and bile duct hyperplasia, most prominently in the subcapsular region, and generally associated with active chronic inflammatory infiltrate with foci of cystic degeneration sometimes present that appeared to originate from foci of vacuolated cells. Nodular hyperplasia accompanied the more severe hepatic lesions and all animals with hepatic lesions also had submucosal fibrosis of the gall bladder. The hepatotoxic effects at 1000 mg/kg bw/day were more marked in males than in females.

Based on higher cholesterol levels and higher liver weights the NOAEL was < 30 mg/kg bw/d for males and 30 mg/kg bw/d for females.

B.6.6.1 Reproductive toxicity

Open point 2.7: RMS to provide a revised table 6.6.1.2 with additional figures and historical control data in order to confirm the NOAELs in the combined rat teratogenicity and reproductive study (based on reporting table 2(16)).

To facilitate the discussion on the combined rat teratogenicity and reproductive study in the expert meeting, the summary of the study as presented in the DAR is copied, and additional figures on the no. of corpora lutea and live foetuses, and the historical control data of these parameters, are presented.

STUDY 2

Characteristics

reference	:	Saegusa, 1988a	exposure	:	9 weeks (males) and 2 weeks (females) premating, during mating (maximum 3 weeks) and for females until day 7 of gestation.
type of study	:	Teratogenicity and reproductive toxicity study	doses	:	0, 100, 300, 500 and 1000 mg/kg bw/day
year of execution	:	1987	vehicle	:	Heated (60 °C), then diluted in corn oil
test substance	:	S-31183 (Pyriproxyfen), batch no. PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Oral	guideline	:	Not indicated, see study design
species	:	Rat Slc:SD (SPF)	acceptability	:	Acceptable
group size	:	24/sex/dose	**NOAEL**males	:	<100 mg/kg bw/day
			NOAELmat	:	100 mg/kg bw/day
			NOAELdev	:	1000 mg/kg bw/day
			teratogenic effects		≥ 1000 mg/kg bw/day

Study design

S-31183 was daily administered by oral gavage prior to mating for a period of 9 weeks (males) and 2 weeks (females) at dose levels of 0, 100, 300, 500 or 1000 mg/kg bw/day. Dosing was continued during the mating period for males and females until the end of mating for males (maximum 3 weeks) or day 7 of gestation for females. During the treatment period clinical signs, body weights, food consumption and water consumption were recorded. Males were sacrificed after the mating period and females were sacrificed on day 21 of gestation, i.e. following a 14-day treatment-free period. All animals were necropsied and subjected to macroscopic examinations. The heart, lungs, liver, spleen, kidneys, adrenal glands, testes including epididymis and thymus were weighed. Reproductive organs (testes, seminal vesicle, prostate, ovaries and uterus) of infertile males and females were subjected to histopathological examinations. At necropsy of dams, the ovaries and uterus were removed. The number of corpora lutea was recorded and the uterus contents examined for the numbers and positions of live and dead foetuses, implantations and early and late implantation loss. Live foetuses were individually weighed as well as their placenta and the foetuses were examined for external anomalies, soft tissue changes (one third of the foetuses) and skeletal changes (two thirds of the foetuses).

Dose levels were selected on the basis of two dose range finding studies (ref.: Saegusa, 1986 and Saegusa, 1987). These studies were not available for evaluation. In the first preliminary study, dose levels of 125, 250, 500 and 1000 mg/kg bw/day were administered by oral gavage to male rats for 28 days and to female rats for 14 days. No deaths occurred. Toxic signs consisted of soft stool or diarrhea, swelling of the periproctal region and salivation in animals receiving 500 or 1000 mg/kg bw/day and a slight decrease of body weight in animals receiving 1000 mg/kg bw/day. In a second study, groups of 8 male and 8 female rats were treated at 1000, 1500 or 2000 mg/kg bw/day for 14 days. All animals died in the 2000 mg/kg dose group, 3 males and 7 females died in the 1500 mg/kg dose group and 2 females died in the 1000 mg/kg dose group. Signs of toxicity were noted in all treated groups and comprised soft stools or diarrhea, erythema and swelling of the periproctal region, hypoactivity, rough hair, salivation, wasting, decreased body weight and food consumption and increased water consumption. At necropsy, dead animals showed congestion of the liver and kidneys, involution of the thymus, enlarged adrenals and hyperemia of the intestinal mucosa and ulceration of the gastric fundus. Necropsy of surviving animals revealed, enlarged liver and kidneys, increased liver, kidneys and adrenal weights and decreased thymus weight.

Results

The results are summarised in table 6.6.1.2 and 6.6.1.3.

Table 6.6.1.2

Dose (mg/kg bw/day)	0		100		300		500		1000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	0/24	0/24	0/24	0/24	0/24	0/24	1[c)]/24	0/24	0/24	2/24	
Clinical signs											
-diarrhoea						9/24	20/23	22/24	24/24	24/24	
-erythema and swelling of periproctal region						1/24	22/23	9/24	24/24	23/24	
-excessive salivation			18/24	1/24	21/24	5/24	24/24	7/24	24/24	8/24	
-excessive lacrimation									3/24		
-hypoactivity										4/24	
-wasting										6/24	
Body weight (gain)					dc	dc[e]	dc	dc[e]	dc	dc[e]	m/f
Food consumption					ic	d(c)[e]	ic	d(c)[e]	ic	d(c)[e]	m
Water consumption			ic		ic		ic	ic[e]	ic	ic[e]	m/f
Organ weights[f]											
-thymus					dc[a]		dc[a,r]		dc[a,r]	ic[r]	m
-liver			ic[r]		ic[a,r]		ic[a,r]		ic[a,r]		m
-kidney			ic[r]	ic[r]	ic[a,r]	ic[r]	ic[r]	ic[r]	ic[a,r]	ic[a,r]	m
-adrenals			ic[a,r]		ic[a,r]		ic[a,r]		ic[a,r]	ic[a,r]	
-spleen										ic[r]	
Pathology[f]											
Macroscopy											
-thymus atrophy					1/24		1/23		12/24		
-liver enlarged					18/24		21/23		22/24	1/24	
-liver dark-red coloured					21/24		23/23		24/24		

Dose (mg/kg bw/day)	0		100		300		500		1000		dr
	m	f	m	f	m	f	m	f	m	f	
-liver congestion										2/24	
-kidney enlarged					1/24		6/23		16/24		
-kidney pitted surface					8/24		22/23		22/24		
-adrenal enlarged					12/24		18/23		24/24	2/24	
No. of animals mated (1st mating)	24		24		24		24		22		
No. of animals copulated (1st mating)	22		24		23		24		21		
Copulated/mated (%)	91.7		100.0		95.8		100.0		95.5		
Pregnant/copulated (%)	90.9		100.0		95.7		95.8		85.7		
No. of animals mated (2nd mating)	2	2			1	1			3[g]	1	
No. of animals copulated (2nd mating)	2	2			1	1			2	1	
Copulated/mated (%)	100.0	100.0			100.0	100.0			66.7	100.0	
Pregnant or impregnated/copulated (%)	100.0	100.0			100.0	100.0			100.0	100.0	
Maternal effects											
Pregnant animals	22		24		23		23		19		
No of corpora lutea									dc		
Body weight (gain)					d(c)		dc[h]		dc[h]		f
Food consumption							(ic)		ic		f
Water consumption					ic		ic		ic		f
Litter response											
Live foetuses			dc						dc		
Placental weight									ic		
Foetal weight[i]	No treatment-related findings										
Post implantation loss	No treatment-related findings										
Sex ratio	No treatment-related findings										
Examination of the foetuses											
External observations	No treatment-related findings										
Skeletal findings	No treatment-related findings										
Visceral findings	No treatment-related findings										

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
c intubation error
e during 14 days pre-mating
f pregnant females after gestation day 7
g two males could not be cohabited in the 1st mating
h body weight gain was noted as increased during the first week of pregnancy
i statistically significantly increased in all treated females, but without a treatment-related distribution

Table 6.6.1.3 No. of corpora lutea and live foetuses and placental weight (g) ± SD and historical control data (range)

Dose (mg/kg bw/day)	0	100	300	500	1000	Historical control data
Maternal effects						
No. of corpora lutea	15.8 ± 1.30	15.4 ± 1.50	15.9 ± 1.18	15.3 ± 1.42	14.2 ± 1.27 **	15.0 (13.7-16.0)
Litter response						
Live foetuses	14.3 ± 2.71	12.6 ± 2.81 *	14.4 ± 1.53	13.1 ± 1.78	12.6 ± 1.67 *	13.2 (11.9-14.4)
Placental weight, m	0.40 ± 0.04	0.42 ± 0.04	0.41 ± 0.04	0.41 ± 0.04	0.44 ± 0.04 **	0.41 (0.38-0.42)
Placental weight, f	0.40 ± 0.04	0.41 ± 0.05	0.41 ± 0.04	0.41 ± 0.04	0.44 ± 0.05 **	0.40 (0.38-0.41)

* Statistically different from control ($p<0.05$)
** Statistically different from control ($p<0.01$)

The historical control data were reported in Annex III of the study report. The historical control data are derived from 12 studies, performed during 1986-1987 in the same laboratory as the study under evaluation.

Acceptability

There is no OECD guideline with an equivalent study design. However, the study is considered acceptable since it adds useful additional information.

Conclusions

Two females in the 1000 mg/kg bw/day group died during the study. There was no treatment-related mortality. Treatment-related clinical signs comprised salivation in males and females at ≥ 100 mg/kg bw/day, diarrhea at ≥ 500 mg/kg bw/day in males and at ≥ 300 mg/kg bw/day in females, erythema and swelling of the periproctal at ≥ 500 mg/kg bw/day in males and females, excessive lacrimation in a few males at 1000 mg/kg bw/day, hypoactivity and wasting in several females at 1000 mg/kg bw/day. Decreased body weights were noted in males and females at ≥ 300 mg/kg bw/day. Increased food consumption was noted in males at ≥ 300 mg/kg bw/day) and in females at 1000 mg/kg bw/day. Water consumption values were increased in males at ≥ 100 mg/kg bw/day and in (pregnant) females at ≥ 300 mg/kg bw/day. At necropsy, treated males showed decreased thymus weights (at ≥ 500 mg/kg bw/day) and increased liver weights (at ≥ 100 mg/kg bw/day), kidney weights (at ≥ 100 mg/kg bw/day) and adrenal weights (at ≥ 100 mg/kg bw/day); high dose females showed increased thymus, kidneys', adrenals' and spleen weights. Treatment-related macroscopic findings in males consisted of thymus atrophy at 1000 mg/kg bw/day, enlarged and dark-red coloured livers (at ≥ 300 mg/kg bw/day), enlarged and pitted surfaced kidneys (at ≥ 300 mg/kg bw/day), enlarged adrenals (at ≥ 300 mg/kg bw/day) ; in females there were only a few gross changes and relationships to treatment could not be established unequivocally.

Reproductive performance was not affected by treatment, but the number of corpora lutea and live foetuses were significantly lower in dams at 1000 mg/kg bw/day. However, the number of corpora lutea and live foetuses was just slightly reduced, not dose-related and within the range of historical

control data, and therefore considered not treatment-related. Placental weights were significantly higher in dams at 1000 mg/kg bw/day, but this increase was slight and no adverse effect was noted in foetuses. In the teratogenicity study in rats (see B.6.6.2, Study 1), no change was observed in placental weights even at 1000 mg/kg bw/day. Therefore, this is considered to be not toxicologically relevant. The body weights of live foetuses were significantly higher at all exposure levels, with no dose-related trend.

External, visceral and skeletal examination of foetuses did not reveal any morphological anomalies or variations that could be attributed to treatment.

Based on organ weight changes and increased water consumption, the NOAEL for parent males is considered to be lower than 100 mg/kg bw/day. Based on the occurrence of diarrhea, decreased body weights and increased water consumption at 300 mg/kg bw/day, the NOAEL for maternal toxicity was considered to be 100 mg/kg bw/day. The NOAEL for developmental toxicity was considered to be 1000 mg/kg bw/day. There were no indications of teratogenic potential at doses up to 1000 mg/kg bw/day.

B.6.6.2 Teratogenicity studies

Open point 2.8: NOAELs in the rat teratogenicity study to be confirmed by the experts. RMS could provide a revised table 6.6.2.1 with additional figures instead of statements in order to ease the discussion (based on reporting table 2(18)).

To facilitate the discussion on the rat teratogenicity study in the expert meeting, the summary of the study as presented in the DAR is copied, and the figures on early implantation loss are revised.

STUDY 1

Characteristics

reference	:	Saegusa, 1988c	exposure	:	Days 7-17 of gestation, gavage (5 ml/kg)
type of study	:	teratogenicity study	doses	:	0, 100, 300 and 1000 mg/kg bw/day
year of execution	:	1986-1987	vehicle	:	Heated (60 °C), then diluted in corn oil
test substance	:	S-31183 (Pyriproxyfen), batch no. PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Oral	guideline	:	Not indicated, see study design
species	:	Rat Slc:SD (SPF)	acceptability	:	acceptable
group size	:	36-47 females/dose	**NOAEL**mat	:	100 mg/kg bw/day
			NOAELdev	:	100 mg/kg bw/day
			teratogenic effects	:	≥ 1000 mg/kg bw/day

Study design

The study was performed in accordance with the OECD 414. S-31183 was daily administered by oral gavage at dose levels of 0, 100, 300 or 1000 mg/kg bw/day, to groups of 36 to 47 pregnant rats from gestation day 7 to day 17 inclusive. On day 21 of gestation, 20 to 23 pregnant dams were subjected to

cesarean section and macroscopic examination. The heart, lungs, liver, spleen, kidneys, adrenal glands, thymus and ovaries were removed, weighed and fixed for possible microscopic examinations. The uterus was removed and the contents examined for the numbers and positions of live and dead foetuses, implantations and early and late implantation loss. Live foetuses were individually weighed as well as their placenta and the foetuses were examined for external anomalies, soft tissue changes (one third of the foetuses) and skeletal changes (two third of the foetuses).
An additional 10 to 13 dams of each group were allowed to deliver naturally and the gestation length was calculated. After birth the F1-offspring was examined for the numbers of live born and stillborn pups, sex, external anomalies and pup viability. In addition, the development of pups was examined by functional, emotional, learning and reproductive ability tests. After culling of each litter, pups not selected for further investigations were prepared for skeletal examinations. At weaning (day 21 postpartum) all dams and F1-offspring were necropsied and subjected to macroscopic examinations. The heart, lungs, liver, spleen, kidneys, adrenal glands (F0), testes including epididymis (F1), thymus (F0), ovaries and brain (F1 selected for tests) were removed, weighed and fixed for possible microscopic examinations.

Dose levels were selected on the basis of a dose range finding study (ref. 1, Saegusa 1987), which was not available for evaluation. In this preliminary study, dose levels of 125, 250, 500 and 1000 mg/kg bw/day were administered to 7 to 8 pregnant rats from day 7 to day 17 of gestation, inclusive. Soft stool or diarrhea as well as erythema and swelling of the periproctal region were noted in females receiving 500 mg/kg bw/day or more. Food consumption was slightly decreased in females receiving 125 or 250 mg/kg bw/day and markedly decreased in females receiving 500 or 1000 mg/kg bw/day. At necropsy, females of the 1000 mg/kg dose group revealed enlargement of the adrenals and involution of the thymus. There were no effects noted on embryo/fetal mortality or fetal body weight.

Results

The results are summarised in table 6.6.2.1.

Table 6.6.2.1

Dose (mg/kg bw/day)	0	100	300	1000	dr
Maternal effects					
No of dams examined	36	36	36	42	
Mortality	0/36	0/36	1[i]/36	12/42	
Clinical signs					
-diarrhea				42	
-erythema and swelling of periproctal region				19	
-hypoactivity			1[j]	10	
-wasting			1[j]	9	
-rough hair				4	
-lacrimation				2	
-hypothermia			1[j]	9	
-blanching of auricle and					

Dose (mg/kg bw/day)	0	100	300	1000	dr
extremity			1[j]	3	
-bloody dirtiness around nose				6	
Body weight (gain)					
-pregnancy			dc	dc	dr
-postpartum			dc	dc[b]	dr
Food consumption					
-pregnancy			dc	dc	dr
-postpartum				ic	dr
Water consumption					
-pregnancy			ic	ic	dr
-postpartum			ic	ic	dr
Pathology					
Macroscopy[f]					
-thymus atrophy			(+)[j]	++	
-liver congestion				++	
-spleen atrophy				++	
-spleen enlargement			(+)[j]	(+)	
-adrenal enlargement			(+)[j]	++	
-kidney congestion				+	
-kidney enlargement				(+)	
-stomach hemorrhage of the mucous membrane				+	
-stomach ulcer				+	
-cecum enlargement				(+)	
Organ weights					
day 21 of pregnancy					
-thymus				dc[a/r]	
-liver			ic[r]	ic[r]	dr
-kidney			ic[r]	ic[a/r]	dr
-adrenals				ic[a/r]	
day 21 postpartum			No treatment-related findings		
Litter response					
No of dams examined	23	23	23	20	
Live foetuses	303	299	299	232	
Litter Size	13.2	13.0	13.0	11.6	
Foetal weight			No treatment-related findings		
Placental weight			No treatment-related findings		
No of Corpora Lutea			No treatment-related findings		
No of implantations			No treatment-related findings		
Post implantation loss					
-early (%)[k]	4.4	6.7	7.7	14.6	
-late			No treatment-related findings		
Sex ratio			No treatment-related findings		
Examination of the foetuses					
External observations			No treatment-related findings		
Skeletal findings					
Abnormality			No treatment-related findings		

Dose (mg/kg bw/day)	0	100	300	1000	dr
Variation Opening of foramen transversarium of the 7th cervical vertebra		i	ic	ic	dr
Visceral findings	No treatment-related findings				
Pup development					
No of dams examined	13	13	13	10	
No of females with live newborn	13	13	12[i]	10	
Delivering rate[g]	No treatment-related findings				
Gestational period	No treatment-related findings				
No of implantations	No treatment-related findings				
No of live newborns / stillborns	No treatment-related findings				
Pup weight	No treatment-related findings				
Pup viability	No treatment-related findings				
External observations	No treatment-related findings				
Physical / sensory development	No treatment-related findings				
Locomotor activity and emotionality	No treatment-related findings				
Motor coordination	No treatment-related findings				
Learning ability	No treatment-related findings				
Reproductive ability	No treatment-related findings				
Visceral examinations[h]	No treatment-related findings				
Skeletal findings	No treatment-related findings				
Organ weights	No treatment-related findings				

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute / relative
b statistically significantly increased body weight gain after delivery
e statistically significantly increased food consumption after delivery
f noted in females found dead. Major findings in dams sacrificed on day 21 of pregnancy: atrophy of the thymus and enlarged adrenals
g (No. of females with live newborns / No. of pregnant females) x 100
h In the top dose a significantly increased number of anomalies was noted in the offspring at 56 days postpartum, which included 5 animals showing dilatation of the renal pelvis. However, no such findings were observed at foetal examinations or any other necropsies.
i The animal was sacrificed, because there was no delivery at day 25 of pregnancy.
j These findings were observed in the sacrificed animal.
k rate of resorbed or dead foetuses

Acceptability

The study is considered acceptable, since the design and conduct were in accordance with or exceeded the requirements of the OECD 414 Guideline. Compared to the OECD 414 of 2001, differences noted were: dosing period from gestation day 7 to 17 (OECD: 6 to 15), no gravid uterus

weight was measured and one third of the fetuses were subjected to visceral examinations and two third to skeletal examinations (OECD: ½ visceral / ½ skeletal).

Conclusions

Maternal toxicity was evident at 1000 mg/kg bw/day by the death of 12 dams, decreased body weights and food consumption, increased water consumption, higher incidences of clinical signs and macroscopic findings and changes in organ weights. Clinical signs included diarrhea, erythema and swelling of the periproctal region, hypoactivity, wasting, rough hair, lacrimation, hypothermia, blanching of auricle and extremity and bloody dirtiness around the nose. Macroscopic findings noted in dams found dead consisted of atrophy of the thymus and spleen, congestion of the liver and kidneys, enlarged adrenals, hemorrhage of mucous membrane of the stomach and ulcer in the stomach. After sacrifice on day 21 of pregnancy, major macroscopic changes were atrophy of the thymus and enlarged adrenals and changes in organ weights comprised a decreased thymus weight (absolute 56% of control; relative 60% of control) and increased weight of the liver (relative 111% of control), kidneys (relative 117% of control) and adrenals (absolute 130% of control; relative 145% of control). No treatment-related changes were detected in dams sacrificed on day 21 postpartum.
In the 300 mg/kg dose group decreased body weights and food consumption and increased water consumption were noted. In addition, a slight increase was noted in liver (relative 105% of control) and kidney (relative 106% of control) weights on day 21 of pregnancy. In the 100 mg/kg dose group no adverse effects of treatment were detected.

There were no significant effects in the mean number of corpora lutea or implantations. Examination of the uterus contents on day 21 of gestation revealed a slight reduction of the litter size and an increase of early implantation loss (not statistically significant). The skeletal examinations of foetuses revealed a significantly increased number of skeletal variations at 1000 mg/kg bw/day. The incidence of foetuses with an opening of the foramen transversarium of the 7th cervical vertebra was significantly higher at 300 and 1000 mg/kg bw/day, with a dose-related trend.

Treatment with S-31183 did not produce adverse effects that could be related to treatment in the postnatal development of pups and in their reproductive performance.

Based on the effects noted in dams, the NOAEL for maternal toxicity was established as being 100 mg/kg bw/day. Based on an increased number of foetuses with an opening of the foramen transversarium of the 7th cervical vertebra, the NOAEL for developmental toxicity was set at 100 mg/kg bw/day. There were no changes in foetal morphology and neonatal development of pups that could be attributed to treatment with S-31183. Therefore, the NOAEL for teratology was considered to exceed 1000 mg/kg bw/day.

B.6.10 SUMMARY OF MAMMALIAN TOXICOLOGY AND PROPOSED ADI, AOEL ARfD AND DRINKING WATER LIMIT (ANNEX IIA 5.11)

Open point 2.12: Derivation of the AOEL to be discussed by the experts (relevant species, relevant study, correction for oral absorption) (based on reporting table 2(28)).

To facilitate the discussion, an overview of all relevant studies is presented in this addendum.

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Semichronic toxicity studies			
13-week, oral, rat (diet)	23.5	118	Cox, 1988
6 months, oral, rat (diet)	24.0	121	Koyama, 1988b
90-days, oral, dog (capsule)	100	300	Nakano, 1988
1-year, oral, dog (capsule)	< 30	30	Chapman, 1991
1-year, oral, dog (capsule)	10	-	Mitchell, 1993
Chronic toxicity studies			
2-year, oral, rat	27.2	138.7	Osheroff, 1991a; Osheroff, 1994; Moore, 1994
78-weeks, oral, mouse	16.4	81.3	Osheroff, 1991b; Cardy et al., 1994; Moore, 1994
Reproduction and teratogenicity studies			
2-generation, oral, rat	Parental: 13.3 Developmental: 66.7 No effects on reproduction	66.7 333.3	Robinson, 1991
Teratogenicity and reproduction, rat	Parental (males): < 100 Maternal: 100 Developmental: 1000 Not teratogenic	100 300 -	Saugusa, 1988a
Teratogenicity, oral, rat	Maternal and developmental: 100 Not teratogenic	300	Saugusa, 1988c
Teratogenicity, oral, rabbit	Maternal: 100 Developmental: 300 Not teratogenic	300 -	Hirohashi, 1988
Peri-post natal study, rat	Maternal and developmental: 100	300	Saugusa, 1988b

OTHER COMMENTS

Open point 2.20: Experts to discuss the relative toxicity of the plant metabolite PYPA ((RS)-2-(2-pyridyloxy)propyl alcohol) in comparison with pyriproxyfen, taking into account that it is proposed as intermediate in the rat metabolic pathway but has not been identified in the rat metabolism studies.
The notifier has provided a position in his comments on the reporting table. (based on reporting table 3 (2) and 2(41)).

Background

In the tomato metabolism study the free and conjugated PYPA metabolite were detected. PYPA and conjugated PYPA account for 0.025 mg/kg (9.4% TRR) in the fruit juice in the metabolism study performed with 3x148 g ai/ha (2x overdose). Pyriproxyfen (mainly present in the pomace) accounts for 0.13 mg/kg (48% TRR). At the expected dose rate of 224 g ai/ha, PYPA and conjugated PYPA will be lower (might be half of the amount found in the metabolism study). The proposed residue definition is pyriproxyfen. Based on the results of the metabolism study, the question arises whether the plant metabolite PYPA (free and conjugated) should be added to the current residue definition.

Statement notifier

Metabolite PYPA was not found in the rat. However, metabolite POP was found in rats. It is suggested by the notifier that the only logic metabolic step is that pyriproxyfen is hydrolysed to POP, thereby releasing PYPA.
Furthermore, it is considered by the RMS that it is also possible that PYPA is released after hydrolyses of 4'OH-pyr.

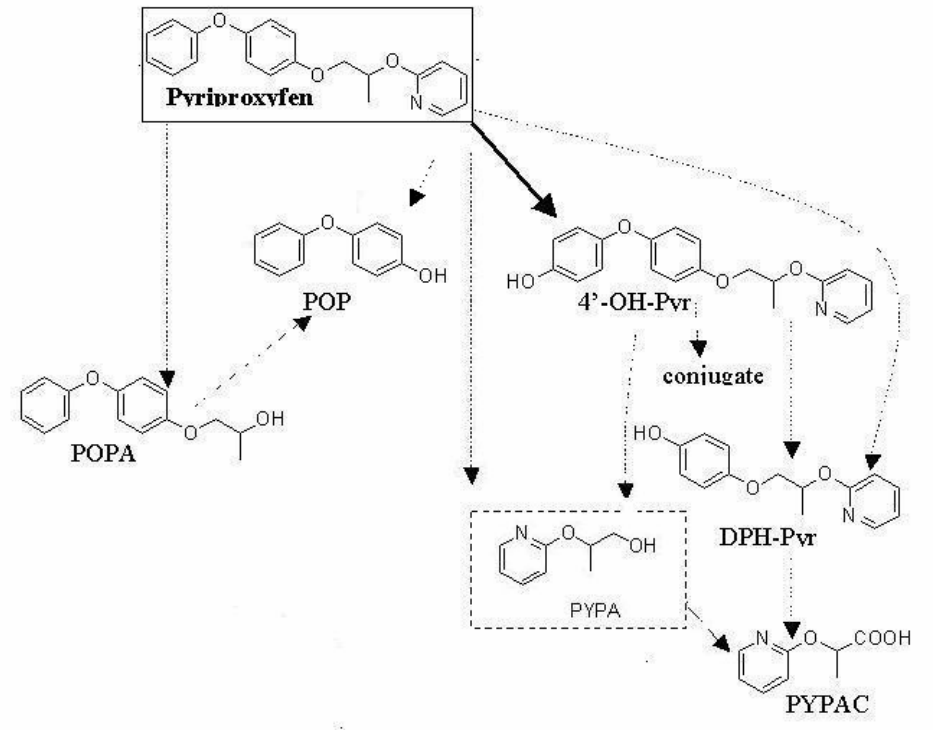


Figure: Degradation of pyriproxyfen in rats: PYPA was not found but is likely to be a metabolic intermediate.

The Expert Meeting on toxicology (PRAPeR 64 d.d. Janaury 21-23th, 2009) is asked to give it's opinion about the likeliness of PYPA being a metabolic intermediate in rats. It is noticed that PYPAC is also formed in hen and goat, and that PYPA is really detected in these species.

B.6.14 EXPOSURE DATA (ANNEX IIIA 7.2)

Product information

Product:	Pyriproxyfen 10EC
Purpose:	Insecticide
Active substance (a.s.):	Pyriproxyfen 100 g a.s./L
	Emulsifiable Concentrate (EC)
Package size:	0.25 L plastic bottle (size opening 41 mm) and 1 L plastic bottle (size opening 40.8 mm)

Pyriproxyfen 10EC is used as an insecticide in cotton, tomato and eggplant, and contains 10% pyriproxyfen. In cotton, Pyriproxyfen 10EC is applied outdoors, by mechanical downward spraying using a tractor-mounted, tractor-pulled and self-propelled ground sprayer. In tomato and eggplant, Pyriproxyfen 10EC is applied indoors, by manual up- and downward spraying. No exposure studies were submitted by the notifier. Exposure data were derived using models.

Internal operator exposure values without and with personal protective equipment (PPE) were calculated using the UK and the German model. For risk assessment purposes, the 75th percentile of the UK-model was used (UK-75th) and the geometric mean of the German model (DE-GM). For the greenhouse application, internal operator exposure values without and with PPE were calculated using the Dutch model. For risk assessment purposes, the 90th percentile of the Dutch model (NL-90th) was used.

For bystander exposure during mechanical spraying outdoors, no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II, 2002 model were used. These values represent the 90th percentile exposure values for bystanders. Since bystanding should as much as possible be prevented and will usually occur incidentally, it cannot be assumed that bystanders will be using any kind of personal protective equipment, therefore the use of this equipment is not considered in bystander risk assessment.
In greenhouses, no bystander exposure during application is considered, as Good Agricultural Practice requires absence of bystanders.

For the use of Pyriproxyfen 10EC, re-entry activities should be considered for harvesting of cotton, and cutting and sorting of tomatoes and eggplants. Worker exposure was estimated using the draft values proposed for the EUROPOEM II model.

For operator, worker and bystander risk assessment the semi-chronic/chronic systemic AOEL based on the 1-year dog study was used (see section B.6.10.5). Dermal absorption of pyriproxyfen was estimated to be 2.5% for the concentrate and 13% for the spray dilution, based on the in vitro study described in section B.6.12. Since the model used for the exposure calculations in the greenhouse

(the Dutch greenhouse model) calculates a combined exposure for mixing/loading and application, a dermal absorption value of 13% will be used for the risk assessment of the indoor applications. For respiratory exposure a default value of 100% was used, i.e. internal exposure equals external exposure.

The basic assumptions, input data and calculations used in the risk assessment are further specified below.

B.6.14.1 Operator exposure (IIIA 7.2.1)

Mechanical downward spraying on cotton

Application technique : tractor mounted equipment, downward spraying (outdoor)

Input data

Concentration a.s. in formulation	:	100 g a.s. /L
Spray volume	:	500 - 800 L/ha
Concentration in spray liquid	:	0.015 kg a.s. /hL
Application rate	:	0.075 kg a.s./ha (0.75 L product/ha)

Manual up- and downward spraying on tomato and eggplant

Application technique : Hand-held spraying equipment (indoor)

Input data

Concentration a.s. in formulation	:	100 g a.s. /L
Spray volume (Northern Europe)	:	800 - 1200 L/ha
Spray volume (Southern Europe)	:	1000 - 1500 L/ha
Concentration in spray liquid (Northern Europe)	:	0.003 kg a.s. /hL
Concentration in spray liquid (Southern Europe)	:	0.0075 kg a.s. /hL
Application rate (Northern Europe)	:	max. 0.03 kg a.s./ha (0.3 L product/ha)
Application rate (Southern Europe)	:	max. 0.1125 kg a.s./ha (1.125 L product/ha)

B.6.14.1.1 Exposure estimates with UK-POEM

The model is largely based on unpublished studies, carried out in the UK by industry and MAFF. 75th percentiles are calculated.

B.6.14.1.1.1 Mechanical downward spraying on cotton without PPE

For the present purpose there are no default values for 250 mL bottles, therefore the model for 1 L bottles is used. Furthermore, using the model for 1 L bottles is more realistic.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	**Pyriproxyfen 10EC**		Active substance	**pyriproxyfen**	
Formulation type	organic solvent-based		a.s. concentration	**100**	mg/ml
Dermal absorption from product	**2.5**	%	Dermal absorption from spray	**13**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.75**	l/ha	Work rate/day	**50**	ha
Application volume	**500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.75	litres product/ha
Work rate	50	ha/day
Number of operations	38	/day
Hand contamination	0.38	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.38	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	500	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 DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.38	ml/day	41.55	ml/day
Concen. of a.s. product or spray	100	mg/ml	0.15	mg/ml
Dermal exposure to a.s.	38	mg/day	6.2325	mg/day
Percent absorbed	2.5	%	13	%
Absorbed dose	0.95	mg/day	0.810225	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.15	mg/ml
Inhalation exposure to a.s.	0.009	mg/day
Percent absorbed	100	%
Absorbed dose	0.009	mg/day

PREDICTED EXPOSURE

Total absorbed dose	1.769225	mg/day
Operator body weight	60	kg
Operator exposure	0.029487083	mg/kg bw/day

B.6.14.1.1.2 Mechanical downward spraying on cotton with PPE

For the present purpose there are no default values for 250 mL bottles, therefore the model for 1 L bottles is used. Furthermore, using the model for 1 L bottles is more realistic.

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	**Pyriproxyfen 10EC**		Active substance	**pyriproxyfen**	
Formulation type	organic solvent-based		a.s. concentration	**100**	mg/ml
Dermal absorption from product	**2.5**	%	Dermal absorption from spray	**13**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0.75**	l/ha	Work rate/day	**50**	ha
Application volume	**500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.75	litres product/ha
Work rate	50	ha/day
Number of operations	38	/day
Hand contamination	0.38	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.038	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	500	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 DERMAL DOSE

	Mix/load		Application		
Dermal exposure	0.038	ml/day		6.45	ml/day
Concen. of a.s. product or spray	100	mg/ml		0.15	mg/ml
Dermal exposure to a.s.	3.8	mg/day		0.9675	mg/day
Percent absorbed	2.5	%		13	%
Absorbed dose	0.095	mg/day		0.125775	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.15	mg/ml
Inhalation exposure to a.s.	0.009	mg/day
Percent absorbed	100	%
Absorbed dose	0.009	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.229775	mg/day
Operator body weight	60	kg
Operator exposure	0.003829583	mg/kg bw/day

B.6.14.1.1.3 Manual spraying on tomato and eggplant indoors

No adequate module available in UK-POEM.

B.6.14.1.2 Exposure estimates with the German model

The German model is based on unpublished studies performed by industry and all carried out in Germany. The chosen statistic is the geometric mean (GM).

