Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

Pyriproxyfen (ISO)

2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)pyridine; 4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether

EC Number: **429-800-1**

CAS Number: **95737-68-1**

Index Number: **613-303-00-3**

Contact details for dossier submitter:

Bureau REACH
National Institute for Public Health and the Environment (RIVM)
The Netherlands
bureau-reach@rivm.nl

Version number: 2.0 **Date: January 2023**

CONTENTS

1 PHYSICAL HAZARDS

1.1 Explosives

1.1.1. Bates, M. (2001) Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10 A14, A15)

Study reference:

Bates, M. (2001) Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10, A14, A15). Sumitomo Chemical Co., Ltd., Unpublished report No.: NNP-0091

Test type

Theoretical assessment and DSC testing, GLP

Detailed study summary and results:

The purpose of the study was the determination of the explosive properties of the test article. The explosive properties of pyriproxyfen were evaluated by consideration of the chemical structure and associated thermodynamic properties by differential scanning calorimetry (DSC).

It is concluded that pyriproxyfen has no explosive properties by theoretical and DSC assessment.

Material and methods

A. Materials:

1.Test article:	Pyriproxyfen
Lot/Batch No.:	80301G
Purity:	97.9%
CAS No.:	95737-68-1

B. Study Design and Methods:

1.In life dates:	10 May 2001 to 18 May 2001
2.Method:	*Consideration of chemical structure* The oxygen balance, various structural parameters (bond groupings) and the exothermic decomposition energy are factors associated with explosive properties. The oxygen balance is calculated, if there is a deficiency of oxygen present, the balance is negative, while an excess of oxygen gives a positive balance and such compounds can act as oxidants or are explosive. Bond groupings known to confer explosive properties will be assessed, typically C-C unsaturated, C-metal, contiguous nitrogen atoms, contiguous oxygen atoms, N-O, N-halogens, O-halogens. *DSC* The exothermic decomposition energy can be measured using DSC. This is a process where a sample and reference are heated under tightly controlled conditions and heat flows into or out of the sample are accurately quantified. A high pressure sealed pan was used in the experiment which started at 20 °C and ramped at 20 °C/ min to 600 °C.

Results

A. Consideration of chemical structure

The test substance has the following molecular formula: $C_{20}H_{19}NO_3$. The calculated oxygen balance is -231.5%. The value is outside the region where there may be a potential for explosivity, and there are no explosive chemical functional groups present in the structure.

B. DSC testing

An initial melting endotherm around 58 °C was observed, followed by a rising baseline from about 280 °C over which a second endotherm around 340 °C and a complex exotherm above approximately 450 °C. The enthalpy of the exotherms were 480 and 1150 J/g respectively but are not considered to be problematic as they are very broad.

Conclusion

Pyriproxyfen does not have explosive properties.

1.2 Flammable gases (including chemically unstable gases)

Pyriproxyfen is a solid at room temperature. Consequently it does not meet the criteria for classification of a flammable gas.

1.3 Oxidising gases

Pyriproxyfen is a solid at room temperature. Consequently it does not meet the criteria for classification of a oxidising gas.

1.4 Gases under pressure

Pyriproxyfen is a solid at room temperature. Consequently it does not meet the criteria for classification as gases under pressure.

1.5 Flammable liquid

Pyriproxyfen is a solid at room temperature. Consequently it does not meet the criteria for classification as a flammable liquid.

1.6 Flammable solids

1.6.1. Bates, M. (2001) Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10, A14, A15)

Study reference:

Bates, M. (2001) Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10, A14, A15). Sumitomo Chemical Co., Ltd., Unpublished report No.: NNP-0091

Test type

EC A.10, GLP

Detailed study summary and results:

The purpose of the study was for the determination of the flammability of the test article. The test article, as supplied was made into a pile, 250 mm long. An attempt to ignite the sample under defined conditions and the burning time were measured.

It is concluded that pyriproxyfen was not flammable under the experimental conditions.

Material and methods

A. Materials:

1.Test article:	Pyriproxyfen
Lot/Batch No.:	80301G
Purity:	97.9%
CAS No.:	95737-68-1

B. Study Design and Methods:

1.In life dates:	10 May 2001 to 18 May 2001
2.Method:	An initial evaluation was performed by forming a small amount of the ground test substance into a cone on a non-combustible, non-porous and low heat conducting (silica) base plate. A flame from a gas burner was applied to the pile and observations made. A full preliminary test (train test) was then performed, wherein test substance was formed into a train 250 mm long by 20 mm wide by 10 mm high and subject to a similar procedure. Due to the low melting point of the test substance, the moisture content was not determinable.

Results

In the initial evaluation, the test substance melted to a liquid which did not ignite. A few sparks of flame and a little white smoke were seen. On cooling, a colourless liquid remained. Closely similar observations were made during the train test. This means that the substance is not classified as highly flammable.

Conclusions

It is concluded that pyriproxyfen was not flammable under the experimental conditions.

1.7 Self-reactive substances

No study conducted. The compound is not explosive, not highly flammable and not oxidising. In addition there are no chemical groups present in the molecule associated with explosive or self-reactive properties (e.g. azides, N-nitroso compounds, aromatic sulfohydrazide). Therefore the substance is not expected to be self-reactive. Consequently it does not meet the criteria for classification as self-reacting.

1.8 Pyrophoric liquids

Pyriproxyfen is a solid at room temperature. Consequently it does not meet the criteria for classification of a pyrophoric liquid.

1.9 Pyrophoric solid

No study conducted. Pyriproxyfen has been handled extensively in air and has never self-ignited. From the chemical structure pyriproxyfen is not expected to be pyrophoric. Consequently it does not meet the criteria for classification as a pyrophoric substance.

1.10 Self-heating substances

1.10.1. Bates, M. (2001) Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10, A14, A15)

Study reference:

Bates, M. (2001) Pyriproxyfen: determination of the physico-chemical properties (92/69/EEC tests A10, A14, A15). Sumitomo Chemical Co., Ltd., Unpublished report No.: NNP-0091

Test type

EC A.15, GLP

Detailed study summary and results:

The purpose of the study was for the determination of the self-heating properties of the test article. The test substance was melted and a known amount of the test substance was injected into a heated open round bottom flask containing air. The contents of the flask were observed until ignition observed. Ignition was not observed up to 400 °C.

It is concluded that pyriproxyfen is not a self-heating substance under the experimental conditions.

Material and methods

A. Materials:

1.Test article:	Pyriproxyfen
Lot/Batch No.:	80301G
Purity:	97.9%
CAS No.:	95737-68-1

B. Study Design and Methods:

1.In life dates:	10 May 2001 to 18 May 2001
2.Method:	The experimental procedure conformed to the requirement of EC A.15. The test substance was melted and the required amounts of material to be tested were added to the test flask by syringe, taking care to avoid the walls of the flask during addition and a timer started. The timer was stopped when a flash or flame was observed, the temperature and lag time were recorded. The test was repeated with different sample amounts until the minimum value of the ignition temperature was obtained. Between each test, the flask was flushed with dry nitrogen. After flushing, a sufficient time interval was allowed before the next sample was added to ensure that the flask contents re-equilibrated with air, and were stabilised at the desired temperature.

Results

No ignition was observed up to 400 °C with 1 mL of test sample at an atmospheric pressure of 97.5 kPa.

Conclusion

In a standard study (EC A.15), pyriproxyfen did not exhibit an auto-ignition temperature up to 400 °C. In accordance with Guidance on the Application of the CLP criteria, substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced. The melting point of pyriproxyfen is 48 – 50 °C, therefore classification as a self-heating substance is not necessary. Consequently, it does not meet the criteria for classification as a self-heating substance.

1.11 Substances which in contact with water emit flammable gases

1.11.1 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)

Study reference:

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., Unpublished report No.: NNP-0094

Test type

EC A.12, GLP

Detailed study summary and results:

The purpose of this study was for the determination of flammability of test item in contact with water upon evolution of flammable gases. The procedures conform to EC method A.12.

Pyriproxyfen was tested and shown not to produce flammable gases when in contact with water, and is considered not to be hazardous.

Material and methods

A. Materials:

1.Test article:	Pyriproxyfen
Lot/Batch No.:	80301G
Purity:	97.9%
CAS No.:	95737-68-1

B. Study Design and Methods:

1.In life dates:	15 August 2002 to 4 September 2002
2.Method:	**Test 1** A small quantity of the test material was placed in a trough containing distilled water, performed in triplicate. **Test 2** A filter paper was floated flat on the surface of distilled water in a suitable vessel. A small quantity of the test item was placed onto the filter paper, performed in triplicate. **Test 3** A pile of test substance was (approximately 2 cm high by 3 cm wide) was formed. Three drops of distilled water were dropped into an indentation made in the top of the pile, performed in triplicate.

Test 4
The test was performed at room temperature and atmospheric pressure. The test substance (10 g) was placed in a conical flask. Distilled water (20 mL) was placed in a blanced-pressure dropping funnel fitted above the conical flask with a side arm. The tap of the dropping funnel was opened to let water into the conical flask and any gas evolution was observed and recorded during a seven hour period, performed in triplicate.

Results

No gas was evolved in any of the tests undertaken.

Conclusion

Pyriproxyfen does not evolve flammable gases when in contact with water.

1.12 Oxidising liquids

Pyriproxyfen is a solid at room temperature. Consequently it does not meet the criteria for classification of an oxidising liquid.

1.13 Oxidising solids

1.13.1 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)

Study reference:

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., Unpublished report No.: NNP-0094

Test type

Theoretical assessment and DSC testing, GLP

Detailed study summary and results:

The purpose of the study was the determination of the oxidising properties of the test article. The oxidising properties of pyriproxyfen were evaluated by consideration of the chemical structure and associated thermodynamic properties by differential scanning calorimetry (DSC).

It is concluded that pyriproxyfen has no oxidising properties by theoretical and DSC assessment

Material and methods

A. Materials:

1.Test article:	Pyriproxyfen
Lot/Batch No.:	80301G
Purity:	97.9%
CAS No.:	95737-68-1

B. Study Design and Methods:

1.In life dates:	15 August 2002 to 4 September 2002

2.Method:

Consideration of chemical structure
The oxygen balance, various structural parameters (bond groupings) and the exothermic decomposition energy are factors associated with oxidising properties. The oxygen balance is calculated, if there is a deficiency of oxygen present, the balance is negative, while an excess of oxygen gives a positive balance and such compounds can act as oxidants or are explosive.
Bond groupings known to confer oxidising properties are described in Bretherick's Handbook of Reactive Chemical Hazards. The presence of such groupings in the chemical structure of the substance was considered.

DSC
The exothermic decomposition energy can be measured using DSC. This is a process where a sample and reference are heated under tightly controlled conditions and heat flows into or out of the sample are accurately quantified. A high pressure sealed pan was used in the experiment which started at 20 °C and ramped at 20 °C/ min to 600 °C.

Results

A. Consideration of chemical structure

The test substance has the following molecular formula: $C_{20}H_{19}NO_3$. The calculated oxygen balance is -231.5%. The value is outside the region where there may be a potential for oxidising capacity. The only oxygen-containing functional groups within the test substance structure are three ether links, which are not regarded as serious hazards for oxidising.

B. DSC testing

There are no sharp decomposition exotherms in the DSC trace.

Conclusion

Pyriproxyfen does not have oxidising properties.

1.14 Organic peroxides

No study conducted, pyriproxyfen is not an organic peroxide.

1.15 Corrosive to metals

No study conducted, based on a lack of redox potential, pyriproxyfen is not expected to be corrosive to metals.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1. CA 5.1.1/01 (1988a): Metabolism study in the rat

Report

CA 5.1.1/01 (1988a)

Metabolism of S-31183 in rats

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNM-80-0001

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment. No qualitative differences were apparent between sexes or dose levels. There was some slight evidence indicating repeated dosing in corn oil enhanced absorption, but the metabolism following repeat dosing was similar. There was no evidence of accumulation in tissues. At least 26 metabolites were detected in faeces and urine, of which 10 were identified. Oral absorption of pyriproxyfen in this study is considered to be 40%.

Characteristics

reference	:	CA 5.1.1/01, 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 ^{14}C-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 B.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 B.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 table B.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 B.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 B.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 B.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 B.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 B.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

*Repeat dose control study (14 daily doses of corn oil followed by a single oral dose of ^{14}C-pyriproxyfen)

Note: values in the table taken from the DAR, which corrected an error in the report.

Conclusions

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. Therefore absorption can be assumed to be ca. 37-39 of the applied dose, based on radiolabel recovered from urine, bile and tissues.

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.

2.1.2. CA 5.1.1/02 (1988b): Tissue distribution study in the rat

Report

CA 5.1.1/02 (1988b)

Metabolism of S-31183 in rats (tissue distribution study)

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNM-80-0002

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment. The group size (3/sex) was lower than the new requirement (4/sex, B.36 toxicokinetics). However, since multiple time points were examined in this study, it is considered not to affect the integrity of the study. After a single oral dose of 2 mg/kg bw, maximum concentration was reached in most tissues 2-8h after dosing, in fat Tmax was 12-24h. Radioactivity in blood, liver and kidney comprised a small amount of unchanged parent and up to seven metabolites.

Characteristics

reference	:	CA 5.1.1/02 1988b	exposure	:	Single by gavage
type of study	:	Distribution	doses	:	2 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	:	no
route	:	oral	guideline	:	Directive 88/302/EEC
species	:	Rat, CD, 7 weeks (at	acceptability	:	acceptable

		administration)
group size	:	3/sex

Study design

The study investigated the distribution of ^{14}C-pyriproxyfen after a single oral dose of 2 mg/kg bw. Seven groups of 3 rats/sex were treated and sacrificed 2, 4, 8, 12, 24, 48 and 72 hours after administration. At sacrifice blood was collected (pooled per sampling time/sex) and the following tissues and organs were taken from all animals: bone, brain, fat, heart, liver, kidney, lung, muscle, spleen, testis, uterus and ovaries. Tissue samples were combusted and radioactivity was measured by LSC. Pooled blood samples were centrifuged and extracted (2X acetone). Supernatant and extracts were radio-assayed by LSC and subjected to TLC co-chromatography for metabolite identification using different solvent systems (fractioning by TLC scraping and LSC). The unextractable fraction was combusted and measured by LSC. One gram samples of liver and kidney (pooled per sampling time) were homogenized (in acetone). The precipitate was extracted (2X acetone) and supernatant, extracts and the unextractable fraction were treated similar to blood samples to identify and quantify metabolites. Fat samples with maximal radioactivity values (M 24 hours; F 12 hours) were treated similar as liver and kidney samples. Polar metabolites were first identified by TLC co-chromatography.

Results

The distribution of radioactivity in tissues is presented in table B.6.1.1-5. Tissue concentrations in male and female rats were comparable. Relatively high concentrations were found in blood, fat, liver and kidney. The highest concentrations (4.5% and 3.6% of total radioactivity in males and females, respectively) were found in the liver 8 hours post-administration. In fat the highest amounts of radioactivity were found after 24 and 12 hours in males and females respectively. Based on the information available half life time was calculated for blood, fat, kidney and liver in males and for fat in females. For liver and kidney a biphasic elimination curve was derived with rapid elimination during the first 48 hours ($T_{1/2}$ 8-10 hours) and slower elimination from 48 to 168 hours ($T_{1/2}$ 23-35 hours). After 72 hours 0.3% of applied radioactivity was still present in fat (0.2% of applied radioactivity) and liver (0.1% of applied radioactivity). In all other tissues the amount of radio activity after 48 hours became ≤ 0.1%.