B.6.14.1.2.1 Mechanical downward spraying on cotton, with and without PPE

= FIELD_CROP_TRACTOR_MOUNTED =

Treated area per day	**A =**	**20**	**ha/d**	at BBA = 20
Use rate	**R =**	***0.075***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	l*a = 0,001	D*a/c = 0,06
l*m	0.0006	0.07	0.008	D*a/h = 0,38	D*a/b = 1,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = l*m x R x A	***0.0006***	0.075	20	0.0009	mg/pers. x d
Ia = l*a x R x A	0.001	0.075	20	0.0015	mg/pers. x d
		I, in total =		**0.0024**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2.4***	0.075	20	3.6	mg/pers. x d
Da/h = D*a/h x R x A	0.38	0.075	20	0.57	mg/pers. x d
Da/c = D*a/c x R x A	0.06	0.075	20	0.09	mg/pers. x d
Da/b = D*a/b x R x A	1.6	0.075	20	2.4	mg/pers. x d
		D, in total =		**6.66**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.0009	-	1	0.0009	mg/pers. x d
Ia =	0.0015	-	1	0.0015	mg/pers. x d
				0.0024	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	3.6	SS 110	0.01	0.036	mg/pers. x d
Da/h =	0.57		1	0.57	mg/pers. x d
Da/c =	0.09		1	0.09	mg/pers. x d
Da/b =	2.4		1	2.4	mg/pers. x d
				3.096	**mg/pers. x d**

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.0009	0.0009	0.0009	0.0009
Inhalation: appl.	100%	0.0015	0.0015	0.0015	0.0015
Dermal: m/l	3%	3.6	0.036	0.09	0.0009
Dermal: appl.	13%	3.06	3.06	0.3978	0.3978
			mg/pers./d:	0.4902	0.4011
kg bw:	70		mg/kg bw/d:	0.00700286	0.00573
syst. AOEL:	0.04		**% of AOEL:**	**17.5071429**	**14.325**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

B.6.14.1.2.2 Manual spraying on tomato and eggplant indoors

No adequate module available in the German model.

B.6.14.1.3 Exposure estimates with the Dutch model

The model is based on studies published in the scientific literature and on studies performed in the Netherlands and indicative 90th percentiles are deduced from the exposure databases.

B.6.14.1.3.1 Manual spraying on tomato and eggplant indoors (Northern Europe)

OPERATOR EXPOSURE — **DUTCH GREENHOUSE MODEL**

form	Pyriproxyfen 10EC
a.s.	pyriproxyfen

Application including mixing and loading

Parameter	Value	Unit	References, comments
MANUAL SPRAYING in greenhouses			
AR Application rate	0.03	kg a.s./ha	summary of intended uses
A Area treated	1	ha/ day	Dutch model
Inhalation Exposure			without PPE
SV Surrogate Exposure Value	1	mg a.s./ kg a.s.	Dutch model
Inhalation Exposure (without PPE)	**0.03**	mg a.s./ day	IE = SV x AR x A
Inhalation Exposure (with PPE)			with PPE
PPE-factor	10		default: 10
Inhalation Exposure (with PPE)	**0.003**	mg a.s./ day	IE(PPE) = (1/PPE factor) x IE
Dermal Exposure			without PPE
SV Surrogate Exposure Value	200	mg a.s./ kg a.s.	Dutch model
Dermal Exposure	**6**	mg a.s./ day	DE = SV x AR x A
Dermal Exposure (with PPE)			with PPE
PPE-factor	10		default (gloves & coverall): 10
Dermal Exposure (with PPE)	**0.6**	mg a.s./ day	DE(PPE) = (1/PPE-factor) x DE
Internal exposure			
IA Inhalation Absorption	100	%	
DA Dermal Absorption	13	%	
AOEL	2.8	mg a.s./ day	based on 70 kg bw

	Without PPE	With PPE	
Internal exposure	[mg a.s. / day]	[mg a.s. / day]	
Inhalation	0.0300	0.0030	IE(int) = IE x (IA/100)
Dermal	0.780	0.078	DE(int) = DE x (DA/100)
Total	**0.810**	**0.081**	**sum**
% AOEL			
Inhalation	1	0	%AOEL = 100 x IE(int) / AOEL
Dermal	28	3	%AOEL = 100 x DE(int) / AOEL
Total	**29**	**3**	**sum**

B.6.14.1.3.2 Manual spraying on tomato and eggplant indoors (Southern Europe)

OPERATOR EXPOSURE — DUTCH GREENHOUSE MODEL

Application including mixing and loading

form	Pyriproxyfen 10EC
a.s.	pyriproxyfen

Parameter	Value	Unit	References, comments
MANUAL SPRAYING in greenhouses			
AR Application rate	0.1125	kg a.s./ha	summary of intended uses
A Area treated	1	ha/ day	Dutch model
Inhalation Exposure			without PPE
SV Surrogate Exposure Value	1	mg a.s./ kg a.s.	Dutch model
Inhalation Exposure (without PPE)	**0.1125**	mg a.s./ day	IE = SV x AR x A
Inhalation Exposure (with PPE)			with PPE
PPE-factor	10		default: 10
Inhalation Exposure (with PPE)	**0.01125**	mg a.s./ day	IE(PPE) = (1/PPE factor) x IE
Dermal Exposure			without PPE
SV Surrogate Exposure Value	200	mg a.s./ kg a.s.	Dutch model
Dermal Exposure	**22.5**	mg a.s./ day	DE = SV x AR x A
Dermal Exposure (with PPE)			with PPE
PPE-factor	10		default (gloves & coverall): 10
Dermal Exposure (with PPE)	**2.25**	mg a.s./ day	DE(PPE) = (1/PPE-factor) x DE
Internal exposure			
IA Inhalation Absorption	100	%	
DA Dermal Absorption	13	%	
AOEL	2.8	mg a.s./ day	based on 70 kg bw

	Without PPE	With PPE	
Internal exposure	[mg a.s. / day]	[mg a.s. / day]	
Inhalation	0.1125	0.0113	IE(int) = IE x (IA/100)
Dermal	2.925	0.293	DE(int) = DE x (DA/100)
Total	**3.038**	**0.304**	**sum**
% AOEL			
Inhalation	4	0	%AOEL = 100 x IE(int) / AOEL
Dermal	104	10	%AOEL = 100 x DE(int) / AOEL
Total	**108**	**11**	**sum**

B.6.14.1.4 Risk assessment for operators

Risk assessment was performed using the 75th percentile from the UK-model (UK-75th), the geometric mean from the German model (DE-GM) and the 90th percentile from the Dutch model (NL-90th).

Table 6.14.1.4-1 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE ***		without PPE	with PPE ***
Mechanical downward spraying on cotton (outdoors)						
UK- 75th	Respiratory	<0.01	<0.01	2.4	<1	<1
	Dermal	1.76	0.22	2.4	73	9
	Total	**1.77**	**0.23**	**2.4**	**74**	**10**
DE- GM	Respiratory	<0.01	<0.01	2.8	<1	<1
	Dermal	0.49	0.40	2.8	17	14
	Total	**0.49**	**0.40**	**2.8**	**18**	**14**
*Manual spraying on tomato and eggplant indoors** (Northern Europe)*						
Dutch-90th	Respiratory	0.03	~~<0.01~~ (0.03)	2.8	1	~~<1~~ (1)
	Dermal	0.78	0.08	2.8	28	3
	Total	**0.81**	**~~0.08~~ 0.11**	**2.8**	**29**	**~~3~~ 4**
*Manual spraying on tomato and eggplant indoors** (Southern Europe)*						
Dutch-90th	Respiratory	0.11	~~0.01~~ (0.11)	2.8	4	~~<1~~ (4)
	Dermal	2.9	0.29	2.8	104	10
	Total	**3.0**	**~~0.30~~ 0.40**	**2.8**	**108**	**~~11~~ 15**

* Assuming a body weight of 60 kg for UK-POEM and 70 kg for other models
** No suitable module available in UK-POEM and German model
*** PPE UK-POEM: gloves during mixing/loading and application
PPE DE-model: gloves during mixing/loading only
Dutch greenhouse model: gloves and coverall during mixing/loading and application (no RPE)

B.6.14.2 Bystander exposure (IIIA 7.2.2)

Mechanical downward spraying on cotton

Input data

Application rate	:	0.075 kg a.s./ha
Spray volume	:	500 L/ha
Dermal exposure	:	0.5% of the application rate (kg/ha) on an body surface of 2 m^2 and dermal absorption of 13%
Inhalation exposure	:	0.03 mL spraying liquid per m^3, a duration of 1 hour, a ventilation rate of 1.25 m^3/hour, and inhalation absorption of 100%.

Manual up- and downward spraying on tomato and eggplant indoors

The presence of bystanders should be prohibited.

B.6.14.2.1 Exposure estimates with EUROPOEM II

The values represent the 90th percentile exposure values for bystanders.

B.6.14.2.1.1 Mechanical downward spraying on cotton

BYSTANDER EXPOSURE			EUROPOEM II MODEL	
form	Pyriproxyfen 10EC		**Outdoor application**	
as	pyriproxyfen			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.075	kg a.s. / ha	summary of intended uses
SV	Spray volume	500	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.03	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.0056**	mg a.s. / day	IE = (ARx1000/SV)xSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.005		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**0.075**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	13	%	
	AOEL	2.4	mg a.s./ day	based on 60 kg bw

	Without PPE	
Internal exposure	[mg a.s./ day]	
Inhalation	0.0056	IE(int) = IE x (IA/100)
Dermal	0.010	DE(int) = DE x (DA/100)
Total	**0.015**	**sum**
% AOEL		
Inhalation	0.2	%AOEL = 100 x IE(int) / AOEL
Dermal	0.4	%AOEL = 100 x DE(int) / AOEL
Total	**0.6**	**sum**

B.6.14.2.2 Risk assessment for bystanders

Table 6.14.2.2-1 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic * (mg a.s./day)	%AOEL
Exposure during mechanical downward spraying on cotton			
Respiratory	0.0056	2.4	0.2
Dermal	0.010	2.4	0.4
Total	**0.015**	**2.4**	**0.6**
Exposure during manual spraying on tomato and eggplant indoors			
The presence of bystanders in greenhouses during spraying should be prohibited.			

* Assuming a body weight of 60 kg

B.6.14.3 Worker exposure (IIIA 7.2.3)

Re-entry activities in cotton

Input data

Application rate : 0.075 kg a.s./ha

Duration of activities : 6 hours

Dermal exposure (transfer coefficient) : 0.45 m^2/hour (worst case TC)

Re-entry activities in tomato and eggplant indoors

Input data

Application rate (Northern Europe) : max. 0.03 kg a.s./ha (0.3 L product/ha)

Application rate (Southern Europe) : max. 0.1125 kg a.s./ha (1.125 L product/ha)

Dermal exposure (transfer coefficient) : 0.45 m^2/hour

Duration of activities : 6 hours

B.6.14.3.1 Exposure estimates with EUROPOEM II

The worker exposure estimates below using EUROPOEM II model differ slightly from the estimation in the DAR, since, based on new insights the default value for dislodgeable foliar residue is used and for cotton, tomato and eggplant it was decided to use a TC of 0.45 m^2/hour.

B.6.14.3.1.1 Re-entry activities in cotton outdoors, based on EUROPOEM II

WORKER EXPOSURE			EUROPOEM II MODEL	
form	Pyriproxyfen 10EC		**Re-entry in the field, DFR model**	
a.s.	pyriproxyfen			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in the field				
AR	Application rate	0.075	kg a.s./ha	summary of intended uses
Worker				
Duration				
T		6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
	Dermal Exposure	**6.075**	mg a.s./ day	DE = DFR x AR x TC x T
Internal exposure				
DA	Dermal Absorption	13	%	
	PPE-factor dermal	0.1		reduction factor
	AOEL	2.8	mg a.s./ day	based on 70 kg bw

	Without PPE	**With PPE**	
Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
Inhalation	-	-	no model available
Dermal	0.790	0.079	DE(int) = DE x (DA/100)
Total	**0.790**	**0.079**	**sum**
% AOEL			
Inhalation	-	-	no model available
Dermal	28	3	%AOEL = 100 x DE(int) / AOEL
Total	**28**	**3**	**sum**

B.6.14.3.1.2 Re-entry activities in tomato and eggplant indoors (Northern Europe), based on EUROPOEM II

WORKER EXPOSURE		EUROPOEM II MODEL		
form	Pyriproxyfen 10EC	**Re-entry in greenhouse, DFR model**		
a.s.	pyriproxyfen			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in the field				
AR	Application rate	0.03	kg a.s./ha	summary of intended uses
Worker				
Duration				
T		6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**2.43**	mg a.s./ day	DE = DFR x AR x TC x T
Internal exposure				
DA	Dermal Absorption	13	%	
	PPE-factor dermal	0.1		reduction factor
	AOEL	2.8	mg a.s./ day	based on 70 kg bw

	Without PPE	**With PPE**	
Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
Inhalation	-	-	no model available
Dermal	0.316	0.032	DE(int) = DE x (DA/100)
Total	**0.316**	**0.032**	**sum**
% AOEL			
Inhalation	-	-	no model available
Dermal	11	1	%AOEL = 100 x DE(int) / AOEL
Total	**11**	**1**	**sum**

B.6.14.3.1.3 Re-entry activities in tomato and eggplant indoors (Southern Europe), based on EUROPOEM II

WORKER EXPOSURE		EUROPOEM II MODEL		
form	Pyriproxyfen 10EC	**Re-entry in greenhouse, DFR model**		
a.s.	pyriproxyfen			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in the field				
AR	Application rate	0.1125	kg a.s./ha	summary of intended uses
Worker				
Duration				
T		6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**9.1125**	mg a.s./ day	DE = DFR x AR x TC x T
Internal exposure				
DA	Dermal Absorption	13	%	
	PPE-factor dermal	0.1		reduction factor
	AOEL	2.8	mg a.s./ day	based on 70 kg bw

	Without PPE	**With PPE**	
Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
Inhalation	-	-	no model available
Dermal	1.185	0.118	DE(int) = DE x (DA/100)
Total	**1.185**	**0.118**	**sum**
% AOEL			
Inhalation	-	-	no model available
Dermal	42	4	%AOEL = 100 x DE(int) / AOEL
Total	**42**	**4**	**sum**

B.6.14.3.2 Risk assessment for workers

Table 6.14.3.2-1 Worker internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Re-entry exposure after mechanical downward spraying on cotton						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	0.79	0.08	2.8	28	3
	Total	**0.79**	**0.08**	**2.8**	**28**	**3**
Re-entry exposure after manual spraying on tomato and eggplant indoors (Northern Europe)						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	0.32	0.03	2.8	11	1
	Total	**0.32**	**0.03**	**2.8**	**11**	**1**
Re-entry exposure after manual spraying on tomato and eggplant indoors (Southern Europe)						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	1.19	0.12	2.8	42	4
	Total	**1.19**	**0.12**	**2.8**	**42**	**4**

* Assuming a body weight of 70 kg
- The inhalation exposure is not quantifiable with this model. However, it can be expected to be lower than respiratory exposure during application.

Pyriproxyfen 10 EC is applied once in cotton and can be applied 1-2 times in tomato and eggplant, with a spray interval of 10 days. The worker exposure has been calculated after one application. It is possible that worker exposure is higher after two applications. Worker exposure after two applications can however not be quantified with the existing models. It is not realistic to assume that after two applications 2 x 100% = 200% will be present on the crop, because in that case there would be no need for a second application. But even the unrealistic worst-case assumption of 200% active substance on the crop would still result in safe uses for the worker without PPE.

B.6.14.4 Conclusions on risk assessments for operators, bystanders and workers

Pyriproxyfen 10EC: mechanical downward spraying on cotton outdoors

- A safe use for operators without PPE was identified using the UK model and the German model (GM).
- A safe use for bystanders was identified using the EUROPOEM II model.
- A safe use for the worker without PPE was identified using the EUROPOEM II model.

Pyriproxyfen 10EC: mechanical downward spraying on tomato and eggplant indoors

- A safe use was identified for operators without PPE in Northern Europe, using the Dutch Greenhouse model. For manual up- and downward spraying on tomato and eggplant in Southern Europe, the AOEL was slightly exceeded (without PPE). Safe uses in Southern Europe were identified with PPE (gloves and coverall), using the Dutch Greenhouse model.
- No bystanders should be allowed in greenhouses during the application.
- A safe use for the worker without PPE was identified in Northern and Southern Europe, using the EUROPOEM II model.

European Commission

PYRIPROXYFEN

REVISED ADDENDUM

VOLUME 3 (B7)

ANNEX B

Rapporteur Member State: The Netherlands

~~**DECEMBER 2008**~~
FEBRUARY 2009

Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of pyriproxyfen in Annex I of Council Directive 91/414/EEC

B.6 Residue date

The Draft Assessment Report was sent to the member states in July 2006. Comments were gathered and the reporting table was sent to EFSA, who composed an evaluation table. The open points identified in this evaluation table are handled in this addendum, which will be dealt with in PRAPeR 65 dd January 22/23rd, 2009. The conlcusions of PRAPeR 64 (toxicology) and 65 (residues) are corrected in this addendum

Open point 3.1: occurrence of metabolite 'PYPA' in rat metabolism

Outcome of PRAPeR 64 on human toxicology: PYPA is part of rat metabolism and therefore it's toxicology is taken into account in the ADI.

Open point 3.3: should gin trash be considered as a feed item?

Cotton gin trash is not taken up in the Feed Table of Appendix G of EU Guidance Document 7031/VI/95 rev.4 dd 22.07.1996 ('Lundehn Document'). Therefore, it was not considered to be a livestock feed item in Europe until now.

In Europe cotton is cultivated in Greece, Bulgaria and Spain. Compared to the global production of cotton, these European countries are minor production sites. However, the by products of cottonseed processing (after separating lints, seeds and hulls) might be used as livestock feed locally. According to the more recent OECD Guidance Document on overview of residue chemistry studies (http://www.olis.oecd.org/olis/2006doc.nsf/LinkTo/NT00003EC2/$FILE/JT03215494.PDF), cotton meal can be fed to cattle, sheep, pig and poultry and cotton gin trash can be fed to cattle. The OECD proposes regional global feed figures for USA/Canada, Australia and Europe. For Europe, gotton gin trash was not considered a feed item for livestock. Only cottonseed meal can be fed to animals.

Open point 3.2: consideration on potential livestock exposure via residues in cotton gin trash.

~~*Residue definition for cotton gin trash.*~~
~~A residue definition for plant material was set for fruits (based on apple and tomato) and for pulses/oil seeds (based on cotton seed). In the metabolism study in cotton, also the residue in cotton gin trash was characterised and identified. Pyriproxyfen parent was the only abundant metabolite in extractable tissue accounting for 45% TRR or 1.53 mg/kg. All other metabolites are < 4.6% TRR or <0.18 mg/kg. It is proposed that the residue definition for cotton gin trash, relevant for livestock feeding, is also pyriproxyfen parent. The maximum level of pyriproxyfen in cotton gin trash was 1.53 mg/kg found in the metabolism study performed at 2N (B7.1.3 of the DAR). To perform a dietary livestock intake calculation, this value is devided by 2 yielding a residue level of 0.77 mg/kg in cotton gin trash at 1N. The maximum residue level found in cotton seed was < 0.01 mg/kg found in field tials with cotton according to GAP (B.7.6 of the DAR).~~

Table: percentage of cotton products in the diet (based on dry weight)

	Cattle (milk)	**Sheep (lamb)**	**Pig (finishing)**	**Poultry (turkey)**
Cotton meal, % of diet	***5%***	***15%***	***10%***	***10%***
% DW	89	89	89	89
Intake kg dw/d	25	1.7	3	0.5

	Cattle (milk)	**Sheep (lamb)**	**Pig (finishing)**	**Poultry (turkey)**
intake kg dry/d	1.25	0.225	0.3	0.05
intake kg fresh/d	1.40	0.25	0.34	0.06
Intake in mg/d based on residue level of 0.01 mg/kg	0.014	0.0025	0.0034	0.0006
Cotton gin trash, % of diet	***5%***	***0***	***0***	***0***
% DW	90	90	90	90
Kg dw/d	25	1.7	3	0.5
intake kg dry/d	1.25			
intake kg fresh/d	1.39			
Intake in mg/d based on a residue level of 0.77 mg/kg	1.07			
Bw	650	75	100	7
Total intake mg/d	1.084	0.0025	0.0034	0.0006
Mg/kg dry feed	0.043	0.0001	0.0011	0.0012
Mg/kg bw/d	0.0006 0.0017 0.000022	0.00003	0.000034	0.000086

Metabolism in goat was found to be identical to that of rats. Therefore, the metabolism study in goat can be used as a model for ruminants (cattle, sheep, goat) and pigs. Highest daily intake was found for cattel and was found to be 0.043 mg/kg dry feed. The trigger value of 0.1 mg/kg dry feed was not exceeded.
In the metabolism study with goat evaluated in B.7.2 of the DAR performed with 5 daily doses with 10 mg/kg dry feed (≥240N), TRR was up to 0.02 mg/kg in meat, 0.49 mg/kg in liver, 0.26 mg/kg in kidney and 0.096 mg/kg in milk. Assuming that residue levels are more or less linear with feeding levels, at a feeding level of 0.043 mg/kg dry feed residues are expected to be 240 fold lower: ≤ 0.01 mg/kg TRR for meat, kidney, milk and liver.

Highest daily intake was found for turkey and was found to be 0.0012 mg/kg dry feed. In the metabolism study with hen evaluated in B.7.2 of the DAR performed with 8 daily doses with 10 mg/kg dry feed (≥8000N), TRR was up to 0.11 mg/kg in meat, 0.69 mg/kg in liver, 0.80 mg/kg in kidney and 0.43 mg/kg in egg yolk and 0.021 mg/kg in egg white. Assuming that residue levels are more or less linear with feeding levels, at a feeding level of 0.0012 mg/kg dry feed residues are expected to be 22 fold lower: ≤ 0.01 mg/kg TRR for meat, liver, kidney, egg yolk and white and abdominal fat, skin and gizzard.

Residue intake was ≤ 0.0012 mg/kg dry feed. Therefore, it is concluded that residues of pyriproxyfen are not likely to occur in animal products if cottonseed (poultry) or cotton gin trash will be used in animal feed. At the moment, a residue definition for animal products and MRLs are not needed.

Open point 3.4: Fate of 4-OH PYR in rotational crops.

Rotational crop study

In the DAR it was concluded: *The maximum levels of radioactivity in radish (leaves and roots), lettuce, wheat forage, wheat grain, straw and chaff after a single application of [U-phenoxyphenyl-^{14}C] and [2,6-pyridyloxy-^{14}C] pyriproxyfen at a rate of 0.198 kg as/ha (0.9-3.3N) to bare soil at 30 days before sowing were 0.007 mg eq/kg (lettuce), 0.011 mg eq/kg (radish leaf), 0.005 mg eq/kg (radish root), 0.011 mg eq/kg (wheat forage), 0.081 mg eq/kg (wheat grain), 0.059 mg eq/kg (wheat straw) and 0.082 mg eq/kg (wheat chaff). Aqueous extractables of wheat straw and chaff contained 5 unidentified regions, all <10% TRR and <0.01 mg eq/kg and 4 unidentified regions, 6.1-12.4% TRR and 0.005-0.01 mg eq/kg, respectively. Other commodities/fractions were not investigated (≤ 0.01 mg eq/kg).*
Therefore, it is unlikely that 4-OH-PYR will occur in rotation crops >0.01 mg/kg

Aerobic soil dissipation study/US field dissipation study

The DT90 value of pyriproxyfen: was 34d (9.2-81d) as was found in aerobic lab studies. The DT90 value of 4-OH-PYR: was 126 (78-234d) as was found in aerobic lab studies. Therefore, 4-OH-PYR is more persistent than parent pyriproxyfen.

The maximum concentration of 4-OH-PYR was found to be 0.9-6.3% of the applied radioactivity in the aerobic soil dissipation study. TRR in soil was found to be 12 mg/kg in the soil rotational crop study at 7-14DAT.
Therefore, the maximum amount of 4-OH-PYR is 0.063*12 = 0.76 mg/kg.
The calculated 4'-OH-PYR maximum plateau concentration was 0.013 in S-EU was reached after 1-year application on tomato. This was in accordance what was found in the USA field dissipation study were maximum concentrations of 4-OH-PYR were 0.007 (0-0.02) mg/kg.

In the rotational crops study it was found that crops planted 30DAT had TRR < 0.01 mg/kg except for cereals in which TRR accounted for 0.011-0.081 mg/kg, however, with no singe fraction >0.01 mg/kg. At longer plant back intervals with lower levels of 4-OH-PYR, 4-OH-PYR is expected to be even lower (< 0.01 mg/kg).

Plant metabolism

Plant metabolism studies in apple, tomato and cotton showed that 4-OH-PYR was part of their metabolic pathway. 4-OH-PYR was always much lower compared to parent pyriproxyfen, indicating that conversion of 4-OH-PYR probably is not the limiting step in metabolism. 4-OH-PYR therefore is not expected to show a potential for accumulation. It is stressed that only metabolism in fruits and oil seeds was investigated (no leaf, root or cereal matrix). If this profile of 4-OH-PYR is representative for all crops and matrices, accumulation of 4-OH-PYR in rotational crops by uptake from the soil is not likely.

Conclusion

Overall, it can be concluded that 4-OH-PYR levels in soil are lower in the field than calculated based on lab DT50 values. No residues of 4-OH-PYR have to be expected in rotational crops since:

- 4-OH-PYR was found as an metabolic intermediate in primary crops 5-10 times lower than parent pyriproxyfen, indicating good metabolism of this metabolite;

- in the rotational crop study no singe fraction exceeded 0.01 mg/kg. At 1-30DAT levels of 4-OH-PYR in the soil are higher compared to 360DAT, therefore at higher DAT lower levels of 4-OH-PYR are expected in rotational crops.

Open point 3.5: MSs to agree with 2 residue trials being sufficient for proving a zero-residue situation.

Two residue trials were provided with 1 application of 70 g pyriproxifen/ha before boll opening (up to BBCH 79). This was within acceptable limits from cGAP (1 application of 75 g pyriproxifen/ha before boll opening). In the metabolism study performed at 2N, residues were maximally 0.13 and 4.4 mg/kg TRR in seeds and gin trash at 28DAT, with pyriproxyfen parent being < 0.01 mg/kg in seeds.
The results show that most of the residue was not penetrating the bolls. These data are consistent and a zero-residue situation is sufficiently proven.

Open point 3.6: Might one of the two isomers be preferentially metabolised?

The residue section contains no information of the specific metabolism of either of the 2 metabolites: all studies were performed with the racemic mixture of both isomers and no stereo-specific determinations were performed. Pyriproxyfen parent is the only relevant residue according to the residue definition for fruits and oilseeds as determined in B7.3. of the DAR. Since the parent molecule contains the asymmetric carbon resulting in both (R)- and (S)-isomerisation, the question still is relevant.

In view of consumer risk assessment, it was questioned whether (R)-pyriproxyfen or (S)-pyriproxyfen might be preferentially metabolised by crops, resulting in one of both isomers being the main residue. The following information was provided by the notifier. Notifier refers to open point 1(6) of the physical chemistry section: (S)-pyriproxyfen is about 8 times more biological active than (R)-pyriproxyfen. However, this is no answer to the question.

Open point 2.21 of the toxicology evaluation table the question was raised whether the (R)- and (S)-isomer are differently toxic. The notifier stated that this is not of concern since all the toxicological studies were performed with the racemic mixture as well. Since this does not show that the isomers have the same toxicity, the question on specific metabolism of either of the 2 metabolites still remains

The expert meeting PRAPeR 65 concluded that in view of the low risk (≤1.5% of the ADI is used) a risk is not likely to occur at the moment. Therefore, It is proposed to perform a bridging study with separated (R)- and (S) isomer when in future new uses use more of the ADI. ~~A Data GAP is proposed.~~

Open point 7 (raised by PRAPeR 65): Update List of Endpoints with EFSA's PRIMo rev.2.

PRAPeR meeting 64 on toxicology decided that an ARfD was not necessary; it was ommited form the List of Endpoints. PRAPeR meeting 65 requested an consumer risk assessment according to EFSA PRIMo rev.2. An ADI of 0.1 mg/kg bw/d, MRLs of 0.3 mg/kg for tomato and aubergine, an MRL of 0.01* mg/kg for cotton seed and MRLs at LOQ (0.01* mg/kg) for

all other plant commodities were put into the model; the five highest outcomes are mentioned below:

diet	% of ADI
WHO Cluster diet B	1.5%
FR toddler	0.9%
DE child	0.9%
UK Toddler	0.8%
NL child	0.8%

All other diets showed lower values of the ADI used.

B.9 Ecotoxicology

Background information

Pyriproxyfen is an existing insecticide proposed for use on tomato and egg plant in Northern Europe (greenhouse, single application or two applications with 10 days interval at 0.02-0.03 kg a.s./ha) and Southern Europe (greenhouse, single application or two applications with 10 days interval at 0.05-0.1125 kg a.s./ha) and on cotton in Southern Europe (single application of 0.075 kg a.s./ha). The proposed formulated product, Pyriproxyfen 10EC, also referred to as Admiral, Atominal and Juvinal, is an EC formulation containing nominally 100 g/L pyriproxyfen.

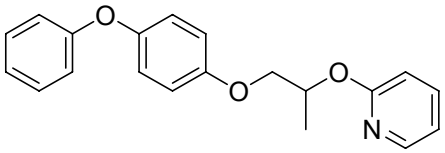
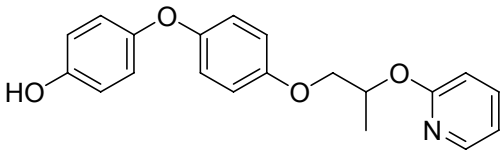
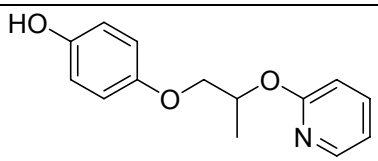
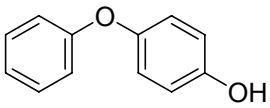
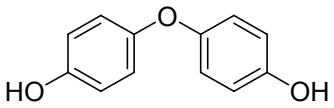
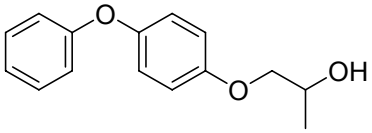
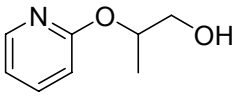
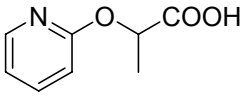
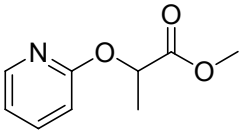
The formulated product was also referred to as Pyriproxyfen 100 g/L, Pyriproxyfen 10% EC, Pyriproxyfen 0.83 EC and S-71639 10 EC, containing 10-11.5% Pyriproxyfen, or 98.0-100 g pyriproxyfen/L. The product S-71639 10 EC is mentioned in Document J as specification 13.

Data were presented on the technical active substance (a.s.) and on the proposed Pyriproxyfen 10EC formulation, as well as on major metabolites.

Table B.9.1 below gives an overview of the major metabolites of pyriproxyfen in the matrices soil, water/sediment and plants. The maximum formation rates in the different matrices, as well as the occurrence of the metabolites in metabolism of rat, goat and hen are included.

Studies summarised below under point B.9.1 to B.9.8 are acceptable unless noted.

Table B.9.1 Overview of metabolites of pyriproxyfen

Metabolite	Structure	Metabolite in	Max. formation %	Found in
pyriproxyfen MW = 321.5 LogPow = 5.37 (pH 5.6) logPow = 5.70(A) LogPow = 5.55(B)				
4'-OH-pyriproxyfen MW = 337.4 LogPow = 5.17(A) LogPow = 5.07(B)		Plants (apple pomace) Soil Water Sediment	11 6.3 4.8 14.8	Goat, hen, rat, mouse
DPH-pyriproxyfen MW = 245.3 LogPow = 3.02(A) LogPow = 3.01(B)		Plants (apple pomace) Soil Water Sediment	1.4 ≤0.5 11.8 4.3	Goat, hen, rat, mouse
POP MW = 186.2 LogPow = 3.64(A) LogPow = 3.57(B)		Plants (apple pomace) Water Sediment	0.5 0.4 0.5	Goat, hen
4'-OH-POP MW = 202.2 LogPow = 3.12(A) LogPow = 3.09(B)		Water Sediment	1.0 0.5	Goat, hen, rat
POPA MW = 244.3 LogPow = 4.18(A) LogPow = 3.57(B)		Plants (apple pomace) Water	0.9 1.0	Goat, hen, rat, mouse
PYPA MW = 153.2 LogPow = 0.67(A) LogPow = 0.78(B)		Plants (tomato juice) Soil Water	2.6 ≤0.8 0.8	Goat, hen
PYPAC MW = 167.2 LogPow = 0.97(A) LogPow = 1.01(B)		Plants (tomato juice) Plants (cotton) Soil Water Sediment	3.8 8.2 8.6 23.6 7.6	Goat, hen, rat
PYPAC-Me MW = 181.2 LogPow = 1.34(A) LogPow = 1.30(B)		Water/sediment	0.7	-

(A) Estimated by RMS using Pallas 3.0 (CompuDrug Chemistry Ltd. 1994,95): 3.18
(B) Estimated by RMS using EPA EPI Suite software

B.9.1 Effects on birds (IIA 8.1; IIIA 10.1)

Studies were conducted under GLP and in compliance with relevant OECD and/or EC guidelines, unless stated.

B.9.1.1 Acute oral toxicity (IIA 8.1.1; IIIA 10.1.1)

B.9.1.1.1 Acute oral toxicity of the active substance

Studies on the acute oral toxicity of pyriproxyfen to Mallard duck and Bobwhite quail have been summarised in Table B.9.2 and B.9.3.

Study 1

reference	:	Roberts N. L. and Hakin B. (1989)	GLP statement	:	Yes
type of study	:	Acute oral toxicity study with the Mallard duck	guideline	:	EPA 71-1 (1982)
year of execution	:	1988	acceptability	:	acceptable
test substance	:	S-31183 technical grade, batch PYG87074, purity 95.3%			

Table B.9.2 Summary of acute oral toxicity of pyriproxyfen to Mallard duck

Purity	Species	Acute oral LD50 (mg a.s./kg bw)	Test Guideline	Reference
95.3%	Mallard duck *Anas platyrhynchos*	>1906	EPA 71-1 (1982)	Roberts N.L. and Hakin B., 1989

Dose levels were 0 (vehicle (corn oil) control), 477, 953 and 1906 mg a.s./kg bw. These dose levels were calculated by the Rapporteur from the reported dose levels, which were expressed in mg technical a.s/kg bw, using the reported purity of 95.3%. Five males and five females per dose level. Fourteen day observation period. One mortality at 953 mg a.s./kg bw. No clinical signs of toxicity. No treatment related effects on body weight and feed consumption. Necropsy (on birds that died and all birds of high dose group) revealed no abnormalities.

Study 2

reference	:	Roberts N.L. *et al.* (1989)	GLP statement	:	Yes
type of study	:	Acute oral toxicity study with the Bobwhite quail	guideline	:	EPA 71-1 (1982)
year of execution	:	1988	acceptability	:	acceptable
test substance	:	S-31183 technical grade, batch PYG87074, purity 95.3%			

Table B.9.3 Summary of acute oral toxicity of pyriproxyfen to Bobwhite quail

Purity	Species	Acute oral LD50 (mg a.s./kg bw)	Test Guideline	Reference
95.3%	Bobwhite quail *Colinus virginianus*	>1906	EPA 71-1 (1982)	Roberts N.L. *et al.*, 1989

Dose levels were 0 (vehicle (corn oil) control), 477, 953 and 1906 mg a.s./kg bw. These dose levels were calculated by the Rapporteur from the reported dose levels, which were expressed in mg technical a.s/kg bw, using the reported purity of 95.3%. Five males and five females per dose level. Fourteen day observation period. No mortality and no clinical signs of toxicity. A decrease in body weight was observed at the highest test dose during day 0-7, with a compensatory increase in body weight during day 7-14. No treatment related effects on feed consumption. Necropsy (on birds of high dose group) revealed no abnormalities.

B.9.1.1.2 Acute oral toxicity of metabolites

No data were submitted.

B.9.1.1.3 Acute oral toxicity of the plant protection product

No data were submitted.

B.9.1.2 Dietary toxicity (IIA 8.1.2)

B.9.1.2.1 Dietary toxicity of the active substance

Study 1

reference	:	Roberts N.L. *et al.* (1989a)	GLP statement	:	yes
type of study	:	Dietary LC50 study with the Mallard duck	guideline	:	EPA 71-2 (1982)
year of execution	:	1988	acceptability	:	acceptable
test substance	:	S-31183 technical grade, batch PYG 87074, purity 95.3%			

In a study on the dietary toxicity of pyriproxyfen (purity 95.3%), four groups of 6-day old Mallard ducks of indeterminate sex (10/group) were fed pyriproxyfen in the diet at analytically confirmed concentrations of 650, 1300, 2600 and 5200 mg/kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. Three control groups of ten 6-day old Mallard ducks received feed treated with the carrier corn oil only.

No mortalities occurred and no clinical signs were noted. Body weight gain and feed consumption in all treated groups were comparable to those in controls. Necropsy (on all birds from the high dose group) revealed no abnormalities.

The 5-day dietary LC50 and NOEC were >5200 mg/kg diet. The corresponding value corrected for test substance purity of 95.3% is >4956 mg a.s./kg diet, equivalent to >1261 mg a.s./kg bw/day.

Comment by RMS
The 5-day dietary LC50 and NOEC were reported in mg technical substance/kg bw. These values were expressed by the Rapporteur in mg a.s./kg bw/day based on the reported mean feed consumption and body weight during study day 0-5.

Study 2

reference	:	Roberts N.L. *et al.* (1989b)	GLP statement	:	yes
type of study	:	Dietary LC50 study with the Bobwhite quail	guideline	:	EPA 71-2 (1982)
year of execution	:	1988	acceptability	:	acceptable
test substance	:	S-31183 technical grade, batch PYG 87074, purity 95.3%			

In a study on the dietary toxicity of pyriproxyfen (purity 95.3%), four groups of 13-day old Bobwhite quails of indeterminate sex (10/group) were fed pyriproxyfen in the diet at analytically confirmed concentrations of 650, 1300, 2600 and 5200 mg/kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. Three control groups of ten 13-day old Bobwhite quails received feed treated with the carrier corn oil only.

Only one bird died during the study in one of the control groups. No clinical signs were noted. Body weight gain and feed consumption were reduced at the highest test dose. Necropsy (on the bird that died and all birds from the high dose group) revealed no abnormalities.

The 5-day dietary LC50 was >5200 mg/kg diet. The corresponding value corrected for test substance purity of 95.3% is >4956 mg a.s./kg diet, equivalent to >863 mg a.s./kg bw/day. The 5-day dietary NOEC was 2600 mg/kg diet (corresponding value corrected for test substance purity 2478 mg a.s./kg diet, equivalent to 445 mg a.s./kg bw/day).

Comment by RMS
The 5-day dietary LC50 and NOEC were reported in mg technical substance/kg bw. These values were expressed by the Rapporteur in mg a.s./kg bw/day based on the reported mean feed consumption and body weight during study day 0-5.

B.9.1.2.2 Dietary toxicity of the plant protection product

No data were submitted.

B.9.1.3 Long term/Reproductive toxicity (IIA 8.1.3)

Study 1

reference	:	Beavers J.B. *et al.* (1994a)	GLP statement	:	yes
type of study	:	Dietary reproductive toxicity study with the Mallard duck	guideline	:	EPA 71-4 (1982)
year of execution	:	1992-1993	acceptability	:	acceptable
test substance	:	Sumilarv T.G., lot no. PYG-87074, purity 95.3%			

Twenty-one-week-old Mallard duck were exposed to pyriproxyfen technical (purity 95.3%) in the diet for 21 weeks. Nominal feed concentrations were 0 (control), 120, 360 and 600 mg/kg feed. There were 16 pairs per dose level, one pair per cage. The birds were maintained in a controlled environmental test room and eggs were collected daily from the onset of egg production (week 13). Hatchlings were identified by wing bands, and maintained for 14 days in brooders according to concentration group. Reproduction endpoints were: number of eggs laid, number of eggs cracked, eggshell thickness, number of viable embryos, number of live 3-week-old embryos, number of hatchlings, hatchling weight, number of 14-day-old survivors, and chick weights at day 14 of age. For eggshell thickness determination, one egg was collected weekly from every other pen throughout the egg laying period.

One hen died in the 120 and and one drake in the 360 mg/kg food group. These mortalities were considered to be unrelated to treatment. No overt signs of toxicity were observed at any concentration tested. There were no treatment-related effects on body weight and feed consumption. Necropsy performed on all adult survivors revealed no abnormalities.

Table B.9.4 Results of a reproductive study on Mallard duck

Nominal concentration (mg/kg feed)	0	120	360	600
Mean measured concentration (mg a.s./kg feed)	nd(A)	122	353	598
Mean parental food consumption (g/bird/day)	132	141	136	145
Mean body weight change males (%)	-0.6	+2.5	-1.5	+0.7
Mean body weight change females (%)	+10	+10	+12	+8.7
Mean egg production (eggs/hen)	49	43	46	44
Total number of eggs set	683	559	595	612
Mean eggshell thickness (mm)	0.394	0.391	0.397	0.404
Percent of eggs laid that were cracked (%)	3	3	3	2
Percent of eggs set that were fertile (%)	84	81	89	94
21-day embryos as percentage of fertile eggs (%)	100	100	99	99
Number hatchlings as percent of 21-day embryos (%)	80	77	80	70
Number hatchlings as percent of eggs set (%)	66	64	71	66
Number 14-day chicks as percent of hatchlings (%)	96	88	97	97
Number 14-day chicks as percent of eggs set (%)	64	61	69	65
Mean body weight (g) of hatchlings	35	35	34	34
Mean body weight (g) of 14-day chicks	302	293	302	289

(A) Not detected (<30 mg/kg diet).

Based on the absence of effects on survival, growth and reproductive parameters, the NOEC in Mallard duck exposed to pyriproxyfen in the diet was >600 mg/kg feed, the highest concentration tested. The corresponding value corrected for test substance purity of 95.3% is >572 mg a.s./kg diet, equivalent to >70.2 and >77.6 mg a.s./kg bw/day for males and females, respectively.

Comments by RMS

The NOECs, expressed as mg a.s./kg bw/day, were not presented in the report. The above values were calculated by the Rapporteur from the analytically confirmed nominal pyriproxyfen concentrations in feed, the mean feed consumption and mean body weight.

Study 2

reference	:	Beavers J.B. *et al.* (1994b)	GLP statement	:	yes
type of study	:	Dietary reproductive toxicity study with the Bobwhite quail	guideline	:	EPA 71-4 (1982)
year of execution	:	1992-1993	acceptability	:	acceptable
test substance	:	Sumilarv T.G., lot no. PYG-87074, purity 95.3%			

Seventeen-week-old Bobwhite quail were exposed to pyriproxyfen technical (purity 95.3%) in the diet for 22 weeks. Nominal feed concentrations were 0 (control), 120, 360 and 600 mg/kg feed. There were 16 pairs per dose level, one pair per cage. The birds were maintained in a controlled environmental test room and eggs were collected daily from the onset of egg production (week 13). Hatchlings were identified by leg bands, and maintained for 14 days in brooders according to concentration group. Reproduction endpoints were: number of eggs laid, number of eggs cracked, eggshell thickness, number of viable embryos, number of live 3-week-old embryos, number of hatchlings, hatchling weight, number of 14-day-old survivors, and chick weights at day 14 of age. For eggshell thickness determination, one egg was collected weekly from every other pen throughout the egg laying period.

One hen and one cock died prior to termination in both the 120 and 360 mg/kg food group, and one hen in the 600 mg/kg food group. These mortalities were considered to be unrelated to treatment. No overt signs of toxicity were observed at any concentration tested. There were no treatment-related effects on body weight and feed consumption. Necropsy performed on all adult survivors revealed no abnormalities.

Table B.9.5 Results of a reproductive study on Bobwhite quail

Nominal concentration (mg/kg feed)	0	120	360	600
Mean measured concentration (mg a.s./kg feed)	nd(A)	123	355	596
Mean parental food consumption (g/bird/day)	27	31	28	30
Mean body weight change males (%)	+6.2	+8.1	+6.2	+8.2
Mean body weight change females (%)	+18	+16	+21	+20
Mean egg production (eggs/hen)	42	39	50	47
Total number of eggs set	589	463	614	621
Mean eggshell thickness (mm)	0.227	0.228	0.233	0.226
Percent of eggs laid that were cracked (%)	3	2	3	1
Percent of eggs set that were fertile (%)	88	90	87	96
21-day embryos as percentage of fertile eggs (%)	99	98	99	99
Number hatchlings as percent of 21-day embryos (%)	94	88	93	97
Number hatchlings as percent of eggs set (%)	81	78	80	93
Number 14-day chicks as percent of hatchlings (%)	69	69	80	80
Number 14-day chicks as percent of eggs set (%)	60	54	63	74
Mean body weight (g) of hatchlings	5.4	5.5	5.3	5.3
Mean body weight (g) of 14-day chicks	20	21	20	21

(A) Not detected (<30 mg/kg diet).

Based on the absence of effects on survival, growth and reproductive parameters, the NOEC in Bobwhite quail exposed to pyriproxyfen in the diet was >600 mg/kg feed, the highest concentration tested. The corresponding value corrected for test substance purity of 95.3% is >572 mg a.s./kg diet, equivalent to >85.0 and >82.8 mg a.s./kg bw/day for males and females, respectively.

Comments by RMS

The NOECs, expressed as mg a.s./kg bw/day, were not presented in the report. The above values were calculated by the Rapporteur from the analytically confirmed nominal pyriproxyfen concentrations in feed, the mean feed consumption and mean body weight.

B.9.1.4 Summary

Acute oral toxicity studies on two species were supplied. The acute LD50 of pyriproxyfen for Mallard duck and Bobwhite quail was >1906 mg a.s./kg body weight.

Short term dietary toxicity studies with pyriproxyfen on two species were supplied. For mallard duck and bobwhite quail, the dietary LC50 was >4956 mg a.s./kg diet, equivalent to >1261 and >863 mg a.s./kg bw/day, respectively.

Reproductive toxicity studies with pyriproxyfen in mallard duck and bobwhite quail were submitted, in which there were no effects on reproductive parameters at the highest tested dose of 572 mg a.s./kg diet, equivalent to 70.2 and 85.0 mg a.s./kg bw/day (males) and 77.6 and 82.8 mg a.s./kg bw/day (females), respectively.

B.9.1.5 Risk assessment for birds

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC (Working Document Sanco/4145/2000, Final 25 September 2002).

B.9.1.5.1 Risk of active substance for birds

Routes of exposure

For the application in tomato and egg plant (glass house), the routes of exposure to birds are considered to be:

- consumption of surface water containing residues of pyriproxyfen (short-term);
- consumption of fish contaminated with residues of pyriproxyfen (long-term).

For the application in cotton, the routes of exposure to birds are considered to be:

- consumption of oversprayed leafy crops with residues of pyriproxyfen (short- and long-term);
- consumption of oversprayed insects with residues of pyriproxyfen (short- and long-term);
- consumption of surface water containing residues of pyriproxyfen (short-term);
- consumption of fish contaminated with residues of pyriproxyfen (long-term);
- consumption of earthworms contaminated with residues of pyriproxyfen (long-term).

Acute risk assessment (standard exposure scenario)

The acute risk assessment is based on a herbivorous bird of body weight (BW) 300 g feeding exclusively on leafy crops, with a Food Intake Ratio (FIR) of 228 g fresh material/day, and a daily water intake (DWI) of 26 mL/day. In addition, an insectivorous bird of body weight (BW) 10 g feeding exclusively on insects, with a DFI of 10.4 g and a DWI of 2.6 mL/day is considered. The acute LD50 is >1906 mg/kg bw. The estimated theoretical exposure (ETE) for the acute time scale is calculated as (FIR/bw)*RUD* *MAF*dose or as PECsw*DWI/BW. In the tables below, PECfeed is equal to RUD*MAF*dose. The residue values for acute intake represent 90th percentile values in agreement with the guidance in Sanco/4145/2000. The PECsw is the worst-case initial PECsw value (FOCUS Step 2, Southern Europe for tomato and egg plant; FOCUS Step 1, Southern Europe for cotton) taken from Section B.8.6.1. The risk assessment for intake of contaminated surface water is based on a small bird (10 g) with a DWI of 2.6 mL/day (worst-case). In a worst-case approach, only the highest application per crop is considered (two applications of 0.1125 kg a.s./ha for tomato and egg plant and 0.075 kg a.s./ha for cotton). Residue values in food and water, ETE and TERa values are presented in Table B.9.6.

Table B.9.6 Acute Toxicity Exposure Ratios for exposure of birds to pyriproxyfen due to consumption of contaminated leafy crops, insects and drinking water

appln.	dose (kg as/ha)	LD50 (mg/kg bw)	Route	PEC_{FEED} or PEC_{WATER} (mg/kg wwt or mg/L)	ETE (mg/kg bw/d)	TERa
Tomato & egg plant EU S	2 x 0.1125	>1906	Water	4.E-05	1.E-05	>2.E+08
Cotton EU S	0.075	>1906	Leafy crops Insects Water	6.5 3.9 2.E-03	5.0 4.1 4.E-04	>384 >470 >5.E+06

TERa values in Table B.9.6 are all far above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to birds is considered to be acceptable.

Short-term risk assessment (standard exposure scenario)

Short-term risk assessment is based on the same birds, weighing 300 g feeding on leafy crops, and weighing 10 g feeding on insects. It is assumed however that in the course of some days birds will not always be exposed to leafy crops or insects contaminated with pyriproxyfen but also to uncontaminated food. Therefore, the arithmetic mean residue values are taken for residues in leafy crops (in agreement with the guidance in Sanco/4145/2000). The LC50 used for risk assessment is >863 mg/kg bw/day. The ETE for the short-term time scale is calculated as PECfeed*FIR/BW, where PECfeed = RUD*MAF*dose. Residue values in food, ETE and TERst values are presented in Table B.9.7.

Table B.9.7 Short-term Toxicity Exposure Ratios for exposure of birds to pyriproxyfen due to consumption of contaminated leafy crops and insects

appln.	dose (kg as/ha)	LC50 (mg/kg bw/d)	route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERst
Cotton EU S	0.075	>863	Leafy crops Insects	3.0 2.2	2.3 2.3	>379 >382

The TERst values in Table B.9.7 are far above the Annex VI 91/414 EEC trigger of 10. Hence, the short-term risk to birds is considered to be acceptable.

Long-term risk assessment (standard exposure scenario)

Long-term risk assessment is based on the same birds weighing 300 g feeding on leafy crops and weighing 10 g feeding on insects, and using arithmetic mean residue values for residues in leafy crops (in agreement with the guidance in Sanco/4145/2000). For the calculation of the concentration in leafy crops, a time window of 3 weeks and a DT50 of 10 days were applied, leading to a TWA correction factor (f_{twa}) of the initial residue values of 0.53. Residues in insects are assumed to be stable in time, and no TWA correction factor is used for this route. The NOEC is 70.2 mg a.s./kg bw/day. The ETE for the long-term time scale is calculated as $PECfeed_{,initial}*f_{twa}*DFI/BW$. Residue values in food, ETE and TERlt values are presented in Table B.9.8.

Table B.9.8 Long-term Toxicity Exposure Ratios for exposure of birds to pyriproxyfen due to consumption of contaminated leafy crops and insects

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
Cotton EU S	0.075	70.2	Leafy crops Insects	1.6 2.2	1.2 2.3	58 31

The TERlt values in Table B.9.8 are above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk is considered to be acceptable.

Long-term risk assessment (bioaccumulation and food chain behaviour)

Earthworms

Long-term risk assessment is based on a bird weighing 100 g feeding exclusively on earthworms, with a daily food intake (DFI) of 113 g fresh material/day. The BCF for earthworms was calculated as $BCF = (0.84+0.01*Pow)/(f_{oc}*Koc)$, where Koc = 21175 L/kg (section B.8.2.1) and f_{oc} = 0.02 (standard value). Pow, calculated from logPow = 5.37 (section B.2.2), was 2E+05. The resulting BCF of pyriproxyfen for earthworms was 5.5 kg earthworm fresh weight per kg soil dry weight. Residue levels in earthworms were calculated from the 21-day TWA PECsoil (0.045 mg/kg dry weight for the 1 x 0.075 kg a.s./ha treatment, section B.8.3.1), as follows: PEC_{FEED}=PECworm = PECsoil*BCFworm.

Fish

Long-term risk assessment is based on a fish-eating bird weighing 1000 g with a daily food intake (DFI) of 206 g fish/day. In a worst-case approach, the BCF of pyriproxyfen for fish is taken to be the value of 1495 L/kg wet weight for radioactivity (pyridinyl-label) (section B.9.2.2.3). Residue levels in fish were calculated from the 21-day TWA PECsw (0.0026 and 0.37 µg/L, respectively, for the 2 x 0.1125 and 1 x 0.075 kg a.s./ha treatment, see section B.8.6.1.) as follows: PEC_{FEED}=PECfish = PECsw*BCFfish.

The NOEC used for risk assessment is 70.2 mg/kg bw/day. The ETE for the long-term time scale is calculated as PEC*DFI/BW. Residue levels in earthworms and fish and ETE and TERlt values are presented in Table B.9.9.

Table B.9.9 Long-term Toxicity Exposure Ratios for exposure of birds to pyriproxyfen due to consumption of contaminated earthworms and fish

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
Tomato & egg plant EU S	2 x 0.1125	70.2	Fish	4.E-03	8.E-04	9.E+04
Cotton EU S	1 x 0.075	70.2	Earthworms Fish	0.25 0.55	0.27 0.11	258 615

TERlt values for consumption of worms and fish in Table B.9.9 are above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk to birds as a result of bio-accumulation in fish and earthworms is considered to be acceptable.

Bio-magnification in terrestrial food chains

The bioaccumulation potential reported in the List of Endpoints of the Toxicology section is stated to be low. Hence, according to the guidance provided in SANCO/4145/2000 – final (25 September 2002), the risk for biomagnification in terrestrial food chains is low.

B.9.1.5.2 Risk of metabolites for birds

According to the the Guidance Document on terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), the risk of plant metabolites to birds should be addressed.

The risk assessment of plant metabolites is based on the guidance provided in the Guidance Document on terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002). It is similar to that for the active substance, but is based on the presence of metabolites in birds administered pyriproxyfen, on estimates of the residue values of the metabolite, and on structural considerations. When a metabolite is found at significant levels in birds after exposure to parent pyriproxyfen, it can be assumed that the toxicity of the metabolite has been covered by studies with parent pyriproxyfen. In case the level of exposure of birds to the metabolite is comparable to that of the parent compound, the toxicity value of the parent compound may be used as a substitute for the toxicity value of the metabolite in the risk assessment of the latter. In case however the level of exposure of birds to the metabolite is much lower than that of the parent compound,

the toxicity value of the parent compound is corrected by a factor which accounts for the difference in exposure level. The corrected value may then be used as a substitute for the toxicity value of the metabolite in the risk assessment of the latter. In the case of exposure to residues in plants, the PECfeed of the parent in plants is corrected for the maximum percentage at which the metabolite is formed in plants and for the ratio of the molecular weights of metabolite and parent pyriproxyfen. The ETE and TER can be calculated and used directly to draw conclusions. When, however, a metabolite is not formed at significant levels in birds, the PEC values are estimated in the same way as for the major (≥10%) metabolite, but conventional calculation of TER values is not possible since no toxic endpoint is available. In such a case, the minimum toxic endpoint, needed to obtain TER above the trigger value is calculated, and the ratio between this calculated toxic endpoint and that of the parent is the factor that the metabolite needs to be more toxic than the parent in order to present a risk for birds. The probability for this to occur can be discussed on the basis of structural considerations, and, if available, on the basis of data on toxicity data of metabolites in other organisms.

B.9.1.5.2.1 Major metabolites

4'-OH-PYRIPROXYFEN

4'-OH-Pyriproxyfen was the only major (>10%) metabolite in plants, which was also found in rat and mouse (section B.6.1), goat and hen (hen: 19% AR, based on 22-25% TRR in excreta containing 89.5% AR and 19-22% TRR in excreta containing 84% AR; section B.7.2.2), orally dosed with parent pyriproxyfen. Based on a mean exposure level to the metabolite of 19% AR for hens dosed with parent compound, the toxicity values of the metabolite for birds are set at a factor of 5.3 below that of the parent. The residues in leafy crops are calculated using the maximum percentage of metabolite formed in plant material (11% in apple pomace) and with corrections for the molecular masses (337.4/321.5). The resulting ETEs and TERs are summarised in Table B.9.10.

Table B.9.10 PECFEED, ETE and TER values for exposure of birds to 4'-OH-pyriproxyfen due to consumption of leafy crops, based on estimated LD50, LC50 and NOEC values

appln.	dose (kg as/ha)	estimated LD50 (mg/kg bw), LC50 (mg/kg bw/day) or NOEC (mg/kg bw/day)	Time scale	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TER
Cotton EU S	1 x 0.075	357	Acute	0.75	0.57	623
		161	Short-term	0.35	0.26	613
		13	Long-term	0.18	0.14	94

The TER values in Table B.9.10 are all above the relevant triggers of 10 (TERa and TERst) and 5 (TERlt). Hence, the acute, short-term and long-term risk to birds as a result of exposure to 4'-OH-pyriproxyfen is considered to be acceptable.

B.9.1.5.2.2 Minor metabolites

According to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), minor (<10%) metabolites

only have to be considered in exceptional cases (e.g. if containing the active moiety of the molecule), and a qualitative approach can be used.

DPH-pyriproxyfen

DPH-pyriproxyfen was a minor metabolite in plants (max. 1.4% in apple pomace), which was also found in rat and mouse (section B.6.1), goat and hen (hen: 4.1% AR, based on 5.4-5.8% TRR in excreta containing 89.5% AR and 2.2-5.2% TRR in excreta containing 84% AR; section B.7.2.2), orally dosed with pyriproxyfen. [February 2009, extra clarification on the value of 4.1% AR: Two residue studies with chicken are available. DPH-PYR has been found in both these studies. Studie 1: 89.5% AR with 5.4 and 5.8 TRR on day 3 and 7, respectively: 3.1 AR DPH-PYR. Studie 2: 84 % AR with 2.2 and 5.2 TRR on day 3 and 7, respectively: 5.0 AR DPH-PYR. On average this gives a value of 4.1% AR DPH-PYR.] The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 94 lower than for the parent. The TERa, TERst and TERlt of this metabolite would only be below the corresponding trigger value (10, 10 and 5, respectively), if the acute, short-term and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 3598, 3543 and 1088, respectively. This is considered unlikely, and the risk is considered to be low.

POP

POP was a minor metabolite in plants (0.5% in apple pomace), which was also found in goat and hen (hen: 1.9% AR, based on 1.8-2.5% TRR in excreta containing 89.5% AR; section B.7.2.2), orally dosed with parent pyriproxyfen. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 345 lower than for the parent. The TERa, TERst and TERlt of this metabolite would only be below the corresponding trigger value (10, 10 and 5, respectively), if the acute, short-term and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 13273, 13071 and 4012, respectively. This is considered unlikely, and the risk is considered to be low.

POPA

POPA was a minor metabolite in plants (0.9% in apple pomace), which was also found in rat and mouse (section B.6.1), goat and hen (hen: 2.0% AR, based on 1.8-2.7% TRR in excreta containing 89.5% AR; section B.7.2.2), orally dosed with parent pyriproxyfen. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 146 lower than for the parent. The TERa, TERst and TERlt of this metabolite would only be below the corresponding trigger value (10, 10 and 5, respectively), if the acute, short-term and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 5620, 5535 and 1699, respectively. This is considered unlikely, and the risk is considered to be low.

PYPA

PYPA was a minor metabolite in plants (2.6% in tomato juice), which was also found in goat and only in traces in hen (hen: <1% AR, based on 5.7% TRR in skin with fat containing 0.03% AR, and 1.1% TRR in gizzard containing 0.60% AR; section B.7.2.2), orally dosed with parent pyriproxyfen. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 81 lower than for the parent. The

TERa, TERst and TERlt of this metabolite would only be below the corresponding trigger value (10, 10 and 5, respectively), if the acute, short-term and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 3102, 3055 and 938, respectively. This is considered unlikely, and the risk is considered to be low.

PYPAC

PYPAC was a minor metabolite in plants (8.2% in cotton; 3.8% in tomato juice), which was also found in rat (section B.6.1), goat and hen (hen: 30% AR, based on 34-37% TRR in excreta containing 84% AR; section B.7.2.2), orally dosed with parent pyriproxyfen. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 23 lower than for the parent. The TERa, TERst and TERlt of this metabolite would only be below the corresponding trigger value (10, 10 and 5, respectively), if the acute, short-term and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 901, 888 and 272, respectively. This is considered unlikely, and the risk is considered to be low.

B.9.1.6 Risk assessment for mammals

Procedures for risk assessment were identical to those for birds (see B.9.1.5), with the exception of the aspects indicated below.

B.9.1.6.1 Risk of active substance for mammals

Acute risk assessment (standard exposure scenario)

The acute risk assessment is based on a herbivorous mammal of body weight (BW) 3000 g feeding exclusively on leafy crops (FIR 832 g/day). Assessment of exposure to pyriproxyfen via intake of contaminated surface water however is based on a small mammal weighing 10 g, with a daily water intake (DWI) of 1.6 mL/day. The lowest acute LD50 is >5000 mg a.s./kg bw for rats (see section B.6.5). The estimated theoretical exposure (ETE) for the acute time scale is calculated as (FIR/bw)*RUD*MAF*dose or as PECsw*DWI/BW. In the tables below, PECfeed is equal to RUD*MAF*dose. The residue values represent 90th percentile values, in agreement with the guidance in Sanco/4145/2000. The PECsw is the initial PECsw value (FOCUS Step 2, Southern Europe for tomato and egg plant; FOCUS Step 1, Southern Europe for cotton) taken from Section B.8.6.1. Residue levels in food and water, and ETE and TERa values are presented in Table B.9.11.

Table B.9.11 Acute Toxicity Exposure Ratios for exposure of mammals to pyriproxyfen due to consumption of contaminated leafy crops and drinking water

appln.	dose (kg as/ha)	LD50 (mg/kg bw)	Route	PEC_{FEED} or PEC_{WATER} (mg/kg wwt or mg/L)	ETE (mg/kg bw/d)	TERa
Tomato & egg plant EU S	2 x 0.1125	>5000	Water	4E-05	6E-06	>8E+08
Cotton EU S	1 x 0.075	>5000	Leafy crops	6.5	1.8	>2763

			Water	2E-03	2E-04	>2E+07

TERa values in Table B.9.11 are all far above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to mammals is considered to be acceptable.

Long-term risk assessment (standard exposure scenario)

Long-term risk assessment is based on the same mammal weighing 3000 g feeding on leafy crops, and using arithmetic mean residue values for residues in leafy crops (RUD = 40). Residue values are time-weighted over a period of 3 weeks assuming a DT50 of 10 days, which leads to a TWA correction factor (f_{twa}) of the initial residue values of 0.53 (in agreement with the guidance in Sanco/4145/2000). The toxicity value used for risk assessment is taken from the 2-generation reproduction study in rat with pyriproxyfen (section B.6.6). The NOAEL (200 mg a.s./kg diet) in this study was lower than the NOAEL in a peri-post natal study in rat, a teratogenicity and reproductive toxicity study in rat and teratogenicity studies in rat and rabbit. The NOAEL in the 2-generation reproduction study was based on effects on liver and kidney weight. In this 2-generation study no haematological parameters were measured. The liver is the primary target organ in mammals, and in (semi)chronic toxicity studies in rat, during which haematological parameters were measured, effects on liver weight were associated with changes in haematological parameters, including reduced red blood cell counts. The latter effect is considered to be ecotoxicologically relevant. The value of 200 mg/kg diet, which was equivalent to 13.3 mg a.s./kg bw/d, is therefore taken to be the ecotoxicologically relevant NOEC in a First Tier assessment. The ETE for the long-term time scale is calculated as $PECfeed_{,initial}$*ftwa*DFI/BW. Residue levels in food, and ETE and TERlt values are presented in Table B.9.12.

Table B.9.12 Long-term Toxicity Exposure Ratios (First Tier) for exposure of mammals to pyriproxyfen due to consumption of contaminated leafy crops

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
Cotton EU S	1 x 0.075	13.3	Leafy crops	1.6	0.44	29.9

The TERlt value in Table B.9.12 is above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk to mammals is considered to be acceptable.

Long-term risk assessment (bioaccumulation and food chain behaviour)

Earthworms

Long-term risk assessment is based on a mammal weighing 10 g feeding exclusively on earthworms, with a daily food intake (DFI) of 14 g fresh material/day. PEC calculations were identical to those for exposure of birds to earthworms (see B.9.1.5.1).

Fish

Long-term risk assessment is based on a fish-eating mammal weighing 3000 g with a daily food intake (DFI) of 390 g fish/day. PEC calculations were identical to those for exposure of birds to fish (see B.9.1.5.1).

The NOEC used for First Tier risk assessment is 13.3 mg/kg bw/day. The estimated theoretical exposure (ETE) for the long-term time scale is calculated as PEC*DFI/BW.

Residue levels in earthworms and fish and ETE and TERlt values are presented in Table B.9.13.

Table B.9.13 Long-term Toxicity Exposure Ratios for exposure of mammals to pyriproxyfen due to consumption of contaminated earthworms and fish

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
Tomato & egg plant EU S	2 x 0.1125	13.3	Fish	4E-03	5E-04	3E+04
Cotton EU S	1 x 0.075	13.3	Earthworms Fish	0.25 0.55	0.35 0.07	38 190

TERlt values for consumption of contaminated fish and earthworms in Table B.9.13 are above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk to mammals due to consumption of contaminated fish and earthworms is considered to be acceptable.

B.9.1.6.2 Risk of plants metabolites for mammals

B.9.1.6.2.1 Major metabolites

4'-OH-PYRIPROXYFEN

4'-OH-Pyriproxyfen was the only major (>10%) metabolite in plants, which was also found in rat and mouse (rat: 23-54% AR in excreta, section B.6.1), goat and hen, orally dosed with parent pyriproxyfen. Based on an exposure level to the metabolite of up to 54.4% AR for rats dosed with parent compound, the toxicity of this metabolite to mammals is considered to have been covered by the studies with the parent compound. Since the acute and long-term risk of pyriproxyfen to mammals was considered to be acceptable (TERa and TERlt of >2763 and 29.9, respectively), and the residues of 4'-OH-pyriproxyfen in plants are about ten times lower than those of parent pyriproxyfen (maximum formation percentage in plants 11%), the risk of this metabolite is considered to be acceptable.

B.9.1.6.2.2 Minor metabolites

DPH-pyriproxyfen

DPH-pyriproxyfen was a minor metabolite in plants (max. 1.4% in apple pomace), which was also found in rat (0.8-1.6% AR; section B.6.1) and mouse, goat and hen orally dosed with pyriproxyfen. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 94 lower than for the parent. The TERa and TERlt of this metabolite would only be below the corresponding trigger value (10 and 5, respectively), if the acute and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 25867 and 556, respectively. This is considered unlikely, and the risk is considered to be low.

POP

POP was a minor metabolite in plants (0.5% in apple pomace), which was also found in goat and hen, orally dosed with parent pyriproxyfen, but not in rat or mouse. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of

formation, will be a factor of 345 lower than for the parent. The TERa and TERlt of this metabolite would only be below the corresponding trigger value (10 and 5, respectively), if the acute and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 95415 and 2073, respectively. This is considered unlikely, and the risk is considered to be low.

POPA

POPA was a minor metabolite in plants (0.9% in apple pomace), which was also found in rat (0.1-0.4% AR; section B.6.1) and mouse, goat and hen orally dosed with parent pyriproxyfen. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 146 lower than for the parent. The TERa and TERlt of this metabolite would only be below the corresponding trigger value (10 and 5, respectively), if the acute and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 40402 and 877, respectively. This is considered unlikely, and the risk is considered to be low.

PYPA

PYPA was a minor metabolite in plants (2.6% in tomato juice), which was also found in goat and hen, orally dosed with parent pyriproxyfen, but not in rat. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 81 lower than for the parent. The TERa and TERlt of this metabolite would only be below the corresponding trigger value (10 and 5, respectively), if the acute and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 22302 and 484, respectively. This is considered unlikely, and the risk is considered to be low.

PYPAC

PYPAC was a minor metabolite in plants (8.2% in cotton; 3.8% in tomato juice), which was also found in rat (1.0-4.9% AR; section B.6.1), goat and hen orally dosed with parent pyriproxyfen. The maximum residue level of this metabolite in plants treated with pyriproxyfen, corrected for the difference in molecular mass with parent pyriproxyfen and for the maximum percentage of formation, will be a factor of 23 lower than for the parent. The TERa and TERlt of this metabolite would only be below the corresponding trigger value (10 and 5, respectively), if the acute and long-term toxicity exceeded that of parent pyriproxyfen by a factor of 6479 and 141, respectively. This is considered unlikely, and the risk is considered to be low.