Table B.6.1.1-5 Maximum residual radioactivity in tissues and organs and elimination half live

Tissue	single oral, 2 mg/kg bw							
	M				F			
	Cmax (ng/g)	Cmax (% AR)	Tmax (h)	T1/2 (h)	Cmax (ng/g)	Cmax (% AR)	Tmax (h)	T1/2 (h)
blood	399	1.4	4	10	86	0.3	8	ne
bone	62	-	8	-	36		4	-
brain	40	<0.1	2	-	34	<0.1	4	-
fat	280	0.7	24	35	461	1.1	12	35
heart	107	<0.1	4	-	58	<0.1	4	-

kidney	395	0.2	8	10/35	158	0.1	4	ne
liver	2440	4.5	8	8/23	2130	3.6	8	ne
lungs	150	<0.1	8	-	60	<0.1	4	-
muscle	47	0.9	8	-	39	0.7	4	-
ovaries	-	-	-	-	109	<0.1	4	-
spleen	72	<0.1	2	-	45	<0.1	4	-
testes	70	<0.1	8	-	-	-	-	-
uterus	-	-	-	-	53	<0.1	8	-
total		7.3	8			5.2	8	0.1

ne not estimated

Results of the metabolite identification in blood, liver and kidney using TLC are presented in table B.6.1.1-6. Extractable activities amounted 52-91% of TRR. In blood 4 metabolites were identified, in kidney 5 and in liver 7. 5",4'-OH-pyriproxyfen sulfate was the main metabolite identified in blood and kidney in males, reaching a maximum after 4 to 8 hours post-administration. Amounts of this metabolite in blood and kidney of females were much lower. 4'-OH-pyriproxyfen sulfate was found in kidney of males and females (maximum between 4 and 8 hours). In the liver of both male and females 4'-OH-pyriproxyfen sulfate and 5",4'-OH-pyriproxyfen sulfate were identified as major metabolites (4'-OH-POPA sulfate was also found in substantial amounts). The concentration of 4'-OH-pyriproxyfen sulfate in blood and liver reached a maximum 2 hours after administration in males. Other metabolites in both sexes and 4'-OH-pyriproxyfen sulfate in females were at maximum 4-8 hours after administration. In samples taken 72 hours after administration metabolite levels were in general low. In fat radioactivity present was identified as the parent compound pyriproxyfen, no metabolites were identified.

There appears to be a sex related difference in blood and kidney probably caused by a difference in oxidation at the 5-position of the pyridine ring.

Table B.6.1.1-6 Metabolite identification (ng/g tissue) in blood, liver and kidney

Metabolites	blood				kidney				liver			
	M		F		M		F		M		F	
	Cmax (ng/g)	Tmax (h)	Cmax (ng/g)	Tmax (h)	Cmax (ng/g)	Tmax (h)	Cmax (ng/g)	Tmax (h)	Cmax (ng/g)	Tmax (h)	Cmax (ng/g)	Tmax (h)
Pyriproxyfen	8	2	10-12	2-24	39	2	27-28	2-4	63	2	40	2
4'-OH-pyriproxyfen					9	4	12	4	68	4	337	8
4'-OH-pyriproxyfen sulfate	14	2	1	8	80-77	4-8	43-45	4-8	770	2	493	8
4'- oxydiphenol sulfate	21	4	5	8	50	8	6-7	4-8	69	8	88	8
5",4'-OH-pyriproxyfen									21	8	8-9	4-8
5",4'-OH-pyriproxyfen sulfate	358	4	37	8	143-153	4-8	28-25	4-8	735	8	568	8
4'-OH-POPA[1]									11	8	15	8
4'-OH-POPA sulfate	24	4	12	8	31-34	4-8	26-30	4-8	138	8	162	8
unidentified	48	4	21	8	74	8	76-78	4-8	214	8	377	8
not extractable	54	4	14	8	94	8	48-47	4-8	863	4	406	4

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

Acceptability

The study is considered acceptable.

2.1.3. CA 5.1.1/03 (1993a): Metabolism study in the rat

Report

CA 5.1.1/03 (1993a)

Metabolism of [pyridyl-2,6-^{14}C]pyriproxyfen in rats (pyridyl (PY)-^{14}C-labeled test material, single oral administration at low and high doses)

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNM-30-0025

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment. No qualitative differences were apparent between sexes or dose levels. There was no evidence of accumulation in tissues. At least 10 metabolites were detected in faeces and urine, of which 9 were identified, accounting for >70% of a single low or high dose.

Characteristics

reference	:	CA 5.1.1/03, 1993a	exposure	:	Single by gavage
type of study	:	metabolism	doses	:	2 and 1000 mg/kg bw
year of	:	1992-1993	vehicle	:	Corn oil

execution					
test substances	:	Pyriproxyfen, lot no KY-1135, chemical purity 100% [Pyridyl-2,6-^{14}C] pyriproxyfen, lot no C-92-001A(C-90-061), radiochemical purity >99%,chemical purity >99%, specific activity 115 mCi/mmol [Phenoxyphenyl-^{14}C] pyriproxyfen, lot no C-92-036A(C-90-058), radiochemical purity >98%,chemical purity >98%, specific activity 73.9 mCi/mmol	GLP statement	:	yes
route	:	oral	guideline	:	EPA 85-1, Directive 88/302/EEC
species	:	Rat, Sprague-Dawley CD, 7 weeks (at administration)	acceptability	:	acceptable
group size	:	See table 6.1.1.1			

Study design

The study investigated the distribution, metabolism and excretion of [pyridyl-2,6-^{14}C] pyriproxyfen after a single oral dose of 2 and 1000 mg/kg bw (study A and B). For comparison of the metabolic profile, male rats were treated with [phenoxyphenyl-^{14}C] pyriproxyfen at a single dose of 2 mg/kg bw (study C). Exposure and sampling of urine, faeces, expired air, cage wash and tissues was as described in table B.6.1.1-7.

Table B.6.1.1-7 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 [pyridyl-2,6-^{14}C] pyriproxyfen	6 hours: urine; day 1, 2, 3, 5 and 7: urine, cage-wash and faeces; day 1 and 2: expired air	Day 7[1]
B (high dose)	5	Single oral dose at 1000 mg/kg bw [pyridyl-2,6-^{14}C] pyriproxyfen	6 hours: urine; day 1, 2, 3, 5 and 7: urine, cage-wash and faeces; day 1 and 2: expired air	Day 7[1]
C (low dose)	2 males	Single oral dose at 2 mg/kg bw [phenoxyphenyl-^{14}C] pyriproxyfen	Day 1 and 2: urine and faeces	Day 2

[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, pancreas, thyroid, adrenal, testis/uterus, ovaries and the remaining carcass.

At termination, blood and selected tissues and organs (see footnote 1 of table B.6.1.1-7) were collected from the animals of study A-B. Clinical signs were observed daily and body weights were determined before administration of the test substance.

In studies A and B, tissues (2 samples of ca. 200 mg) and carcass were combusted and analysed by LSC. Blood was centrifuged and fractions of cells and plasma were subjected to combustion/LSC. Faeces of individual rats at each collection time (day 0-2) were extracted (2X acetone). Extracts were combined per rat and analysed by TLC and HPLC for metabolite identification (details see below). Residues were combusted and analysed by LSC. The 3-5 and 5-7 day faeces of individual rats were homogenised (water) and combusted for radio-assay. Radioactivity in urine and cage-washings of individual rats on each collection time (day 0-3) were individually assayed by LSC. The 3-5 and 5-7 day urine and cage-wash samples were combined per rat per collection time and measured by LSC. Urine sampled during day 0-2 was combined per rat and subjected to TLC and HPLC analyses for metabolites identification (details see below). For study C combined 0-2 day faecal and urine samples (per rat) were analysed by TLC and HPLC. Trapped expired air (10% NaOH) was radio-assayed by LSC.

Metabolite identification:

Faecal extracts (day 0-2) were fractionated (methanol, acetonitrile, acetic acid and water) by HPLC. Urine samples (day 0-2) of study B were fractionated (water/acetonitrile/acetic acid) by HPLC. Metabolites from (sub)fractions were isolated by TLC and polar metabolites were subjected to enzymatic hydrolysis.

Results

On day 1 loose stool/diarrhoea was observed in all animals at 1000 mg/kg bw. No other treatment related symptoms were observed.

Data on excretion are presented in table B.6.1.1-8. At the end of the study (7 days post-dosing), overall excretion for all groups was found to be 93-99% AR.

After a single oral dose of 2 and 1000 mg/kg bw, radioactivity recovered after 7 days (range for males and females) represented 6-12% AR (urine and cage wash) and 85-93% AR (faeces). Excretion within the first 24 hours after dose administration was essentially 54-70% AR. The amount of radioactivity retained in tissues and residual carcass of male and female rats was low (0.1-0.2% AR).

There were no remarkable differences between patterns of absorption and excretion of sexes.

During the first day no clear difference between excretion of [pyridyl-2,6-^{14}C] and [phenoxyphenyl-^{14}C] labelled pyriproxyfen was found.

Table B.6.1.1-8 Excretion of radioactivity (% AR) in rats after single oral exposure to ^{14}C-pyriproxyfen

	period	**single oral, 2 mg/kg bw** [pyridyl-2,6-^{14}C]		**single oral, 1000 mg/kg bw** [pyridyl-2,6-^{14}C]		**single oral, 2 mg/kg bw** [phenoxyphenyl-^{14}C]
sample	(d)	M	F	M	F	M
urine	0-1	5	4	6	8	9

	0-7	6	5	8	12	
faeces	0-1	65	54	61	46	76
	0-7	86	93	89	85	
air	0-1	0.4	0.3	0.2	0.1	
	0-2	0.5	0.4	0.3	0.2	
tissues	7	0.2	0.1	0.2	0.2	
total	0-7	93	99	97	97	

The distribution of radioactivity in tissues and carcass for all groups 7 days post-dose is presented in table B.6.1.1-9. 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 oral dose of 2 mg/kg bw or 1000 mg/kg bw. Tissue concentrations were in general very low (<0.1% AR).

Table B.6.1.1-9 Residual radioactivity in tissues and organs in % AR and ng pyriproxyfen equivalents/gram tissue taken at 7 days post-radiolabelled-dose.

	single oral, 2 mg/kg bw				single oral, 1000 mg/kg bw			
	M		F		M		F	
tissue	**conc**	**% AR**	**conc**	**% AR**	**conc**	**% AR**	**conc**	**% AR**
adrenals	5	0	5	0	1300	0	1500	0
blood	2	0	2	0	1900	0	1200	0
- cells	4	-	3	-	2800	-	2000	-
- plasma	<1	-	<1	-	700	-	400	-
bone	<1	-	<1	-	400	-	300	-
brain	<1	0	<1	0	<200	-	300[1]	0
fat	14	0	15	0	6300	0	6000	0
heart	1[1]	0	<1	0	500	0	400	0
kidney	6	0	5	0	3100	0	2700	0
liver	9	0	7	0	4500	0	3300	0
lungs	2	0	2	0	900	0	600	0
muscle	1	-	<1	-	300	-	300	
ovaries	-	-	<4	-	-	-	800	0
pancreas	1	0	2	0	500	0	400	0
spleen	2	0	2	0	800	0	600	0
testes	<1	-	-	-	400	0	-	-
thyroid	<17	-	<23	-	395000[1]	0	<3100	-
uterus	-	-	2	0	-	-	400	0
carcass	2	0.1	1	0.1	1700	0.1	1800	0.1
total		0.2		0.1		0.2		0.2

1. data from one rat

Results of the metabolite identification in urine and faeces using HPLC/TLC are presented in table B.6.1.1-10.

In faeces 9 metabolites were identified and in urine 5. 4'-OH-pyriproxyfen was the major metabolite in faeces, representing on average 23-38% and 46-47% of the total administered radioactivity in males and females, respectively. The parent compound accounted for 33-35% of the total radioactivity administered in males and for 21-22% in females. In low dosed males 5",4'-OH-pyriproxyfen was identified in small amounts (7% AR). 2'-OH-pyriproxyfen was identified in faeces of low dose animals (1.8-2.8% AR) and to lesser extent in high dose animals (0.2% AR). All other metabolites were <2% of the administered radioactivity. Concentrations of conjugated metabolites (sulfate or glucuronide) were increased in the faeces of high dosed animals (1.1-1.6% AR) compared to low dose animals (0.2-0.4% AR).

In urine PYPAC (1.0-3.0% in males and 1.7-4.9% in females) and 4'-OH-pyriproxyfen (5.6% in females at 1000 mg/kg bw) were identified. Other metabolites were present in minor amounts (<4% of administered radioactivity). Pyriproxyfen and 4'-OH-pyriproxyfen were only detected in animals at 1000 mg/kg bw. According to the author of the report this was related to contamination of the urine with faeces during sampling caused by the presence of soft stool and diarrhea in the high dose group.

In general in urine 2-4% of radioactivity could not be identified. In faeces up to 14% of the administered radioactivity could not be identified (or was not extractable). In females hydroxylation at the 4'-position of the terminal phenyl ring was higher than in males in both dose groups. In males more of the parent compound was found than in females.

Table B.6.1.1-10 Urinary and faecal metabolite identification (% AR; mean for males and females)

	single oral, 2 mg/kg				single oral, 1000 mg/kg			
	urine		Faeces		urine		faeces	
Metabolites	M	F	M	F	M	F	M	F
Pyriproxyfen	ND	ND	35	21	1.3	2.7	33	22
5"-OH-pyriproxyfen	-	-	0.3	0.3	-	-	0.1	0.1
2'-OH-pyriproxyfen	-	-	1.8	2.8	-	-	0.2	0.2
4'-OH-pyriproxyfen	ND	ND	23	47	1.0	5.6	38	46
5",4'-OH-pyriproxyfen	-	-	7.2	1.2	-	-	0.3	0.4
DPH-pyriproxyfen[1]	-	-	0.8	1.1	-	-	1.6	1.2
PYPAC[2]	1.0	1.7	-	-	3.0	4.9	-	-
4'-OH-pyriproxyfen sulfate	0.4	0.3	0.4	0.4	0.2	0.8	1.6	1.2
4'-OH-pyriproxyfen glucuronide	-	-	0.3	0.2	-	-	1.1	1.1
5",4'-OH-pyriproxyfen sulfate	ND	ND	0.3	0.2	0.1	0.2	0.9	0.3
unidentified	4.1	3.0	3.9	2.6	2.9	2.4	0.4	0.5
not extractable	-	-	10	11	-	-	8.4	4.6
total RA chromatographed	5.5	5	83	88	8.5	17	86	78
total RA identified	1.4	2	69	74	5.6	14	77	73

1. DPH –pyriproxyfen = 4-hydroxyphenyl (RS)-2-(2-pyridyloxy)propyl ether
2. PYPAC = (RS)-2-(2-pyridyloxy)propionic acid

ND =not detected

Acceptability

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

2.1.4. CA 5.1.1/04 (1993b): Metabolism study in the rat

Report

CA 5.1.1/04 (1993b)

Metabolism of phenoxyphenyl-^{14}C-pyriproxyfen in rats (high dose, ^{14}C-concentrations in tissues)

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNM-30-0028

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment. The group size (3/sex) was lower than the new requirements of 4/sex. However, since the study examines multiple time points, this is considered not the affect the integrity of the study. Following a single oral dose of 1000 mg/kg bw, maximum concentration was reached 2-8 hours after dosing in most tissues and within 12-24h after dosing for fat. Thereafter, the radioactivity declined in all tissues.

Characteristics

reference	:	CA 5.1.1/04., 1993b	exposure	:	Single by gavage
type of study	:	Distribution	doses	:	1000 mg/kg bw
year of execution	:	1992-1993	vehicle	:	Corn oil
test substances	:	Pyriproxyfen, lot no KY-1135, chemical purity 100.0% [Phenoxyphenyl-^{14}C] pyriproxyfen, lot no C-92-036A(C-90-058), radiochemical purity >98%, specific activity 73.9 mCi/mmol	GLP statement	:	yes
route	:	oral	guideline	:	EPA 85-1, Directive 88/302/EEC
species	:	Rat, CD, 7 weeks(at administration)	acceptability	:	acceptable
group size	:	3/sex			

Study design

The study investigated the distribution of ^{14}C-pyriproxyfen after a single oral dose of 1000 mg/kg bw. Seven groups of 3 rats/sex were treated and sacrificed 2, 4, 8, 12, 24, 48 and 72 hours after administration. At sacrifice the following tissues and organs were taken from all animals: blood bone, brain, fat, heart, liver, kidney, lung, muscle, spleen, testis, uterus and ovaries. Tissue samples were combusted and radioactivity was measured by LSC.

Results

The distribution of radioactivity in tissues is presented in table B.6.1.1-11. Tissue concentrations in male and female rats were comparable. The highest concentration was found in the liver of males 4-8 hours post

administration (1.3% of total radioactivity). In females only 0.6% of applied radioactivity was recovered from the liver. Peak concentrations in fat appeared in males and females 24 and 12 hours post-administration (0.7 and 0.9% of total radio activity). Blood concentration in males was higher than in females. In general radioactivity levels were very low. Based on the information available half live time was calculated to be 12-17 hours for all tissues except brain ($T_{1/2}$ 5-7 hours), fat ($T_{1/2}$ 23-35 hours) and heart ($T_{1/2}$ 9-12 hours). Half lives for males were in general shorter than for females.

After 72 hours 0.2 and 0.3% of applied radioactivity was still present in males and females (in fat and liver). In all other tissues the amount of radio activity after 24 hours became below 0.1%.

Table B.6.1.1-11 Maximum residual radioactivity in tissues and organs % AR and [µg pyriproxyfen equivalents/gram tissue (contents)] and elimination half live

Tissue	single oral, 1000 mg/kg bw							
	M				F			
	Cmax (µg/g)	Cmax (% AR)	Tmax (h)	T1/2 (h)	Cmax (µg/g)	Cmax (% AR)	Tmax (h)	T1/2 (h)
blood	70	0.4	8	12	11-12	0.1	2-8	12
bone	12-14	-	4-8	12	7	-	2	17
brain	16	0	4	5	7-8	0	2-8	7
fat	155	0.7	24	23	170	0.9	12	35
heart	35	0	4	9	14-18	0	2-8	12
kidney	83	0.1	4	12	31-34	0	2-8	17
liver	295-323	1.3	4-8	12	140-155	0.6	2-8	17
lungs	60	0	4	12	15-20	0	2-8	14
muscle	21	0	4	12	7	NI	8	12
ovaries	-	-	-	-	32	0	8	17
spleen	17	0	4	17	7-9	0	2-8	14
testes	17	0	4	12	-	-	-	-
uterus	-	-	-	-	10	0	4-8	12
total		2.3	4			1.4	12	

NI=not indicated

Acceptability

The study is considered acceptable.

2.1.5. CA 5.1.1/05 (1995): Comparative metabolism study in the rat and mouse

Report

CA 5.1.1/05 (1995)

Metabolism of pyriproxyfen 2. Comparison of *in vivo* metabolism between rats and mice

Journal of Agriculture and Food Chemistry, Volume 43, pages 2681-2686

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment.

	Insufficient reporting of experimental methods and not all data collected were reported. However, as stated in the original DAR, the study is considered acceptable for interspecies comparison. There were no remarkable differences between patterns of absorption and excretion between sexes. At the high dose more radioactivity was excreted in urine. In mice, elimination via urine is higher than in rats. Metabolism in mice is comparable to that in rats.

Characteristics

reference	:	CA 5.1.1/05., 1995	exposure	:	Single by gavage
type of study	:	excretion, metabolism	doses	:	2 and 1000 mg/kg bw
year of execution	:	not specified	vehicle	:	Corn oil
test substances	:	[Pyridyl-2,6-^{14}C] pyriproxyfen, radiochemical purity >98%, specific activity 4.27 GBq/mmol [Phenoxyphenyl-^{14}C] pyriproxyfen, radiochemical purity >98%, specific activity 2.73 GBq/mmol	GLP statement	:	no
route	:	oral	guideline	:	not specified
species	:	Mouse, ICR, 7 weeks (at administration)	acceptability	:	acceptable
group size	:	3/sex			

Study design

The study investigated the metabolism and excretion of [pyridyl-2,6-^{14}C] pyriproxyfen and [phenoxyphenyl-^{14}C] pyriproxyfen after a single oral dose of 2 and 1000 mg/kg bw in mice. Pooled samples of extracted faeces and urine were radio assayed by LSC. Metabolites in excreta (day 0-2) were identified by HPLC/TLC.
In the same publication the results of the rat study (Isobe, 1988a; Yoshino, 1993a) were included for interspecies comparison. The description of the tests was poorly (most likely similar to Yoshino, 1993a) and therefore only the main results are included in the summary.

Results

Cumulative excretion over the 7 day period is presented in table B.6.1.1-12. Only data on exposure to [phenoxyphenyl-^{14}C] pyriproxyfen were reported.
After a single oral dose of 2 mg/kg bw radioactivity recovered after 7 days (range for males and females) represented 10-27% AR (urine) and 78-90% AR (faeces). At 1000 mg/kg bw ranges were 35-37% AR in

urine and 64-65% AR in faeces. There were no remarkable differences between patterns of absorption and excretion of sexes. At the high dose more radioactivity was excreted in urine.

Table B.6.1.1-12 Excretion of radioactivity (% AR) in mice after single oral exposure to ^{14}C-pyriproxyfen

	period	**single oral, 2 mg/kg bw** [phenoxyphenyl-^{14}C]		**single oral, 1000 mg/kg bw** [phenoxyphenyl-^{14}C]	
sample	(d)	**M**	**F**	**M**	**F**
urine	0-7	10	27	37	35
faeces	0-7	90	78	64	65
total	0-7	100	105	102	100

Results of the metabolite identification in urine and faeces using HPLC/TLC are presented in table B.6.1.1-13 (data after administration of [phenoxyphenyl-^{14}C] pyriproxyfen).

In faeces 11 metabolites were identified and in urine 7. 4'-OH-pyriproxyfen was the major metabolite in faeces, representing on average 36-38% and 13-15% of the total administered radioactivity in low and high dose animals, respectively. The parent compound accounted for 12-20% of the total radioactivity administered at 2 mg/kg bw and for 23-25% at 1000 mg/kg bw. DPH-pyriproxyfen (2.7-3.1% AR and 2.6-2.9% AR) and POPA (1.4-3.4% AR and 2.2-2.6% AR) were found in both males and females. In males POPA-sulfate was identified at 1.1-3.8% of administered radioactivity. In addition 4'-OH-pyriproxyfen glucuronide accounted for 1.9% AR in high dose males. In low dose animals 5"-OH-pyriproxyfen accounted for 1.3-2.1% of the applied radioactivity. All other metabolites in faeces were <1% of the administered radioactivity.

In urine POPA-sulfate (3.1-3.9% in males and 2.6-5.9% in females), 4'-OH-pyriproxyfen (2.6-4.6% in females) and 4'-OH-pyriproxyfen glucuronide (2.9-28% in males and 13-18% in females) were the main metabolites. Other metabolites were present in minor amounts (<1% of administered radioactivity). The parent compound pyriproxyfen was only identified in very low amounts in animals at 1000 mg/kg bw.

In general in urine 2-6% of radioactivity could not be identified. In faeces up to 19% of the administered radioactivity could not be identified (or was not extractable). Hydroxylation at the 4'-position of the terminal phenyl ring was the major metabolic reaction. In high dose males glucuronidation was more extensive than in females and low dose males and females.

Table B.6.1.1-13 Urinary and faecal metabolite identification (% AR; mean for males and females) in mice after single oral exposure to [phenoxyphenyl-^{14}C]pyriproxyfen

	single oral, 2 mg/kg				**single oral, 1000 mg/kg**			
	urine		**Faeces**		**urine**		**faeces**	
Metabolites	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Pyriproxyfen	0	0	20	12	0.2	0.1	25	23
5"-OH-pyriproxyfen	-	-	1.3	2.1	-	-	0.7	0.7
2'-OH-pyriproxyfen	-	-	0.4	0.5	-	-	0.3	0.3
4'-phenoxyphenol	0.1	0.1	0.8	0.4	0	0.6	0.2	0.2
4'-OH-pyriproxyfen	0.7	2.6	38	36	0.3	4.6	15	13
DPH-pyriproxyfen[1]	0.2	0.1	3.1	2.9	0.1	1.8	2.7	2.6

POPA[2]	0.2	0.1	3.4	2.6	0	0.2	1.4	2.2
4'-oxydiphenol	-	-	0.6	0.3	-	-	0.2	0.2
5",4'-OH-pyriproxyfen	0.1	0	0.6	1.2	0	0.1	0.4	0.4
4'-OH-POPA	-	-	0.9	1.1	-	-	0.4	0.7
POPA sulfate	3.1	5.9	3.8	0.5	3.9	2.6	1.1	0.8
4'-OH-pyriproxyfen glucuronide	2.9	13	0.4	0.2	28	18	1.9	0.3
unidentified	2.4	4.6	5.3	2.7	2.0	5.8	2.9	3.4
not extractable	-	-	11	9.6	-	-	11	16
total RA chromatographed	9.7	26	90	72	35	34	64	64
total RA identified	7.3	22	73	60	33	28	49	44

1. DPH–pyriproxyfen = 4-hydroxyphenyl (RS)-2-(2-pyridyloxy)propyl ether
2. POPA = 4-phenoxyphenyl(RS)-2-(2-hydroxy)propyl ether

Acceptability

Despite the limited details, the study is considered acceptable for interspecies comparison. The totals of the metabolites were recalculated by the reviewer in the original DAR, because the totals in the report were not in agreement with values for individual metabolites. No results on mice after administration of [pyridyl-2,6-^{14}C] pyriproxyfen were reported.

Overall conclusion on oral absorption

Following the commenting round, the applicant was requested to provide additional information to substanctiate the overall oral absorption value of 40%. The applicant provided the following:

An oral absorption value of 40% has been used for pyriproxyfen risk assessment and derivation of the AOEL. This absorption value is based on the amount of radioactivity present in the urine and bile of bile duct cannulated rats administered a single oral dose of 2 mg/kg bw ^{14}C-pyriproxyfen. The actual sum of administered radioactivity present in urine and bile from these rats is 36.5% and 38.2% dose in males and females, respectively. The radioactivity present in residual carcass and cage wash, which is usually also included in the estimation of absorbed dose, has not been added in this case since these measurements were not made during the study, therefore the resulting actual oral absorption values are likely to be an underestimate. In order to substantiate the oral absorption value of 40%, an overview of the relevant excretion data from the bile duct cannulated animals and groups of intact animals are tabulated below.

− Table:

Overview of excretion data from rats administered a single oral dose of ^{14}C-pyriproxifen (2 mg/kg bw)

Matrix	Radioactivity in sample expressed as % administered dose			
	Bile duct cannulation study	Tissue distribution study (48 h time point)	Excretion Study (0-48 h)	Excretion Study (0-48 h)

	(0-48 h) CA 5.1.1-01 (1988a)		CA 5.1.1-02 (1988b)		CA 5.1.1-04 (1993b)		CA 5.1.1-01 (1988a)	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine	2.7	1.7	[a]	[a]	7.0	10.9	7.9	5.0
Bile	33.8	36.5	[a]	[a]	-	-	-	-
Cage wash	not done	not done	[a]	[a]	included with urine	included with urine	included with urine	included with urine
Tissues[b]	-	-	0.7	1.0	[c]	[c]	[c]	[c]
Residual carcass	-	-	max 0.3	max 0.3	[c]	[c]	[c]	[c]
Faeces	38.4	51.3	[a]	[a]	85.6	77.8	87.8	89.9
GI contents	5.0	0.7	[a]	[a]	[c]	[c]	[c]	[c]
Recovery	79.9	90.2	[a]	[a]	92.9[d]	88.9[d]	95.8[d]	94.9[d]

- sample not taken

a Excreta not collected in the tissue distribution study

b Tissues analysed: blood, brain, fat, heart, kidney, liver lung, muscle, spleen, ovary/uterus or testis

c Animals not sacrificed until 7 days after dosing

d Recovery value not complete since animals not sacrificed until 7 days after dosing

From the Table, it is apparent that at the 48 hour time point, it is likely that radioactivity in tissues and residual carcass would account for approximately 1% of the administered dose in the bile duct cannulated animals based on the amount of radioactivity present in tissues and carcass from the tissue distribution study. Adding this amount to the bile duct cannulation data gives values of 38% and 39% dose in males and females, respectively for radioactivity in urine, bile and tissues/carcass. The data in Table 1 also show that urinary excretion values are slightly higher in the intact animals where cage washing was included in the experimental design compared to the bile duct cannulation experiment where no cage wash took place. In the absence of separate cage wash data it is not possible to definitively attribute the difference in urinary excretion values solely to cage wash but it could be a contributory factor.

Furthermore this value is also supported by estimation of oral absorption by other means. Since there was no parent compound present in bile, it can be assumed that the amount of parent in the faeces of intact animals must represent unabsorbed dose. At the low dose level this represents 31%-37% administered dose giving an oral absorption value of 63-69%, which is higher than that obtained from bile duct cannulation rats. A possible explanation of the difference in the absorption values between intact and cannulated rats is the influence of bile on the oral absorption of pyriproxyfen. The dependence on the presence of bile salts for oral absorption has been reported for a number of compounds and this may also apply to pyriproxyfen (refs 1-3). In this case the oral absorption calculated from the bile duct cannulated rats is likely to be an underestimate and a better estimation could be made by using urinary excretion values from intact rather than cannulated rats.

Overall, we can conclude that the oral absorption rate of 40% is still an underestimated value and can be used for pyriproxyfen risk assessment and derivation of the AOEL.

References

Ref 1: Holm, R.; Tønsberg, H.; Jørgensen, E.B.; Abedinpour, P.; Farsad, S.; Müllertz, A. Influence of bile on the absorption of halofantrine from lipid-based formulations. Eur. J. Pharm. Biopharm. 2012, 81, 281−287.
Ref 2: van Hasselt, P. M.; Janssens, G. E. P. J.; Slot, T. K.; van der Ham, M.; Minderhoud, T. C.; Talelli, M.; Akkermans, L. M.; Rijcken, C. J. F.; van Nostrum, C. F. The influence of bile acids on the oral bioavailability of vitamin K encapsulated in polymeric micelles. J. Controlled Release 2009, 133, 161−168.
Ref 3: Humberstone, A. J.; Porter, C. J. H.; Charman, W. N. A physicochemical basis for the effect of food on the absolute oral bioavailability of halofantrine. J. Pharm. Sci. 1996, 85, 525−529.