B.9.2 Effects on aquatic organisms (IIA 8.2, IIIA 10.2)

B.9.2.1 Acute toxicity to aquatic life (IIA 8.2)

Studies were conducted in compliance with relevant OECD and/or EC guidelines, unless stated.

B.9.2.1.1 Acute toxicity of the active substance

Studies on the acute toxicity of pyriproxyfen to aquatic life are summarised in Table B.9.14.

reference	:	Bowman J.H. (1989a)	GLP statement	:	yes
type of study	:	Acute toxicity study in rainbow trout	guideline	:	EPA 72-1
year of execution	:	1989	acceptability	:	acceptable
test substance	:	Sumilarv T.G., lot no. PYG-87074, purity 95.3%			
reference	:	Bowman J.H. (1989b)	GLP statement	:	yes
type of study	:	Acute toxicity study in bluegill	guideline	:	EPA 72-1
year of execution	:	1989	acceptability	:	acceptable
test substance	:	Sumilarv T.G., lot no. PYG-87074, purity 95.3%			
reference	:	Burgess D. (1989)	GLP statement	:	yes
type of study	:	Acute toxicity study in *Daphnia magna*	guideline	:	USEPA 72-2
year of execution	:	1989	acceptability	:	acceptable
test substance	:	Sumilarv T.G., lot no. PYG-87074, purity 95.3%			
reference	:	Blasberg J.W. *et al.* (1991)	GLP statement	:	yes
type of study	:	Algal growth inhibition	guideline	:	OECD 201
year of execution	:	1991	acceptability	:	acceptable
test substance	:	Pyriproxyfen, lot no. 007024, purity 97.2%			
reference	:	Hoberg J.R. (1996)	GLP statement	:	yes
type of study	:	Toxicity to duckweed	guideline	:	USEPA FIFRA 122-2 & 123-2
year of execution	:	1996	acceptability	:	acceptable
test substance	:	Pyriproxyfen (Sumilarv TG), lot no. 50401G, purity 98.4%			

Table B.9.14 The acute toxicity of pyriproxyfen to aquatic life

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC_{50} in mg a.s./L (95% CL)	NOEC mg a.s./L	Test guideline	Ref
a) Fish (IIA 8.2.1)						
Salmo gairdneri	Flow through 96 hours (95.3%) in acetone	0 h: 67-94[(B)] 96 h: 52-111[(B)]	24, 48, 72 and 96 h: >0.325[A)]	24 and 48 h: ≥0.325 72 and 96 h: 0.102[(A)]	EPA 72-1	Bowman J.H., 1989a
Lepomis macrochirus	Flow through 96 hours (95.3%) in acetone	0 h: 75-95[(B)] 96 h: 64-75[(B)]	24, 48, 72 and 96 h: >0.270[A)]	24, 48, 72 and 96 h: ≥0.270[(A)]	EPA 72-1	Bowman J.H., 1989b
b) Invertebrates (IIA 8.2.4)						
Daphnia magna	Flow-through 48 hours (95.3%) in acetone	69-90 (0 h) 44-80 (48 h)	24 h: 0.54 (0.43-0.60) 48 h: 0.40 (0.35-0.46)[(A)]	24 h: 0.19 48 h: 0.043[(A)]	USEPA 72-2	Burgess D., 1989
c) Algae (IIA 8.2.6)						
Selenastrum capricornutum (green alga)	Static 72 hours (97.2%) in acetone	0 h: 80-88 72 h: 57-78	72 h EbC50 0.094 (0.051-0.17) ErC50 0.15 (0.063-0.34)[(C)]	72 h NOEbC 0.025 NOErC 0.05[(C)]	OECD 201	Blasberg J.W. *et al.*, 1991

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC_{50} in mg a.s./L (95% CL)	NOEC mg a.s./L	Test guideline	Ref
d) Aquatic plants (IIA 8.2.8)						
Lemna gibba G3 (duckweed)	Semi-static 14 days (98.4%) in acetone	61-100 (new) 24-41 (old)(D)	14-day EbC50 and ErC50 >0.18(A)(E)	14-day NOEbC and NOErC ≥0.18(A)(E)	USEPA FIFRA 122-2 & 123-2	Hoberg J.R., 1996

(A) Based on mean measured concentrations.
(B) Nominal test concentrations of 22.5, 45, 90, 180 and 360 µg a.s./L. At the highest test concentration a surface film was observed (solubility limit in distilled water 367 µg/L at 25°C).
(C) Based on nominal concentrations, since initial measured concentrations were ≥80% of nominals. This evaluation procedure is in agreement with the guidance provided in the Guidance Document on Aquatic Ecotoxicology (Sanco/3268/2001 rev. 4 (final) of 17 October 2002), point 2.1.4. EbC50, ErC50, NOEbC and NOErC values were estimated by the Rapporteur from the reported raw data on cell counts using the procedures in OECD 201, as the report presented only EC50 and NOEC values based on the percentage reduction of absolute cell numbers relative to the pooled control.
(D) Based on analysis of fresh and aged solutions of one 3-day renewal interval (day 6-9).
(E) No effect on growth rate, frond biomass and frond dry weight at the highest tested concentration.

B.9.2.1.2 Acute toxicity of metabolites

Studies on the acute toxicity of the metabolite PYPAC are summarised in Table B.9.15.

reference	:	Putt A.E. (2000a)	GLP statement	:	yes
type of study	:	Acute toxicity study in rainbow trout	guideline	:	EEC C.1, OECD 203
year of execution	:	2000	acceptability	:	acceptable
test substance	:	PYPAC, lot no. 9610351-1, purity 100%			
reference	:	Putt A.E. (2000c)	GLP statement	:	yes
type of study	:	Acute toxicity study in *Daphnia magna*	guideline	:	EEC C.2, OECD 202
year of execution	:	2000	acceptability	:	acceptable
test substance	:	PYPAC, lot no. 9610351-1, purity 100%			
reference	:	Hoberg J.R. (2000a)	GLP statement	:	yes
type of study	:	Algal growth inhibition	guideline	:	EEC C.3, OECD 201
year of execution	:	2000	acceptability	:	acceptable
test substance	:	PYPAC, lot no. 9610351-1, purity 100%			

Table B.9.15 The acute toxicity of PYPAC to aquatic life

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC_{50} in mg a.s./L (95% CL)	NOEC mg a.s./L	Test guideline	Ref
a) Fish (IIA 8.2.1)						
Oncorhynchus mykiss	Static 96 hours limit test (100%)	91-95%	24, 48, 72 and 96 h: >93(A)	24, 48, 72 and 96 h: ≥93(A)(B)	EEC C.1, OECD 203	Putt A.E., 2000a
b) Invertebrates (IIA 8.2.4)						
Daphnia magna	Static 48 hours limit test (100%)	94-96%	24 and 48 h: >95(A)	24 and 48 h: ≥95(A)	EEC C.2, OECD 202	Putt A.E., 2000c
c) Algae (IIA 8.2.6)						
Pseudokirchneri-ella subcapitata (green alga)	Static 72 hours (100%)	98-109%	72 h EbC50 26 (16-36) ErC50 30 (18-42)(A)	72 h NOEbC 9.8 NOErC 22(A)	EEC C.3, OECD 201	Hoberg J.R., 2000a

(A) Based on mean measured concentrations.
(B) 10% mortality in the only tested concentration of nominal 100 mg/L was considered to be acceptable since the guidelines state that 10% mortality in the control is acceptable.

Studies on the acute toxicity of 4'-OH-pyriproxyfen are summarised in Table B.9.16.

reference	:	Putt A.E. (2000b)	GLP statement	:	yes
type of study	:	Acute toxicity study in rainbow trout	guideline	:	EEC C.1, OECD 203
year of execution	:	2000	acceptability	:	acceptable
test substance	:	4'-OH-pyriproxyfen, lot no. MN-96183, purity 98.6%			
reference	:	Putt A.E. (2000d)	GLP statement	:	yes
type of study	:	Acute toxicity study in *Daphnia magna*	guideline	:	EEC C.2, OECD 202
year of execution	:	2000	acceptability	:	acceptable
test substance	:	4'-OH-pyriproxyfen, lot no. MN-96183, purity 98.6%			
reference	:	Hoberg J.R. (2000b)	GLP statement	:	yes
type of study	:	Algal growth inhibition	guideline	:	EEC C.3, OECD 201
year of execution	:	2000	acceptability	:	acceptable
test substance	:	4'-OH-pyriproxyfen, lot no. MN-96183, purity 98.6%			

Table B.9.16 The acute toxicity of 4'-OH-pyriproxyfen to aquatic life

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC_{50} in mg a.s./L (95% CL)	NOEC mg a.s./L	Test guideline	Ref
a) Fish (IIA 8.2.1)						
Oncorhynchus mykiss	Flow-though 96 hours (98.6%) in DMF	70-131 (0 h) 55-100 (96 h)	24 h: 0.33 (0.26-0.41) 48 & 72h: 0.29 (0.22-0.45) 96 h: 0.27 (0.15-0.45)(A)	24, 48, 72 and 96 h: 0.15(A)	EEC C.1, OECD 203	Putt A.E., 2000b
b) Invertebrates (IIA 8.2.4)						
Daphnia magna	Flow-through 48 hours (98.6%) in DMF	97-144	24 h: 2.1 (1.1-2.2) 48 h: 1.8 (1.1-2.2)(A)	24 h: 1.1 48 h: 0.36(A)	EEC C.2, OECD 202	Putt A.E., 2000d
c) Algae (IIA 8.2.6)						
Pseudokirchneri-	Static	78-120	72 h EbC50 and	72 h	EEC C.3,	Hoberg J.R.,

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC_{50} in mg a.s./L (95% CL)	NOEC mg a.s./L	Test guideline	Ref
ella subcapitata (green alga)	72 hours (98.6%) in DMF	(0 h) <5-95 (72 h)(C)	ErC50 >2.5(B)	NOEbC and NOErC 0.50(B)	OECD 201	2000b

(A) Based on mean measured concentrations; individual measured concentrations deviated by <20% from the mean.
(B) Based on initial measured concentrations.
(C) At nominal concentrations of 0.0020, 0.0078, 0.032, 0.13, 0.50 and 2.0 mg/L, measured concentrations represented <21%, <5%, 49%, 66%, 70% and 95%, respectively, of nominal concentrations, after 72 hours.

B.9.2.1.3 Acute toxicity of the plant protection product (IIIA 10.2.1)

All studies under this heading were conducted with the S-71639 10 EC formulation.

Studies on the acute toxicity of the S-71639 10 EC formulation (10.4% w/w pyriproxyfen) for fish, *Daphnia* and algae are summarised in Table B.9.17.

reference	:	Sword M.C. & Stratton J. (1992)	GLP statement	:	yes
type of study	:	Acute toxicity study in rainbow trout	guideline	:	USEPA 72-1, OECD 203
year of execution	:	1992	acceptability	:	acceptable
test substance	:	S-71639 10 EC, lot no. #C611L13, 10.4% w/w pyriproxyfen			
reference	:	Blackmore G.C. & Stratton Veltri J. (1992)	GLP statement	:	yes
type of study	:	Acute toxicity study in *Daphnia magna*	guideline	:	USEPA 72-2, OECD 202
year of execution	:	1992	acceptability	:	acceptable
test substance	:	S-71639 10 EC, lot no. #C611L13, 10.4% w/w pyriproxyfen			
reference	:	Blasberg J.W. *et al.* (1992)	GLP statement	:	yes
type of study	:	Algal growth inhibition	guideline	:	OECD 201
year of execution	:	1992	acceptability	:	acceptable
test substance	:	S-71639 10 EC, lot no. #C611L13, 10.4% w/w pyriproxyfen			

Table B.9.17 The acute toxicity of S-71639 10 EC formulation (10.4% w/w pyriproxyfen) to fish, invertebrates and algae.

Species	Test type and duration. (content of a.s.)	Actual concn. (as % of nominal)	LC/EC_{50} in mg/L (95% CL)	NOEC mg/L	Test guideline	Ref
a) Fish (IIIA 10.2.1)						
Oncorhynchus mykiss	Static 96 hours (10.4%)	0 h: 103-113 96 h: 73-89	24 h: 0.48 (0.24-0.75) a.s. 4.6 (2.3-7.2) formn 48 h: 0.31 (0.24-0.48) a.s. 3.0 (2.3-4.6) formn 72 h: 0.24 (0.12-0.48) a.s. 2.3 (1.2-4.6) formn 96 h: 0.22 (0.12-0.48) a.s. 2.1 (1.2-4.6) formn (A)	24, 48, 72 and 96 h: 0.068 a.s. 0.65 formn(A)(B)	OECD 203	Sword M.C. & Stratton J., 1992
b) Invertebrates (IIIA 10.2.1)						

Species	Test type and duration. (content of a.s.)	Actual concn. (as % of nominal)	LC/EC_{50} in mg/L (95% CL)	NOEC mg/L	Test guideline	Ref
Daphnia magna	Static 48 hours (10.4%)	0 h: 115-140 96 h: 85-105	24 h: >0.26 a.s. >2.5 formn 48 h: 0.19 (0.11-0.26) a.s. 1.8 (1.1-2.5) formn(A)(C)	24 h: 0.058 a.s. 0.56 formn 48 h: 0.028 a.s. 0.27 formn(A)(C)	USEPA 72-2, OECD 202	Blackmore G.C. & Stratton Veltri J., 1992
c) Algae (IIIA 10.2.1)						
Selenastrum capricornutum (green alga)	Static 72 hours (10.4%)	0 h: 103-120 72 h: 45-88(E)	72 h EbC50 0.074 (0.037-0.15) a.s. 0.71 (0.36-1.4) formn ErC50 0.11 (0.058-0.21) a.s. 1.1 (0.56-2.1) formn(D)	72 h NOEbC 0.026 a.s. 0.25 formn NOErC 0.052 a.s. 0.5 formn(D)	OECD 201	Blasberg J.W. *et al.*, 1992

(A) Based on mean measured concentrations.

(B) The abnormal effects of dark discolouration, surfacing, laboured respiration, and fish on the bottom of the test chamber, noted at mean measured concentrations of 1.2 mg formulation/L and above, were also observed in the formulation blank, which had concentrations of formulants equivalent to that in the highest test concentration of mean measured concentration 7.2 mg formn/L.

(C) No immobility and abnormalities were observed in the formulation blank, which had concentrations of formulants equivalent to that in the highest test concentration.

(D) Based on nominal concentrations, since initial measured concentrations were ≥80% of nominals. This evaluation procedure is in agreement with the guidance provided in the Guidance Document on Aquatic Ecotoxicology (Sanco/3268/2001 rev. 4 (final) of 17 Octover 2002), point 2.1.4. EbC50, ErC50, NOEbC and NOErC values were estimated by the Rapporteur from the reported raw data on cell counts using the procedures in OECD 201, as the report presented only EC50 and NOEC values based on % reduction of absolute cell numbers relative to the pooled control.

(E) At nominal concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 mg formn/L, measured concentrations represented 88%, 78%, 74%, 85% and 45%, respectively, of nominal concentrations, after 72 hours.

Comment by RMS

The S-71639 10 EC formulation is mentioned in document J, Specification No.13. Since acute risk assessment indicated no risk, further questions are redundant.

B.9.2.2 Chronic toxicity

B.9.2.2.1 Chronic toxicity of the active substance

B.9.2.2.1.1 Fish (IIA 8.2.2)

reference	:	Rhodes J.E. & Cramer D.. (1991)	GLP statement	:	yes
type of study	:	Chronic toxicity study in rainbow trout (ELS)	guideline	:	EPA 72-4
year of execution	:	1991	acceptability	:	acceptable
test substance	:	Sumilarv T.G., lot no. 007024, 97.2%			

A 95-day fish early life stage flow-through study was undertaken with eggs, larvae and juveniles of rainbow trout (*Oncorhynchus mykiss*). Newly fertilised eggs (<3 hours post-fertilisation, four replicates/concentration, 35 eggs/replicate) were exposed to pyriproxyfen technical (97.2% pure) at nominal concentrations of 1.9, 3.8, 7.5, 15 and 30 µg a.s./L plus control and solvent control (DMF). Mean measured concentrations of pyriproxyfen were 1.8, 4.3, 6.7, 14 and 26 µg a.s./L, representing 87 to 113% of nominal. Water quality parameters were: temperature (9.8-11.3°C), dissolved oxygen (64-98% of saturation) and pH (8.0 to 8.6).

Dissolved oxygen levels below 75% saturation were intermittently observed in the DMF control and the test concentrations beginning study day 84, but not in the control (with the highest biomass loading), and are likely to be due to the presence of DMF. No impact on study validity is suspected since no adverse biological effects were detected during this period. The total duration of the exposure period was 95 days. Sac-fry were thinned to 15 per replicate at day 36 to give a total of 60 individuals per concentration. Survival and fish behaviour was monitored daily, fish length on day 35 post-hatch and fish length and weight at 61 days post-hatch.

Egg hatchability, time to hatch and time to initiation of swim-up were not affected at any test concentration when compared to the pooled control group. At 35 and 61 days post-hatch, fry survival was significantly reduced relative to the pooled control at the 14 µg a.s./L mean measured concentration. This reduction however was due to a reduced survival rate in a single replicate only, and since no effects on survival were apparent at the highest mean measured test concentration of 26 µg a.s./L, the reduced survival at 14 µg a.s./L is considered to be unrelated to the treatment. It was stated in the report that test compound-related abnormalities were observed in the 6.7, 14 and 26 µg a.s./L treatment, which included fish resting on the bottom of the test chamber, quiescence, discolouration, spinal curvature and irregular respiration.

Fish length at 35 days post-hatch, and fish length and weight at 61 days post-hatch, were significantly reduced in the 6.7, 14 and 26 µg a.s./L treatment, when compared to the pooled control group.

Based on the most sensitive parameter fish growth, the NOEC was 4.3 µg a.s./L, and the LOEC 6.7 µg a.s./L.

The study was in accordance with GLP and FIFRA 72-4.

B.9.2.2.1.2 Invertebrates (IIA 8.2.5)

Study 1

reference	:	Blakemore G.C. *et al.* (1992)	GLP statement	:	yes
type of study	:	Chronic toxicity study in *Daphnia magna*	guideline	:	EPA 72-4
year of execution	:	1991	acceptability	:	acceptable
test substance	:	pyridyl-2,6-14C-Pyriproxyfen, sp.act. 817000 dpm/µg, radiochemical purity 100%, chemical purity not reported			

The chronic toxicity of pyridyl-2,6-14C-pyriproxyfen (radiochemical purity 100%) to *Daphnia magna* was assessed in two 21-day flow-through studies. First instar daphnids (<24 hours old, 40 per treatment, 10 per replicate) were used to initiate the study. In study #1, the nominal concentrations were 2.4, 4.8, 10, 20 and 40 ng/L, and the mean measured concentrations, based on LSC, were 1.8, 4.4, 7.1, 15 and 31 ng eq./L, representing 71 to 92% of nominal. In study #2, the nominal concentrations were 18, 36, 75, 150 and 300 ng/L, and the mean measured concentrations, based on LSC, were 20, 27, 56, 120 and 240 ng eq./L, representing 75 to 111% of nominal. Each study included a control and vehicle (DMF) control. The radioactivity in the test solutions was characterised by GC in the highest test concentration of study #2 on day 7, 14 and 21 and found to consist of 80, 101 and 113% parent pyriproxyfen, respectively. Water quality parameters were: temperature (19-20°C), dissolved oxygen (≥90% of saturation) and pH (8.2 to 8.4).

In study #1, none of the parameters investigated (survival, time to first brood, no. of live young per adult per reproduction day, adult daphnia length after 21 days) was significantly affected at any test concentration when compared to the pooled control group. The author of the report however stated that there was a slight trend towards an adverse effect on adult daphnia length and lower no. of live young per adult per reproduction day value at the highest mean measured test concentration of 31 ng/L (reductions of 2.6% and 3.3% respectively). According to the author of the report, this correlated well with the findings in study #2, which gave a reported LOEC of 27 ng/L. The author of the report therefore concluded that in study #1 the 31 ng/L level adversely affected *Daphnia magna* and concluded NOEC and LOEC values of 15 and 31 ng/L, respectively.

In study #2, survival of adult *Daphnia magna*, was not affected at any test concentration when compared to the pooled control group. The time to first brood was significantly affected at the three highest test concentrations, when compared to the pooled control group. Adult daphnia length after 21 days was significantly affected at all test concentrations, when compared to the pooled control group (control and vehicle control were not significantly different, mean±SD 4.10±0.13 and 4.03±0.16 mm, respectively). When compared to the vehicle control alone, adult daphnia length was significantly reduced at the four highest test concentrations (mean lengths decreasing from 3.88 to 3.33 mm), but not at mean measured test concentration 20 ng/L (mean±SD 3.93±0.14 mm). The latter concentration was therefore considered the NOEC for this parameter by the author of the report. The no. of live young per adult per reproduction day was significantly affected at all test concentrations, when compared to the pooled control group (control and vehicle control were not significantly different, mean±SD 9.02±0.99 and 8.09±0.87 young per adult per reproduction day, respectively). When compared to the vehicle control alone, the no. of live young per adult per reproduction day was significantly reduced at the four highest test concentrations (means decreasing from 5.99 to 1.58 young per adult per reproduction day), but not at mean measured test concentration 20 ng/L (mean±SD 7.43±0.61 mm). Therefore also for this parameter the author of the report considered the latter concentration to be the NOEC.

It was reported that all young produced during study #1 and #2 appeared normal.

The author of the report concluded that the combined NOEC and LOEC (based on reproduction and Daphnia length) from both studies was 15 and 27 ng/L. The NOEC value of 15 ng/L is accepted, the LOEC however is set by the Rapporteur at 20 ng/L (see comment below).

The study was in accordance with GLP and EPA 72-4.

Comment by RMS

Slight differences in results of similar toxicity tests conducted at different times are not uncommon and may be due to e.g., slight differences in sensitivity of test organisms, fluctuations of test concentrations, etc. Study #1 firmly established the NOEC to be 15 ng/L, possibly 31 ng/L. At the latter level of 31 ng/L (highest tested concentration), there may have been slight effects which were not statistically significant, and a firm LOEC could therefore not be concluded from study #1. Study #2 firmly established the LOEC to be 27 ng/L. In study #2, at the lowest tested level of 20 ng/L, slight significant effects were found relative to the pooled control, but not relative to the vehicle control. The concentration of DMF in the vehicle control was a factor of 17 lower than in the 20 ng/L test concentration. In addition, an effect of the vehicle DMF on reproduction and daphnia length is not likely, given the results

for both controls from study #1. The statistically significant effects on Daphnia length and no. of live young per adult per reproduction day at 20 ng/L observed in study #2 relative to the pooled control are therefore taken into consideration when deriving the LOEC, given also the fact that a clear downward trend existed for both parameters over the entire concentration range tested. Based on the above rational, the reported NOEC of 15 ng/L is accepted, but the LOEC is set at 20 ng/L.

Study 2

reference	:	Kagoshima M. *et al.* (1995)	GLP statement	:	no
type of study	:	Chronic toxicity study in *Daphnia pulex*	guideline	:	OECD 202(II)
year of execution	:	not reported	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen technical, lot no. PTG-86011, 97.2% pure			

The chronic toxicity of pyriproxyfen technical (97.2% pure) to *Daphnia pulex* was assessed in three 21-day semi-static studies. First instar daphnids (<24 hours old, 40 per treatment, 10 per replicate) were used to initiate the study. The nominal concentrations were 3.75, 7.5, 15, 30, 60 and 120 µg/L in study #1, 0.12, 0.24, 0.47, 0.94 and 1.88 µg/L in study #2, and 0.01, 0.03 and 0.06 µg/L in study #3. Each study included a control and study #1 and #2 also a vehicle control (DMSO + HCO-40, 1:1). The test concentrations were not analytically confirmed during the study. After 21 days of exposure to pyriproxyfen, surviving individuals from 0.12 and 1.88 µg/L ((lowest and highest concentration in study #2) and from all exposure groups in study #3 were transferred to fresh water and the test was continued for 21 days under the same conditions to investigate recovery. Water quality parameters were: temperature (19-21°C), dissolved oxygen (≥80% of saturation) and pH (7.4 to 8.3).

During exposure to pyriproxyfen, signs of intoxication and 97.5% mortality were observed by day 4 at 120 µg/L, but at lower concentrations there were no treatment related effects on survival and behaviour. The time to fist brood was significantly delayed at 0.24 µg/L and above. The no. of live young per adult per reproduction day was significantly reduced at 0.06 µg/L and above. There was no effect on the number of exuvia at any test concentration. The adult daphnia length was significantly reduced at 0.03 µg/L and above. Based on reduced body length, the LOEC was 0.03 µg/L and the NOEC 0.01 µg/L.

During the 21-day recovery test, the survival rates in the treatments of study #3 were 67.5-89.7% and exceeded that of the control (55.0%). The survival rate in study #2 was low (7.5, 40.0 an 12.5% in control, 0.12 and 1.88 µg/L, respectively). In both studies, there were no adverse effects on the no. of live young per adult per reproduction day in any treatment (114.1-191.8 no. of live young per adult per reproduction day in the treatments versus 124.5-127.0 no. of live young per adult per reproduction day in the controls). Hence the effect on this parameter was reversible upon transference of the organisms to fresh water. There was no effect on the number of exuvia at any test concentration. Daphnia length however was still significantly reduced at 0.06 µg/L and above.

Comment by RMS

The report of this non-GLP study lacked documentation on: the date of the report and the time of conduct of the study; the signatures of the study director; individual daily data on reproduction; methods of statistical analysis; identification of the vehicle and the concentration of the vehicle in the test solutions. The lack of individual data on reproduction and on methods of statistical analysis hampered evaluation of results on reproduction (in study # 3, reductions in mean no. of offspring of 13% and 16% at 0.01 and 0.03 µg/L were

marked as not statistically significant). Generally however test procedures complied with OECD 202 (II), with one major exception: the actual test concentrations were not verified by chemical analysis. Therefore the study result is not taken to the List of Endpoints, but may be used as supplementary information.

Study 3

reference	:	Hagino S. & Matsuda T. (1992a)	GLP statement	:	no
type of study	:	Chronic toxicity study in *Asellus hilgendorfii*	guideline	:	-
year of execution	:	1992	acceptability	:	**not acceptable**
test substance	:	S-31183 (pyriproxyfen) technical, lot no. PTG-86011, 97.2% pure			

The effect of pyriproxyfen technical (97.2% pure) on survival and growth of the crustacean *Asellus hilgendorfii* (Asellidae, Isopoda) was assessed in a 21-day semi-static study. Juveniles on the first day of free living life were used to initiate the study. The test was conducted at 25°C in 5 L aquariums containing 2 L of test solution (one per treatment) of pyriproxyfen in dechlorinated water with 20 test organisms. The nominal concentrations were 0.1, 1 and 10 µg/L including a control. The test concentrations were not analytically confirmed during the study. Observations were made on the size of the test organisms (weekly), signs of toxicity and survival rate.

There were no treatment related effects on survival and behaviour. Results of length measurement are shown in Table B.9.18. It was reported that "*mean body lengths after 2 weeks at 1.0 an 10 µg/L tended to be a little shorter than in the control group, but significant differences were not observed. No significant differences of the body length were also observed between the treatment and the control groups on other observation periods.*" The author of the report concluded that the NOEC was 10 µg/L, the highest concentration tested. This conclusion is not accepted (see comment below).

Table B.9.18 Mean (SD) body length of *Asellus hilgendorfii* (mm) during 21-day exposure to pyriproxyfen

day	control	0.1 µg/L	1 µg/L	10 µg/L
0	1.00 (0.05)	1.00 (0.03)	1.00 (0.04)	0.99 (0.04)
7	2.06 (0.45)	2.05 (0.47)	1.86 (0.24)	1.94 (0.45)
14	3.02 (0.50)	3.09 (0.77)	2.85 (0.59)	2.60 (0.69)
21	3.27 (0.80)	3.10 (0.81)	3.26 (0.77)	3.08 (0.76)

Comment by RMS

The report of this non-GLP study lacked documentation on:feeding frequency; measurement of water quality and physico-chemical parameters (pH, oxygen, temperature) during the test. The methods of statistical analysis of body length data, if any, were not identified. For this reason, the reported conclusion that there was no "significant" effect on body length can not be supported, in view of reductions of 5.6 an 14% after 14 days at 1 and 10 µg/L respectively. In view of the lack of documentation on the above points and the unreplicated test design, and since the actual test concentrations were not verified by chemical analysis, the study result is not taken to the List of Endpoints, but may be used as supplementary information.

Study 4

reference	:	Hagino S. & Matsuda T. (1992b)	GLP statement	:	no
type of study	:	Chronic toxicity study in *Asellus hilgendorfii*	guideline	:	-
year of execution	:	1992	acceptability	:	**not acceptable**
test substance	:	S-31183 (pyriproxyfen) technical, lot no. PTG-86011, 97.2% pure			

The effect of pyriproxyfen technical (97.2% pure) on reproduction of the crustacean *Asellus hilgendorfii* (Asellidae, Isopoda) was assessed in a 19-24 days semi-static study at 25°C. First delivery females (21 days old) were used to initiate the study. The test was started in 5 L aquariums containing 2 L of test solution (ten per treatment) of pyriproxyfen in dechlorinated water and one pair of test organisms. After at least 10 days of exposure, the egg brooding female was transferred to another beaker with 300 mL of treated test solution. Animals brooding earlier than 10 days were eliminated to avoid underestimation of the effect of the test chemical on ovarian maturation Exposure continued until juveniles started free living (about 9 days later). The nominal concentrations were 0.1, 1 and 10 µg/L including a control. The test concentrations were not analytically confirmed during the study. Observations were made on the size of the test organisms, signs of toxicity, survival and the number of hatched juveniles.

There were no treatment related effects on survival, behaviour, body length of females an number of hatched juveniles. The author of the report concluded that the NOEC was 10 µg/L, the highest concentration tested. This conclusion is not accepted (see comment below).

Comment by RMS

The report of this non-GLP study lacked documentation on: the name and signatures of the study director; feeding frequency; measurement of water quality and physico-chemical parameters (pH, oxygen, temperature) during the test. In view of the lack of documentation on the above points, and since the actual test concentrations were not verified by chemical analysis, the study result is not taken to the List of Endpoints, but may be used as supplementary information.

Study 5

reference	:	Hagino S. & Matsuda T. (1992c)	GLP statement	:	no
type of study	:	Chronic toxicity study in *Tigriopus japonicus*	guideline	:	-
year of execution	:	1992	acceptability	:	**not acceptable**
test substance	:	S-31183 (pyriproxyfen) technical, lot no. PTG-86011, 97.2% pure			

The effect of pyriproxyfen technical (97.2% pure) on survival and growth of the marine crustacean *Tigriopus japonicus* (Harpacticoida, Copepoda) was assessed in a 5-day static study at 25°C. The test was initiated by placing ten females (immediately after brooding) in a petri dish containing 50 mL of test solution of pyriproxyfen in 30‰ artificial seawater. The hatched larvae were distributed over 2 dishes (50 larvae each) containing test solution. The total test duration was 5 days. The nominal concentrations were 0.1, 1 and 10 µg/L including a vehicle (DMSO + HCO-40) and seawater control. The test concentrations were not analytically confirmed during the study. Observations were made on the number of metamorphoses of nauplii to copepods on day 2 after hatching, the sex ratio on day 5 after hatching, survival rate and size of the test organisms.

All nauplii transformed to copepodids, and there were no treatment related effects on survival, sex ratio and body length and behaviour. The author of the report concluded that the NOEC was 10 µg/L, the highest concentration tested. This conclusion is not accepted (see comment below).

Comment by RMS
The report of this non-GLP study lacked documentation on: the name and signatures of the study director; measurement of water quality and physico-chemical parameters (pH, oxygen, temperature) during the test. In view of the lack of documentation on the above points, the unreplicated test design (initial exposure of all females in one single dish), and since the actual test concentrations were not verified by chemical analysis, the study result is not taken to the List of Endpoints, but may be used as supplementary information.

Study 6

reference	:	Hagino S. & Matsuda T. (1992d)	GLP statement	:	no
type of study	:	Chronic toxicity study in *Tigriopus japonicus*	guideline	:	-
year of execution	:	1992	acceptability	:	**not acceptable**
test substance	:	S-31183 (pyriproxyfen) technical, lot no. PTG-86011, 97.2% pure			

The effect of pyriproxyfen technical (97.2% pure) on reproduction of the marine crustacean *Tigriopus japonicus* (Harpacticoida, Copepoda) was assessed in a 8-day semi-static study at 25°C. The test was initiated by placing adult females in a petri dish containing test solution of pyriproxyfen in 30‰ artificial seawater. Females after the first brood were transferred individually into a 20 mL vial containing the same test solution (10 replicates per treatment). The total test duration was 8 days. The nominal concentrations were 0.1, 1 and 10 µg/L including a vehicle (DMSO + HCO-40) and seawater control. The test concentrations were not analytically confirmed during the study. Observations were made on survival an behaviour of adults, and the number of larvae hatching between the first to the third brood.

There were no treatment related effects on behaviour and survival, and number of hatched larvae. The author of the report concluded that the NOEC was 10 µg/L, the highest concentration tested. This conclusion is not accepted (see comment below).

Comment by RMS
The report of this non-GLP study lacked documentation on: the name and signatures of the study director; measurement of water quality and physico-chemical parameters (pH, oxygen, temperature) during the test. In view of the lack of documentation on the above points, the unreplicated test design (initial exposure of all females in one single dish), and since the actual test concentrations were not verified by chemical analysis, the study result is not taken to the List of Endpoints, but may be used as supplementary information.

Study 7

reference	:	Machado M.W. (1995)	GLP statement	:	yes
type of study	:	Chronic toxicity study in *Mysidopsis bahia*	guideline	:	EPA 72-4
year of execution	:	1995	acceptability	:	acceptable
test substance	:	Sumilarv (pyriproxyfen), lot no. PYG-87074, purity 95.3%			

The chronic toxicity of pyriproxyfen (purity 95.3%) to the marine invertebrate *Mysidopsis bahia* was assessed in a 28-day flow-through study. First instar mysids (<24 hours old) were used to initiate the study. Each test aquarium contained two retention chambers with 15 mysids each, and each concentration was tested in two replicate aquariums. The nominal concentrations were 0.63, 1.3, 2.5, 5.0 and 10 µg a.s./L, including untreated and solvent (acetone) control. The mean measured concentrations were 0.48, 0.81, 1.6, 3.1 and 6.4 µg

a.s./L, representing 62 to 77% of nominal. Water quality parameters were: temperature (26-27°C), salinity 24-26‰, dissolved oxygen (77-114% of saturation) and pH (8.0 to 8.5).