RMS NL: In the bile cannulated rats in study 1, the percentage found in bile was 34% in males and 37% in females. Combined with the amount found in urine (3% males, 2% females), this results in oral absorption values of 37% for males and 38% for females. In these rats no measurements of carcass or cage wash were done. In study 2 (CA 5.1.1/02, 1988b) residueal carcass represented max 0.3% of the administered dose. No specific cage wash data are available; in studies 1 (non bile cannulated rats) and 4 cage wash was measured together with urine, resulting in values of 7.9% for males and 5.0% for females. These urine values are much higher than the ones found in the bile cannulated rats in study 1 in which cage wash samples were not included (2.7% males and 1.7% females).
No specific data on carcass or cage wash are available for the bile cannulated rats, however, data from the other animals indicated that up to 1% can be found in carcass and higher urine values are found when cage wash was included in these samples. These findings indicate that the oral absorption in the bile cannulated rats is likely to be higher than the values solely based on bile and urine (when carcass and cage was would be included). Therefore, RMS considers it justified to round the found values to an overall oral absorption value of 40%.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity-oral route

3.1.1 Animal data

3.1.1.1 CA 5.2.1/01 (1987a): Acute oral toxicity study in the rat

Report

CA 5.2.1/01 (1987a)

Acute oral toxicity of S-31183 in rats

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0005

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added.

Characteristics

reference	:	CA 5.2.1/01, 1987a	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	0, 1000, 2500 and 5000 mg/kg bw (both sexes)
year of execution	:	1986	vehicle	:	Corn oil
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 401
species	:	Rat, Sprague-Dawley	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 5000 mg /kg bw (both sexes)

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Description:

Lot/Batch No.: PTG-86011

Purity: 97.2%

CAS No.: 95737-68-1 (of active ingredient)

Stability of test compound: Confirmed stable for the duration of the study

2. Vehicle and/or positive control: corn oil

3.Test animals:

Species:	Rat
Strain:	Sprague Dawley
Age:	6 weeks
Weight at dosing:	♂: 232 – 256 g; ♀: 156 – 180 g
Source:	Charles River Japan Inc.,Kanagawa, Japan
Acclimation period:	12 days

B. Study Design and Methods:

1.In life dates:	21 February 1986 to 28 January 1987
2.Animal assignment and treatment:	The test article was suspended in corn oil and administered to groups of 5 male and 5 female fasted Sprague-Dawley rats by oral gavage at dose levels of 0, 1000, 2500 and 5000 mg/kg bw (dose volumes of: 2, 5 and 10 mL/kg bw, respectively). The observation period was 14 days post-exposure.
3.Statistics:	none

C. Methods:

1.Observations:	Undertaken at 10 and 30 minutes, 1, 2 and 4 hours post dosing on day 0 and then once daily for 14 days.
2.Body weights:	Recorded on study day 0, 7 and 14.
3.Food consumption:	Not undertaken.
4.Gross pathology:	All of the organs/tissues were examined macroscopically. No histopathology was performed.

Results

Mortality: No mortality occurred.

Symptoms of toxicity:

Decreased motor activity was observed in males at the 2500 mg/kg bw level. Within one day these animals were normal. Decreased motor activity, soft faeces and diarrhoea were observed in males and females at the 5000 mg/kg bw level. Within two days these animals were normal. No treatment-related findings were noted in the animals in the control group and in the 1000 mg/kg bw group.

Body weight: A significant decrease of body weight and body weight gain was noted among the males given 5000 mg/kg bw at day 7 of the study. Body weight of these animals returned to control values at day 14. A significant decrease of body weight and body weight gain was noted among the females given 5000 mg/kg bw throughout the observation period. No treatment related findings were noted in the animals of other dose groups.

Pathology: White substance in urinary bladder and uterine horn distended with fluid in some animals in all groups including control animals. However these changes have been usually found in this strain of rats and are not considered treatment related.

Acceptability

The study is considered acceptable.

Conclusions

The acute oral LD_{50} of pyriproxifen was found to be greater than 5000 mg/kg bw in males and females.

3.1.1.2 CA 5.2.1/02 (1987b): Acute oral toxicity study in the mouse

Report

CA 5.2.1/02 (1987b)

Acute oral toxicity of S-31183 in mice

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0014 and Amendment (1993a, NN-70-0110)

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added.

Characteristics

reference	:	CA 5.2.1/02, 1987b	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	0, 1000, 2000 and 5000 mg/kg bw (both sexes)
year of execution	:	1987	vehicle	:	Corn oil
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 401
species	:	Mouse, ICR	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 5000 mg/kg bw (both sexes)

Study design

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Description:

Lot/Batch No.: PTG-86011

Purity:	97.2%
CAS No.:	95737-68-1 (of active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study
2.Vehicle and/or positive control:	corn oil
3.Test animals:	
Species:	Mouse
Strain:	ICR
Age:	5 weeks
Weight at dosing:	♂: 25.3 - 31.6 g; ♀: 19.4 - 24.2 g
Source:	Charles Rives Japan Inc.
Acclimation period:	11 days

B. Study Design and Methods:

1.In life dates:	3 February 1987 to 17 February 1987
2.Animal assignment and treatment:	The test article was suspended in corn oil and administered to groups of 5 male and 5 female fasted ICR mice by oral gavage at dose levels of 0, 1000, 2000 and 5000 mg/kg bw (dose volume of 10 mL/kg bw). The observation period was 14 days post-exposure.
3.Statistics:	none

C. Methods:

1.Observations:	Undertaken at 10 and 30 minutes, 1, 2 and 4 hours post dosing on day 0 and then once daily for 14 days.
2.Body weights:	Recorded on study day 0, 7 and 14.
3.Food consumption:	Not undertaken.
4.Gross pathology:	All of the organs/tissues were examined macroscopically. No histopathology was performed.

Results

Mortality: 2/5 males given 2000 and 5000 mg/kg bw were found dead within 2 days after treatment. 1/5 females given 5000 mg/kg were found dead within 1 day after treatment. No further mortality occurred.

Symptoms of toxicity: Excretion of an oily substance was noted in a male at the 1000 mg/kg bw level, but this is considered as not treatment-related. Decreased motor activity, ataxic gait and irregular respiration was observed in males at the 2000 mg/kg bw and 5000 mg/kg bw levels. In females at the 5000 mg/kg bw level decreased motor activity, ataxic gait and irregular respiration was observed with one female dying one day

after dosing. No treatment-related findings were noted in the females at the 2000 and 1000 mg/kg bw group. Within five days all surviving animals were normal.

Body weight: A significant decrease of body weight gain was noted among the males given 5000 mg/kg bw at day 7 of the study. Body weight of these animals returned to control values at day 14. A significant increase of body weight was noted among the females given 5000 mg/kg bw on day 7. Body weight of these animals returned to control values at day 14. Therefore this effect is considered as not treatment-related. No treatment related findings were noted in the animals of other dose groups.

Pathology: Vacuolation on the renal surface was found in one dead male of the top-dose group. Autolysis of the intestine was observed in both sexes, at 2000 and 5000 mg/kg in males and in females only at the top dose of 5000 mg/kg bw. White substance in urinary bladder in males (control group), fluid filled cyst surrounded in the ovary and uterine horn distended with fluid was observed in the sacrificed females (control and 5000 mg/kg bw group). However these changes are considered as not treatment related.

Acceptability

The study is considered acceptable.

Conclusions

The acute oral LD_{50} of pyriproxifen in mice was found to be greater than 5000 mg/kg bw in males and females.

3.1.1.3 CA 5.2.1/03 (1986): Acute oral toxicity study in the dog

Report

CA 5.2.1/03 (1986)

Acute oral toxicity of S-31183 in dogs

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-60-0012

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added.

Characteristics

reference	:	CA 5.2.1/03, 1986	exposure	:	Once by capsules
type of study	:	Acute oral toxicity study	doses	:	0, 500, 1500 and 5000 mg/kg bw (both sexes)
year of execution	:	1986	vehicle	:	gelatine capsule
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 401

species	:	Dog	acceptability	:	Acceptable as range finding study
group size	:	1/sex/dose	**LD_{50}**	:	-

Study design

The study was performed in accordance with OECD 401 (1987), except that only 1 animal per group was used instead of 5 animals per group. The test substance was filled into hard gelatin capsules and administered orally.

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Description:	
Lot/Batch No.:	PTG-86011
Purity:	97.2%
CAS No.:	95737-68-1 (of active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study
2.Vehicle and/or positive control:	Gelatin capsule
3.Test animals:	
Species:	Dog
Strain:	Beagle
Age:	6 months
Weight at dosing:	♂: 8.1 – 9.8 kg; ♀: 6.3 – 7.6 kg
Source:	White Eagle Laboratories Inc. (USA)
Acclimation period:	

B. Study Design and Methods:

1.In life dates:	30 January 1986 to February 15 1986
2.Animal assignment and treatment:	The test article was filled into hard gelatin capsules in quantities of 1500 mg/capsule and force administered once orally to 1 male and 1 female dog (fasted) per dose level (0, 500, 1500 and 5000 mg/kg bw). The observation period was 14 days post-exposure.
3.Statistics:	none

C. Methods:

1.Observations:	Undertaken up to 2 hours after administration, thereafter, at 1-2 hour intervals up to 8 hours after administration. At 24 hours and onward, animals were observed more than twice every day, at least once in the

	morning and once in the afternoon. The period of observation was 2 weeks after administration.
2.Body weights:	Recorded on study days -7, -2, 4, 7, 11 and 14.
3.Food consumption:	The leftover of food was measured every day for 5 days before administration and up to 2 weeks after administration.
4. Haematology	On the day before administration and at day 1, 7 and 14 blood was collected and parameters were measured.
5. Blood biochemistry	Serum was separated from the remainder of the blood collected as described under 4. And tested for biochemistry parameters.
6.Gross pathology:	All of the organs/tissues were examined macroscopically and selected organs were weighed.

Results

Mortality: No mortality occurred.

Symptoms of toxicity: Vomiting was observed within 1 day after administration at the 5000 mg/kg bw level in both sexes, but since this was also observed in the control group this is considered as not treatment-related. In all male dose groups soft or mucosal faeces were noticed. This was not noted in females. No other symptoms of toxicity were noticed.

Food consumption: No treatment related findings.

Body weight: No treatment related findings.

Haematology: No treatment related findings.

Blood biochemistry: No treatment related findings.

Pathology: In no organs, except for the testes and prostate, abnormalities were seen on organ weight and its ratio to body weight. In one male in the 1500 mg/kg bw group, depression of the lymphatic tissue and scattering of white spots were observed. Scattering of red spots on the mucosa of the gastric part, testes and prostate was observed and scattering of red spots on the mucosa of the uterine horn in the control group alone or the control and dose groups. No further findings.

Acceptability

Although only 1 animal per group was used instead of 5 animals per group, the study is considered acceptable as a range finding study. An LD_{50} cannot be derived.

Conclusions

Administration of doses of 500, 1500 and 5000 mg/kg bw to dogs, did not result in mortality. In all treated animals soft or mucosal faeces were noted. No changes in food consumption, body weight, haematology, blood

chemistry and organ weights were noted. No treatment-related changes were noted at post-mortem necropsy at all dose levels.

3.1.2 Human data

No human data on the acute oral toxicity of pyriproxyfen are available.

3.1.3 Other data

No other data on the acute oral toxicity of pyriproxyfen are available.

3.2 Acute toxicity-dermal route

3.2.1 Animal data

3.2.1.1 CA 5.2.2/01 (1987c): Acute dermal toxicity study in the rat

Report

CA 5.2.2/01 (1987c)

Acute dermal toxicity of S-31183 in rats

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0006

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added.

Characteristics

reference	:	CA 5.2.2/01, 1987c	exposure	:	24 hours on a skin area of 30 cm^2 (semi-occlusive exposure)
type of study	:	Acute dermal toxicity study	doses	:	0, 2000 mg/kg bw (both sexes) (limit test)
year of execution	:	1986 - 1987	vehicle	:	Corn oil
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 402
species	:	Rat, Sprague-Dawley	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 2000 mg/kg bw (both sexes)

Study design

Materials and methods

A. Materials:

1.Test Material: S-31183 (pyriproxyfen)

Description:	
Lot/Batch No.:	PTG-86011
Purity:	97.2%
CAS No.:	95737-68-1 (of active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study
2. Vehicle and/or positive control:	Corn oil
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Age:	6 weeks
Weight at dosing:	♂: 226 – 257 g; ♀: 153 – 169 g
Source:	Charles River Japan Inc.
Acclimation period:	12 days

B. Study Design and Methods:

1. In life dates:	10 September 1986 to 24 September 1986
2. Animal assignment and treatment:	The dorsal hair (5 x 10 cm^2) was shaved by an electric clipper just before administration. The test article was suspended in corn oil and applied dermally to groups of 5 male and 5 female rats at dose levels of 0 and 2000 mg/kg bw (administration volume of 5 mL/kg bw). The animals were fixed for 1 hour after application to prevent the animals from receiving the test material orally. The tape was taken away after 24 hours and the area was cleaned. The observation period was 14 days post-exposure.
3. Statistics:	Mean body weights were compared using the t-test. The incidence of gross pathological findings was compared with the control group using the Fisher exact test.

C. Methods:

1. Observations:	Undertaken at 10 and 30 minutes, 1, 2 and 4 hours post dosing on day 0 and then once daily for 14 days.
2. Body weights:	Recorded on study days 0, 7 and 14.
3. Food consumption:	Not conducted
4. Gross pathology:	All of the organs/tissues were examined macroscopically. No histopathology was performed.

Results

Mortality: No mortality occurred.

Symptoms of toxicity: No treatment related findings.

Body weight: No treatment related findings.

Pathology: White substance in the urinary bladder and uterine horn distended with fluid in some animals. However these changes are not treatment related since they have been usually found in this strain of rats.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD_{50} of pyriproxifen in rats was found to be greater than 2000 mg/kg bw in males and females.

3.2.1.2 CA 5.2.2/02 (1987d): Acute dermal toxicity study in the mouse

Report

CA 5.2.2/02 (1987d)

Acute dermal toxicity of S-31183 in mice

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0015 and Amendment (1993b, NN-70-0111)

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added.

Characteristics

reference	:	CA 5.2.2/02, 1987d	exposure	:	24 hours on a skin area of 4.5 cm^2 (semi-occlusive exposure)
type of study	:	Acute dermal toxicity study	doses	:	0, 2000 mg/kg bw (both sexes) (limit test)
year of execution	:	1987	vehicle	:	Corn oil
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 402
species	:	Mouse, ICR	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 2000 mg/kg bw (both sexes)

Study design

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Description:	
Lot/Batch No.:	PTG-86011
Purity:	97.2%
CAS No.:	95737-68-1 (of active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study
2.Vehicle and/or positive control:	Corn oil
3.Test animals:	
Species:	Mice
Strain:	ICR
Age:	5 weeks
Weight at dosing:	♂: 30.6 – 35.0 g; ♀: 22.4 – 25.5 g
Source:	Charles River Japan Inc.
Acclimation period:	11 days

B. Study Design and Methods:

1.In life dates:	3 February 1987 to 17 February 1987
2.Animal assignment and treatment:	The dorsal hair (2 x 3 cm^2) was shaved by an electric clipper just before administration. The test article was suspended in corn oil and applied dermally to groups of 5 male and 5 female mice at dose levels of 0 and 2000 mg/kg bw (administration volume of 10 mL/kg bw). The tape was taken away after 24 hours and the area was cleaned. The observation period was 14 days post-exposure.
3.Statistics:	Mean body weights were compared using the t-test. The incidence of gross pathological findings was compared with the control group using the Fisher exact test.