Survival was unaffected at all test concentrations The number of offspring per female per reproduction day was significantly lower at mean measured concentrations of 1.6 µg a.s./L and above, when compared with the pooled control. Terminal body length and weight of male and female mysids was unaffected at any test concentration when compared to the pooled control group. Based on adverse effects on reproductive success, the NOEC and LOEC values were identified to be 0.81 and 1.6 µg a.s./L, respectively.

The study was in accordance with GLP and EPA 72-4.

B.9.2.2.1.3 Effects on sediment dwelling organisms (IIA 8.2.7)

Reference	:	Putt A.E. (2003)	GLP statement	:	yes
type of study	:	Chronic toxicity study in *Chironomus riparius*	guideline	:	draft OECD 219 (spiked water)
year of execution	:	2002	acceptability	:	acceptable
test substance	:	Pyriproxyfen, lot no. 80301G, 97.9% pure; pyridyl-2,6-14C-pyriproxyfen, lot no.RIS 99031, radiochemical purity 98.9%, sp. act. 13.0 MBq/mg			

The chronic toxicity of pyridyl-2,6-14C-pyriproxyfen (radiochemical purity 98.9%; isotopically diluted for the three highest concentrations with non-radiolabeled pyriproxygen, 97.9% pure) to *Chironomus riparius* (2 day old, 1st instar larvae) was assessed in a 28 day water/sediment system under static conditions. Nominal test concentrations were 2.5, 5, 10, 20 and 40 µg a.s./L. The test was performed in 600 mL glass beakers, containing approximately 1.5 cm of natural sediment (2.4% oc, 94% sand, 6% silt, 0% clay, pH 6.2, 2 mm sieved) and 6 cm of overlying water (well water). There were four replicates for each test concentration and for the untreated and solvent (acetone) control, each with 20 midge larvae. Four additional replicates with midge larvae were set up at each concentration for analytical purposes. Midge larvae were added to the test system one day prior to the addition of test substance. Treatment was performed by spiking the water with an aliquot of a solution of the test substance in acetone.

Analysis of the stock solutions used to spike the water resulted in measured concentrations ranging from 107 to 113% of the nominal concentrations. One hour after application, the concentrations of the test substance (determined by LSC) in the overlying water were 2.2, 4.5, 9.6, 19 and 35 µg a.s./L, representing 88-96% of nominal. During the remainder of the study, the concentrations in the overlying water ranged from 14-27% of nominal. At 2.5, 10 and 40 µg a.s./L nominal, respectively, measured concentrations in the pore water (determined by LSC) were 0.057, 0.38 and 0.77 µg eq../L at 1 hour, increasing to 0.66, 2.7 an 9.4 µg eq./L after 28 days. One hour after application, the concentrations of the test substance (determined by extraction/LSC) in the sediment of nominal test concentrations 2.5, 5, 10, 20 and 40 µg eq./L nominal, respectively, were <LOQ (i.e. <0.606), 1.6, 2.7, 5.0 and 10 µg eq./kg, representing 9.9-13% of the applied RA. Residues in sediment increased by day 7 (19-36% AR), and decreased slightly during the remainder of the study to 9-13% AR on day 28. HPLC analysis showed that at 1 hour after treatment ≥98% and 83-100% of the RA in overlying water and sediment, respectively, consisted of parent pyriproxyfen. After 7 days, parent pyriproxyfen was no longer detected in the overlying water, PYPAC being the main water metabolite (68-100% of the RA in water, 16-23% of AR). The metabolite 4'-OH-

pyriproxyfen was not detected in the overlying water at any concentration and time point. HPLC analysis of the sediment extracts of day 7, 14 and 28 showed that ≤41% of the RA in sediment represented parent pyriproxyfen, the remainder being associated with PYPAC (up to 22%), 4'-OH-pyriproxyfen (up to 40%) and other unidentified degradates (up to 100%).

Statistical endpoints were calculated using the nominal concentrations in the overlying water. Water quality parameters were: temperature (19-22°C), dissolved oxygen (7.2-9.3 mg/L) and pH (7.6 to 8.2). Following 28 days of exposure, mean percent emergence was unaffected at all concentrations, when compared to the controls. At nominal concentrations of 20 and 40 µg a.s./L, however, the development rate for male and female midges (combined) was significantly lower than in the pooled control.

Observations on emergence were continued beyond 28 days until 7 consecutive days without emergence were achieved, in order to assess whether midge mortality or significant delays in midge emergence had occurred. Only 2 midges emerged beyond day 28, one each at 20 and 40 µg a.s./L.

Based on reduced development rate, the NOEC for emergence is 10 µg a.s./L, and the LOEC 20 µg a.s./L. The 28-day EC50 was >40 µg a.s./L, the highest concentration tested.

The study was in accordance with GLP and the draft OECD 219 guideline. There were no significant deviations from the protocol.

B.9.2.2.1.4 Microcosm and mesocosm studies (IIIA 10.2.2)

Reference	:	van Wijngaarden R.P.A (2004)	GLP statement	:	yes
type of study	:	indoor microcosm study	guideline	:	not stated
year of execution	:	2003	acceptability	:	acceptable
test substance	:	Pyriproxyfen 10EC, lot no. B0200001, 102 g a.s./L			

The biological effects of a single application of Pyriproxyfen 10EC on a plankton-dominated community consisting of algae, cladocerans, copepods, ostracods, and rotifers, was investigated in indoor nutrient-rich microcosms, in compliance with GLP.

Methods

Design and treatment

Each microcosm consisted of an all glass cylinder (25 cm diameter, 35 cm high, volume 18 L) to which a 2 cm thick layer of mixed sediment (1.5% organic carbon, 9% particles <106 µm, 91% particles >106 µm) and 14.7 L of water (that was filtered through a 0.5 mm mesh net) from an uncontaminated eutrophic ditch were added. After settling for 6-7 days, plankton seeding communities and daphnids ((sub)adults, all belonging to *Daphnia* group *galeata*, ten per microcosm), collected from an uncontaminated ditch or pond, were added, 36-37 days before treatment with Pyriproxyfen 10EC. To suppress periphyton growth, 5 snails per system (*Lymnaea stagnalis*, (sub(adults)) were introduced into each microcosm. The microsoms were kept under the following conditions: 18-22°C; 14 hours of artificial daylight per day; addition of 0.09 mg N/L and 0.015 mg P/L twice a week; gentle air flow over the water surface to prevent growth of a bacterial layer; replenishment of lost water with demineralised water. The randomly allocated treatments consisted of a single application of Pyriproxyfen 10EC,

nominal treatment levels 0, 0.02, 0.08, 0.32, 1.2, 5 and 20 µg a.s./L. Control microcosms were treated with water only. The dosage solution was applied below the water surface, following which the water was gently stirred. Each treatment was tested in three replicate microcosms. Effects on zooplankton, (species composition and abundance), phytoplankton (chlorophyll-a), and community metabolism were measured, as well as some water quality parameters. An additional microcosm was set up at 5 µg a.s./L to investigate dissipation of pyriproxyfen. Two further microcosms were used to provide control samples for the chemical analysis of pyriproxyfen.

Sampling

Samples of water for determination of pyriproxyfen concentration were taken from all dosing solutions, from each microcosm within 1 hour after treatment, and from the 5 µg a.s./L fate microcosm at day
-1, <1 h and 1, 3, 7, 14 days after application, and every two weeks afterwards. Pyriproxyfen was extracted from water using hexane and analysed by GC-MS (LOD and LOQ 0.006 and 0.02 µg a.s./L respectively). Samples for determination of water quality (bicarbonate, N-total, ammonium, nitrate, P-total and orthophosphate) were taken from each microcosm on the day before application and 49 days after application. Oxygen levels, conductivity, pH and temperature were measured in the morning and afternoon in each microcosm 7 and 1-0 days before application and 2, 8, 15, 22, 29, 36, 43, 50 and 57 days after application. Species composition of the zooplankton was determined to the lowest practical taxonomic level in the filtrate (55 µm) of a 1 L composite sample collected from several points in each microcosm 8 and 1 days before application and 3, 7, 14, 21, 28, 35, 42, 49 and 56 days after application. Chlorophyll-a content of the phytoplankton was measured photometrically in the filtrate (1.2 µm) of a 0.1 L sample collected from each microcosm at the same time zooplankton was sampled. The filtered water from zooplankton and chlorophyll-a sampling was poured back into the microcosm from which it was originally collected.

Data analysis

Results are based on the nominal concentrations of the active ingredient of the test substance. Prior to statistical analysis, zooplankton data were ln(a*x*+1) transformed, where *x* is the abundance value. Univariate statistics using the multiple t-test of Williams were used to test differences at taxon or parameter level ($p \leq 0.05$; one sided) and to calculate the NOEC. For the analysis on the community level, multivariate analysis using Principal Response Curves (PRC) was used. For the calculation of community NOECs per sampling date the Williams test was applied to the sample scores of the first principal component of each sampling date. The statistical significance of treatment effects at the community level was tested using a Monte-Carlo permutation procedure. The significance of the effects of the treatment regime on the zooplankton community for each sampling date was tested by Monte Carlo permutation tests per sampling date.

Results and conclusions

Residue analysis

Mean recovery of pyriproxyfen from untreated microcosm water fortified at 0.02, 0.5, 5 and 20 µg a.s./L (number of samples 4, 8, 11 and 2 respectively) and analysed concurrently with study samples was in the range 92-103% (RSD 5-14%). Levels of pyriproxifen in dosing solutions ranged between 96 and 105% of nominal. The level of pyriproxifen in samples of microcosm water collected within 1 hour after treatment ranged between 100 and 108% of nominal at nominal concentrations of 0.32-20 µg a.s./L, was 138% at 0.08 µg a.s./L nominal, and 415% at 0.02 µg a.s./L nominal. At the latter concentration, pyriproxyfen concentrations in duplicate samples from individual microscosms differed by a factor of 2.3, 7.7 and 13.4. According to the author of the report, the high variation was considered to be an artefact of sampling at very low concentrations. There is no impact on the study validity, since conclusions are based on nominal concentrations, and measured concentrations in general exceeded nominal concentrations, and since NOECs and LOECs were established at a concentration with acceptable analytical confirmation.

In the additional fate microcosm (5 µg a.s./L), pyriproxyfen concentrations in the water decreased from 5.1 µg a.s./L at <1 h to 0.012 µg a.s./L after 7 days (not detectable afterwards). The first order DT50 and DT90 value was 0.8 and 2.6 days respectively (r^2 0.99).

Environmental conditions

Mean ± SD minimum and maximum water temperatures were 18.6 ± 0.4 and 20.6 ± 0.5°C. Bicarbonate levels were lower on day 49 (mean 8.6-13 mg/L) than on day -1 (mean 60-74 mg/L). Inorganic N levels were higher on day 49 (mean N-total 30-47 mg/L; mean ammonium 1.2-1.4 mg/L) than on day -1 (mean 0.90-1.1 mg/L; mean ammonium <0.04-0.09 mg/L). Inorganic phosphate levels were at or near the limit of detection throughout the study. Statistical analysis showed no treatment related effects on these parameters (NOECs ≥20 µg a.s./L).

Chlorophyll

Concentrations were very low throughout the test (generally below or at the LOD of 8.7 µg/L, always below the LOQ of 29 µg/L). Due to the low values, a NOEC calculation could not be performed, but inspection of the data suggested that no treatment related response occurred (NOEC ≥20 µg a.s./L).

Community metabolism

No concentration related effects were observed on temperature, oxygen concentration, pH and conductivity. Some isolated statistically significant deviations were found (oxygen and pH, day 36, 20 µg a.s./L; afternoon pH, day 15, all concentrations) but considered to have no ecological significance (isolated occurrence, relationship with treatment unclear). Oxygen production (maximum minus minimum oxygen level) was occasionally reduced compared to the control, but in all cases oxygen levels remained above 10 mg/L. Since saturated oxygen concentrations at 18 to 21ºC are 9.2 to 8.7 mg O_2/L the incidental reduction is considered not to have had any ecologically adverse effects. $NOEC_{community\ metabolism} \geq 20$ µg a.s./L.

Biological parameters

Zooplankton species richness in the microcosms: 24 taxa, comprising of 14 taxa of rotifera, 6 taxa of cladocera, 3 taxa of copepoda and 1 taxon of ostracoda (no taxonomical groups identified). Abundance of four rotifera taxa (*A. fissa*, *K. cochlearis*, *P. remata*, *T. tigris*), two cladocera taxa (*D. galeata*, *S. vetulus*) and the total cladoceran, ostracodan and rotifera populations was sufficiently high to provide valid data.

In Table B.9.19 a summary is presented of consistent NOECs (William's test, $p<0.05$) per sampling date. An effect was considered to be consistent when statistical significant deviations pointed in the same direction for at least two consecutive sampling dates. Concentrations >NOEC showed significant increases (↑) or reductions (↓). The blank cells indicate no statistical significance at the highest tested concentration of 20 µg a.s./L. Statistically significant changes which had a doubtful relationship to the treatment (e.g., observed at a single sampling date only and/or involving very low numbers or irregular abundances) are not included.

Table B.9.19 NOECs (William's test, p<0.05) per sampling date for zooplankton populations and community (nominal treatment levels, µg a.s./L). Concentrations >NOEC showed significant increases (↑) or reductions (↓). NOECs based on poor data are not shown.

	NOEC (µg a.s./L) at sampling day:										
Parameter	**-8**	**-1**	**3**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**
Cladocera											
Daphnia gr. *galeata*			5 (↓)	1.2 (↓)	5 (↓)	5 (↓)	5 (↓)				
Cladocera total			5 (↓)	5 (↓)		5 (↓)	0.32 (↓)[B]				
Ostracoda							5 (↓)[D]	5 (↓)[D]			
Copepoda						no treatment related effects					
Rotifera											
Anuraeopsis fissa			0.02 (↑)[E]	5 (↑)	5 (↑)	5 (↑)	5 (↑)				
Polyartha remata			5 (↑)	5 (↑)	5 (↑)	5 (↑)	5 (↑)	5 (↑)			
Keratella quadrata	5 (↓)[C]				5 (↑)					5 (↑)	5 (↑)
Rotifera total			5 (↑)	5 (↑)	5 (↑)	5 (↑)					
Community[A]			5	5	5	5					

(A) Determined by William's test applied to the sample scores of the first principal component of each sampling date (p<0.05).
(B) No clear concentration-response relationship; isolated observation late in study.
(C) Before treatment, not treatment related.
(D) Low and scattered abundance numbers; no concentration-response relationship; NOEC not valid because data not suitable for adequate analysis.
(E) One isolated observation, no concentration-response relationship.

No consistent treatment related effects on ostracods and copepods were recorded. Treatment with Pyriproxyfen 10EC caused a direct negative effect on the abundance of the most sensitive zooplankton species *Daphnia* group *galeata* at 20 µg a.s./L (reduction on the first sampling (day 3) and day 28; recovery within 35 days) and at 5 µg a.s./L (reduction on day 7, recovery within 1 week). Based on the latter effect, the $NOEC_{population}$ was 1.2 µg a.s./L. Consistent reductions of the total cladoceran population occurred only during the first week after treatment at 20 µg a.s./L (NOEC 5 µg a.s./L). The assumed reduced grazing pressure on algae by cladocerans at 20 µg a.s./L did not result in significant increases of algal chlorophyll-a, probably due to increased rotifer abundance (*A. fissa*, day 7-28; *P. remata*, day 3-35; *K. quadrata*, day 14 and 49-56; total rotifers, day 3-21). This increase of rotifers is likely to be a secondary effect. It is assumed that larger daphnids suppress the smaller planktonic rotifer species both through competition for algae as a shared food source and through mechanical interference when rotifers are swept into the branchial chambers of feeding daphnids. Most rotifer populations were back to normal levels within 42 days. Abundance of *K. quadrata* however, was higher until study end at 20 µg a.s./L.

PRC analysis confirmed the results of univariate analysis. The community response was mainly dominated by the increase in rotifers (in particular the cluster of *P. remata*, *A. fissa*, *K. quadrata* and *T. tigris*), whilst responses of *D.* group. *galeata* were negatively correlated with the treatment regime. The $NOEC_{community}$ according to Williams test applied to the sample scores of the first principal component of each sampling date was 5 µg a.s./L, based on a statistically significant effects at 20 µg a.s./L between day 0 and 21 (recovery on day 28).

The statistically significant effect at 5 µg a.s./L on sampling day 7 only (decrease of abundance of *D.* group *galeata*) was short lasting (recovery within 1 week). The NOEC for the microcosm study is set at 5 µg a.s./L., based on a clear long-term indirect effect (increase in *K. quadrata*) with no full recovery within 8 weeks. However, the indirect effect may be

transitory and may not pose a significant ecological risk to natural aquatic ecosystems. The ecological function and community structure in the field situation may not be significantly affected, which may mean that the No Observed Ecologically Adverse Effect Concentration (NOEAEC) is higher than the NOEC. Since however no evidence was presented that the observed indirect effect does not have a serious ecological meaning, uncertainty remains. Therefore NOEAEC is set at 5 µg a.s./L.

Conclusion

Following single treatment of plankton-dominated indoor microsocms with Pyriproxyfen 10EC (analytically confirmed nominal treatment levels of 0, 0.02, 0.08, 0.32, 1.2, 5 and 20 µg a.s./L), no concentration related effects were observed on water quality and community metabolism. Levels of Chlorophyll-a were low, and comparable in all treatments and the control. No effects were observed at concentrations up to and including 1.2 µg a.s./L. A slight transient direct negative effect on the most sensitive zooplankton species *Daphnia* group *galeata* was the only effect at 5 µg a.s./L. It was observed only on one single sampling date and is considered to be non-significant. At 20 µg a.s./L, clear but transient effects on total cladocerans and on *D.* group *galeata* occurred, and clear indirect effects (increased abundances) on total rotifers and individual taxa (transient except for *K. quadrata*).

The No Observed Effect Concentration (NOEC) is 5 µg a.s./L, based on effects on total cladocerans and on *D.* group *galeata,* as well as on a clear long-term indirect effect (increase in *K. quadrata*) with no full recovery within 8 weeks. The $NOEC_{community}$ determined by PRC analysis was 5 µg a.s./L (transient effect at 20 µg a.s./L).

The No Observed Ecologically Adverse Effect Concentration (NOEAEC) is 5 µg a.s./L.

February 2009: At Praper 63, a concern was raised whether during the study there had been sufficient algae to provide food for the daphnids. RMS consulted the institute which preformed the study (Alterra, The Netherlands) by phone and the outcome was discussed in the meeting.

The organic matter content was not measured during the study, but instead the chlorophyll-a levels were measured. These levels were generally low. However, in this study only algae and groups feeding on algae were present, while predators of daphnids were absent. This means that daphnids could feed on algae unrestrictedly. Nutrients were added to the microcosms weekly. Since a clear increase in number of daphnids was seen in the control (at least up to day 28), and since levels of daphnids throughout the study were at acceptable levels (according to expert judgment), it is concluded that there must have been sufficient algae in the study. The chlorophyll-a levels were just low because the algae were grazed off immediately by the daphnids. The meeting agreed that the study can be used for risk assessment of crustaceans.

Comment by RMS

It is noted that laboratory testing shows a very low chronic NOEC for *D. magna* of 0,015 µg a.s./L in a continous flow system. This raises some concern, because *D.magna* was not present in de microcosm. However, on the basis of expert judgment and supplemental information from the notifier (see below) it is concluded that the microcosm study design is appropiate to negate the chronic effect on *D. magna.*

D. galeata is more relevant for the environment at risk from pyriproxyfen than *D. magna*. Furthermore, *D. magna* and *D. galeata* are two species from the same taxonomic group, *D. galeata* is smaller than *D. magna* and is therefore expected to usually have a higher sensitivity to toxic substances. This expectation is confirmed by the articles submitted by the notifier. Comparison of sensivity of the two species from these articles gives sufficient indication that

D.magna and *D.galatea* are at least equally sensitive to toxic substances. Therefore, the microcosm study can be used for the risk assessment.

Supplemental information

Three studies on cladocerans show that:

- *D. magna* withstands higher copper levels than *D. galeata* on short- as well as on long-term copper stress (Koivisto *et al.*, 1992[2]*;*
- endpoints of toxicity of pentachlorophenol for *D. galeata mendotae* were acutely about 3.5 times lower and chronically about 7 times lower than for *D. magna* (Stphenson *et al.* 1991[3]*);*
- semsitivity of *D. galeata* to *λ-cyhalothrin* is assessed and compared to sensitivity of *D magna,* assessed in a different study (not submitted by the notifier). EC50 is three times lower for *D. galeata*. NOEC is not given (Schroer *et al.* 2004[4]).

Futhermore, two microcosm studies were submitted in which *D. galeata* was present as a relevant species but *D. magna* was not[5], [6]. Indeed, according to expert judgment *D. galeata* is more often present in microcosm studies than *D. magna.*

B.9.2.2.2 Chronic toxicity of metabolites

B.9.2.2.2.1 Fish (IIA 8.2.2)

No data were submitted.

B.9.2.2.2.2 Aquatic invertebrates (IIA 8.2.5)

No data were submitted.

---

[2] *Comparison of five cladoceran species in short- and long-term copper exposure*. Koivisto, S, M. Ketola & M. Walls, 1992. Hydrobiologia 248: 125-136.

[3] *Acute toxicity of pure pentachlorophenol and a technical formulation to three species of Daphnia*. Stephenson, G.L., N.K. Kaushik & K.R. Solomon, 1991. Archives of environmental contamination and toxicology 20: 73-80.

[4] *Comparison of laboratory single species and field population-level effects of the pyrethroid insecticide λ-cyhalothrin on freshwater invertebrates*. Schroer, A.F.W., J.D.M.Belgers, T.C.M.Brock, A.M.Matser, S.J.Maud and P.J. van den Brink, 2004. Archives of environmental contamination and toxicology 46: 324-335.

[5] *Effects of the insectide Dursban 4E (active ingredient: chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery.* Brink, P.J. van den, R.P.A. van Wijngaarden, W.G.H. Lucassen, T.C.M.Brock and P.Leeuwangh, 1996. Environmental toxicology and chemistry 15, no. 7: 1143-1153

[6] *Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: a microcosm study.* Wijngaarden, R.P.van, J.G.M.Cuppen, G.H.P.Arts, S.J.H.Crum, M.W.van den Hoorn, P.J.van den Brink and T.C.M.Brock, 2004. Environmental toxicology and chemistry vol 23 no 6: 1479-1498

B.9.2.2.2.3 Effects on sediment dwelling organisms (IIA 8.2.7)

No data were submitted.

B.9.2.2.3 Bioaccumulation (IIA 8.2.3)

Reference	:	Pate H.O. *et al.* (1990)	GLP statement	:	yes
type of study	:	Bioconcentration in fish	guideline	:	EPA 165-4
year of execution	:	1990	acceptability	:	acceptable
test substance	:	[U-14C-phenoxyphenyl]-pyriproxyfen, chemical purity 98.9%, radiochemical purity >99%, sp. act. 181 µCi/mg; [2,6-pyridyl-14C]-pyriproxyfen, chemical purity >99%, radiochemical purity 99.1%, sp. act. 257 µCi/mg; unlabeled pyriproxyfen, lot no. NN-1, 100% pure			
Reference	:	Steginsky C.A. *et al.* (1994)	GLP statement	:	yes
type of study	:	Characterization of metabolites in water and fish	guideline	:	EPA 165-4
year of execution	:	1990-1991	acceptability	:	acceptable
test substance	:	[U-14C-phenoxyphenyl]-pyriproxyfen, chemical purity 98.9%, radiochemical purity 100%, sp. act. 181 µCi/mg; [2,6-pyridyl-14C]-pyriproxyfen, chemical purity >99%, radiochemical purity 99.2%, sp. act. 257 µCi/mg; unlabeled pyriproxyfen, lot no. NN-1, 100% pure			
Reference	:	Fujisawa T. et al. (1999)	GLP statement	:	not applicable (calculation)
type of study	:	Calculation of CT90s	guideline	:	-
year of execution	:	1999	acceptability	:	acceptable
test substance	:	[U-14C-phenoxyphenyl]-pyriproxyfen, chemical purity 98.9%, radiochemical purity >99%, sp. act. 181 µCi/mg; [2,6-pyridyl-14C]-pyriproxyfen, chemical purity >99%, radiochemical purity 99.1%, sp. act. 257 µCi/mg; unlabeled pyriproxyfen, lot no. NN-1, 100% pure			

Two groups of 250 Bluegill sunfish (*Lepomis macrochirus*) were exposed separately to [U-14C-phenoxyphenyl] (abbreviated 14C-PP) or [2,6-pyridyl-14C]-pyriproxyfen (abbreviated 14C-PYR) (radiochemical purity >99%) for 28 days in a flow-through system, followed by 14 days of depuration. Nominal concentrations were 20 µg/L for both labels, plus vehicle (DMF, 0.04 mL/L) control. The mean measured radioactivity concentrations in the water during exposure were 19.1 and 20.0 µg eq./L (standard deviation 2.3 and 2.7 µg eq./L) for the 14C-PP and 14C-PYR treatment, respectively. Taking into consideration the mean percentage of parent compound determined in water samples on day 21 and 28 during the uptake phase (72.8% and 89.8%, respectively, for PP- and PYR-label), the mean measured concentrations of pyriproxyfen in the water during exposure were 13.9 and 16.2 µg a.s./L for PP- and PYR-label respectively. Water quality parameters were: temperature (20.4-22.1°C), dissolved oxygen (≥68% of saturation) and pH (6.4-7.1). Water was sampled daily, and six fish were sampled on days 3, 7, 14, 21 and 28 of uptake and 1, 3, 7, 10 and 14 of depuration. Total radioactive residues in water (by LSC), three whole fish, and fillets and viscera from three more fish (all by combustion/LSC), were determined at all sampling times. Lipid content of fish was not determined.

Extraction and metabolite identification was performed on samples of water from days 0, 21 and 28 of uptake and on edible and non-edible portions of additional fish sampled on day 21 and 28 of uptake. The fillet and viscera samples were extracted with methanol, leaving only

4.1-7.5% (PP-label) or 11.6-24.6% (PYR-label) of the initial Total Radioactivity Residues (TRR) in post-extraction solids (PES).

Bligh-Dyer lipid extraction and subsequent hexane extraction of the day-28 PES from the PP- and PYR-label, respectively, released only 0.6% and 1.5% of the 14C, but subsequent protease treatment solubilised 2.5% and 14.3%. Conjugated metabolites in extracts were identified by enzyme hydrolysis (β-glucuronidase, aryl sulfatase) followed by extraction of the incubate with organic solvent. Water samples were extracted with acidified ethyl acetate/ethanol. The extracts were analysed by normal phase 1D- and 2D-TLC with confirmation by reversed phase HPLC. Compound identity was based on co-chromatography with reference standards.

No abnormal behaviour was observed for control and test fish throughout the study. Whereas no fish mortality occurred during depuration, 7%, 4% and 4% of the fish died during uptake in control, 14C-PP and 14C-PYR treatments. A steady-state situation for ^{14}C concentration in whole fish appeared to be reached within 3 days of exposure for both labels. The reported steady-state biological concentration factor (BCF) for whole fish, calculated by dividing the mean 14C-concentration during day 3-28 by the overall mean 14C-concentration in the water, was 1379 and 1495 L/kg wet weight for 14C-PP- and 14C-PYR-pyriproxyfen, respectively. After 14 days in uncontaminated water, 98.1% and 89.6% of the radioactivity present at the end of the uptake phase was cleared from whole fish from the 14C-PP and 14C-PYR exposure group, respectively. For the 14C-PP and 14C-PYR label, respectively, the CT50 (whole fish) was 0.86 and 1.63 days. These CT50 values were determined by first order regression analysis on day 0-3 data points (depuration after 3 days 90.3 and 71.0%), which gave r^2 values of 0.97-0.98. The CT90 values were determined by a nonlinear least squares kinetic MULTI modelling programme and found to be 3.4 and 8.4 days.

Nearly all of the radioactivity from the treated day 21 and 28 water samples was extractable into organic solvents and consisted mainly of pyriproxyfen (on day 21 and 28, respectively, 61.1% and 84.5% (PP-label) and 68.5% and 93.0% (PYR-label)). Pyriproxyfen was the main component of the TRR in edible portions of fish (47.1-48.2% an 31.4-38.9% TRR for PP- and PYR-label respectively), and it represented 23.3-24.0 and 18.3-21.8% TRR in the non-edible portions for PP- and PYR-label respectively. Taken into consideration the proportion of edibles and non-edibles in whole fish (46.6% and 53.4%, respectively), pyripoxyfen represented on average 34.8% and 27.1% of the TRR in whole fish from the PP- and PYR-label treatments, respectively.

Metabolites detected at levels >10% TRR in fish tissues from PP-label were 4'-OH-pyriproxyfen sulfate (10.7-15.5 and 7.2-12.2% TRR in edibles and non-edibles, respectively), 4',5''-OH-pyriproxyfen sulfate (7.7-13.3 and 30.0-34.0% TRR in edibles and non-edibles, respectively), 4'-OH-pyriproxyfen glucuronide (5.4-6.8 and 11.5-12.7% TRR in edibles and non-edibles, respectively), and 4,4'-oxydiphenol sulfate (3.7-9.0 and 11.0-12.2% TRR in edibles and non-edibles, respectively). Metabolites detected at levels >10% TRR in tissues from PYR-label were 4'-OH-pyriproxyfen sulfate (15.0-18.0 and 15.7-16.8% TRR in edibles and non-edibles, respectively), and the sum of the unresolved 4',5''-OH-pyriproxyfen sulfate and 5-OH-PYPAC sulfate (5.4-11.5 and 22.7-34.6% TRR in edibles and non-edibles, respectively).

Taking into consideration the mean percentage of parent pyriproxifen in water during uptake (72.8 and 80.8% TRR for PP- and PYR-label, respectively) and in whole fish (34.8 and 27.1%

TRR for PP- and PYR-label, respectively), the BCFs for radioactivity (1379 and 1495 L/kg wet weight for 14C-PP- and 14C-PYR-label, respectively, mean 1437 L/kg) correspond with values of 660 and 501 L/kg wet weight for the active substance (mean value 581 L/kg wet weight).

The study was in accordance with GLP and EPA 165-4.

B.9.2.3 Risk assessment

B.9.2.3.1 Risk assessment of the active substance

B.9.2.3.1.1 Acute risk

Measurement of treatment concentrations during the course of the flow-through tests with technical pyriproxyfen in fish (2 species) and *Daphnia magna* showed that concentrations of pyriproxyfen were maintained within acceptable limits, and results were based on mean measured concentrations. In the static test in green algae (*Selenastrum capricornutum*), pyriproxyfen was not stable, and results were based on analytically confirmed nominal concentrations. In the semi-static test with pyriproxyfen in *Lemna gibba*, concentrations decreased during the renewal intervals, and endpoints based on mean measured concentrations were calculated.

Static tests with the proposed Pyriproxyfen 10EC formulation were also submitted. In the test with fish (1 species) and *Daphnia magna* (1 study), analytical measurements showed that initial and mean measured concentrations of pyriproxyfen were >80% of nominals, and results were based on mean measured concentrations. In the test with algae (1 species), analytical measurements showed that initial concentrations of pyriproxyfen were >80% of nominals, and although they declined to <80% of nominals during the test period, results were based on nominal concentrations. The results of these tests (see Table B.9.17) showed that the formulation is of comparable toxicity to fish, *Daphnia* and algae.
Acute risk assessment will be based on the lowest LC/EC50 values for each group of organisms. Acute risk assessment will therefore be based on the results of the studies with the formulated product for fish, *Daphnia* and algae, and on the result of the study with the active substance for *Lemna.*

For the initial risk assessment the following endpoints will be used:
96 hr LC50 for rainbow trout (*Oncorhynchus mykiss*) of 0.22 mg a.s./L;
48 hr EC50 for *Daphnia magna* of 0.19 mg a.s./L;
72 hr EC50 for *Selenastrum capricornutum* of 0.074 mg a.s./L;
14 day EC50 for *Lemna gibba* of >0.18 mg a.s./L.

The acute TERs for spray drift at 1 metre for fish, *Daphnia magna*, algae and *Lemna* are shown in Table B.9.20. The PECsw value for spray drift at 1 metre was taken from section B.8.6.1. These values were calculated for the single application at 0.075 kg a.s./ha in cotton using FOCUS STEP 1 and for two applications at 0.1125 kg a.s./ha in tomato (worst-case for this crop). No drift percentages are available at EU level for the greenhouse scenario. In the Netherlands a drift rate of 0.1% is used (emission via condensation water and evaporation/volatilisation). PECsw values for the greenhouse applications were calculated for this drift rate of 0.1% using FOCUS STEP 2 (as opposed to FOCUS STEP 1 no run off/drainage, which is considered appropriate for greenhouse treatments).

Table B.9.20 Acute TERs for pyriproxyfen from spray drift at 1 m for fish, *Daphnia*, algae and *Lemna*

Crop	dose (kg a.s./ha)	dist. (m)	% drift	LC/EC50 (µg as/L)				PECsw (µg a.s./L)	TER			
				fish	*Daphnia*	algae	*Lemna*		fish	*Daphnia*	algae	*Lemna*
T & E	2x0.1125	1	0.1	220	190	74	>180	0.0382	5765	4979	1939	>4717
Cotton	1x0.075	1	2.77[1]	220	190	74	>180	1.5449	142	123	48	>117

T & E, tomato and egg plant EU S [1] 2.77% spray drift + 10% run-off and drainage

The acute TERs for fish and *Daphnia magna* are above the relevant Annex VI triggers of 100, and the TERs for algae and *Lemna* are above the relevant Annex VI triggers of 10. Hence the acute risk from the proposed use should be low for fish, *Daphnia*, algae and aquatic plants.

B.9.2.3.1.2 Long-term risk

The proposed use in cotton involves a single seasonal application (at 0.075 kg a.s./ha) only, while the use in tomato and egg plant involves one or two applications at 0.1125 kg a.s./ha. In water/sediment studies, pyriproxyfen dissipated from the water column with a DT50 of 1.4-1.7 days, hence <2 days. According to the Guidance Document on Aquatic Ecotoxicology (Working Document, Sanco/3268/2001), chronic exposure to pyriproxyfen is therefore likely to occur. Chronic toxicity data for the technical substance and the proposed formulated product were submitted and are summarised in Table B.9.21 and Table B.9.22. A first-tier chronic risk assessment has been performed for fresh water organisms, which was based on the data for the active substance. Given the nature of the proposed uses, it is unlikely that pyriproxyfen will reach the marine environment, and a risk assessment for the marine organism *M. bahia* is not performed.