C. Methods:

1.Observations:	Undertaken at 10 and 30 minutes, 1, 2 and 4 hours post dosing on day 0 and then once daily for 14 days.
2.Body weights:	Recorded on study days 0, 7 and 14.
3.Food consumption:	Not conducted

4.Gross pathology: All of the organs/tissues were examined macroscopically. No histopathology was performed.

Results

Mortality: No mortality occurred.

Symptoms of toxicity: No treatment related findings.

Body weight: No treatment related findings.

Pathology. White substance in the urinary bladder was seen in 2 animals in the control group and uterine horn distended with fluid was observed in 3 females of the 2000 mg/kg bw group. However these changes are usually seen in mice and therefore appeared unrelated to treatment.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD_{50} of pyriproxifen in mice was found to be greater than 2000 mg/kg bw in males and females.

3.2.2 Human data

No human data on the acute dermal toxicity of pyriproxyfen are available.

3.2.3 Other data

No other data on the acute dermal toxicity of pyriproxyfen are available.

3.3 Acute toxicity-inhalation route

3.3.1 Animal data

3.3.1.1 CA 5.2.3/01 (1987): Acute inhalation toxicity study in the rat

Report

CA 5.2.3/01 (1987)

Acute inhalation toxicity of S-31183 in rats

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0022

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added.

Characteristics

reference	:	CA 5.3.2/01, 1987	exposure	:	4 hours, whole body
type of study	:	Acute inhalation toxicity study	doses	:	0, 0.6, 1.3 mg/L (0, 600, 1300 mg/m^3), MMAD 0.75-0.86 µm, GSD 1.35-1.55 µm
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	:	In accordance with OECD 403
species	:	Rats, Sprague Dawley	acceptability	:	Acceptable.
group size	:	5/sex/dose	**LD_{50}**	:	> 1.3 mg/L

Study design

The study was performed in accordance with OECD 403 (1981). A concentration of 1.3 mg/L was the maximum attainable concentration.

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Description:

Lot/Batch No.: PTG-86011

Purity: 97.0%

CAS No.: 95737-68-1 (of active ingredient)

Stability of test compound: Confirmed stable for the duration of the study

2. Vehicle and/or positive control: Corn oil

3. Test animals:

Species: Rats

Strain: Sprague-Dawley

Age: 5 weeks

Weight at dosing: ♂: 172 – 207 g; ♀: 140 – 174 g

Source: Clea Japan Inc.

Acclimation period: 8 days

B. Study Design and Methods:

1. In life dates: 10 February 1987 to 24 February 1987

2.Animal assignment and treatment:	In a preliminary study, the rats were exposed to mists generated by spraying 50% solution, the maximum concentration that could be sprayed, at a rate of 0.24 mL/min for 4 hours. No animals died, therefore, the high dose level for the main study was set at 50% solution exposure and the low dose was set at 25% solution exposure. The actual aerial concentration was measured 1 and 3 hours after the beginning of the exposure. The distribution of aerodynamic diameter of mist particles in the exposure chamber was measured from 30 to 40 minutes after the beginning of the exposure.
3.Statistics:	Body weight data were examined by F-test, and statistically compared to the control group by Student's t-test in the case of 1) without significant difference in F-test and by Fisher-Behrens test in the case of 2) with significant difference in F-test.

C. Methods:

1.Observations:	Undertaken at 30 minutes, 1, 2, 3 and 4 hours of exposure and one hour after the end of the exposure, and daily thereafter for 14 days.
2.Body weights:	Recorded on study days 0, 3, 7 and 14.
3.Food consumption:	Not conducted
4.Gross pathology:	All of the organs/tissues were examined macroscopically. Histopathology was performed on respiratory organs.

Results

Aerial concentration and particle size: The mean aerial concentrations of pyriproxifen were 600 mg/m^3 for the low dose group and 1300 mg/m^3 for the high-dose group. The mean aerodynamic diameters of mist particles were 0.83 μm in the control group, 0.86 μm in the low dose group and 0.75 μm in the high dose group.

Mortality: No mortality occurred.

Symptoms of toxicity: Salivation was noticed in 2/5 males and 1/5 females in the 1.3 mg/L dose group. Urinary incontinence was noticed in 2/5 females four hours after the beginning of exposure. All animals appeared normal one hour after the end of exposure.

Body weight: Slight decrease of weight gain was noted in males in the 1.3 mg/L dose group three days after the end of exposure. Body weight of these animals returned to control values at day 7. No treatment related findings were noted in the animals of other dose groups.

Pathology: Brown points on the lung surface were observed in 1/5 males in the 0.6 mg/L dose group and 1/5 females in the vehicle dose group. White substance in the lumen of the urinary bladder was observed in some males and uterine horn distended with fluid was observed in some females. However these changes were noted among all groups without a dose-relationship.

In the lungs some minimal changes were observed in one or two males or females of each group.

Histopathology: No remarkable changes were observed to the nasal cavity, except minimal to slight prominence of goblet cells in the respiratory epithelium found in four males and one female in the vehicle control group. No remarkable changes to the trachea were observed. In the lungs, minimal changes including accumulation of eosinophilic crystals, focal congestion, focal haemorrhage, focal interstitial pneumonia, perivascular aggregation of eosinophilic cells and thickening of alveolar walls were sporadically observed in one or two males and females of each group. No other changes were seen in any group. Accumulation of eosinophilic crystals and focal congestion in the lungs were observed only in the high dose group. These findings were very slight and were regarded as incidental. It was thought, therefore, that inhalation of pyriproxifen had no histopathological effect on the respiratory organs.

Acceptability

The study is considered acceptable.

Conclusions

The acute inhalation LD_{50} of pyriproxifen in rats was found to be greater than 1.3 mg/L for males and females.

3.3.1.2 CA 5.2.3/02 (1987e): Acute inhalation toxicity study in the mouse

Report

CA 5.2.3/02 (1987e)

Acute inhalation toxicity of S-31183 in mice

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0023 and Addendum (1995, NN-50-0131)

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added.

Characteristics

reference	:	CA 5.2.3/02, 1987e	exposure	:	4 hours, whole body
type of study	:	Acute inhalation toxicity study	doses	:	0, 0.6, 1.3 mg/L (0, 600, 1300 mg/m^3), MMAD 0.75–0.86 µm, GSD 1.35-1.55 µm
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	:	In accordance with OECD 403
species	:	Mouse, ICR	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 1.3 mg/L

Study design

The study was performed in accordance with OECD 403 (1981). A concentration of 1.3 mg/L was the maximum attainable concentration.

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Description:	
Lot/Batch No.:	PTG-86011
Purity:	97.0%
CAS No.:	95737-68-1 (of active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study
2.Vehicle and/or positive control:	Corn oil
3.Test animals:	
Species:	Mice
Strain:	ICR
Age:	5 weeks
Weight at dosing:	♂: 28.0 – 34.7 g; ♀: 23.9 – 28.4 g
Source:	Clea Japan Inc.
Acclimation period:	8 days

B. Study Design and Methods:

1.In life dates:	10 February 1987 to 24 February 1987
2.Animal assignment and treatment:	In a preliminary study, the rats were exposed to mists generated by spraying 50% solution, the maximum concentration that could be sprayed, at a rate of 0.21 mL/min for 4 hours. No animals died, therefore, the high dose level for the main study was set at 50% solution exposure and the low dose was set at 25% solution exposure. The actual aerial concentration was measured 1 and 3 hours after the beginning of the exposure. The distribution of aerodynamic diameter of mist particles in the exposure chamber was measured five times from 30 to 40 minutes after the beginning of the exposure.

3.Statistics:	Body weight data were examined by F-test. If the variances were equal, the group mean values were compared to the vehicle control group using Students t-test and, if the variances were unequal, Fisher-Behrens test was used.

C. Methods:

1.Observations:	Undertaken at 30 minutes, 1, 2, 3 and 4 hours of exposure and then at hourly intervals until 4 hours post-exposure. Thereafter once daily during 14 days.
2.Body weights:	Recorded on study days 0, 3, 7 and 14.
3.Food consumption:	Not conducted
4.Gross pathology:	All of the organs/tissues were examined macroscopically. Histopathology was performed on respiratory organs.

Results

Aerial concentration and particle size: The mean aerial concentrations of pyriproxifen were 600 mg/m^3 for the low dose group and 1300 mg/m^3 for the high-dose group. The mean aerodynamic diameters of mist particles were 0.83 µm in the control group, 0.86 µm in the low dose group and 0.75 µm in the high dose group.

Mortality: No mortality occurred.

Symptoms of toxicity: Irregular respiration was noticed 2 hours after the beginning of exposure in one to two animals in males and females of the 1.3 mg/L dose group. These animals appeared normal one hour after the end of exposure. A rough coat was noticed from the end of exposure in all animals in all dose groups except for the negative control group and disappeared one day after exposure. This finding is not considered to be treatment-related in the absence of a dose response relationship.

Body weight: No treatment related findings.

Gross pathology: White substance in the urinary bladder was observed in some males of all groups and uterine horn distended with fluid was observed in some females in all groups. However these changes are not considered as treatment related in the absence of a dose-response relationship.

Histopathology: No treatment-related changes were observed in the respiratory organ, except for pulmonary lesions of minimal agonal haemorrhage that are considered incidental (one male in vehicle control group and one female in the high dose group).

Acceptability

The study is considered acceptable.

Conclusions

The acute inhalation LD_{50} of pyriproxifen in mice was found to be greater than 1.3 mg/L for males and females.

3.3.2 Human data

No human data on the acute inhalation toxicity of pyriproxyfen are available

3.3.3 Other data

No other data on the acute inhalation toxicity of pyriproxyfen are available

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 CA 5.2.4/01 (1987f): Skin irritation study in the rabbit

Report

CA 5.2.4/01 (1987f)

Primary eye and skin irritation tests with S-31183 in rabbits

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0004

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added.

Characteristics

reference	:	CA 5.2.4/01, 1987f	exposure	:	4 hours, occlusive, application area 6.25 cm^2
type of study	:	skin irritation study	doses	:	0.5 g
year of execution	:	1986 - 1987	vehicle	:	None
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 404
species	:	Rabbits, New Zealand White	acceptability	:	Acceptable
group size	:	3 rabbits/sex	**Effect**	:	Not skin irritating

Study design

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Description:

Lot/Batch No.: PTG-86011

Purity:	97.2%
CAS No.:	95737-68-1 (of active ingredient)
Stability of test compound:	Confirmed stable for the duration of the study
2. Vehicle and/or positive control:	none
3. Test animals:	
Species:	Rabbits
Strain:	New Zealand White
Age:	6 – 12 weeks
Weight at dosing:	2.26 – 2.78 kg
Source:	Nihon Dobutsu Co. (Osaka, Japan)
Acclimation period:	19-28 days

B. Study Design and Methods:

1. In life dates:	16 December 1986 to 19 December 1986
2. Animal assignment and treatment:	The hair was clipped with an electric clipper to prepare two application sites on both sides of the median line of the back (ca. 15 x 15 cm). One of the sites was abraded using a needle, the other site remained untreated. The scratch was deep enough to disturb the stratum corneum, but not to damage the dermis or cause bleeding. The test material (500 mg) was spread on a patch moistened with saline and applied to the skin with an occlusive tape for 4 hours. Thereafter, the patches were removed and the treated area was wiped to remove any remaining material.
3. Statistics:	Not performed

C. Methods:

1. Observations:	Skin reactions were scored at 4.5, 24, 48 and 72 hours after application according to the method by Draize.

Results

The study was performed in accordance with OECD 404 with the exception that the test substance was applied under an occlusive patch. Test substance was applied on an intact and an abraded skin site of each animal. The results of the intact and abraded skin do not differ. Only the results of the intact skin are presented, since these results are used for classification purposes.

The results are summarised in the following table:

Table B.6.2.4-1 Skin irritation scores of six animals

Scores observed after	4.5 hour	24 hours	48 hours	72 hours
Erythema (intact skin)	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)
Oedema (intact skin)	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)

( ): mean value

Acceptability

The test substance was applied under an occlusive dressing instead of a semi-occlusive dressing. However, this method of application was considered to be a worst-case condition.

Conclusions

Pyriproxifen was not-irritating in rabbits.

3.4.2 Human data

No human data on the skin irritation potential of pyriproxyfen are available.

3.4.3 Other data

There was no evidence of marked local effects in a repeated dose dermal toxicity study with pyriproxyfen.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 CA 5.2.5/01 (1987f): Eye irritation study in the rabbit

Report

CA 5.2.5/01 (1987f)

Primary eye and skin irritation tests with S-31183 in rabbits

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0004

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added.

Characteristics

reference	:	CA 5.2.5/01, 1987f	exposure	:	Single instillation
type of study	:	Eye irritation study	doses	:	100 mg
year of execution	:	1986 - 1987	vehicle	:	None
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Eye	guideline	:	In accordance with OECD 405
species	:	Rabbits, New Zealand White	acceptability	:	Acceptable
group size	:	3 rabbits/sex	**Effect**	:	Not eye irritating

Study design
Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Description:

Lot/Batch No.: PTG-86011

Purity: 97.2%

CAS No.: 95737-68-1 (of active ingredient)

Stability of test compound: Confirmed stable for the duration of the study

2. Vehicle and/or positive control: none

3. Test animals:

Species: Rabbits

Strain: New Zealand White

Age: 6 – 12 weeks

Weight at dosing: 2.26 – 2.78 kg

Source: Nihon Dobutsu Co. (Osaka, Japan)

Acclimation period: 19-28 days

B. Study Design and Methods:

1. In life dates: 16 December 1986 to 19 December 1986

2. Animal assignment and treatment: The test material (100 mg) was placed on the everted lower lid of one side eyes of 6 rabbits (3 males and 3 females). The upper and lower eye lids were gently held together for 1 second. The other side eyes

remained untreated and served as control. The treated eyes remained unwashed.

3.Statistics: Not performed.

C. Methods:

1.Observations: Reading of ocular lesions was conducted 1, 24, 48 and 72 hours after treatment. The grading and scoring of irritating reactions were performed in accordance with the scale of Draize.

Results
The results are summarised in the following table:

Table B.6.2.5-1 Treated-unrinsed eyes (6 animals)

Scores observed after	1 hour	24 hours	48 hours	72 hours
Cornea/opacity	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)
Iris	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)
Conjunctiva redness	1, 1, 1, 1, 1, 1	0, 0, 0, 0, 1, 1 (0.33)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)
Conjunctiva chemosis	1, 1, 1, 1, 2, 2	0, 0, 0, 0, 0, 1 (0.17)	0, 0, 0, 0, 0, 0 (0)	0, 0, 0, 0, 0, 0 (0)

( ): mean values

Acceptability
The study is acceptable.

Conclusions
Pyriproxifen was found to be not eye-irritating in rabbits.

3.5.2 Human data

No human data on the eye irritation potential of pyriproxyfen are available.

3.5.3 Other data

No other data on the eye irritation potential of pyriproxyfen are available.

3.6 Respiratory sensitisation

3.6.1 Animal data

No animal data on the respiratory sensitisation of pyriproxyfen are available.

3.6.2 Human data

No human data on the respiratory sensitisation of pyriproxyfen are available.

3.6.3 Other data

No other data on the respiratory sensitisation of pyriproxyfen are available.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 CA 5.2.6/01 (1987g): Sensitisation study (Maximisation) in the guinea pig

Report

CA 5.2.6/01 (1987g)

Skin sensitisation test with S-31183 in guinea pigs

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0003 and Amendment (1995, NNT-50-0130)

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment.

Characteristics

reference	:	CA 5.2.6/01, 1987g	exposure	:	Intradermal and topical induction, topical challenge (occlusive, 24 h)
type of study	:	Skin sensitization study (GPMT)	doses	:	0.5% intradermal induction 25% topical induction 25% challenge
year of execution	:	1986	vehicle	:	Corn oil
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	dermal	guideline	:	OECD 406
species	:	Guinea pig, Dunkin/Hartley	acceptability	:	Acceptable
group size	:	20 controls (males) 20 test animals (males)	**Effect**	:	Not sensitising

Study design

The study was conducted according to Magnusson and Kligman. DNCB was used as the positive control.
According to OECD 406, the concentration of test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest non-irritant dose. The appropriate concentrations can be determined from a pilot study.
Dose levels for this study were based on the results of a range-finding study using 0.1, 0.25, 0.5 and 1% for intradermal injections and 25% for topical applications. A test substance concentration of 25% was stated to be the maximum concentration for sensitisation in general (Magnussen and Kligman, J. Invest. Derm 1969). In this paper the following is stated: “challenge by topical application. Provided there is no irritation, solids are incorporated in petrolatum at 25% concentration and liquids are used as is.” Slight erythema was noted after 0.5% intradermal application. Slight erythema and swelling were noted after intradermal injection with 1.0%. No skin reaction was noted after 25% topical application.
For this reason, 25% petrolatum ointment was considered an adequate concentration for the challenge treatment.
This was not completely in line with OECD 406, where it is indicated that the highest non-irritant dose should be used; hwoever, it is in line with the article by Magnussen and Kligman (1969).
Intradermal induction was performed with 0.5% test substance in corn oil (or 0.05% DNCB in corn oil). Topical induction was initiated 6 days after intradermal induction with treatment of 10% concentration of sodium lauryl sulphate. One day later this was followed by dermal application of 25% test substance in petrolatum. Fourteen days after the 2nd induction, challenge was performed with dermal application using 25% test substance in petrolatum. The challenge site was observed 24 and 48 hours after challenge exposure. The skin reations of erythema and swelling were scored.

Results

Following topical challenge with 25% test substance no skin reactions as erythema and swelling were noted in the test and control animals treated with S-31183. Sensitisation of this strain was positively tested with DNCB. One animal in the test group and one animal in the control group died. Anal prolapse and enterocele were found in these animals at necropsy. It was not believed that the deaths were attributed to administration of the test material or the solvents.

Acceptability

The study is considered acceptable

Conclusions

Under the conditions of the study, the test substance S-31183 showed no skin sensitization.

3.7.2 Human data

No human data on the skin sensitisation potential of pyriproxyfen are available.

3.7.3 Other data

No other data on the skin sensitisation potential of pyriproxyfen are available.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 CA 5.4.1.1/01 (1988): Bacterial mutagenicity study (Ames test)

Report

CA 5.4.1.1/01 (1988)

Reverse mutation test of S-31183 in bacterial systems.

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0034

Previous evaluation	In DAR (2005)
Evaluation RMS	The study does not fulfil the requirements of the 1197 OECD 471 guideline, since no historical control data were included. In addition, strain TA1538 was included which is not in the OECD471 guideline and duplicate plating was undertaken rather than triplicate plating (wihtout a scientific justification). However, these differences from the OECD guideline do not influence the results and conclusion of this study. This study is considered acceptable. Agreed with the original conclusion, pyriproxifen is not mutagenic in this Ames test.

Characteristics

reference	:	CA 5.4.1.1/01, 1988	Exposure	:	In vitro
type of study	:	Bacterial reverse mutation (Ames)	Doses	:	0, 0.01, 0.05, 0.1, 0.5, 1 and 5 mg/plate
year of execution	:	1988	Vehicle	:	DMSO
test substance	:	S-31183 (pyriproxifen), batch PTG-86011, purity 97.2%.	GLP statement	:	Yes
Guideline	:	in accordance with OECD 471	Acceptability	:	acceptable
			Conclusion	:	negative

Study design

The potential of pyriproxifen to induce bacterial mutation was evaluated in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100; and *Escherichia coli* strain WP2uvrA. The test was conducted with and without metabolic activation using an S9 fraction. Methyl methanesulfonate, 2-nitroflurone, sodium azide, ICR-191 and N-ethyl-N'-nitro-N-nitrosoguanidine served as the positive controls for the non-activated assay, while 2-aminoanthracene (2AA) and benzo(a)pyrene served as the positive controls for the activated assay.

Results

Precipitation of the test compound was observed at the dose level of 5 mg/plate without S9 mix. Pyriproxifen did not induce any significant increases of revertant colonies in the 6 test strains both with and without S9-mix. The positive control chemicals induced marked increases in the number of revertant colonies under the conditions tested.

Table B.6.4.1-1 Results without metabolic activation

Conc. (µg/plate)	TA98	TA100	TA1535	TA1537	TA1538	WP2uvrA
Experiment I						
Vehicle control	30	77	8	7	12	18
Pyriproxifen						
10	31	85	10	6	11	23
50	36	93	10	7	11	18
100	27	90	11	9	9	20
500	25	81	9	7	9	20
1000	30	82	11	5	9	18
5000	29	87	10	14	13	18
Positive control	520	445	388	1288	536	428
Experiment II						
Vehicle control	31	81	14	14	7	16
Pyriproxifen						
10	27	77	18	14	14	24
50	29	66	12	11	10	18
100	26	76	12	7	5	13
500	25	68	10	11	7	16
1000	27	73	12	11	11	26
5000	31	66	7	9	8	21
Positive control	395	302	304	1370	611	316

Table B.6.4.1-2 Results with metabolic activation

Conc. (µg/plate)	TA98	TA100	TA1535	TA1537	TA1538	WP2uvrA
Experiment I						
Vehicle control	48	68	11	25	37	17
Pyriproxifen						

10	49	80	10	22	44	18
50	50	107	8	20	35	24
100	30	74	11	24	34	22
500	39	86	7	22	32	26
1000	41	83	10	25	30	22
5000	37	79	15	32	27	21
Positive control	674	954	179	169	218	610
Experiment II						
Vehicle control	49	68	9	36	32	18
Pyriproxifen						
10	46	74	8	34	46	22
50	42	73	12	35	23	17
100	44	58	12	38	21	12
500	44	65	9	28	24	28
1000	39	70	10	32	33	21
5000	39	66	8	26	25	19
Positive control	433	558	143	149	118	451

Acceptability

The study was performed in accordance with 40 CFR 160. The study does not fulfil the requirements of the more recent OECD 471 guideline of 1997, since the spontaneous revertant colonies yield of the strains was not verified with the laboratory's historical control data. However, the study was considered acceptable.

Conclusion

S-31183 did not induce point mutations in *S. typhimurium* and *E.coli*.

3.8.1.2 CA 5.4.1.2/01 (1989): Clastogenicity study in CHO cells

Report

CA 5.4.1.2/01 (1989)

In vitro chromosomal aberration test of pyriproxyfen in Chinese hamster ovary cells (CHO-K1)

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0054

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. The exposure time in the presence of S9 mix were marginally short (2h) compared to the current guideline requirements (3-6h). Short-term treatment in the absence of metabolic activation was not included. Twohundred metaphases were scored per test concentration rather than the current

	requirements of 300 metaphases. RMS still considers this study to be acceptable.

Characteristics

reference/notifier	:	CA 5.4.1.2/01, 1989	Exposure	:	In vitro
type of study	:	In vitro mammalian chromosome aberration test	Doses	:	10, 30, 100, 300 µg/ml
year of execution	:	1989	Vehicle	:	DMSO
test substance	:	S-31183 (pyriproxifen), Batch PTG-86011, purity 97.2%	GLP statement	:	yes
Guideline	:	OECD 473	Acceptability	:	acceptable
			Conclusion	:	Negative

Study design

A. Materials:

1.Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PTG-086011

Purity: 97.2%

CAS No.: 95737-68-1

Stability of test compound: Confirmed stable for the duration of the study

2.Control materials:

Vehicle/final concentration DMSO (dimethyl sulphoxide) / 1% (v/v)

3.Test organism: Chinese hamster ovary cells (CHO-K1), obtained from Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan).

4.In life dates: 23 March 1989 to 19 May 1989

5.Treatment: Cultured CHO were exposed to the test article in the presence and absence of S9 mix (rat PCB). Without S9 mix cells were exposed continuously for 18 or 24 hours without recovery and with S9 mix exposure was limited to 2 hours, with cells harvested 22 hours later. On the basis of the results of the preliminary toxicity test the following

concentrations (in duplicate) were selected in order that an appropriate range of toxicity was observed:
-S9: 0, 10, 30, 100 µg/mL
+S9: 0, 30, 100, 300 µg/mL
Positive controls were included (mitomycin C and cyclophosphamide).
From 200 metaphases/dose level with 20 centromeres, chromosome number, all chromosomes normal or some aberrant, and specific types and numbers of aberrations were recorded.

Results

Preliminary cytotoxicity test:

In the absence of S9, marked decreases in relative mitotic index (MI) were observed at 30 µg/mL (69%) and 100 µg/mL (79%) compared to the solvent control. Marked cell cycle delay was observed at 30 µg/mL (M1/M1+ were 44%/37%) and at 100 µg/mL (66%/27%), compared to the solvent control. A 300 µg/mL a decrease in relative MI (77%) and cell cycle delay (74%/23%) were comparable with the data obtained at 100 µg/mL. Based on these data, 100 µg/mL was selected as the top dose and treatment periods were decided to be 18 and 24 hours.

In the presence of S9, marked decreased in relative MI were observed at 100 µg/mL (40%) and 300 µg/mL (69%) compared to the solvent control. Moderate cell cycle delay was observed at 100 µg/mL (43% / 19%), with marked cell cycle delay observed at 300 µg/mL (81% / 16%). Based on these results 300 µg/mL was selected as the maximum dose, with recovery times of 16 and 22 hours post the 2 hour treatment.

Chromosome aberration test:

In the chromosomal aberration test, cells were treated with pyriproxyfen at dose levels of 0, 10, 30, 100 µg/mL for 18 and 24 hours in the absence of metabolic activation. Results are shown in Table B.6.4.1-3. In the presence of S9 cells were treated at dose levels of 0, 30, 100 and 100 µg/mL. Results are shown in Table B.6.4.1-4.

Table B.6.4.1-3: Overview of chromosomal aberrations in the absence of S9

18 h exposure			24 h exposure		
Conc. (µg/mL)	**MI (%) [Rel. MI]**	**Cells with aberrations (%)[a]**	**Dose level (µg/mL)**	**MI (%) [Rel. MI]**	**Cells with aberrations (%)[a]**
Untreated	6.3 [-]	2.5 [2.0]	Untreated	6.3 [-]	1.0 [0.0]
0	6.3 [-]	0.5 [0.5]	0	5.1 [-]	0.5 [0.0]
10	3.8 [40%]	0.5 [0.5]	10	3.4 [33%]	1.5 [1.5]
30	2.9 [54%]	2.0 [2.0]	30	3.1 [39%]	1.0 [1.0]
100	2.0 [68%]	1.0 [0.5]	100	2.7 [47%]	1.5 [1.0]

Positive control	2.2 [65%]	41.0** [33.0**]	Positive control	2.4 [53%]	61.0** [58.0**]

** $p \leq 0.01$
MI: mitotic index
Rel. MI: relative mitotic index
[a]Cells with aberrations: +gaps [-gaps]
Positive control: Mitomycin C (0.02 µg/mL)

Table B.6.4.1-4: Overview of chromosomal aberrations in the presence of S9

2 h exposure and 16h culture (experiment 1)			2 h exposure and 22h culture (experiment 2)		
Conc. (µg/mL)	**MI (%) [Rel. MI]**	**Cells with aberrations (%)[a]**	**Conc. (µg/mL)**	**MI (%) [Rel. MI]**	**Cells with aberrations (%)[a]**
Untreated	5.8 [-]	1.0 [0.5]	Untreated	8.9 [-]	2.0 [1.0]
0	6.4 [-]	2.5 [2.0]	0	7.8 [-]	2.5 [2.0]
30	2.9 [54%]	1.5 [0.5]	30	8.6 [-10%]	2.5 [2.5]
100	3.0 [53%]	0.5 [0.0]	100	2.3 [71%]	2.0 [1.5]
300	3.2 [50%]	2.5 [1.5]	300	1.7 [78%]	1.0 [1.0]
Positive control	3.5 [45%]	35.0** [34.0**]	Positive control	3.5 [55%]	57.0** [52.0**]

** $p \leq 0.01$
MI: mitotic index
Rel. MI: relative mitotic index
[a]Cells with aberrations: +gaps [-gaps]
Positive control: cyclophosphamide (50 µg/mL)

Acceptability

The study was performed in accordance with 40 CFR part 160, and fulfils the requirements of the more recent OECD 473 guideline of 1997. Therefore, the study is considered acceptable.

Conclusion

The test substance did not induce chromosome aberrations in Chinese hamster ovary (CHO).

3.8.1.3 CA 5.4.1.3/01 (1990): Mammalian cell gene mutation assay (HPRT assay)

Report

CA 5.4.1.3/01 (1990) *In vitro* gene mutation test of S-31183 in V79 Chinese hamster cells

Sumitomo Chemical Co. Ltd

Unpublished Report No.: NNT-90-0067

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment. The compliance of this study considering the more recent OECD 476 guideline (1997) was reconsidered.

Characteristics

reference/notifi : CA 5.4.1.3/01, 1990 Exposure : In vitro

er					
type of study	:	*In vitro* gene mutation in mammalian cells	Doses	:	10, 30, 100, 300 µg/ml
year of execution	:	1990	Vehicle	:	DMSO
test substance	:	S-31183 (pyriproxifen), Batch PYG-87074, purity 95.3%	GLP statement	:	yes
Guideline	:	OECD 476	Acceptability	:	acceptable
			Conclusion	:	Negative

Study design

Materials and methods

A. Materials:

1.Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PTG-87074

Purity: 95.3%

CAS No.: 95737-68-1

Stability of test compound: Confirmed stable for the duration of the study

2.Control materials:

Vehicle/final concentration DMSO (dimethyl sulphoxide) / 1% (v/v)

3.Test organism: Chinese hamster V79 cells, obtained from the Institute of Medical Science, University of Tokyo.

4.In life dates: 26 February 1990 to 5 April 1990

5.Treatment: Cultured V79 cells, 1 x 10^6 cells/dish treated in the absence and presence of metabolic activation. Exposure treatments were 5 hours in duration.

Dose levels used in the main test:

- S9: 0, 10, 30, 300 µg/mL

+S9: 0, 3, 10, 30, 100 µg/mL

Duplicate plates/dose level were used.

Positive controls were included (ethyl methanesulfonate (EMS) and 9,10-dimethyl-1,20benzathracene (DMBA)). The main test was repeated.

Results

Cytotoxicity assay:

Precipitate was observed at 300 µg/mL in both the absence and presence of S9. In the absence of S9, marginal cytotoxicity, as measured by relative survival (RS) was observed at 300 µg/mL (mean RS 75%). In accordance with the requirements of OECD 476, in the absence of cytotoxicity, the presence of precipitate is deemed to be an acceptable limiting factor for dose level selection. In the presence of S9, marked cytotoxicity was observed at 100 and 300 µg/mL (RS 16% and 7%, respectively). With the intention of reducing RS to between 10-20%, a maximum dose of 100 µg/mL was selected for the mutation experiment.