Table B.9.21 The chronic toxicity of pyriproxyfen to aquatic life

Species	Type of test	NOEC (µg a.s./L)
Oncorhynchus mykiss	95-day fish early life stage test	4.3
Daphnia magna	21-day study	0.015
Mysidopsis bahia	28-day study	0.81
Chironomus riparius	28-day study (spiked water)	10

Table B.9.22 The chronic toxicity of Pyriproxyfen 10EC to aquatic life in a microcosm study

Type of test	NOEC (µg a.s./L)
8-week microcosm study	5.0

The long-term TERs assuming constant exposure to the initial PECsw (taken from section B.8.6.1), and based on the NOEC values of the active substance, are shown in Table B.9.23 and Table B.9.24.

Table B.9.23 Long-term TERs for pyriproxyfen assuming constant exposure to the initial PECs

Crop	Dose (kg a.s./ha)	dist. (m)	% drift	NOEC (µg as/L)			PECsw (µg a.s./L)	TER		
				fish	*Daphnia*	*C. riparius*		fish	*Daphnia*	*C. riparius*
T & E	2x0.1125	1	0.1	4.3	0.015	10	0.0382	113	**0.39**	262
Cotton	1x0.075	1	2.77[1]	4.3	0.015	10	1.5449	**2.8**	**0.01**	**6.5**

T & E, tomato and egg plant EU S [1] 2.77% spray drift + 10% run-off and drainage

The long-term TER for fish, *Daphnia* and *C. riparius* are all below the Annex VI trigger of 10, except for the TER for fish and *C. riparius* for the use in tomato and egg plant. Therefore, the long-term risk assessment needs to be refined.

In the refined risk assessment, the initial PECsw values for the application in cotton have been calculated according to FOCUS STEP 2 for Southern Europe, June-September application, which involves a lower runoff and drainage percentage (3% instead of 10%, see section B.8.6.1). For tomato and egg plant, refinement of the risk for Daphnia using FOCUS was not possible. Refinement using 21-day TWA instead of the initial PECsw values is considered not appropriate in this case, since in the microcosm study (single application, DT50 (water) 0.8 days) reductions of the population of the cladoceran *Daphnia* group *galeata* were observed, hence it cannot be excluded that effects on *Daphnia* result from exposure occurring early on in the exposure period. The resulting long-term TERs for treatment of cotton are shown in Table B.9.24.

Table B.9.24 Refined long-term TERs for pyriproxyfen assuming constant exposure to the initial PECs (FOCUS step 2) for cotton

Crop	Dose (kg a.s./ha)	dist. (m)	% drift	NOEC (µg as/L)			PECsw (µg a.s./L)	TER		
				fish	*Daphnia*	*C. riparius*		fish	*Daphnia*	*C. riparius*
Cotton	1x0.075	1	2.77[1]	4.3	0.015	10	0.6898	**6.2**	**0.02**	14

[1] 2.77% spray drift + 3% run-off and drainage

The long-term TER for *C. riparius* in the refined risk assessment for treatment of cotton is above the Annex VI trigger of 10. Hence, the long-term risk for sediment-dwelling organisms should be low. The long-term TERs for fish and *Daphnia magna* are still below the trigger of 10. Hence, the risk for these organisms needs to be further refined.

Further refinement of the risk assessment for the use in cotton is based on FOCUS step 3 PECsw values, calculated for the only relevant scenario for cotton (ditch scenario D6), with application in September. More detailed information on parameter selection for STEP 3 calculations may be found in volume 3, section B.8.6.1. The resulting long-term TERs are shown in Table B.9.25a.

Table B.9.25a Refined long-term TERs for pyriproxyfen assuming constant exposure to the initial PECs (FOCUS step 3) for cotton

Crop	dose (kg a.s./ha)	dist. (m)	% drift	NOEC (µg as/L)		PECsw (µg a.s./L)	TER	
				fish	*Daphnia*		fish	*Daphnia*
Cotton	1x0.075	1.3[2]	2.77[1]	4.3	0.015	~~0.393~~ 0.381	11	**0.04**

[1] 2.77% spray drift + drainage dependent on substance characteristics [2] Standard distance to the crop in Step 3, D6 ditch

The long-term TER for fish in the refined risk assessment is above the Annex VI trigger of 10. Hence, the long-term risk for fish should be low. The long-term TERs for *Daphnia magna* is still below the trigger of 10. Hence, also for the use in cotton the risk assessment for this organism needs to be further refined.

For further refinement of the assessment, data from a microcosm study is available. Following single treatment of plankton-dominated indoor microsocms with Pyriproxyfen 10EC no effects were observed at initial concentrations up to and including 1.2 µg a.s./L. A slight transient direct negative effect on the most sensitive zooplankton species *Daphnia* group *galeata* was the only effect at 5 µg a.s./L. It was observed only on one single sampling date and is considered to be non-significant. At the highest tested concentration of 20 µg a.s./L, clear but transient effects on total cladocerans and on *D.* group *galeata* occurred, and clear indirect effects (increased abundances) on total rotifers and individual taxa (effect transient except for *K. quadrata*). The No Observed Effect Concentration (NOEC) is 5 µg a.s./L, based on effects on total cladocerans and on *D.* group *galeata,* as well as on a clear long-term indirect effect (increase in *K. quadrata*) with no full recovery within 8 weeks. The $NOEC_{community}$ determined by PRC analysis was 5 µg a.s./L (transient effect at 20 µg a.s./L). The No Observed Ecologically Adverse Effect Concentration (NOEAEC) is 5 µg a.s./L. From this value, an Ecologically Acceptable Concentration (EAC) can be derived, taking into account the amount of data and the types of effects seen at the NOEAEC. As at the concentration of 5 µg a.s./L only slight effects were seen, a safety factor is considered not necessary and the EAC can be set at 5 µg a.s./L.
The treatment regime in the microcosm study (single application) does not reflect the exposure regime of surface water for the use in tomato and eggplant (2 applications with 10 days' interval), so the EAC of 5 µg a.s./L can only be used for refinement of the risk assessment of use in cotton.

Table B.9.25b Refined long-term TERs for pyriproxyfen assuming constant exposure to the initial PECs (FOCUS step 3) for cotton, using the EAC from the microcosm study

Crop	dose (kg a.s./ha)	dist. (m)	% drift	EAC (µg as/L)	PECsw (µg a.s./L)	TER
Cotton	1x0.075	1.3	2.77	5	~~0.393~~ 0.381	13

The long-term TER based on the EAC from the microcosm in the refined risk assessment is above the trigger of 1. Hence, the long-term risk for all water organisms including *Daphnia* for the use in cotton should be low.
It has not been shown that the long-term risk for *Daphnia* for the use in tomato and egg plant is low. However, in the case the risk assessment can be carried out using a weight-of-evidence approach. Considering that the TER of cotton from table B.9.25b is 13 times higher than the trigger and that the PECsw from tomato and eggplant is at least ten times lower than the PECsw for cotton (0.0382 at FOCUS Step2 vs. 0.381 µg a.s./L at FOCUS Step 3), there is a safety margin of 130. It is not expected that the effect of the second application in tomato and eggplant is this big. Therefore, the long-term risk for tomato and eggplant should be low.

February 2008: At Praper 63 a concern was raised that the risk to aquatic insects might not have been sufficiently addressed. This was set as a data gap for the notifier. The assessment factor on the NOEAEC of 5.0 µg as/Lmicrocosm study will be determined when information on the toxicity to insects is available

B.9.2.3.1.3 Bioaccumulation

In a bio-concentration study with 14C-pyriproxyfen under flow-through conditions, the BCFs for radioactivity were 1379 and 1495 L/kg wet weight for 14C-phenoxyphenyl- and 14C-pyridinyl-label, respectively, and the corresponding BCFs for pyriproxyfen were 660 and 501 L/kg wet weight. The Annex VI trigger factor is 100 L/kg for non-readily biodegradable substances. Pyriproxyfen was not readily biodegradable in a closed bottle test (this type of test is however considered to be less suitable to determine ready biodegradability of poorly soluble substances like pyriproxyfen). Pyriproxyfen has a DT90 in the whole system of a water/sediment system of ≥12.7 days. The risk for bioaccumulation should therefore be considered. In birds and mammals, the risk of bioaccumulation due to the intake of contaminated fish, based on the worst case BCF value of 1495 L/kg, was considered to be low (section B.9.1.5.2 and B.9.1.6.2). Therefore, bioaccumulation is considered to be of no concern.

B.9.2.3.2 Risk assessment of metabolites

DPH-PYR and PYPAC were shown to be major (>10%) metabolites in the water phase of a water/sediment system (Section B.8.4.3.2), with maximum levels of respectively 11.8 and 23.6% AR in water and 4.3% and 7.6% AR in sediment. 4'-OH-pyriproxyfen was a major (>10%) metabolite in sediment (maximum 4.8% and 14.8% AR in water and sediment, respectively; section B.8.4.3.2). In addition, POP, 4'-OH-POP, POPA, PYPA and PYPAC-Me were found at 0.2-1.6% AR in the water phase and POP and 4'-OH-POP at 0.5% AR in the sediment phase. The risk of these metabolites must be considered.

B.9.2.3.2.1 Acute risk

PYPAC

The LC/EC50s for PYPAC are summarised in Table B.9.26.

Table B.9.26 The acute toxicity of PYPAC to aquatic life

Species	Test duration	LC/EC50 (mg/L)
Oncorhynchus mykiss	96 hours	>93
Daphnia magna	48 hours	>95
Pseudokirchneriella subcapitata	72 hours	26

Acute risk assessment will be based on these LC/EC50 values. The worst case TERs for this metabolite resulting from spray drift at 1 m are summarised in Table B.9.27. The PECsw presented in the table are taken from section B.8.5.1.

Table B.9.27 Acute TERs for PYPAC from spray drift at 1 m

		LC/EC50 (µg/L)			PECsw	TER		
crop	% drift	fish	*Daphnia*	Algae	(µg/L)	fish	*Daphnia*	Algae
T & E	0.1	>93000	>95000	26000	0.0110	>8.E+06	>9.E+06	2.E+06
Cotton	2.759	>93000	>95000	26000	1.2000	>8.E+04	>8.E+04	2.E+04

T & E, tomato and egg plant EU S

The acute TERs of PYPAC for fish, *Daphnia* and algae are all far above the relevant Annex VI triggers (100, 100 and 10, respectively). Hence the acute risk from the proposed use should be low.

DPH-PYR

Pyriproxyfen was the main component of the residue in water-sediment studies treated with pyriproxyfen, and the maximum level of DPH-PYR in water and sediment was 11.8 and 4.3% AR, respectively. No studies on the aquatic toxicity of DPH-PYR are available. DPH-PYR was a major (>10%) metabolite in the water phase of water-sediment systems. Acute toxicity tests with aquatic organisms were conducted with the metabolites PYPAC and 4'-OH-pyriproxyfen, but the molecular structure of DPH-PYR differs too much from that of these metabolites to allow extrapolation. Since exposure of aquatic organisms to this metabolite during the microcosm study is likely to have occurred, the risk of DPH-PYR is covered by the results of the microcosm study. Therefore, the risk of DPH-PYR for aquatic organisms should be low.

4'-OH-PYRIPROXYFEN

The LC/EC50s for 4'-OH-pyriproxyfen are summarised in Table B.9.28.

Table B.9.28 The acute toxicity of 4'-OH-pyriproxyfen to aquatic life

Species	Test duration	LC/EC50 (mg/L)
Oncorhynchus mykiss	96 hours	0.27
Daphnia magna	48 hours	1.8
Pseudokirchneriella subcapitata	72 hours	>2.5

Acute risk assessment will be based on these LC/EC50 values. The worst case TERs for this metabolite resulting from spray drift at 1 m are summarised in Table B.9.29. The PECsw presented in the table are taken from section B.8.5.1.

Table B.9.29 Acute TERs for 4'-OH-pyriproxyfen from spray drift at 1 m (0.1 and 2.77%)

		LC/EC50 (µg/L)			PECsw	TER		
crop	% drift	fish	*Daphnia*	Algae	(µg/L)	fish	*Daphnia*	Algae
T & E	0.1	270	1800	>2500	0.0075	4.E+4	2.E+05	>3.E+05
Cotton	2.77	270	1800	>2500	0.4854	556	3708	>5150

T & E, tomato and egg plant EU S

The acute TERs of 4'-OH-pyriproxyfen for fish, *Daphnia* and algae are all above the relevant Annex VI triggers (100, 100 and 10, respectively). Hence the acute risk from the proposed use should be low.

POP, 4'-OH-POP, POPA, PYPAC-Me and PYPA

No studies on the aquatic toxicity of POP, 4'-OH-POP, POPA, PYPAC-Me or PYPA are available. The maximum levels of any single metabolite in water and sediment was 1.6 and 0.5% AR, respectively. Given the high safety margins calculated for the acute risk for parent pyriproxyfen (TERa based on worst case STEP 1 FOCUS PECsw ≥123 for fish and daphnia and ≥48 for algae and aquatic plants), and the low levels of these metabolites, the acute risk of these minor metabolites is considered to be low.

B.9.2.3.2.2 Long-term risk

According to the Guidance Document on Aquatic Ecotoxicology (Working Document, Sanco/3268/2001), chronic toxicity testing is only required where the metabolite is acutely more toxic than the parent compound. For metabolites in sediment, it is stated that testing is required where a metabolite is present in the sediment at a level of more than 10% of the parent applied radioactivity at day 14 or later.

PYPAC and 4'-OH-pyriproxyfen

PYPAC and 4'-OH-pyriproxyfen are not more acutely toxic than parent pyriproxyfen. Therefore, chronic testing is not required for these metabolites. 4'-OH-pyriproxyfen was a major metabolite in sediment, while PYPAC was a minor metabolite in sediment. Both metabolites were not detected at levels >10% AR after 14 days in water/sediment studies. Therefore, chronic testing with sediment-dwelling organisms is not required.

DPH-PYR

DPH-PYR was a major metabolite in water (max. 11.8% AR), but not in sediment. The DT50 for dissipation from the water column of water-sediment studies is >2 days, hence chronic exposure is relevant. No acute toxicity studies with DPH-PYR were performed and its acute

toxicity cannot be compared with that of parent pyriproxyfen. Since exposure of aquatic organisms to this metabolite during the microcosm study is likely to have occurred, the risk of DPH-PYR is covered by the results of the microcosm study. The risk of DPH-PYR should therefore be low.

PYPA and PYPAC-Me

PYPA and PYPAC-Me were minor metabolites in water (max. 1.6% AR), but not detected in sediment. No studies on the acute toxicity of PYPA or PYPAC-Me are available. Considering the close similarity between the molecular structures of PYPA, PYPAC-Me and PYPAC, and the much lower toxicity of PYPAC (L(E)C50 values >93, >95 and 26 mg a.s./L for fish, *Daphnia* and algae, respectively) compared to that of pyriproxyfen (L(E)C50 values >0.27, 0.40 and >0.18 mg a.s./L, respectively), it is not considered likely that the acute toxicity of PYPA or PYPAC-Me will be higher than that of pyriproxyfen. The chronic risk of these metabolites to aquatic organisms should therefore be low.

POP, 4'-OH-POPand POPA

POP, 4'-OH-POP and POPA were minor metabolites in water (max. 1.6% AR), but not detected in sediment. No studies on the acute toxicity of these minor metabolites are available. In the water phase of the various water-sediment systems, POP and POPA were either not detected or only detected twice, between day 1 and 3, whilst 4'-OH-POP was only detected once. Considering the short-lived appearance of these metabolites, chronic exposure is considered to be of no concern.

Conclusion long-term risk assessment of metabolites

Chronic exposure to metabolites is considered to be of no concern.

B.9.2.3.2.3 Bioaccumulation

No experimentally determined BCF values in fish are available for 4'-OH-pyriproxyfen, DPH-PYR, POP, 4'-OH-POP, POPA, PYPA, PYPAC and PYPAC-Me. LogPow values were estimated by the RMS using EPA EPI Suite software and Pallas 3.0 software (see Table B.9.1). The BCF for fish can be estimated according to the formula logBCF=0.85*logPow-0.7 (USES 2.0). The worst-case estimated logPow values and estimated BCF values are presented in Table B.9.30.

Table B.9.30 LogPow and BCF-values for metabolites of pyriproxyfen

Metabolite	logPow	BCF (L/kg)
4'-OH-pyriproxyfen	5.17(A)	4949
DPH-PYR	3.02(A)	74
POP	3.64(A)	248
4'-OH-POP	3.12(A)	90
POPA	4.18(A)	713
PYPA	0.78(B)	0.9
PYPAC	1.01(B)	1.4
PYPAC-Me	1.34(A)	2.7

(A) Estimated by RMS using Pallas 3.0 (CompuDrug Chemistry Ltd. 1994,95): 3.18
(B) Estimated by RMS using EPA EPI Suite software

The estimated BCF values are all below the Annex VI trigger of 100, except those of 4'-OH-pyriproxyfen, POP and POPA. POP and POPA however are both short-lived metabolites in the water-phase (only detected twice, between day 1 and 3), and their risk for bioaccumulation should be low. The metabolite 4'-OH-pyriproxyfen does not meet the trigger for a study into direct long-term effects in fish (EC50 >0.1 mg/L). Parent pyriproxyfen has a higher logPow (logPow = 5.70) than 4'-OH-pyriproxyfen (logPow = 5.17). The 21-day PECsw of pyriproxyfen in surface water is about a factor of 10 higher than that of 4'-OH-pyriproxyfen. The risk for bioaccumulation in birds and mammals was considered to be low for parent pyriproxyfen. Therefore the risk of secondary poisoning of birds and mammals with the metabolite should also be low. Biomagnification in aquatic food chains should be of no concern as the DT90 for dissipation of 4'-OH-pyriproxyfen from the water phase of water-sediment systems is <10 days, and hence below the trigger of 100 days stated in the Guidance Document on Aquatic Ecotoxicology of 17 October 2002. The risk of bioaccumulation of 4'-OH-pyriproxyfen is considered to be low.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

The effect of exposure to pyriproxyfen for other terrestrial invertebrates has been determined for mammals (see section B.9.1.6). No further data were submitted and no studies judged necessary.

B.9.4 Effects on bees (IIA 8.3.1, IIIA 10.4)

Studies were conducted in compliance with relevant OECD and/or EPPO guidelines under GLP, unless stated.

B.9.4.1 Toxicity

B.9.4.1.1 Toxicity of the active substance

Studies on the acute contact and oral toxicity of pyriproxyfen are summarised in Table B.9.31.

Reference	:	Hoberg J.R. (2001)	GLP statement	:	yes
type of study	:	Acute contact and oral toxicity study with honey bee	guideline	:	EPPO 170 (1992), OECD 213. OECD 214
year of execution	:	2000	acceptability	:	acceptable under conditions
test substance	:	Pyriproxyfen, Lot No. 00303G, purity 99.7%			
Reference	:	Atkins E.L. (1989)	GLP statement	:	no
type of study	:	Bee adult toxicity dusting test	guideline	:	-
year of execution	:	1989	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen, Code 315-89, purity 96.6% technical			

Table B.9.31 The acute contact and oral toxicity of pyriproxyfen to honeybees (*Apis mellifera*)

Test type (purity of test substance)	LD50 (µg a.s./bee)	Test guideline	Reference
acute contact, 48 hr (99.7%) in acetone	>100(A)	EPPO 170 (1992) OECD 214 (1998)	Hoberg J.R., 2001
acute oral, 48 hr (99.7%) in acetone	>100(A)	EPPO 170 (1992) OECD 213 (1998)	Hoberg J.R., 2001
acute contact (B), 96 hr (96.6%) in acetone mixed with attayclay diluent	>100	-	Atkins E.L., 1989

(A) See comment 1 below.
(B) Bees were dusted with a dried, sieved, blended sample using a vacuum duster. See also comment 2 below.

Comment 1, concerning Hoberg J.R., 2001
The test was performed with pure pyriproxyfen, with stated purity of 99.7%. The specifications of pyriproxyfen technical, provided by the notifier, state a purity of ≥97.0%. In Commission Directive 96/12/EC (15 March 1996), point 8 (Ecotoxicological studies), article vi, it is stated that if testing is done using active substance the material used should be of that specification that will be used in the manufacture of preparations to be authorized. It is possible that pyriproxyfen technical, as it will be present in the product, contains up to 3% relevant inpurities (possibly toxic to bees), while these inpurities have not been tested in the study. Therefore, the study result is not accepted in its current form. Further studies are not needed since acceptable data for the toxicity of the formulation to honey bees are available.

Comment 2, concerning Atkins E.L., 1989
(1) The test was performed according to Atkins E.L. *et al.*, 1975. The method of treatment (dusting) deviated from that mentioned in the current day guidelines. The endpoint is not useful for risk assessment.
(2) Current guidelines (EPPO (2000), OECD 214 (1998) and EC C.16 (2001)) all state that a toxic standard should be included and that the LD50 of the toxic standard should meet a specified range in order for a test to be valid. The sensitivity of the bees and the test procedures were not verified using a toxic standard in the above test, which, therefore, is not acceptable.

B.9.4.1.1.1 Bee brood studies

Reference	:	Hagino S. (1992)	GLP statement	:	no
type of study	:	Bee brood test with honey bee	guideline	:	-
year of execution	:	1992	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen, S-31183, Lot No PTG-86011, purity 97.2%			

In a laboratory honey bee brood test, conducted in Japan between October 13 and December 18, 1992, bee colonies (one per treatment, each consisting of 1 hive, 1 queen and 5000-10000 workers) were exposed for 6 days to pyriproxyfen (97.2% pure) mixed with pollen. Pyriproxyfen was dissolved in DMSO and polyoxyethylene hydrogenated oil and mixed with pollen at nominal doses of 1, 5 and 25 mg a.s./kg pollen. In addition to treated pollen, bees were fed with untreated 50% sucrose solution. Following the 6-day exposure period, bees were fed untreated pollen and untreated 50% sucrose solution and were observed for 14 more days. Frames containing sealed brood were kept in an incubator (35°C) to observe morphological effects and emergence. Emerged bees were counted and weighed daily, amorphogenic effects were assessed daily, feed consumption was determined weekly, and the number of worker bees and maturity of the queens was assessed at the end of the test. Pyriproxyfen concentrations in the diet were measured at the start and end of exposure in all treatments and ranged between 90 and 106% of the nominal concentrations. Pyriproxyfen was not detected in honey produced by the bees, nor in bee brood, at any concentration. Residues measured in worker bees were not detectable (<0.1 mg/kg bw) at the nominal treatment concentration of 1 mg a.s./kg pollen. At nominal concentrations of 5 and 25 mg a.s./kg pollen, respectively, residues measured in worker bees were 0.6 and 2.0 mg a.s./kg bw on day 6 of exposure, and 0.1 and 0.8 mg a.s./kg bw 2 weeks after termination of the treatment.

No abnormalities were observed on rows of hairs on the inner side of the first tarsal segument of the hind legs, mandibles, curvature of centris, color of body, abdomen or compound eyes. The queen continued oviposition during the test at 25 mg a.s./kg. Further results are summarised in Table B.9.32.

Table B.9.32 Effects of pyriproxyfen (97.2%) to brood of honey bees (*Apis mellifera*)

Parameter	1 mg a.s./kg	5 mg a.s./kg	25 mg a.s./kg
Emergence rate (%) of normal bees(A)	91.0	97.7	92.7
Average body weight of newly emerged bees (mg)			
Sealed brood	107	106	98
4 day old larva	98	100	76
1-6 day old larva	88	95	98
Egg – larva	n.m.	n.m.	97
Oocyte – egg	n.m.	n.m.	90
No. of worker bees at end	5900	10100	8100
Body weight queen (mg)	271	270	280
Ovary weight queen (mg)	52.6	44.9	52.3
Feed consumption			
Pollen (mg/bee)	68	58	73
Sucrose solution (ml/bee)	0.76	0.43	0.47

n.m. not measured

(A) Some of the newly emerged bees exposed during the sealed or larval stage had incompletely expanded wings. The emergence rates including these abnormal bees were 94.6, 97.9 and 95.9% for 1, 5 and 25 mg a.s./kg.

The report stated that pyriproxyfen did not cause adverse effects on honey bee colonies at the doses tested.

Comments by RMS

The test was not GLP compliant (although performed in 1992) and not performed according to the current guideline. This guideline (EPPO 1992) states that a toxic standard and a blank control should be included. The sensitivity and the test procedures were not verified using a toxic standard in the above test. The results will not be taken to the List of Endpoints, therefore, but may be used as supplementary information.

B.9.4.1.2 Toxicity of the plant protection product

A study on the acute oral and contact toxicity of Pyriproxyfen 10% EC to honey bees (Study 1) is summarised in Table B.9.33. A study on the acute contact toxicity of Pyriproxyfen 0.83 EC on three bee species (honey bee (*Apis mellifera*), alfalfa leafcutter bee (*Megachile rotundata* (F.) and alkali bee (*Nomia melanderi* Cockerell) (Study 2) is summarised in Table B.9.34.

Study 1

Reference	:	Hoberg J.R. (2002)	GLP statement	:	yes
type of study	:	Acute contact and acute oral test with honey bee	guideline	:	EPPO 170 (1992), OECD 213, OECD 214
year of execution	:	2001	acceptability	:	acceptable
test substance	:	Pyriproxyfen 10% EC, B0100005, 10.3 % w/v			

Table B.9.33 The acute contact and oral toxicity of Pyriproxyfen 10% EC to honeybees (*Apis mellifera*)

Test type (content of a.s.)	LD50 (µg a.s./bee)	Test guideline	Reference
acute contact, 48 hours (10.3 % w/v)	>100	EPPO (1992) OECD 214	Hoberg J.R., 2002
acute oral, 48 hours (10.3 % w/v)	74 (95% C.I.: 52-100)	EPPO (1992) OECD 213	Hoberg J.R., 2002

Study 2

Reference	:	Mayer D.F. (1995)	GLP statement	:	no
type of study	:	Acute contact study with honey bee, leafcutter bee and alkali bee	guideline	:	-
year of execution	:	1994	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen 0.83 EC			

Table B.9.34 The acute contact toxicity of Pyriproxyfen 0.83 EC on three bee species (honey bee, leafcutter bee and alkali bee)

Test type (content of a.s.)	LD50 (µg a.s./bee)	Test guideline	Reference
acute contact, 24 hours (content a.s. not specified)	>0.53 (A)	-	Mayer D.F., 1995

(A) Calculated by the Rapporteur using a dosage volume of 2 µL/bee (reported value: 2 L/bee) and the reported concentration of the dosing of 0.26 g a.i./L).

Comments by RMS on study 2

The report was not signed, the test was not GLP compliant and not performed according to the current guideline (24 hours exposure instead of 48 hours; vehicle water instead of acetone). Current guidelines (EPPO (2000), OECD 214 (1998) and EC C.16 (2001)) all state that a toxic standard should be included and that the LD50 of the toxic standard should meet a specified range in order for a test to be valid. The sensitivity of the bees and the test procedures were not verified using a toxic standard in the above test. The results were only presented as a statement ("no significant differences in mortality"). The result is not accepted.

B.9.4.1.2.1 Bee brood studies

Study 1

Reference	:	Mayer D.F. (1995)	GLP statement	:	no
type of study	:	Bee brood test with honey bees	guideline	:	-
year of execution	:	1994	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen 0.83 EC			

In a honey bee brood test, bee colonies (four per treatment) were fed a sugar syrup solution in water containing Pyriproxyfen 0.83 EC at a nominal dose of 124 g a.s./ha in 95 L/ha (equivalent to a concentration of 1305 mg a.s./L) on 30 August 1994. Four colonies were fed untreated syrup. After 12 days, randomly selected capped bee brood (300/colony) were opened to assess abnormal development.

Results are summarised in Table B.9.35.

Table B.9.35 Effects of pyriproxyfen to brood of honey bees (*Apis mellifera*)

Parameter	control	124 g a.s./ha
Number of live adults	11	13
Number of dead adults	0	2
Number of live pupae	265	116 *
Number of dead pupae	0	94 *
Number of live larvae	25	74 *
Number of dead larvae	0	7

* Significantly different from control at 5% level

Pyriproxyfen fed to honey bee colonies in a syrup solution resulted in increased pupa mortality and increased number of live larvae.

Comments by RMS

The report was not signed and the test was not GLP compliant (although conducted in 1994). Main guideline deviations were: no toxic standard, test duration too short (EPPO, 1992). The dose was stated to be equal to 124 g a.i./ha, but it was not verifiable how this figure was arrived at. The information on test design, test procedures and test results was essentially limited to what is included in this summary. The lack of information on test design and test results (e.g. no data for individual hives) render the result not reliable enough for inclusion in the List of Endpoints. The results however may be used as supplementary information as they suggest that a nominal dose of 124 g a.i./ha (equivalent to 1305 mg a.s./L) fed to a bee colony causes increased pupal mortality.

Study 2

reference	:	Wael L. de *et al*. (1992)	GLP statement	:	no
type of study	:	Bee brood test with bumble bees	guideline	:	-
year of execution	:	1992	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen (100 g/L EC), purity 100 g pyriproxyfen/L			

In a laboratory bumble bee (*Bombus terrestris*) brood study, bee colonies (one per treatment) were exposed for 24 hours to Pyriproxyfen 100 g/L EC in a sugar syrup solution in water at nominal concentrations of 0.2, 2 and 20 mg a.s./L. Two trials were performed. The first trial included all three doses of pyriproxyfen, a negative control and two reference products (Cyromazine at 85 mg a.s./L, reported to be harmless, and Fenoxycarb at 100 mg a.s./L, reported to be the toxic standard). The second trial included only the highest dose of pyriproxyfen and a toxic standard (Teflubenzuron at 150 mg a.s./L). Each colony contained

one queen and 30-50 workers. The hives were kept in a climate room at 29°C and 60% RH. Consumption of the sucrose solution was monitored on a daily basis. Dead adults and larvae were counted and removed on a daily basis, and colour photographs were taken daily for a period of 30 days to establish age and development of the colony.

Based on the photographs, the development of the various stages was reported to be normal for all treatments except for teflubenzuron. Further results are summarised in Table B.9.36.

Table B.9.36 Effects of pyriproxyfen to brood of bumble bees (*Bombus terrestris*)

	First trial						Second trial	
Parameter	Control	Pyripro-xyfen 0.2 mg a.s./L	Pyripro-xyfen 2 mg a.s./L	Pyripro-xyfen 20 mg a.s./L	Cyroma-zine 85 mg a.s./L	Fenoxy-carb 100 mg a.s./L	Pyripro-xyfen 20 mg a.s./L	Tefluben-zuron 150 mg a.s./L
Adults exposed	30	32	50	50	32	43	28	40
Sucrose consumption (g/colony)								
Before treatment	17	18	25	25	14	30	13	16
During treatment	16	17	25	27	20	30	17	20
After treatment	19	26	33	52	36	57	56	17
Number of dead adults	5	16(A)	24(A)	48	18	54	27	115(A)
Number of dead larvae	61	73	64	82	92	64	32	97

(A) Including the queen.

The report stated that pyriproxyfen has no adverse effect on the development of the brood of bumble bees, but this conclusion is not accepted (see below).

Comments by RMS

The test was not GLP compliant (although performed in 1992) and deviated from the current guideline (EPPO, 1992) on several points. Only one replicate per treatment was employed instead of three, and development of brood was determined by photographs instead of by counting cells with larvae and eggs, and no data on living larvae were recorded. The EPPO 1992 guideline states that a toxic standard and a blank control should be included. In the first trial, the toxic standard did not produce the expected response, and no reliable conclusions could be drawn. In the second trial, the positive control may have given the expected response, but since a negative control was not included, the results of the second trial cannot be evaluated either. The study result is therefore not accepted.

B.9.4.1.3 Residue tests (IIIA 10.4.2)

Reference	:	Mayer D.F. (1995)	GLP statement	:	no
type of study	:	Residue study with honey bee, leaf cutter bee and alkali bee	guideline	:	-
year of execution	:	1994	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen 0.83 EC			

In a residual contact toxicity study, three bee species (honey bee (*Apis mellifera*), alfalfa leafcutter bee (*Megachile rotundata*) and alkali bee (*Nomia melanderi* Cockerell)) were exposed for 24 hours to field treated alfalfa leaves containing 2- and 8-hours dried residue of Pyriproxyfen 0.83 EC (reported dose information: 234 L/ha) and a blank control . The test was conducted in wire screen cages with 500 cm^2 detached foliage and 50% sucrose solution, with four replicates per treatment (50/20/20 honey/leafcutter/alkali bees per replicate, respectively).

The report stated that there were no significant differences in mortality of treated bees as compared to the untreated control.

Comments by RMS
The application dose of the alfalfa was not further specified in the report than "234 L/ha", and mortality data were not provided. The report was not signed and the test was not in compliance with GLP and not conducted according to the current guideline. This guideline (EPPO 2000), which gives general guidance on the performance of bee toxicity studies, states that a toxic standard should be included and that the LD50 of the toxic standard should meet a specified range in order for a test to be valid. No mortality was seen in the treatment group, so it is not known if exposure took place. The sensitivity of the bees and the test procedures were not verified using a toxic standard in the above test, which, therefore, is not acceptable.

B.9.4.1.4 Field tests (IIIA 10.4.4)

Study 1

reference	:	Mayer, D.F. (1995)	GLP statement	:	no
type of study	:	Field study with honey bees	guideline	:	-
year of execution	:	1994	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen 0.83 EC			

A field study was performed in April (pear) and August (dutch clover) 1994 to assess the effects of Pyriproxyfen (0.83 EC) on the behaviour and mortality of bees and the development of bee colonies. The treatment was assigned to one pear orchard measuring 2.4 ha and to one clover field measuring 1.6 ha. Only one half of the pear orchard and the clover field were treated. The treatment rate of pyriproxyfen was 124 g a.s./ha in both experiments. Four colonies with Todd dead bee traps were placed adjacent to the treated part of the area four to six days prior to application. The treatments were made in 379 or 189 L of water per acre (pear and dutch clover, respectively), when the pear was in 65% open bloom and the dutch clover was in full bloom. Each experiment included a negative control (for foraging assessments only). Before application, the colonies were opened and 100 cells containing eggs or young larvae on one frame per colony were marked using stick pins.

Assessments of mortality in the Todd traps were made daily for 10 (clover) or 11 (pear) days following application. At +11DAT (pear) and +12 DAT (clover), the marked cells were

examined, and at +12DAT, 2 brood frames containing capped brood were taken from each colony. Assessments of abnormal development of immatures were made from 150 randomly selected capped brood from each of these frames.