Gene mutation assay:

In the absence and presence of S9-mix, the mutation frequencies were under threshold of those of the vehicle control groups. No dose-dependent increases in the mutation frequencies of the treated groups were observed. The positive control chemical induced marked increases in the mutation frequency.

Table B.6.4.1-5: Overview of mammalian gene mutation frequency

5 h exposure in the absence of S9					5 h exposure in the presence of S9				
Conc. (µg/mL)	**Relative survival (%)**		**Mutant frequency**		**Dose level (µg/mL)**	**Relative survival (%)**		**Mutant frequency**	
	Expt 1	**Expt 2**	**Expt 1**	**Expt 2**		**Expt 1**	**Expt 2**	**Expt 1**	**Expt 2**
0	100	100	5.2	9.6	0	100	100	6.0	4.6
10	104	103	4.5	3.2	3	93	91	4.8	3.4
30	110	102	4.5	6.1	10	84	84	6.0	10.7
100	91	104	6.9	5.2	30	56	57	9.3	1.7
300[ppt]	61	91	5.7	2.8	100	12	16	4.4	11.3
Positive control	105	110	196.8 *	184.2 *	Positive control	78	71	240.3 *	247.7 *

MI: mitotic index
Mutant frequency: mutants/10^6 viable cells
PPT: precipitate observed

Positive control: -S9 ethyl methanesulphonate (200 µg/mL); +S9 9,10-dimehtyl-1,2-benzanthracene (5 µg/mL)
* higher than 3-fold of the vehicle control

Acceptability

No guidelines are mentioned in the report, however the study was performed in accordance with OECD 476 of 1984. The study does not fulfil the requirements of the more recent OECD 476 guideline of 1997, such as cytotoxicity criteria and number of dose levels tested. However, the study is considered acceptable.

RMS view during the renewal: The notifier argued the following: The EU review incorrectly stated that the requirements of OECD 476 (1997) were not fulfilled in respect of cytotoxicity. This view is not shared by the notifier. In the presence of metabolic activation, the limiting factor was cytotoxicity, measured in terms

of relative survival. In the absence of metabolic activation the limiting factor was not cytotoxicity (where relative survival was reduced to 61% and 91% in two independent tests), but precipitate. The presence of precipitate during treatment is deemed to be an acceptable limiting factor for dose level selection. The study is therefore deemed to be acceptable meeting the requirements of the relevant test guideline.
RMS agrees with this argumentation and considers this study to comply to OECD 476.

Conclusion

S-31183 did not induce gene mutations in mammalian cells.

3.8.1.4 CA 5.4.1.3/02 (1988): UDS assay in HeLa cells

Report

CA 5.4.1.3/02 (1988)
Assessment of unscheduled DNA repair synthesis in mammalian cells after exposure to S-31183
Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-91-0053

Previous evaluation	In DAR (2005)
Evaluation RMS	No quantitative measure of toxicity was undertaken in this study, with rather a qualitative assessment performed. Although OECD 486 (1986) was deleted from the OECD guidelines for testing of chemical on 2 April 2014, this study provides supporting information for the risk assessment. The positive control 2AA used for the study with S9 mix gave strange results at the high dose (40 µg/mL) in the second study compared to the first study. This finding is not addressed in the study report and no possible reason is given. The study is considered as supplemental.

Characteristics

reference/notifier	:	CA 5.4.1.3/02, 1988	Exposure	:	In vitro
type of study	:	*In vitro* unscheduled DNA synthesis	Doses	:	Range 0.1 – 204.8 µg/ml
year of execution	:	1988	Vehicle	:	DMSO
test substance	:	S-31183 (pyriproxifen), Batch PYG-87074, purity 95.3%	GLP statement	:	yes
Guideline	:	OECD 482	Acceptability	:	acceptable

Conclusion : Negative

Study design

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Lot/Batch No.:	PYG-87074
Purity:	95.3%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2.Control materials:	
Vehicle/final concentration	DMSO (dimethyl sulphoxide) / 1% (v/v)
3.Test organism:	HeLa S3 cells
4.In life dates:	11 February 1988 to 6 April 1988
5.Treatment:	Cultured HeLa cells were exposed to the test article in the presence and absence of S9 mix (rat PCB). In both the absence and presence of S9, cells were exposed to the test article at various concentrations for 180 minutes before being harvested and processed for autoradiography. On the basis of the results of the preliminary toxicity test the following concentrations (in duplicate) were selected in order that an appropriate range of toxicity was observed: -S9/+S9: 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, 102.4, 204.8 µg/mL. The maximum tested concentration was limited by solubility of the test agent in the culture medium. Positive controls were included (4-nitroquinoline-1-oxide and 2-aminoanthracene) and were tested at five dose levels. From 100 nuclei count the mean number of grains were calculated. No concurrent measure of toxicity was performed.

Results

The highest dose tested proved toxic resulting in cell death and subsequent sloughing of cells from coverslips or inhibition of normal S-phase synthesis of DNA.

Without S9-mix, pyriproxifen did not cause any significant increases in the gross or net nuclear grain count at any dose level in either test.

In the presence of S9-mix, a single marginal statistically significant increase in the gross nuclear grain count was obtained at once concentration of pyriproxifen in the second test only. In the first test, small but statistically significant increases in the net nuclear grain count were obtained at several test concentrations. These aparent increases are considered to result from an unusually low vehicle control net grain count (-30 grains per 100 nuclei) rather than being indicative of DNA repair induction especially as there were no significant increases in the corresponding gross nuclear grain count in this test. In the second test, only one marginal statistically significant increase in net grain count was obtained, again without an accompanying significant increase in the gross nuclear grain count.

Both positive control agents caused large highly significant increases in both net and gross nuclear grain counts in both tests.

Table B.6.4.1-6 Results for the incubation without S9

compound	Concen-tration (µg/mL)	Grains per 100 nuclei		Net grains per 100 nuclei		% Nuclei with >3 net grains	
		Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
DMSO	-	130	146	-6	29	1.3	1.5
Pyriproxifen	0.1	124	123	17	16	0	1.5
	0.2	146	132	-19	-9	3	0.5
	0.4	119	149	7	-3	0.5	0.5
	0.8	128	112	-12	-9	0.5	0.5
	1.6	138	109	-5	-3	2	0.5
	3.2	152	-[s]	25	-	1	-
	6.4	-[s]	-[s]	-	-	-	-
	12.8	-[s]	-[s]	-	-	-	-
	25.6	-[s]	-[s]	-	-	-	-
	51.2	-[s]	-[s]	-	-	-	-
	102.4	-[s]	-[s]	-	-	-	-
	204.8[p]	-[s]	-[s]	-	-	-	-
4NQO	0.02	1647***	1924***	1481***	1820***	100	100
	0.04	2354***	2663***	2211***	2560***	100	100
	0.08	2854***	3659***	2710***	3541***	100	100
	0.16	3737***	3208***	3547***	3116***	100	100
	0.32	3204***	3802***	3065***	3663***	100	100

Statistically significant at * p<0.05, **p<0.01, ***p<0.001.

[s]Severe inhibition of S-phase incorporation of ^{3}HTdR, [p]precipitate

Table B.6.4.1-7 Results for the incubation with S9

Compound	Concen-tration (µg/mL)	Grains per 100 nuclei		Net grains per 100 nuclei		% Nuclei with >3 net grains	
		Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
DMSO	-	161	158	-30	-22	1.4	1.8
Pyriproxifen	0.1	165	180	-19	17	2	4
	0.2	133	145	-31	-37	1	0.5
	0.4	209	204*	12*	-6	2	2
	0.8	167	134	6*	-41	0.5	1
	1.6	164	174	27**	-19	2.5	2
	3.2	152	161	3*	-10	0.5	1
	6.4	150	167	-7	9	0	1
	12.8	136	180	31**	18	1.5	3.5
	25.6	135	162	-4	25*	1.5	3
	51.2	133	166	7*	30	2	2
	102.4	113	132	3*	-5	0.5	0.5
	204.8[p]	-[s]	-[s]	-	-	-	-
2AA	2.5	279*	321***	37	135**	5.5	9.5
	5	349***	413***	158***	268***	16.5	27
	10	623***	707***	429***	526***	39	50
	20	691***	860***	493***	627***	54	65
	40	962***	243***	769***	16	79	3.5

Statistically significant at * p<0.05, **p<0.01, ***p<0.001.
[s]Severe inhibition of S-phase incorporation of ^{3}HTdR, [p]precipitate

Acceptability

The study is considered acceptable.

Conclusion

S-31183 did not cause DNA damage *in vitro*.

RMS NL conclusion during renewal: Even though OECD 482 (1986) was deleted, this study still provides supplemental information. Pyriproxifen did not cause DNA damage in this *in vitro* assay.

3.8.2 Animal data

3.8.2.1 CA 5.4.2/01 (1991): Mouse bone marrow micronucleus assay

Report

CA 5.4.2/01 (1991)

Mouse micronucleus test on S-31183

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-11-0082

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. The dose tested was 5000 mg/kg bw instead of the 2000 mg/kg bw described in the OECD guideline (in absence of toxicity). In the study report, this is explained by stating this is the limit dosage recommended by the US EPA and the Japanese EPA, MOHW and MITI and the dosage was therefore considered appropriate. Animals were dosed once. The OECD guideline indicates that when dosing once, samples of bone marrow should be taken at least twice, starting not earlier than 24h after treatment but not extending beyond 48h after treatment. The bone marrow was sampled three times, however, the last sampling time was after 72h for which no explanation is given in the study report. At the 48h-interval, a decrease in polychromatic/normochromatic erythrocytes was seen in the animals treated with pyriproxifen. This indicates that the bone marrow was reached by the test compound in this assay.

Characteristics

Reference	:	CA 5.4.2/01, 1991	exposure	:	Via gavage
Type of study	:	In vivo micronucleus assay	dose	:	0 and 5000 mg/kg bw
year of execution	:	1990	vehicle	:	Corn oil
test substance	:	S-31183 (pyriproxifen), Batch PYG-87074, purity 95.3%	GLP statement	:	yes
route	:	oral	guideline	:	OECD 474
species	:	Mice, CD-1	acceptability	:	Acceptable
			Conclusion	:	Negative

Study design

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PYG-87074

Purity: 95.3%

CAS No.: 95737-68-1

Stability of test compound: Confirmed stable for the duration of the study

2. Test animals:

Species:	Mice
Strain:	CD-1
Age:	35 - 42 days
Weight at dosing:	♂: 19 – 25 g; ♀: 18 – 23 g
Source:	Charles River UK, Limited (Kent, UK)
Acclimation period:	4 - 5 days

B. Study Design and Methods

1.In life dates:	22 August 1990 to 5 November 1990
2.Animal assignment and treatment:	A preliminary toxicity assay was performed with increasing doses up to 5000 mg/kg bw. Ten male and ten female mice were used for this experiment. Very limited toxicity was shown, therefore, the dosage of 5000 mg/kg bw was chosen for the micronucleus test. On arrival animals were weighed and were randomly assigned ti groups. All animals were dosed orally by intragastric gavage with the standard volume of 10 ml/kg bw. The animals were deprived of diet overnight prior to and for two hours after oral dosing. For the vehicle control group, 15 mice/sex were used. For the pyriproxifen group (5000 mg/kg bw), 20 animals/sex were used (including 5 satellite animals). For the positive control group (mitomycin C), 5 animals/sex were used.
3.Statistics	Non-parametric statistical methods based on rank were used to analyze the results. For a comparison of an individual treated group with a concurrent control group, Wilcoxon's sum of ranks test was used.

C. methods

Animals were examined regularly and mortalities or clinical signs over the 72 hour period were recorded. Five males and five females from the negtive control and test compound groups were sacrificed 24, 48 and 72 hours after dosing. The positive control group was sacrificed 24 hours after dosing.The femurs were cleared from tissue and one epiphysis was removed from each bone. Stained bone marrow smears were examined by light microscopy to determine the incidence of micronucleated cells per 1000 polychromatic erythrocytes per animal. The ratio of polychromatic to normochromatic erythrocytes for each animal was assessed by examination of at least 1000 erythrocytes.

Results

Preliminary toxicity test

No mortalities were obtained during this part of the test. Only minor signs of toxicity were observed: hunched posture and pilo-erection shortly after dosing (up to 7 hrs, not seen tehreafter). Therefore, a dosage of 5000 mg/kg bw was chosen for the micronucleus test.

Micronucleus test

One animal (female, in satellite group) died after treatment with the test agent. Post-mortem examination did not reveal any abnormalities and there was no sign of mis-dosing. Clinical signs seen in the animals treated with pyriproxifen included hunched posture and pilo-erection. No clinical signs were obtained for the positive control group.

Pyriproxifen did not cause any statistically significant increases in the number of micronucleated polychromatic erythrocytes. The positive control mitomycin C did cause highly significant increases in the frequency of micronucleated polychromatic eythrocytes.

Pyriproxifen did not cause any substantial increases in the incidence of micronucleated normochromatic erythrocytes. At the 48h sampling time, a small but statistically significant decrease in the p/n ratio was obtained for mice treated with pyriproxifen. This decrease may be evidence of slight bone marrow cell toxicity/depression at the 48 hour sampling time, although no other such decreases were obtained at the other two sampling times.

Table B.6.4.2-1 Results micronucleus test

Sampling time	Compound	Dosage (mg/kg bw)	Ratio p/n	Incidence mnp (number per 1000 cells)	Incidence mnn (number per 1000 cells)
24 hour	Vehicle control	-	1.011	0.0	0.0
	Pyriproxifen	5000	1.015	0.3	0.6
	Positive control	12	1.070	29.9*	3.0
48 hour	Vehicle control	-	0.933	0.4	0.2
	Pyriproxifen	5000	0.719*	0.5	0.3
72 hour	Vehicle control	-	1.632	0.3	0.2
	Pyriproxifen	5000	1.495	0.6	0.5

*Statistically different from control value ($p<0.05$)
p/n ratio: Ratio of polychromatic to normochromatic erythrocytes
mnp: number of micronucleated polychromatic erythorcytes
mnn: number of micronucleated normochromatic erythrocytes

Acceptability

The study is considered acceptable.

Conclusions

Only 1000 instead of at least 2000 immature erythrocytes per animal were scored for the incidence of micronucleated immature erythrocytes. However, no effects were observed and it is concluded that S-31183 did not induce micronuclei in mouse bone marrow cells.

RMS NL conclusion during renewal: Table B.6.4.2-1 gives the combined results for males and females. The applicant provided the following statement: "*Only 1000 PCE/animal were scored rather than the current guideline requirement of 4000 PCE. However in the absence of sex differences, male and female data can be combined for greater statistical power, to provide 10000 PCE/group scored. No statistically increase in micronucleated PCEs was observed in pyriproxyfen treated groups, and all the incidences of micronucleated PCEs were within the laboratory historical control range (0.01-0.25%).*"

RMS considers it acceptable to have combined results for males and females. The study report does give individual animal data which indeed shows no sex differences. No effects for S-31183 were observed for either males or females nor when the two sexes were combined. In addition, the positive control did show a marked increase in micronuclei. It is agreed with the original conclusion that S-31183 did not induce micronuclei in mouse bone marrow cells.

3.8.3 Human data

No human data on the germ cell mutagenicity of pyriproxyfen are available.