Foraging assessments were only made once during the study. In the afternoon of the day of treatment, the mean number of foraging honey bees/tree/30 seconds was 4.3 and 4.5 in the treated and untreated part of the pear orchard, respectively. On the day after treatment, the mean number of foraging honey bees/12.5 m^2/30 seconds was 2.2 and 2.4 in the treated and untreated part of the clover field, respectively. In both experiments, the number of dead bees in the Todd traps was stated to be from normal die-off (25-125 bees per day), and no immature or deformed adult bees were found.

Results on adult and brood mortality at +12DAT of immature honey bees in selected capped cells are summarised in Table B.9.37.

Table B.9.37 Effects of Pyriproxifen 0.83 EC in the field on immature honey bees (*Apis mellifera*) in selected capped cells

Parameter	Pear	Dutch clover
Number of live adults	120	190
Number of dead adults	1	1
Number of live pupae	473	395
Number of dead pupae	1	0
Number of live larvae	8	2
Number of dead larvae	0	1

The report stated that pyriproxyfen applied to blooming pears and white dutch clover at a rate of 124 g a.s./ha is not hazardous to adult or immature honey bees.

Comment by RMS
The report was not signed and the test was not GLP compliant (although conducted in 1994) and not performed according to the current guideline. This guideline (EPPO 2000) states that a blank control should be included and that exposure at the time of application should be convincingly demonstrated by the use of a reference product or from foraging assessments. An untreated control was not included for brood cell assessment, and the exposure and the test procedures were not adequately verified in the above test, which, therefore, is not acceptable.

Study 2

reference	:	Barth M. (2003)	GLP statement	:	yes
type of study	:	Field study with honey bee	guideline	:	EPPO 170 (2000)
year of execution	:	2002	acceptability	:	acceptable
test substance	:	Pyriproxyfen 10% EC, batch B0200001, 98.0-99.0 g a.s./L			

A field study was performed in August 2002 in Germany to assess the effects of the proposed Pyriproxyfen 10% EC formulation on the behaviour and mortality of bees and the development of bee colonies. Each treatment was assigned to one plot measuring 100 x 53 m (water treated control) and 113 x 48 m (pyriproxyfen) covered by *Phacelia tanacetifolia*. Treated and control plot were 4 km apart. The treatment rate of Pyriproxyfen 10% EC was 75 g a.s./ha, and the experiment included a water treated control. In each plot four hives were

introduced four days before application, each with 60000-80000 worker bees with a queen and 11 brood frames. The treatments were made in 500 L water/ha, when the *Phacelia* crop was in full bloom, and bees were actively visiting flowers. The treatment rates were confirmed to be within 6% of nominal by weighing spray liquids prior to and after treatment. The hives were removed from the plots to the apiary at +15DAT.

Starting three days prior to treatment, assessments of flight activity and foraging intensity were made once daily until +14DAT (frequently on the day of treatment). Starting three days before treatment, assessments of mortality and behaviour were made once daily, frequently on the day of treatment and at +1 to +14DAT, +21DAT, +28DAT, +36DAT, +42DAT, +49DAT and +59DAT. Colony weight was determined on the day before treatment and at +7DAT, +14DAT, +21DAT, +28DAT, +36DAT, +42DAT, +49DAT and +59DAT. Assessments on brood and comb status (health, brood area size, presence and number of eggs) and colony strength were made the day before treatment and at +7DAT, +13DAT, +21DAT, +28DAT, +36DAT, +42DAT, +49DAT and +60DAT. On the day before application and day 28 after application, at least 100 cells containing newly laid eggs and 100 cells containing young larvae were marked on one brood comb of each control and test item to assess the individual development of single eggs and larvae over two successive brood cycles, with assessments the day before treatment and at +7DAT, +13DAT, +21DAT, +28DAT, +36DAT, +42DAT, +49DAT and +60DAT.

The following parameters were not different for the Pyriproxyfen 10% EC treated hives and the untreated control: adult bee mortality, flight activity, foraging intensity, behaviour, colony weight, colony strength, health, overall brood size area and queen bee activity.
Results for brood development in the single cell assessments are summarised in Table B.9.38.

Table B.9.38 Effects of Pyriproxyfen 10% EC (75 g a.s./ha) to honeybees under field conditions

Parameter	First cycle (28-days)[A]		Second cycle (32-days)[A]	
	Control	Pyriproxyfen	Control	Pyriproxyfen
Egg into adult development (%)	68 (84)[B]	94	80	75
Larvae into adult development (%)	78 (89)[B]	90	83	76

(A) First cycle comprises evaluations on -1, 7, 13, 21 and 28 days after treatment, second cycle comprises evaluations on 28, 36, 42, 49, and 60 days after treatment,

(B) Between brackets is the mean calculated without the fourth colony, in which the queen was not found on day 7 and a new queen was introduced.

Pyriproxyfen 10% EC did not affect mortality of adults or juvenile stages, overall colony performance or survival and development of eggs and larvae through to adult emergence.

This study was performed in accordance with GLP and the EPPO 170 guideline.

B.9.4.2 Risk assessment

B.9.4.2.1 Acute risk of the active substance

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). Data on the acute toxicity of the active substance pyriproxyfen and formulated products to honeybees are presented in Tables B.9.31, B.9.33 and B.9.34. The studies performed with the active substance and Pyriproxyfen 0.83 EC were not acceptable, since in one study, the specification of the tested substance differed from the specification of the substance used in the proposed formulation, and in the other studies the sensitivity of the bees and the precision of the test procedure was not verified using a toxic standard. The calculation of hazard quotients (HQs) is therefore based on the acute oral and contact LD50 values, determined for the proposed pyriproxyfen 10EC formulation (74 and >100 µg a.s./bee, respectively). The hazard quotients for the treatment of tomato, egg plant and cotton are presented in Table B.9.39.

Table B.9.39 Hazard quotients for honey bees using laboratory toxicity studies on Pyriproxyfen 10EC formulation

Crop	dose (g a.s./ha)	oral toxicity		contact toxicity		Annex VI trigger
		LD50 (µg a.s./bee)	HQ	LD50 (µg a.s./bee)	HQ	
Tomato & egg plant EU N	30	74	0.41	>100	<0.30	50
Tomato & egg plant EU S	112.5	74	1.5	>100	<1.1	50
Cotton EU S	75	74	1.0	>100	<0.75	50

The HQs are well below the trigger of 50 specified in Annex VI of 91/414 EC. The acute risk to honeybees is therefore considered to be acceptable.

B.9.4.2.2 Risk of plant metabolites

Pyriproxyfen was the main component of the residue in plants, the maximum level of a single identified compound in any of the investigated plant matrices (apple, tomato, cotton) being 11% of the Total Radioactive Residue (TRR). Given the very low levels of individual metabolites, and the large safety margins calculated for parent pyriproxyfen, the risk of metabolites is considered low. In addition, in a field study at a dose of 75 g a.s./ha with a 60-day observation period, no effects at all were observed, including adult and juvenile mortality. Effects of metabolites were covered in this study.

B.9.4.2.3 Risk for bee brood

According to Commission Directive 96/12/EC, amending Council Directive 91/414/EEC, effects on bee brood must be carried out when the active substance may act as an insect growth regulator. The risk to bee brood should therefore be considered.
Studies on the toxicity of the active substance and formulated products were submitted. All studies, except one, were not acceptable for one or more of the following reasons: no toxic standard; no adequate response of toxic standard; no untreated control; insufficient information on test design and test results. Therefore, the risk assessment will be based on the field study with Pyriproxyfen 10% EC. Mortality of adults or juvenile stages, overall colony performance or survival and development of eggs and larvae through to adult emergence did not differ from the untreated control at a dose of 75 g a.s./ha. Therefore, the risk for bee brood due to exposure on cotton in Southern Europe (1 X 75 g a.s./ha), and on tomato and egg plant in Northern Europe (1-2 X 30 g a.s./ha) should be low. The dose in the field study (75 g a.s./ha) was too low to address the risk due to exposure on tomato and egg plant in Southern Europe (1-2 X 112.5 g a.s./ha). Increased pupa mortality and increased number of live larvae were found at a dose of 124 g a.s./ha in a bee brood study with Pyriproxyfen 0.83 EC (study not acceptable because of incomplete description of test design and results, used as supplementary information; it is noted that the concentration in the spray liquid in this single dose rate test is much higher than that of the proposed use in S-Europe). **The notifier is therefore requested to provide additional information for the risk assessment of Pyriproxyfen 10EC for bee brood at a treatment regime equivalent to that of tomato and egg plant in Southern Europe. If no supplemental information is provided, the risk for use on tomato and egg plant in Southern Europe should be addressed at member-state level, e.g. by including a warning-phrase on the label.**

B.9.4.2.4 Risk of plant metabolites

Pyriproxyfen was the main component of the residue in plants, the maximum level of a single identified compound in any of the investigated plant matrices (apple, tomato, cotton) being 11% of the Total Radioactive Residue (TRR). Given the very low levels of individual metabolites, and the fact that in a field study at a dose of 75 g a.s./ha with a 60-day observation period, no effects at all were observed, including adult and juvenile mortality, the risk of metabolites is considered low.

B.9.5 Effects on other arthropod species (IIA 8.3.2, IIIA 10.5)

B.9.5.1 Laboratory toxicity studies

Studies have been submitted on the toxicity of Pyriproxyfen 10% EC (10.3-11.5% pyriproxyfen) to non-target terrestrial arthropods. These data have been summarised in Table B.9.40. All tests were conducted in accordance with GLP. A reference product was included in each test and gave an adequate response unless stated. Differences in effect from the untreated control were not statistically significant unless indicated.

reference	:	Nienstedt K.M. (2001a)	GLP statement	:	yes
type of study	:	Laboratory toxicity study with *Aphidius rhopalosiphi*	guideline	:	IOBC (Polgar, 1988) & Mead-Briggs (1992) & Mead-Briggs *et al.* (1999)
year of execution	:	2000	acceptability	:	acceptable
test substance		Pyriproxyfen 10% EC, Lot No. VHE-018EC-8, 11.5% pyriproxyfen			
reference	:	Nienstedt K.M. (2001b)	GLP statement	:	yes
type of study	:	Laboratory toxicity study with *Typhlodromus pyri* Scheuten	guideline	:	Louis & Ufer (1995) & Blümel *et al*. (2000)
year of execution	:	2000	acceptability	:	acceptable
test substance	:	Pyriproxyfen 10% EC, Lot No. VHE-018EC-8, 11.5% pyriproxyfen			
reference	:	Kollmann S.I. (2003)	GLP statement	:	yes
type of study	:	Laboratory toxicity study with *Orius laevigatus*	guideline	:	IOBC (Bakker *et al*, 2000)
year of execution	:	2001-2002	acceptability	:	acceptable
test substance	:	Pyriproxyfen 10% EC, Lot No. B0100005, 10.3% pyriproxyfen			
reference	:	Nienstedt K.M. (2003a)	GLP statement	:	yes
type of study	:	Extended toxicity study with aged residue with *Typhlodromus pyri* Scheuten	guideline	:	IOBC (Oomen 1988 & Overmeer 1988 & Blümel *et al.* 2000)
year of execution	:	2002	acceptability	:	acceptable
test substance	:	Pyriproxyfen 10% EC, Lot No. B0100005, 10.3% pyriproxyfen			
reference	:	Nienstedt K.M. (2003b)	GLP statement	:	yes
type of study	:	Extended toxicity study with aged residue with *Chrysoperla carnea* Steph.	guideline	:	IOBC (Vogt *et* al. 2000)
year of execution	:	2002	acceptability	:	acceptable
test substance	:	Pyriproxyfen 10% EC, Lot No. B0100005, 10.3% pyriproxyfen			

Table B.9.40 Effects of Pyriproxyfen 10% EC on non-target terrestrial arthropods under laboratory conditions

Species	Test type, substrate & duration	Appln. (g a.s./ha)	Effect(s)[A]	Test guideline	Ref
Aphidius rhopalosiphi	laboratory, exposure to dry residue on glass for 48 hrs followed by 11 day fecundity assessment	control 31.25 62.5 125 250 500	Mortality (%) / mummies per female / reduction of reproduction[B]: 0 / 23 / - 0 / 15 / 35 0 / 17 / 25 13 / 7.0* / 70 63 / n.d. / - 97 / n.d. / - LR50 (g a.s./ha): 213 (95% C.I.: 151-265) ER50 (g a.s./ha): 81[C]	IOBC (Polgar, 1988) & Mead-Briggs (1992) & Mead-Briggs *et al.* (1999)	Nienstedt K.M., 2001a
Typhlodromus pyri	laboratory, exposure to dry residue on glass for 14 days including 7 days fecundity assessment	control 3.75 7.5 15 30 60	Mortality (%) / corrected mortality[D] / reproduction[E] / reduction of reproduction[B]: 10 / - / 6.8 / - 16 / 6.7 / 6.2 / 9 2.0 / -8.9[F] / 4.0* / 42 31 / 23 / 0.98* / 86 87 / 86 / - / 100 / 100 / - / LR50 (g a.s./ha): 20 (95% C.I.: 18-21)[G] ER50 (g a.s./ha): 8.1[C]	Louis & Ufer, (1995) & Blümel *et al*. (2000)	Nienstedt K.M., 2001b
Orius laevigatus	laboratory, glass substrate, exposure to dry residue for 9 days followed by 11 days fecundity assessment	control 28.13 56.25 112.5 225 450	Mortality (%) / corrected mortality[D] / reproduction[E] / hatching rate (%) / reduction of reproduction (%)[H]: 14 / - / 18 / 89 / - 10 / -5.0[F] / 17 / 69 / 27 16 / 1.7 / 20 / 76 / 5.1 29 / 17 / 19 / 79 / 6.3 34 / 23 / 27 / 80 / -35[F] 36 / 25 / 25 / 72 / -12[F] LR50/ER50 (g a.s./ha): > 450	IOBC (Bakker *at al.* 2000)	Kollmann S.I., 2003

* Significantly different from the control at 5% level. N.d., not detected

(A) Effects are adverse effects, i.e. X% effect on mortality means X% more mortality and Y% effect on reproduction means Y% less reproduction. As a consequence, -Z% effect on reproduction means an increase in reproduction.

(B) Reduction = 100%*(1-R), where R = Rt/Rc, Rt and Rc = average number of mummies or eggs per treated or control female, where applicable calculated by RMS

(C) Calculated by RMS using regression analysis (log-linear).

(D) According to Abbott formula, calculated by RMS where applicable.

(E) Mean number of eggs per female.

(F) Note: negative effect %, hence no adverse effect.

(G) Calculated by RMS using corrected mortality data. The reported value was 17 g a.s./ha, which may have been based on mortality data.

(H) Reduction = 100%*(1-Rt/Rc), where R (Rt and Rc) = (hatching rate (%)/100)*Ft/Fc, Ft and Fc being the number of eggs produced per treated or control female.

Species	Test type, substrate & duration	Appln. (g a.s./ha)	Effect(s)[A]	Test guideline	Ref
Typhlodromus pyri	Extended laboratory with aging (0, 7 & 14 days under semi-field conditions) of residues on field-sprayed potted grape plants, exposure to dry residue on leaf disks for 14 days including a 7 days fecundity assessment in the lab.		Mortality (%) / corrected mortality[D] / reproduction[E] / reduction of reproduction[B]:	IOBC (Oomen 1988 & Overmeer 1988 & Blümel *et al*. 2000)	Nienstedt K.M., 2003a
			0 d aging:		
		control	6.0 / - / 10 / -		
		75	4.0 / -2.1[F] / 7.5* / 26		
		191.3	7.0 / 1.1 / 5.6* / 45		
		225	12 / 6.4 / 4.6* / 55		
			LR50 (g a.s./ha): >225		
			ER50 (g a.s./ha): 205[C]		
			7 d aging:		
		control	13 / - / 9.3 / -		
		75	4.0* / -10[F] / 7.7 / 18		
		191.3	13 / 0 / 7.5 / 19		
		225	7.0 / -6.9[F] / 7.7 / 18		
			LR50/ER50 (g a.s./ha): >225		
			14 d aging:		
		control	7.0 / - / 8.8 / -		
		75	5.0 / -2.2[F] / 7.5* / 15		
		191.3	7.5 / 0.5 / 6.4* / 27		
		225	13 / 6.5 / 7.2* / 18		
			LR50/ER50 (g a.s./ha): >225		
Chrysoperla carnea	Extended laboratory with aging (0, 7 & 14 days under semi-field conditions) of residues on field-sprayed potted grape plants, exposure to dry residue on grape leaf sectors until 5 days after pupation followed by fecundity assessment in the lab. Total duration 34 days.		Mortality (%) / corrected mortality[D] / reproduction[E] / hatching rate (%)/ reduction of reproduction (%)[H]	IOBC (Vogt *et al*. 2000)	Nienstedt K.M., 2003b
			0 d aging:		
		control	9.7 / - / 35 / 97 / -		
		75	10 / 0.4 / 36 / 97 / -2.9[F]		
		191.3	33* / 26 / 34 / 88 / 12		
		225	43* / 37 / 37 / 87 / 4.8		
			LR50/ER50 (g a.s./ha): >225		
			7 d aging:		
		control	0 / - / 34 / 99 / -		
		75	13 / 13 / 30 / 97 / 13		
		191.3	41* / 41 / 30 / 98 / 12		
		225	17* / 17 / 26 / 91 / 28		
			LR50/ER50 (g a.s./ha): >225		
			14 d aging:		
		control	3.5 / - / 29 / 95 / -		
		75	0 / -3.6[F] / 26 / 98 / 7.8		
		191.3	25* / 22 / 28 / 98 / -1.9[F]		
		225	14 / 11 / 33 / 96 / -14[F]		
			LR50/ER50 (g a.s./ha): >225		

* Significantly different from the control at 5% level. N.d., not detected

(C) Effects are adverse effects, i.e. X% effect on mortality means X% more mortality and Y% effect on reproduction means Y% less reproduction. As a consequence, -Z% effect on reproduction means an increase in reproduction.

(D) Reduction = 100%*(1-R), where R = Rt/Rc, Rt and Rc = average number of mummies or eggs per treated or control female, where applicable calculated by RMS

(C) Calculated by RMS using regression analysis (log-linear).

(D) According to Abbott formula, calculated by RMS where applicable.

(E) Mean number of eggs per female.

(F) Note: negative effect %, hence no adverse effect.

(H) Reduction = 100%*(1-Rt/Rc), where R (Rt and Rc) = (hatching rate (%)/100)*Ft/Fc, Ft and Fc being the number of eggs produced per treated or control female.

B.9.5.2 Semi-field and field studies

No study was submitted.

B.9.5.3 Risk assessment

B.9.5.3.1 Risk of the active substance

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002) and ESCORT 2. For the proposed use in tomato and egg plant (single application rate of 0.02-0.03 or 0.05-0.1125 kg a.s./ha and two applications of the same doses with a 10-day interval) and cotton (single application rate of 0.075 kg a.s./ha), it is assumed for in-field and off-field environments that 100 and 2.77% (90^{th} percentile, 1 application, 1 m buffer zone) of the dose will be deposited, respectively. As the proposed use involves a single seasonal treatment as well as two treatments with a 10-day interval, the maximum recommended dose for glass substrate during dose-response laboratory tests for parasitiods and predatory mites will be 191.3 g a.s./ha (applying a MAF of 1.7). Table B.9.41 below summarises the results of laboratory testing with non-target arthropod species.

Table B.9.41 Summary of effects of Pyriproxyfen 10EC on non-target arthropod species during laboratory testing

Species	Test type, Substrate	Max. recommended test dose (g a.s./ha)	Actual dose tested (g a.s./ha)	Overall effect (%) at respective dose in test and LR50 and ER50 (reproduction) values
Aphidius rhopalosiphi	Laboratory, glass	191.3	31.25; 62.5; 125; 250; 500	0; 0; 13; 63; 97 (% mortality) 35; 25; 70; n.a.(A); n.a. (A) (% reduction of reproduction) LR50 (g a.s./ha): 213 (95% C.I. 151-265) ER50 (g a.s./ha): 81
Typhlodromus pyri	Laboratory, glass	191.3	3.75; 7.5; 15; 30; 60	6.7; -8.9(B); 23; 86; 100 (% mortality) 9; 42; 86; n.a. (A); n.a. (A) (% reduction of reproduction) LR50 (g a.s./ha): 20 (95% C.I. 18-21) ER50 (g a.s./ha): 8.1
Orius laevigatus	Laboratory, glass	191.3	28.13; 56.25; 112.5; 225; 450	-5.0(B);1.7; 17; 23; 25 (% mortality) 27; 5.1; 6.3; -35(B); -12(B) (% reduction of reproduction) LR50 & ER50 (g a.s./ha): >450
Typhlodromus pyri	Extended laboratory, plant leaves(C)	191.3	75; 191.3; 225 (0 d aging)	-2.1(B); 1.1; 6.4 (% mortality) 26; 45; 55 (% reduction of reproduction) LR50 (g a.s./ha): >225 ER50 (g a.s./ha): 205
			75; 191.3; 225 (7 d aging)	-10(B); 0; -6.9(B) (% mortality) 18; 19; 18 (% reduction of reproduction) LR50 and ER50 (g a.s./ha): >225
			75; 191.3; 225 (14 d aging)	-2.2(B); 0.5; 6.5 (% mortality) 15; 27; 18 (% reduction of reproduction) LR50 and ER50 (g a.s./ha): >225
Chrysoperla carnea	Extended laboratory, plant leaves(C)	191.3	75; 191.3; 225 (0 d aging)	0.4; 26; 37 (% mortality) -2.9(B); 12; 4.8 (% reduction of reproduction) LR50 & ER50 (g a.s./ha): >225
			75; 191.3; 225 (7 d aging)	13; 41; 17 (% mortality) 13; 12; 28 (% reduction of reproduction)

Species	Test type, Substrate	Max. recommended test dose (g a.s./ha)	Actual dose tested (g a.s./ha)	Overall effect (%) at respective dose in test and LR50 and ER50 (reproduction) values
				LR50 & ER50 (g a.s./ha): >225
			75; 191.3; 225 (14 d aging)	-3.6(B); 22; 11 (% mortality) 7.8; -1.9(B); -14(B) (% reduction of reproduction) LR50 & ER50 (g a.s./ha): >225

(A) n.a. = not applicable (insufficient survivors from initial phase to assess reproduction).
(B) Note: negative effect %, hence no adverse effect.
(C) Exposure to residues on leaf disks of field-sprayed potted grape plants in the lab, aged for 0, 7 and 14 days under semi-field conditions.

According to Annex II of 91/414, data must be submitted on two sensitive species and two species that are relevant to the intended use. Annex II refers to the SETAC Guidance Document on Regulatory Testing Procedures for Pesticides with Non-target Arthropods (Barrett *et al*., 1994) as a source of guidance for testing. Since several limitations have been identified in this Guidance document it is proposed that the risk to non-target arthropods both in and off-field should be adequately addressed according to the recommendations of ESCORT 2. For products with a special mode of action (IGR mode of action), testing should be conducted with *T. pyri* and one other species. Data have been submitted on *Typhlodromus pyri* and three additional species: *Aphidius rhopalosiphi* (a sensitive species), and *Orius laevigatus* and *Chrysoperla carnea* (both species for which juvenile stages are tested).

A Tier I assessment is performed using the data from the laboratory studies with *T. pyri, A. rhopalosiphi* and *O.laevigatus*. For this standard risk assessment, the following scenarios are used to describe the exposure in-field and off-field. For both the key input is the nominal field application rate supplemented by various factors:
in-field exposure= Application rate * MAF
off-field exposure= Application rate * MAF * (drift factor / vegetation distribution factor)
As the proposed worst-case use of the Pyriproxyfen 10EC formulation involves 2 applications with a 10-day interval at the highest dose (tomato and egg plant in Southern Europe, 112.5 g a.s./ha), the multiple application factor (MAF) for the maximum dose is 1.7. The drift factor for off-field exposure was based on the 90th percentile drift data and corrected by a "vegetation distribution factor" of 10%.

The LR50 for *Typhlodromus pyri, Aphidius rhopalosiphi* and *O. laevigatus* on fresh dried residue on glass was determined as 20, 213 and >450 g a.s./ha, respectively. The ER50 values for *T. pyri, A. rhopalosiphi* and *O. Laevigatus* were 8.1, 81 and >450 g a.s./ha., respectively. These values were used for hazard quotient (HQ) calculation for in-field and off-field exposure: in-field HQ= in-field exposure/LR50 and off-field exposure= (off-field exposure/LR50)*uncertainty factor. An uncertainty (safety) factor of 10 was included in the off-field Hazard Quotient calculations to account for uncertainty with the extrapolation from *Typhlodromus pyri, Aphidius rhopalosiphi* and *O. laevigatus* as indicator species, to all off-

field non-target arthropods. ESCORT 2 states that for IGRs a trigger of 50% is used for both lethal and sublethal effects. Hence the risk is considered acceptable if HQ <1. Table B.9.42 below summarises the results.

Table B.9.42 Risk to *Typhlodromus pyri*, *Aphidius rhopalosiphi* and *Orius laevigatus* as a result of in-field (0 m) and off-field (1 m) exposure of the proposed Pyriproxyfen 10EC formulation in tomato and egg plant in Northern and Southern Europe and in cotton in Southern Europe (Hazard Quotient Approach; vegetation distribution factor off-field of 10), **based on LR50 and ER50 values from inert substrate**

Crop / species	dose g as/ha	distance (m)	% drift	Exposure (g a.s./ha)	LR50 (g a.s./ha)	ER50 (g a.s./ha)	HQ lethal	HQ sublethal	trigger value
Typhlodromus pyri									
Tomato EU N(A)	2 x 30	0	-	51	20	8.1	**2.6**	**6.3**	1
		1	NA	NA	20	8.1	NA	NA	1
Tomato EU S(A)	2 x 112.5	0	-	191	20	8.1	**9.6**	**24**	1
		1	NA	NA	20	8.1	NA	NA	1
Cotton EU S	1 x 75	0	-	75	20	8.1	**3.8**	**9.3**	1
		1	2.77	0.21	20	8.1	0.10	0.26	1
Aphidius rhopalosiphi									
Tomato EU N(A)	2 x 30	0	-	51	213	81	0.24	0.63	1
		1	NA	NA	213	81	NA	NA	1
Tomato EU S(A)	2 x 112.5	0	-	191	213	81	0.90	**2.4**	1
		1	NA	NA	213	81	NA	NA	1
Cotton EU S	1 x 75	0	-	75	213	81	0.35	**0.93**	1
		1	2.77	0.21	213	81	1E-2	**3E-2**	1
Orius laevigatus									
Tomato EU N(A)	2 x 30	0	-	51	>450	>450	<0.11	<0.11	1
		1	NA	NA	>450	>450	NA	NA	1
Tomato EU S(A)	2 x 112.5	0	-	191	>450	>450	<0.42	<0.42	1
		1	NA	NA	>450	>450	NA	NA	1
Cotton EU S	1 x 75	0	-	75	>450	>450	<0.17	**<0.17**	1
		1	2.77	0.21			5E-3	**5E-3**	1

EU N and EU S, Northern Europe and Southern Europe, respectively
(A) Also applies to egg plant.

The HQs for off-field exposure are all below the trigger of 1 for application in tomato, egg plant and cotton, using a buffer zone of at least 1 m. In extended laboratory studies with *Chrysoperla carnea* and *Typhlodromus pyri*, effects on mortality and reproduction were below 50% at a dose of 75 g a.s./ha, which exceeds the maximum off-field dose by a factor of 360. Therefore, the off-field risk to non-target arthropods is considered to be acceptable.

Since the sublethal and lethal HQs for in-field exposure for *T. pyri*, and the sublethal HQ for in-field exposure for *A. rhopalosiphi* for application in tomato and egg plant in Southern Europe are above 1, an in-field risk is assumed to be present.

The guidance document (Sanco/10329/2002 rev 2 final, referring to ESCORT 2) states that for products with a special mode of action (IGR mode of action), testing should focus on those

stages likely to demonstrate effects, i.e. juvenile stages. In these tests, ESCORT 2 proposes to use a 50% trigger value for both lethal and sub-lethal endpoints for both in-field and off-field exposure (i.e. HQ<1). Testing with *T. pyri* and one other species is required. To address the concern for in-field exposure, arisen from the Tier I assessment, data from the extended laboratory study with *T. pyri* and *C. carnea* is used for a higher tier risk assessment.

For this higher tier risk assessment, scenarios are similar to those used in the Tier I assessment. In the extended laboratory study with *T. pyri* exposed to leaves from field-sprayed plants, the ER50 and LR50 values for fresh residues were 205 and >225 g a.s./ha, respectively. In the extended laboratory study with *C. carnea* exposed to leaves from field-sprayed plants, the ER50 and LR50 values for fresh residues were >225 g a.s./ha. These values were used for hazard quotient calculation for in-field and off-field exposure as described above. The risk is considered acceptable if HQ <1 for IGRs (ESCORT 2). Table B.9.43 below summarises the results.

Table B.9.43 Risk to *Typhlodromus pyri* and *Chrysoperla carnea* as a result of in-field (0 m) exposure of the proposed Pyriproxyfen 10EC formulation in tomato and egg plant in Northern and Southern Europe and in cotton in Southern Europe (Hazard Quotient Approach), **based on LR50 and ER50 values on plant leaves**

Crop / species	dose g as/ha	distance (m)	% drift	Exposure (g a.s./ha)	LR50 (g a.s./ha)	ER50 (g a.s./ha)	HQ lethal	HQ sublethal	trigger value
Typhlodromus pyri									
Tomato EU N(A)	2 x 30	0	-	51	>225	205	<0.23	0.25	1
Tomato EU S(A)	2 x 112.5	0	-	191	>225	205	<0.85	0.93	1
Cotton EU S	1 x 75	0	-	75	>225	205	<0.33	0.37	1
Chrysoperla carnea									
Tomato EU N(A)	2 x 30	0	-	51	>225	>225	<0.23	<0.23	1
Tomato EU S(A)	2 x 112.5	0	-	191	>225	>225	<0.85	<0.85	1
Cotton EU S	1 x 75	0	-	75	>225	>225	<0.33	<0.33	1

The HQs for in-field exposure are all below the trigger of 1 for application in tomato, egg plant and cotton. In addition, in the laboratory study on inert substrate with *Orius laevigatus*, mortality and reduction of reproduction were below the trigger of 50% at a dose of 450 g a.s./ha (25% mortality and 15% more reproduction). Therefore, the in-field risk to non-target arthropods is considered to be acceptable.

B.9.5.3.2 Risk of metabolites

Pyriproxyfen was the main component of the residue in plants, the maximum level of a single identified compound in any of the investigated plant matrices (apple, tomato, cotton) being 11% of the Total Radioactive Residue (TRR). Given the very low levels of individual metabolites, and the low risk for parent pyriproxyfen, the risk of metabolites is considered low. In addition, in two extended laboratory studies (one of which with the sensitive indicator species *T. pyri*), performed at a dose of 225 g a.s./ha with aging of residues for 7 and 14 days,

no effects at all were observed, including reproduction. Effects of plant metabolites were covered in these studies.

B.9.6 Effects on earthworms (IIA 8.4, IIIA 10.6.1)

B.9.6.1 Toxicity

B.9.6.1.1 Toxicity of the active substance

Reference	:	Dijk, A. van (1988)	GLP statement	:	yes
type of study	:	Acute toxicity to earthworms	guideline	:	OECD 207
year of execution	:	1987	acceptability	:	acceptable
test substance	:	S-31183, batch PTG-86012, purity 99.0%			

In an acute toxicity study, earthworms (*Eisenia fetida*) were exposed to pyriproxyfen (purity 99.0%) for 14 days in artificial soil. The test was conducted in glass jars covered with glass lids containing 750 g of moist soil. The test substance was added to the soil as a mixture with Tween 80. Nominal soil concentrations of 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil and a blank and solvent control were tested in 4 replicates of 10 worms each. Jars were maintained at 21.5°C under continuous light. The study was conducted according to OECD 207 and GLP.

Results are summarised in Table B.9.44.

Table B.9.44 Acute toxicity of pyriproxyfen (purity 99.0%) to *Eisenia fetida*

Nominal concentration (mg a.s./kg soil d.w.)	Mortality at 14 days (%)	Mean weight change over 14 days (%)
Control	2.5	-12.1
Tween 80 control	2.5	+1.3
62.5	0	+0.3
125	5	-9.8
250	2.5	-18.2*
500	2.5	-20.6 *
1000	2.5	-32.6*

* Significantly different from the Tween 80 control at the 5% level

Based on significant effects on weight change, the NOEC is 125 mg a.s./kg dry soil. The 14 day LC50 is >1000 mg a.s./kg dry soil.

B.9.6.1.2 Toxicity of metabolites (IIA 8.4)

No data were submitted.

B.9.6.1.3 Toxicity of the plant protection product (IIIA 10.6.1)

No data were submitted.

B.9.6.2 Risk assessment

B.9.6.2.1 Risk assessment of the active substance

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). For the proposed uses, the initial soil PEC has been determined according to the criteria detailed in Section B.8.3 of the Environmental Fate and Behaviour Section, taking into account 80% crop interception for tomato and egg plant and 40% crop interception for cotton. An acute toxicity study has been supplied on the active substance (14-day LC50 >1000 mg a.s./kg). Risk assessment will be based on this LC50 value. Since pyriproxyfen has a logPow of 5.37 (pH 5.6), it is necessary to reduce the LC50 value by a factor of 2. This is to take account of the relatively high organic matter content of the artificial test soil compared with field soil (EPPO guidelines). The corrected LC50 is annotated as $LC50_{corr}$. Chronic toxicity/reproductive studies on technical pyriproxyfen or Pyriproxyfen 10EC were not submitted, since multiple (>2) applications of the plant protection product will not be made and the (mean) DT90 of pyriproxyfen is ≤100 days (Section B.8.1). The acute TER is presented in Table B.9.45.

Table B.9.45 Acute risk pyriproxyfen to earthworms

appln.	dose (kg a.s./ha)	$LC50_{corr}$ (mg a.s./kg)	PECs (mg a.s./kg)	Acute TER	Annex VI trigger 91/414 EEC
Tomato and egg plant EU S	2 x 0.1125	500	0.055	9091	10
Cotton EU S	1 x 0.075	500	0.060	8333	10

The TER values in Table B.9.45 are far above the Annex VI 91/414 EEC trigger of 10. Hence the acute risk of pyriproxyfen to earthworms is considered to be acceptable.

B.9.6.2.2 Risk assessment of metabolites

Pyriproxyfen was the main component of the residue in soil. The maximum level of the main degradation products 4'-OH-pyriproxyfen and PYPAC in any of the investigated soils was 6.3% and 8.6% AR, respectively. Also DPH-pyriproxyfen (≤0.5% AR), PYPA (≤0.8% AR) and 2-OH-PY (≤0.1% AR) were identified in soil treated with pyriproxyfen. Given the very low levels of individual metabolites in soil, and the fact that the TERs for the parent compound (8333-9091) were clearly on the safe side, the risk of metabolites is considered to be low.

Chronic toxicity/reproductive studies on metabolites of pyriproxyfen were not submitted. These studies are not required when the field DT90 <100 days (Sanco/10329/2002). Field DT90 values are not available for metabolites; therefore, the assessment will be based on available DT90 lab values. For PYPAC only one DT90 (lab) value is available (118 days at 20°C). For 4'-OH-pyriproxyfen four DT90 (lab) values are available (78-234 days at 20°C, mean 126 days). The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002) does not provide guidance for the case where the DT90 is between 100 and 365 days and the number of applications is less than three. In the update of the HTB (Dutch

Manual for Authorisation of Pesticides), the Netherlands will propose to require chronic/reproductive data in cases where the 100 days <field DT90 <365 days, if the TERa ≤100. Considering the large safety margin calculated for the acute risk of pyriproxyfen (TERa = 8333-9091), and the much lower levels of metabolites compared to those of the parent compound, it is considered unlikely that the TERa values for 4'-OH-pyriproxyfen and PYPAC will be ≤100. Therefore, chronic/reproductive data are not required.

B.9.7 Effects on soil non-target macro-organisms (IIIA 10.6.2)

The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Sanco 10329/2002, rev 2 final) states that testing is not required if the field DT90 values in soil are less than 100 days. Field DT90 values are not available for pyriproxyfen and metabolites; therefore, the assessment will be based on available DT90 lab values. The laboratory DT90 values for pyriproxyfen are <100 days (Section B.8.1). Hence further testing for the active substance is not required.

For 4'-OH-pyriproxyfen four DT90 (lab) values are available (78-234 days at 20°C, mean 126 days). The DT90 (lab, 20°C) values for PYPAC in one soil is 118 days (section B.8.1). Testing of metabolites is not necessary if effects on soil micro-organisms are <25% and the TERlt for earthworms is >5. Chronic data for earthworms were not required for this metabolite (section B.9.6.2.2). The effects of 4'-OH-pyriproxyfen and PYPAC to soil micro-organisms are assumed to be covered by studies with parent pyriproxyfen, since the maximum percentage of formation of these metabolites in laboratory incubated soil was reached within 14 days. Reliable data on effects of pyriproxyfen on soil micro-organisms are however not available and this has been marked as a data requirement. **After submission of data on the effects of pyriproxyfen on soil micro-organisms, the need for testing with 4'-OH-pyriproxyfen and PYPAC on non-target macro-organisms should be reconsidered.**

Given the very low levels of the three other minor metabolites DPH-pyriproxyfen (≤0.5% AR), PYPA (≤0.8% AR) and 2-OH-PY (≤0.1% AR) the risk of these compounds to soil non-target macro-organisms is considered to be low.

B.6.1.1 B.9.8 Effects on soil non-target micro-organisms (IIA 8.5, IIIA 10.7)

B.9.8.1 Toxicity

B.9.8.1.1 Toxicity of the active substance (IIA 8.5)

Study 1

Reference	:	Vonk J.W. (1987)	GLP statement	:	Yes
type of study	:	Soil microflora test (nitrification)	guideline	:	Dutch guideline form A, H.4.1 (1983)
year of execution	:	1987	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen, batch PTG-86012, purity 99.0%			

Data were submitted from a laboratory study on the effect of pyriproxyfen on microbial nitrification in a loamy sand and a loam soil.

Batches of a loamy sand soil (3.1, 7.9 and 84.5% particles in the <2, 2-50 and >50 µm size class, pH (KCl) 5.4, 4.5% organic carbon, 2 mm sieved) and of a loam soil (22.7, 40.6 and 26.7% particles in the <2, 2-50 and >50 µm size class, pH (KCl) 7.3, 2.1% organic carbon, 2 mm sieved) were adjusted to 0.32 bar moisture content (pF = 2.5) and pre-incubated for 1 week at 20°C. Aliquots (100 g) were dispensed into conical flasks and treated with the test substance mixed with sand at 1.4 or 14 mg/kg d.w.. Control soil was left untreated. The soils were amended with lucerne meal (0.5% w/w). The soil was adjusted to pF 2.5, and the flasks (2 per treatment) were plugged with cotton wool and incubated for 6 weeks in the dark at 20±1°C. On day 0, and after 1, 2, 4 and 6 weeks, the soil was removed from the container and extracted with potassium chloride. Ammonium, nitrite and nitrate were analysed by means of a Technicon Autoanalyser.

It was stated in the report that nitrite levels in any sample were <0.1 mg/kg. Ammonium levels in control and treated soils were low (3.9-6.7 mg/kg on day 0, decreasing to 0.8-2.0 mg/kg after 4-6 weeks). Nitrate levels in control soils steadily increased from 8.1-11 mg/kg on day 0 to 58-69 mg/kg after 6 weeks. The reported evaluation was based on absolute nitrate values. Absolute nitrate-N values differed from untreated soil by ≤12 and ≤15% at any evaluation day for the treated loamy sand and loam soil, respectively. The OECD 216 guideline for the nitrification test states that evaluation should be based on nitrate formation rates. Results for pyriproxyfen based on nitrate formation rates are summarised in Table B.9.46.

Table B.9.46 Effect of pyriproxyfen on the nitrification rate in a sandy loam and a loam soil

treatment rate (mg a.s./kg soil d.w.)	Parameter	% deviation from control on week:				
		0	1	2	4	6
1.4 (loamy sand)	nitrate-N	n.a.	2	8	-18	63
14 (loamy sand)	nitrate-N	n.a.	-8	10	6	23
1.4 (loam)	nitrate-N	n.a.	-4	9	-34	-14
14 (loam)	nitrate-N	n.a.	-23	-6	-36	43

In the loamy sand soil, effects on the rate of nitrate-N formation were ≤18% within the first 4 weeks of incubation, but after 6 weeks increases relative to the untreated control of 63% and 23% were recorded in the 1.4 and 14 mg/kg treatment, respectively. In view of the inverse relationship with the dose, these differences are considered to be unrelated to the treatment with pyriproxyfen. The NOEC in this soil is 14 mg/kg. In the loam soil, effects on the rate of nitrate-N formation were ≤23% within the first 2 weeks of incubation, but after 4 weeks reductions relative to the untreated control of 34% and 36% were recorded in the 1.4 and 14 mg/kg treatment, respectively. After 6 weeks, a reduction of only 14% was observed in the 1.4 mg/kg treatment, and an increase of 43% in the 14 mg/kg treatment. As the test was not continued beyond 6 weeks for the 14 mg/kg treatment, in order to establish that the effect after 6 weeks was reversible, the NOEC in this soil is 1.4 mg/kg.

Comments by RMS

(1) Both soils were collected from agricultural research stations, but the report lacked documentation on the dates of collection of the soils in the field, the storage of the soils, and the history of the soil. Although the study was conducted in 1987, the reported data on soil characteristics were generated in 1980. Characterisation data generated in 1980, in particular pH and organic carbon content, may not be correct anymore for soils used seven years later. Unless the report is amended with data adequately addressing these issues, the study results cannot be accepted.
(2) The report did not clarify whether soil moisture was maintained at adequate levels throughout incubation. Nitrate formation however proceeded at a steady rate, suggesting that moisture levels were adequate.
(3) NB notifier calculated different nitrate formation rates and a different NOEC. This has not been checked by the RMS as the study is not acceptable anyway and new studies will be submitted.

Study 2

Reference	:	Vonk J.W. and van Dijk C.P. (1989)	GLP statement	:	Yes
type of study	:	Soil microflora test (respiration)	guideline	:	Dutch guideline form A, H.4.1 (1983)
year of execution	:	1987-1988	acceptability	:	**not acceptable**
test substance	:	Pyriproxyfen, lot no. PTG 86012, purity 99.0%			

Data were submitted from a laboratory study on the effect of pyriproxyfen on respiration of a loamy sand and a loam soil.

Batches of a loamy sand soil (3.1, 7.9 and 84.5% particles in the <2, 2-50 and >50 µm size class, pH (KCl) 5.4, 4.5% organic carbon, 2 mm sieved) and of a loam soil (22.7, 40.6 and 26.7% particles in the <2, 2-50 and >50 µm size class, pH (KCl) 7.3, 2.1% organic carbon, 2 mm sieved) were adjusted to 0.32 bar moisture content (pF = 2.5) and pre-incubated for 1 week at ambient temperature. Aliquots (200 g wet weight) were dispensed into conical flasks and treated with the test substance mixed with sand at 1.4 or 14 mg/kg d.w.. Control soil was left untreated. Two tests were conducted, one with and another one without amendment of soils with lucerne meal (0.5% w/w). The soil was adjusted to pF 2.5, and the flasks (4 per treatment, 3 for the control) were connected to a soil respiration apparatus (incubation for 30 days in the dark at 20±2°C). Carbon dioxide-free humidified air was continuously passed through each flask. Evolution of carbon dioxide was measured by a CO_2-analyser every two hours. Reported evaluation was based on the total amount of CO_2 evolved every 24 hours.

In control loamy sand and loam soil, respectively, unamended with lucerne, the amounts of CO_2 evolved were 4.8 and 22.9 mL CO_2/kg soil d.w. on day 1 and increased throughout the study to 181 and 246 mL/kg soil on day 29. In control loamy sand and loam soil, respectively, amended with lucerne, the amounts of CO_2 evolved were 111 and 150 mL CO_2/kg soil d.w. on day 1 and increased throughout the study to 1301 and 1287 mL/kg soil on day 29. Short-term respiration of treated unamended and amended soil, defined as the total amounts of CO evolved at a particular sampling day, differed from untreated soil by ≤9% at all sampling points and for both soils. None of the differences between treated and control soil were statistically significant at the 5% level.

Comments by RMS

(1) Both soils were collected from agricultural research stations, but the report lacked documentation on the dates of collection of the soils in the field, the storage of the soils, and the history of the soil. Although the study was conducted in 1987, the reported data on soil characteristics were generated in 1980. Characterisation data generated in 1980, in particular pH and organic carbon content, may not be correct anymore for soils used seven years later. For the study to be valid these issues should be adequately addressed.
(2) The report did not clarify whether soil moisture was maintained at adequate levels throughout incubation.
(3) The OECD 217 guideline on effects of test compounds on soil microbial respiration states that the short-term induced respiration rate should be determined in incubated soils after amendment with an appropriate amount of glucose (i.e. that amount giving the maximum response as determined in preliminary testing). In the above study, evaluation was based on comparison of the mean 24-hour respiration rate in incubated soil, without amendment with glucose. This procedure may not be sensitive enough to detect effects of test compounds. No reference substance with a known inhibitory effect on soil respiration was included to verify the sensitivity of the test procedure.
(4) Overall assessment: considering the comments in point (1) (soil history not reported; data on soil characteristics possibly not valid) and point (3) (test procedure disagrees with that of OECD 217, no reference substance included to verify sensitivity of test procedures), the study result is not accepted.

B.9.8.1.2 Toxicity of metabolites (IIA 8.5)

No data were submitted.

B.9.8.1.3 Toxicity of the plant protection product (IIA 8.5, IIIA 10.7)

No data were submitted.

B.9.8.2 Risk assessment

B.9.8.2.1 Risk assessment of the active substance

Two studies on the toxicity of pyriproxyfen were submitted, but found to be not acceptable. **The notifier is therefore requested to provide data for a risk assessment of the active substance for soil microflora (i.e. studies on respiration and nitrification (or statement to justify the validity of the submitted nitrification test)).**

In a **preliminary** risk assessment, the results of the unacceptable studies are considered. Respiration of a loamy sand and a loam soil treated with pyriproxyfen up to 14 mg a.s./kg soil did not differ from untreated soils by greater than 25% after 28 days. The NOEC for nitrification of a loamy sand soil treated with pyriproxyfen was 14 mg a.s./kg soil, but that of a treated loam soil was 1.4 mg a.s./kg, based on effects >25% at 14 mg a.s./kg after 6 weeks (last sampling point).

The worst case initial soil PEC of pyriproxyfen (0.060 mg a.s./kg, section B.8.3) is a factor of 23 below the highest test concentration of pyriproxyfen without lasting effects on soil microbial activity (1.4 mg a.s./kg). Based on this **preliminary** assessment, the risk of pyriproxyfen for soil microflora would be acceptable.

B.9.8.2.2 Risk assessment of metabolites

In laboratory incubated soil, the maximum level of the main degradation products 4'-OH-pyriproxyfen and PYPAC in any of the investigated soils was 6.3% and 8.6% AR, respectively. No separate studies on the effects of metabolites of pyriproxyfen to soil micro-organisms have been submitted. The effects of 4'-OH-pyriproxyfen and PYPAC to soil micro-organisms are assumed to be covered by studies with parent pyriproxyfen, since the maximum percentage of formation of these metabolites in laboratory incubated soil was reached within 14 days. Reliable data on effects of pyriproxyfen on soil micro-organisms are however not available and this has been marked as a data requirement. Further data for these two metabolites are therefore not required.

Given the very low levels of the three other minor metabolites DPH-pyriproxyfen (≤0.5% AR), PYPA (≤0.8% AR) and 2-OH-PY (≤0.1% AR) the risk of these compounds to soil micro-organisms is considered to be low.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6, IIIA 10.8)

B.9.9.1 Screening tests

Study 1

Reference	:	Umeda K. (2001)	GLP statement	:	no
type of study	:	Insecticidal activity	guideline	:	
year of execution	:	2001	acceptability	:	acceptable as supplementary information
test substance	:	Pyriproxyfen (100 g/L EC), batch C611, 10%			

In a screening study, small brown planthopper (*Laodelphax striatellus*), two-spotted spider mite (*Tetranychus urticae*) and common mosquito (*Culex pipiens pallens*) were exposed to Pyriproxyfen (100 g/L EC) and to a control.
Planthoppers (10 adults) were exposed to dried residues on sprayed potted rice plants (500 mg a.s./L) for two days. Mortality in control and treated planthoppers was 0%.
Mites (20-30 nymphs and adults), situated on potted kidney bean plants, were sprayed with pyriproxyfen (500 mg a.s./L). Damage of leaves, assessed after 7 days, was >80% for control and treated plants, indicating a high activity of the two-spotted spider mite.
Common mosquitos (20 last-instar larvae) were immersed in a solution of 3.5 mg a.s./L. Mortality, assessed one day after treatment, was <10% in control and treated mosquitos.

Results are summarised in Table B.9.47.

Table B.9.47 The toxicity of pyriproxyfen (100 g/l EC) to insects

Species	Application (g a.s./L)	NOEC (g a.s./L)	EC50 (g a.s./L)	Test guideline	Ref
L. striatellus	0.5	0.5	>0.5	-	Umeda K., 2001
T. urticae	0.5	0.5	>0.5		
C. pipiens pallens	0.0035	0.0035	> 0.0035		

The study was not GLP compliant or in accordance with a guideline. The study result is therefore not taken to the List of Endpoints, but may be considered as supplementary information.

Study 2

reference	:	Oguri Y. (2001)	GLP statement	:	no
type of study	:	Fungicidal activity	guideline	:	
year of execution	:	2001	acceptability	:	acceptable as supplementary information
test substance	:	Pyriproxyfen (100 g/L EC), batch C611, 10%			

In a screening study, seedlings of cucumber, tomato, apple, peanut and Japanese radish were treated with pyriproxyfen at a nominal dose of 500 mg a.s./L and an untreated control (100 g/L EC). Before or after spraying, control and treated plants were inoculated with fungal pathogens. After 3-14 days of incubation the disease control on the seedlings was scored using indices (0, 1, 2, 3 and 4: size of infected area >75%, 50-75%, 30-50%, 10-30%, <10% of that in untreated plot, respectively; 5, infected area is not observed at all).

The results are summarised in Table B.9.48.

Table B.9.48 The effects of pyriproxyfen to fungi

host	pathogen	Index
Cucumber	Cucumber gray mold (*Botrytis cinerea*) Cucumber anthracnose (*Colletotrichum lagenarium*) Cucumber downey mildew (*Pseudopernospora cubensis*)	0 0 0
Tomato	Tomato late blight (*Phytophthora infestans*)	0
Apple	Apple scab (*Venturia inaequalis*)	0
Peanut	Peanut brown spot (*Mycosphaerella arachidicola*)	0
Japanese radish	Japanese radish bacterial soft rot (*Erwinia aroideae*)	0

Pyriproxyfen (100 g/l EC) did not show a fungicidal activity.

The studies were not GLP compliant or in accordance with a guideline. The study result is therefore not taken to the List of Endpoints, but may be considered as supplementary information.

Study 3

reference	:	Mito N. (2001)	GLP statement	:	no
type of study	:	Herbicidal activity	guideline	:	
year of execution	:	2001	acceptability	:	acceptable as supplementary information
test substance	:	Pyriproxyfen (100 g/L EC), batch C611, 10%			

In a screening study, Pyriproxyfen (100 g/L EC) was applied to barnyard grass (*Echinochloa crus-galli*), oat (*Avena sativa*), velvetleaf (*Abutilon theophrasti*) and radish (*Raphanus sativus*) 7 days after seeding at a nominal dose of 8000 g a.s./ha. A negative control was included. Each treatment was tested in one replicate containing plants of one species, which were kept in a greenhouse. Temperature was 25°C (by night) to 30°C (by day). Assessments of herbicidal activity were made19 days after treatment using an injury scale (0: no injury, to 10: complete mortality). No effects were observed on oat, while very weak effects were observed on barnyard grass and velvetleaf and weak effects were observed on radish.

Results are summarised in Table B.9.49.

Table B.9.49 The effects of Pyriproxyfen (100 g/L EC) to plants

	INDEX	
	Control	Pyriproxyfen 8000 g a.s./ha
Barnyard grass	0	1
Oat	0	0
Velvetleaf	0	1
Radish	0	2

Pyriproxyfen (100 g/l EC) did not show any herbicidal activity.

The study was not GLP compliant. The study result is therefore not taken to the List of Endpoints, but may be considered as supplementary information.

B.9.9.2 Risk assessment

According to Annex II of 91/414, data from preliminary tests used to assess the biological activity and the possible impact on non-target species, both flora and fauna, should be provided. Since no specific data requirements are given, procedures for risk assessment were conducted in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). According to the Working Document (Sanco/10329/2002 rev 2 final, 17 October 2002) assessment for plants is performed using ecotoxicological relevant endpoints for plants (germination, biomass production and survival) of at least 6 species from different taxa. Data were submitted of Tier I initial laboratory screening on 4 plant species, 7 fungal species and 3 insect species. Table B.9.50 below summarises the results.

Table B.9.50 Summary of effects of Pyriproxyfen 100 g/L EC on non-target organisms during laboratory screening studies

Species	treatment	endpoint	NOER or NOEC (g a.s./ha or mg a.s./L)	ER50 or EC50 (g a.s./ha or mg a.s./L)
Plants				
Barnyard grass	Post-emergence	Damage/mortality	≥8000	>8000
Oat	Post-emergence	Damage/mortality	≥8000	>8000
Velvetleaf	Post-emergence	Damage/mortality	≥8000	>8000
Radish	Post-emergence	Damage/mortality	≥8000	>8000
Insects				
Small brown planthopper	Dried residues on plants	Mortality	≥500 mg a.s./L	>500 mg a.s./L
Two-spotted spider mite	Sprayed while on plants	Insect activity	≥500 mg a.s./L	>500 mg a.s./L
Common mosquito	Immersion in solution	Mortality	≥3.5 mg a.s./L	>3.5 mg a.s./L
Fungi				
Cucumber gray mold	Sprayed plants	Disease control	≥500 mg a.s./L	>500 mg a.s./L
Cucumber anthracnose	Sprayed plants	Disease control	≥500 mg a.s./L	>500 mg a.s./L
Cucumber downey mildew	Sprayed plants	Disease control	≥500 mg a.s./L	>500 mg a.s./L
Tomato late blight	Sprayed plants	Disease control	≥500 mg a.s./L	>500 mg a.s./L
Apple scab	Sprayed plants	Disease control	≥500 mg a.s./L	>500 mg a.s./L
Peanut brown spot	Sprayed plants	Disease control	≥500 mg a.s./L	>500 mg a.s./L
Japanese radish bacterial soft rot	Sprayed plants	Disease control	≥500 mg a.s./L	>500 mg a.s./L

A Tier I assessment is performed according to the recommendations in the Working Document (Sanco/10329/2002). A preliminary risk assessment is based on the available data of initial laboratory screening. The risk is considered acceptable if less than 50% effect is observed at

the maximum application rate (191.3 g a.s./ha, calculated from two applications at the rate of 112.5 g a.s./ha with a 10-day interval). Effects were well below 50% in all four plant species at a dose of 8000 g a.s./ha, a dose 42 times higher than the maximum application rate. The number of species tested is less than six, but pyriproxyfen is an insect hormone antagonist and not a herbicide, and herbicidal effects are not anticipated. Therefore, the likelihood for terrestrial plant effects is considered to be low, and further testing on plants is not required. The risk for plants is considered to be acceptable.

The maximum spray liquid concentration for the proposed Pyriproxyfen 10EC formulation is 150 mg a.s./L. No effects >50% were observed in screening studies with fungi (7 species) and insects (2 species) at a dose of 500 mg a.s./L. Therefore, the risk for fungi and insects is considered to be acceptable.

B.9.10 Effects on biological methods for sewage treatment (IIA 8.7)

B.9.10.1 Effects of the active substance

reference	:	L'Haridon J. (2001)	GLP statement	:	yes
type of study	:	Activated sludge, respiration inhibition test	guideline	:	OECD 209
year of execution	:	2000	acceptability	:	acceptable
test substance	:	Pyriproxyfen, batch 00303G, purity 99.7%			

The effect of pyriproxyfen (99.7% purity) on the rate of respiration of bacterial populations from activated sludge was determined after a contact time of 3 hours. Pyriproxyfen was added to the test vessels as a suspension in dechlorinated water (1, 3.16 and 10 mg a.s./L) or by direct addition (31.6 and 100 mg a.s./L) and toxicity was tested at 20-23°C in one flask/concentration, with untreated controls in duplicate flasks. The sludge was obtained from a water treatment works containig effluent from predominantly domestic origin (final concentration in test 1.6 g solids/L). At the end of the contact time, the oxygen consumption was measured for 10 minutes.

After 3 hours, the respiratory rate in the duplicate control flasks was 44.0 and 45.0 mg O_2/L/hour. At 100 mg a.s./L, the inhibition percentage of the respiratory rate was 2%. The EC50 is >100 mg a.s./L.

The concurrently determined EC50 of the reference product 3,5-dichlorophenol was in the acceptable range of 5 to 30 mg/L.

The study was conducted according to OECD 209 and in compliance with GLP.

B.9.10.2 Effects of the plant protection product

No data were submitted.

B.9.10.3 Risk assessment

Given the nature of the proposed uses, it is likely that pyriproxyfen can reach sewage treatment works. The compound caused no inhibition of the bacterial respiratory rate in the study submitted (EC50 >100 mg a.s./L). Consequently the risk to sewage treatment processes is acceptable.

B.9.11 References relied on

References for the active substance

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.1.1/01	Roberts, N.L., Hakin, B.	1989	The avian single-dose oral LD_{50} study of S-31183 to the mallard duck Sumitomo Chemical Co., Ltd. Report No. NNW-91-0027 GLP, Unpublished	N	SUM
IIA, 8.1.1/02	Roberts, N.L., Hakin, B., Rodgers, M.	1989	The avian single-dose oral LD_{50} study of S-31183 to the bobwhite quail Sumitomo Chemical Co., Ltd. Report No. NNW-91-0028 GLP, Unpublished	N	SUM
IIA, 8.1.2/01	Roberts, N.L., Hakin, B., Howlett, C., Anderson, A., Dawe, I.S.	1988a	A dietary LC_{50} study on S-31183 to the mallard duck Sumitomo Chemical Co., Ltd. Report No. NNW-91-0025 GLP, Unpublished	N	SUM
IIA, 8.1.2/02	Roberts, N.L., Hakin, B., Howlett, C., Anderson, A., Dawe., I.S.	1988b	A dietary LC_{50} study on S-31183 to the bobwhite quail Sumitomo Chemical Co., Ltd. Report No. NNW-91-0026 GLP, Unpublished	N	SUM
IIA, 8.1.3/01	Beavers, J.B., Sipler, O., Marselas, G.A., Jaber, M.	1994a	Sumilarv T.G.; A reproduction study with the mallard Sumitomo Chemical Co., Ltd. Report No. NNW-41-0115 GLP, Unpublished	Y	SUM
IIA, 8.1.3/02	Beavers, J.B., Sipler, O., Marselas, G.A., Jaber, M.	1994b	Sumilarv T.G.; A reproduction study with the bobwhite Sumitomo Chemical Co., Ltd. Report No. NNW-41-0114 GLP, Unpublished	Y	SUM
IIA, 8.2.1/01	Bowman, J.H.	1989a	Acute flow-through toxicity of Sumilarv to rainbow trout (*Salmo gairdneri*) Sumitomo Chemical Co., Ltd. Report No. NNW-91-0035 GLP, Unpublished	N	SUM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.2.1/02	Bowman, J.H.	1989b	Acute flow-through toxicity of Sumilarv to bluegill (*Lepomis macrochirus*) Sumitomo Chemical Co., Ltd. Report No. NNW-91-0034 GLP, Unpublished	N	SUM
IIA, 8.2.1/03	Putt, A.E.	2000a	PYPAC - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static conditions Sumitomo Chemical Co. Ltd., Report No. NNW-0141 GLP, Unpublished	Y	SUM
IIA, 8.2.1/04	Putt, A.E.	2000b	4'-OH-Pyriproxyfen - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions Sumitomo Chemical Co. Ltd., Report No. NNW-0143 GLP, Unpublished	Y	SUM
IIA, 8.2.2.2/01	Rhodes, J.E., Cramer, D.	1991	Early life-stage toxicity of Sumilarv technical to the rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions Sumitomo Chemical Co., Ltd. Report No. NNW-11-0062 GLP, Unpublished	Y	SUM
IIA, 8.2.3/01	Pate, H.O., McIntyre, D. O., Maciorowski, A.F.	1993	Uptake, depuration and bioconcentration of ^{14}C-pyriproxyfen in bluegill sunfish Sumitomo Chemical Co., Ltd. Report No. NNM-31-0027 GLP, Unpublished	Y*	SUM
IIA, 8.2.3/02	Steginsky C. A., Powell, J. M., Chang, J. S.	1994	Characterization of ^{14}C-residues in bluegill sunfish treated with ^{14}C-pyriproxyfen Sumitomo Chemical Co., Ltd. Report No. NNM-41-0031 GLP, Unpublished	Y	SUM
IIA, 8.2.3/03	Fujisawa, T., Nambu, K., Nishioka, K., Takimoto, Y.	1999	Calculation of ^{14}C-pyriproxyfen clearance time (CT_{90}, CT_{95}) in bluegill sunfish Sumitomo Chemical Co., Ltd. Report No. NNM-0061 Not GLP, Unpublished	Y	SUM
IIA, 8.2.4/01	Burgess, D.	1989	Acute flow-through toxicity of Sumilarv to *Daphnia magna* Sumitomo Chemical Co., Ltd. Report No. NNW-91-0036 GLP, Unpublished	N	SUM
IIA, 8.2.4/02	Putt, A.E.	2000c	PYPAC - Acute toxicity to daphnids (*Daphnia magna*) under static conditions Sumitomo Chemical Co., Ltd. Report No. NNW-0140 GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.2.4/03	Putt, A.E.	2000d	4'-OH-Pyriproxyfen - Acute toxicity to water fleas, (*Daphnia magna*) under flow-through conditions Sumitomo Chemical Co., Ltd. Report No. NNW-0144 GLP, Unpublished	Y	SUM
IIA, 8.2.5/01	Blakemore, G.C., Butzlaff, T.S., Stuerman, L.	1992	Chronic toxicity of ^{14}C-Sumilarv to *Daphnia magna* under flow-through test conditions Sumitomo Chemical Co., Ltd. Report No. NNW-21-0075 GLP, Unpublished	N	SUM
IIA, 8.2.5/02 8.2.7/01	Putt, A.E.	2003	Pyriproxyfen - The full life-cycle toxicity to midge (*Chironomus riparius*) under static conditions Sumitomo Chemical Co., Ltd. Report No. NNW-0157 GLP, Unpublished	Y	SUM
IIA, 8.2.5/07	Machado, M.W.	1995	Sumilarv (pyriproxyfen) - Chronic toxicity to mysids (*Mysidopsis bahia*) under flow-through test conditions Sumitomo Chemical Co., Ltd. Report No. NNW-51-0119 GLP, Unpublished	Y	SUM
IIA, 8.2.6/01	Blasberg, J.W., Hicks, S.L., Cramer, D.L.	1991	Acute toxicity of pyriproxyfen to *Selenastrum capricornutum* Prinz Sumitomo Chemical Co., Ltd. Report No. NNW-11-0068 GLP, Unpublished	N	SUM
IIA, 8.2.6/02	Hoberg, J.R.	2000a	PYPAC - Toxicity to the freshwater green alga, *Pseudokirchneriella subcapitata* Sumitomo Chemical Co., Ltd. Report No. NNW-0142 GLP, Unpublished	Y	SUM
IIA, 8.2.6/03	Hoberg, J.R.	2000b	4'-OH-Pyriproxyfen - Toxicity to the freshwater green alga, *Pseudokirchneriella subcapitata* Sumitomo Chemical Co., Ltd. Report No. NNW-0145 GLP, Unpublished	Y	SUM
IIA, 8.2.8/01	Hoberg, J.R.	1996	Pyriproxyfen - Toxicity to duckweed, *Lemna gibba* Sumitomo Chemical Co., Ltd. Report No. NNW-0126 GLP, Unpublished	Y	SUM
IIA, 8.3.1.1/01	Hoberg, J.R.	2001	Pyriproxyfen - Acute contact and oral toxicity tests with honey bees (*Apis mellifera*) Sumitomo Chemical Co. Ltd., Report No. NNW-0149 GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.3.2/01 10.5.1/01	Nienstedt, K.M.	2001a	Pyriproxyfen 10% EC: Dose response toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Sumitomo Chemical Co. Ltd., Report No. NNW-0147 GLP, Unpublished	Y	SUM
IIA, 8.3.2/02 10.5.1/02	Nienstedt, K.M.	2001b	Pyriproxyfen 10% EC: Dose response toxicity test with the predacious mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Sumitomo Chemical Co. Ltd., Report No. NNW-0146 GLP, Unpublished	Y	SUM
IIA, 8.3.2/03 10.5.1/03	Kollmann, S.I.	2003	Pyriproxyfen 10% EC: Dose response toxicity test with the predatory bug, *Orius laevigatus* (Heteropterai: Anthocoridae) Sumitomo Chemical Co., Ltd. Report No. NNW-0158 GLP, Unpublished	Y	SUM
IIA, 8.3.2/04 10.5.1/04	Nienstedt, K.M.	2003a	Pyriproxyfen 10% EC: Aged residue toxicity test on grapevines held under semi-field conditions exposing *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) under laboratory conditions Sumitomo Chemical Co. Ltd., Report No. NNW-0165 GLP, Unpublished	Y	SUM
IIA, 8.3.2/05 10.5.1/05	Nienstedt, K.M.	2003b	Pyriproxyfen 10% EC: Aged residue toxicity test on grapevines held under semi-field conditions exposing *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) under laboratory conditions Sumitomo Chemical Co. Ltd., Report No. NNW-0166 GLP, Unpublished	Y	SUM
IIA, 8.4.1/01	Van Dijk, A.	1988	Acute toxicity (LC_{50}) study of S-31183 to earthworms Sumitomo Chemical Co., Ltd. Report No. NNW-71-0012 GLP, Unpublished	N	SUM
IIA, 8.6/01 10.8/01	Umeda, K.	2001	Evaluation of insecticidal activity of pyriproxyfen Sumitomo Chemical Co., Ltd., Report No. NNG-0072 Not GLP, Unpublished	Y	SUM
IIA, 8.6/02 10.8/02	Oguri, Y.	2001	Evaluation of fungicidal activity of pyriproxyfen Sumitomo Chemical Co., Ltd., Report No. NNG-0073 Not GLP, Unpublished	Y	SUM
IIA, 8.6/03 10.8/03	Mito, N.	2001	Evaluation of herbicidal activity of pyriproxyfen Sumitomo Chemical Co., Ltd., Report No. NNG-0071 Not GLP, Unpublished	Y	SUM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA, 8.7/01	L'Haridon, J.	2001	Activated sludge, respiration inhibition test Sumitomo Chemical Co., Ltd., Report No. NNW-0148 GLP, Unpublishe	Y	SUM

References for the plant protection product

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA, 10.2.1/01	Sword, M.C., Stratton, J.	1992	Static acute toxicity of S-71639 10EC to rainbow trout (*Oncorhynchus mykiss*) Sumitomo Chemical Co., Ltd., Report No. NNW-21-0076 GLP, Unpublished	Y	SUM
IIIA, 10.2.1/02	Blakemore, G.C., Veltri, J.S.	1992	Acute toxicity of S-71639 10EC to *Daphnia magna* Sumitomo Chemical Co., Ltd., Report No. NNW-21-0096 GLP, Unpublished	Y	SUM
IIIA, 10.2.1/03	Blasberg, J.W., Hicks, S.L., Stratton, J.	1992	Acute toxicity of S-71639 10EC to *Selenastrum capricornutum* Printz Sumitomo Chemical Co., Ltd., Report No. NNW-21-0077 GLP, Unpublished	Y	SUM
IIIA, 10.2.2/02	Van Wijngaarden, R.P.A.	2004	Pyriproxyfen 10EC: Plankton-dominated microcosm experiment Sumitomo Chemical Co., Ltd., Report No. NNW-0171 GLP, Unpublished	Y	SUM
IIIA, 10.4.1/01	Hoberg, J.R.	2002	Pyriproxyfen 10% EC - Acute contact and oral toxicity tests with honey bees (*Apis mellifera*) Sumitomo Chemical Co., Ltd., Report No. NNW-0153 GLP, Unpublished	Y	SUM
IIIA, 10.4.4/02	Barth, M.	2003	Effects of pyriproxyfen 10% EC on the honeybee *Apis mellifera* L. under field conditions with additional assessments on colony and brood development Sumitomo Chemical Co., Ltd., Report No. NNW-0167 GLP, Unpublished	Y	SUM