3.8.4 Other data

No other data on the germ cell mutagenicity of pyriproxyfen are available.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 CA 5.5.1/01 (1991a): Oral (dietary) carcinogenicity study in the rat

Report

CA 5.5.1/10 (1991a)

Combined chronic toxicity and oncogenicity study in rats with S-31183

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-11-0085

Report

Addendum to the final report: Combined chronic toxicity and oncogenicity study in rats with S-31183 (1994). Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-41-0112

Report

Amendment 1 & 2 to the final report: Combined chronic toxicity and oncogenicity study in rats with S-31183 (1994). Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-41-0113

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

reference	:	CA 5.1.1/01, 1991a[1]	exposure	:	104-weeks, diet
type of study	:	combined toxicity/carcinogenicity study	doses	:	0, 120, 600 and 3000 ppm[2]
year of execution	:	1988 – 1990	vehicle	:	None
test substance	:	Pyriproxyfen (S-31183), batch no. PYG 87074, purity 95.3%	GLP statement	:	Yes
route	:	Oral	guideline	:	OECD guideline 453
species	:	Crl:CD®BR Sprague-Dawley rats	acceptability	:	Acceptable
group size	:	50/sex/group for the main study 30/sex/group for the satellite study	**NOAEL**syst NOAELcarc	:	27.2 mg/kg bw/day 138 mg/kg bw/day (highest dose tested)

1 In an addendum mean and summary data on the results were reported (1994, NNT-41-0112). In an amendment, correction pages on the gross pathology incidence summary were provided (1994, NNT-41-0113).

2 Equal to 0, 5.4, 27.3 and 138.0 mg/kg bw/day in males and 0, 7.0, 35 and 182.7 mg/kg bw/day in females in the main study. Equal to 0, 0, 5.4, 27.2 and 138.7 mg/kg bw/day in males and 0, 7.0, 34.4 and 177.9 mg/kg bw/day in females in the satellite study.

Study design

The study was performed in accordance with OECD 453. Clinical pathology evaluations (haematology, clinical chemistry and urinalysis) were performed on 10 animals/sex/satellite group during weeks 13 (except blood chemistry), 26, 52, 78 and 104. Following week 52 of the study, 10 animals/sex/satellite group were subjected to complete necropsy. After 104 weeks, all remaining animals were subjected to complete gross necropsy.

Materials and methods

A. Materials:

1.Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PYG-87074

Purity: 95.3%

CAS No.: 95737-68-1

Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Rat
Strain:	Sprague-Dawley, Crl:CD®BR
Age:	6 weeks
Weight at dosing:	♂: 186.4 – 240.5 g; ♀: 136.2 – 182.1 g
Source:	Charles River Laboratories Inc. (New York, USA)
Acclimation period:	13 days

B. Study Design and Methods

1.In life dates:	3 May 1988 to 8 May 1990.
2.Animal assignment and treatment:	Animals were randomly assigned to different treatment groups (50 animals/sex/dose), with the following dose levels: 0, 120, 600 or 3000 ppm. The dietary mixtures with test compound were prepared weekly.
3.Statistics	Absolute body weight, food consumption, water consumption, clinical pathology data, oran weight data were compared statistically. If variances of untransformed data were heterogeneous, analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed routinely at the 5% two-tailed probability level.
C. methods	
1.Observations	Rats were observed for mortality and moribundity twice daily. A careful cageside observation for indications of toxic effects was performed once a day. A hands-on physical examination was conducted each week.
2.Body weights	Body weights were measured and recorded prior to treatment, weekly for weeks 1 to 14 and then once every four weeks.
3.Food and water consumption	Food consumption was measured and recoreded weekly for weeks 1 to 14 and once every four weeks thereafter. Water consumption was measured twice weekly at 3- and 4-day intervals to yield a composite weekly water consumption value for weeks 1 to 14, and for once every 4 weeks thereafter.

4.Ophthalmoscopic examination	Prior to treatment, at the end of week 52 and at week 104, an indirect ophthalmoscopic examination was performed.
5.Clinical pathology	During weeks 13, 26, 52, 78 and 104 of treatment, 10 animals/sex/satellite group were placed in urine collection racks for clinical sampling. Blood was collected for haematology and serum chemistry. Urine specimens were collected during the overnight fast in individual urine collection cages and specimens were examined by urinalysis.
6.Terminal studies	Necropsies were performed on all animals. Following 52 weeks of treatment, 10 animals/sex/satellite group were weighed anesthesized and necropsied. At termination (week 105) all surviving main study animals were sacrificed. Organ weights were recorded and histopathology was performed.

Results

Analytical chemistry: Stability of the test material in the diet was demonstrated for 14 days. Homogeneous mixes were shown at all dietary levels. Verification of dietary levels, performed on every fourth mix, revealed values within ± 10% of target concentration and were considered acceptable for this study.

Observations: Survival was 62, 90, 58 and 54% for males and 48, 50, 51 and 72% for females for the control and 120, 600 and 3000 ppm groups, respectively. Survival rates showed no apparent dose-related effects. No clinical signs that could be attributed to treatment were observed.

Body weight: Mean body weight for the high dose males and females were generally lower than control values throughout the study. When compared statistically, mean values were significantly lower than the controls for 600 ppm females at week 13 (<10%), 3000 ppm males at weeks 13, 26, 50 (<10%) and for 3000 ppm females at weeks 13, 26, 50 and 78 (-12 to -14%). In addition, growth rates for high dose males and high- and mid-dose females were significantly lower than control values.

Table B.6.5-1: Mean body weights (in grams)

	Males				Females			
Dose (ppm)	**Week 13**	**Week 26**	**Week 50**	**Week 102**	**Week 13**	**Week 26**	**Week 50**	**Week 102**
0	570.0	638.6	687.3	635.0	319.5	366.0	445.8	464.9
120	556.5	623.3	690.7	621.2	308.9	356.7	429.4	496.3
600	563.3	624.9	691.3	631.9	305.8*	363.1	425.1	459.9
3000	537.9*	591.7*	655.3*	631.4	281.4*	322.3*	383.7*	433.4

*Significantly different from control value (p≤0.05)

Table B.6.5-2: Mean body weights gains

	Males	Females

	0 ppm	120 ppm	600 ppm	3000 ppm	0 ppm	120 ppm	600 ppm	3000 ppm
Wk 1-13	353.9 g	342.3 g (-3.3%)	348.9 g (-1.5%)	322.2 g (--9%)	157.6 g	149 g (-5.5%)	145.6 g (-7.6%)	122.5 g (-22%)
Wk 1-26	422.5 g	409.1 g (-3.2%)	410.5 g (-2.8%)	376 g (-11%)	204.1 g	196.8 g (-3.6%)	202.9 g (-0.6%)	163.4 g (-20%)
Wk 1-50	471.2 g	476.5 g (+1%)	476.9 g (+1.2%)	439.6 g (-6.7%)	283.9 g	269.5 g (-5%)	264.9 g (-6.7%)	224.8 g (-21%)
Wk 1-102	418.9 g	407 g (-2.8%)	417.5 g (-0.04%)	415.7 g (-0.08%)	303 g	336.4 g (+11%)	299.7 g (-1%)	274.5 g (-9.5%)

Food and water consumption: Mean food consumption values for high dose rats was generally lower than controls up to week 70, at which time the food consumption for the female control group started to decline at a faster rate than the female treated groups.Mean values were significantly decreased at week 50 and increased at week 102 for the 120 and 600 ppm females. Significant decreases were noted for the 3000 ppm males at week 26 and foor 3000 ppm females at weeks 13, 26 and 50.

Statistical evaluation of mean water consumption values revealed significantly lower values in females in all treated groups at week 13. The differences noted were 11% for 120 ppm group, 13.5% for the 600 ppm group and 16.5% for the 3000 ppm group.

Ophthalmoscopy: No treatment-related findings.

Haematology and clinical chemistry: A statistically significant increase in mean eosinophil count was noted in males in week 13 given 3000 ppm. A statistically significant increase in mean segmented neutrophil count was noted at 600 ppm in females in week 104. As these findings were not observed in the other sampling intervals, were not dose-related and/or not observed in the semichronic toxicity studies with rats, these findings were considered incidental and not toxicologically relevant. No treatment-related changes in haematology parameters were observed.

Serum cholesterol was statistically significant increased in males at 3000 ppm in weeks 26 and 52 (149 and 147% of controls, respectively). Serum cholesterol was slightly increased in males in week 78 (119% of controls). No significant difference from control in serum cholesterol was noted in males at week 104. In females serum cholesterol was statistically significant increased in week 26 only (145% of controls). The changes observed in cholesterol point to perturbations in liver lipid metabolism at 3000 ppm.

Gamma-glutamyl transferase activity was significantly increased in males at 3000 ppm in week 104 and in females at 120, 600 and 3000 ppm in weeks 26 and 52 only. Alkaline phosphatase activity was significantly increased in males at all dose groups in weeks 26, 52 and 78. Observed changes were increased 111-166% of control values, however, the activity (87-104 U/L) remained within the historical control range (45 - 114 U/L). Although the changes in gamma-glutamyl transferase activity and alkaline phosphatase activity were slight, both changes point to hepatobiliary effects. However, as these changes in clinical biochemistry at 120 and 600 ppm were not accompanied by changes in liver weight or an increased incidence of histopathological changes and did not occur throughout the study period, observed changes in glutamyl transferase activity and alkaline

phosphatase activity at 120 and 600 ppm were not considered toxicologically relevant. A decrease in globulin (71% of controls) and an increase in the albumin/globulin ratio (146% of controls) were noted in females at 3000 ppm in week 104. Further statistically significant changes in clinical biochemistry were considered incidental findings, since they were only slight, not dose-related or did not occur consistently over the sampling times.

Table B.6.5-3: Haematology and clinical chemistry findings

	0		120		600		3000	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Haematology								
Eosinophils								
-week 13	0.1	0.1	0.1	0.1	0.1	0.1	0.3*	0.1
-week 52	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
-week 104	0.1	0.1	0.1	0.1	0.2	0.0	0.1	0.1
Segmented neutrophils								
-week 52	3.2	1.6	2.5	1.7	3.0	2.2	2.5	0.9
-week 104	5.3	3.0	5.9	3.0	9.2	5.2*	7.6	2.5
Clinical chemistry								
Cholesterol								
-week 26	90	90	78	108	99	101	134*	131*
-week 52	103	121	109	173	120	113	151*	144
-week 78	135	168	130	130	122	126	161	142
-week 104	193	132	108	136	89*	143	134	133
γ-GT								
-week 26	1	0	1	1*	1	2*	1	2*
-week 52	1	0	1	1	1	1*	1	1*
-week 78	1	0	1	1	1	1	2	1
-week 104	0	0	1	0	0	1	2*	1
Alk. phos								
-week 26	71	43	85	44	92*	39	104*	53
-week 52	67	33	87*	39	99*	34	100*	40
-week 78	61	40	93*	47	91*	35	101*	51
-week 104	81	43	80	45	114	60	90	44
Globulin								
-week 26	2.2	1.8	2.1	1.8	2.2	2.0	2.0	1.5
-week 52	2.2	2.2	2.2	2.0	2.3	2.1	2.1	1.9
-week 78	2.9	2.9	2.8	2.4	3.0	2.4	2.7	2.4
-week 104	2.9	3.1	3.5	3.0	3.8	3.1	3.0	2.2*
A/G ratio								
-week 26	2.25	3.28	2.38	3.43	2.31	2.84	2.60	4.32*
-week 52	2.17	2.74	2.17	3.02	2.15	2.68	2.30	3.18
-week 78	1.57	1.91	1.59	2.42	1.50	2.30	1.70	2.31
-week 104	1.46	1.67	1.15	1.75	0.96*	1.59	1.43	2.43*

*Significantly different from control ($p \leq 0.05$)

Urinalysis: A slight increase in pH in males at 120 and 3000 ppm was noted in week 78 (6% for both dose groups). This finding was considered incidental as no dose-response was observed and occurred only in week 78. A non-statistically significant increase in urinary protein was noted in females at 3000 ppm in weeks 26 and 52 (300% and 90%, resepctively). As this finding was not consistent over the study period, it was not considered toxicologically relevant.

Gross pathology: There were no apparent treatment-related gross pathology findings noted.

Organ weights: At interim sacrifice after 52 weeks, absolute liver weight was increased in males and females at 3000 ppm (115 and 113% of controls, respectively) and relative liver weight was statistically significantly increased in females at 3000 ppm (120% of controls). At terminal sacrifice absolute liver weight were slightly increased in males and females of the high dose group (108 and 107% of controls, respectively) and relative liver weight was increased in females at 3000 ppm (127% of controls).

Table B.6.5-4: Organ weight findings

Parameter	0 ppm		120 ppm		600 ppm		3000 ppm	
	m	f	m	f	m	f	m	f
Absolute organ weights (g)								
Liver								
-week 52	16.39	10.08	16.46	9.95	16.11	10.08	18.88	11.34
-week 104	15.62	12.33	15.39	11.92	16.81	12.14	19.96	13.22
Kidney								
-week 52	4.16	2.52	4.35	2.55	4.31	2.57	4.60	2.67
-week 104	4.40	3.35	4.24	3.06	4.89	3.11	4.23	3.04
Testis								
-week 52	3.90	-	3.73	-	3.51	-	3.71	-
-week 104	3.26	-	3.78	-	3.28	-	4.36	-
Thyroid								
-week 52	0.035	0.031	0.037	0.029	0.039	0.031	0.042	0.031
-week 104	0.067	0.040	0.043	0.040	0.047	0.039	0.043	0.040
Ovaries								
-week 52	-	0.104	-	0.101	-	0.108	-	0.100
-week 104	-	0.152	-	0.168	-	0.125	-	0.113
Organ-to-body-weight ratios (g)								
Liver								
-week 52	2.583	2.480	2.418	2.532	2.416	2.471	2.726	2.969*
-week 104	2.548	2.766	2.404	2.719	2.960	2.988	2.879	3.522
Kidney								
-week 52	0.655	0.622	0.641	0.653	0.648	0.630	0.669	0.701
-week 104	0.722	0.771	0.671	0.708	0.852	0.777	0.719	0.825
Testis								
-week 52	0.614	-	0.551	-	0.525	-	0.542	-
-week 104	0.534	-	0.601	-	0.559	-	0.736	-
Thyroid								

-week 52	0.0055	0.0077	0.0054	0.0075	0.0059	0.0076	0.0060	0..0083
-week 104	0.0109	0.0089	0.0070	0.0092	0.0081	0.0097	0.0075	0.105
Ovaries								
-week 52	-	0.0255	-	0.0259	-	0.0264	-	0.0270
-week 104	-	0.0337	-	0.0392	-	0.0310	-	0.0298

*Significantly different from control ($p \leq 0.05$)

Histopathology: At post-mortem necropsy, an increased incidence of dark areas in the liver was noted in females at 3000 ppm.

Treatment-related histopathological changes were noted in the liver at 3000 ppm. A slightly increased incidence of liver necrosis was noted in males at 3000 ppm that died during the study. Liver necrosis was only noted in one surviving animal at 600 ppm. Treatment-related increased incidences in neoplastic lesions were not observed during histopathological examination.

Table B.6.5-5 Pathology findings

	0 ppm		**120 ppm**		**600 ppm**		**3000 ppm**	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Pathology								
macroscopy								
- liver, dark area[1]	2/28	1/21	3/23	0/23	5/28	3/23	2/27	11/34
Microscopy								
non-neoplastic lesions								
- liver, necrosis[2]	0/22	4/29	2/17	3/27	3/22	4/27	8/23	4/16

[1]Data of terminal sacrifice of the main study. No increased incidence was noted in unscheduled deaths or satellite groups.

[2]Data of the unscheduled deaths of the main study.

Table B.6.5-6 Neoplastic findings

Parameters	♂ (ppm)				♀ (ppm)			
	0	120	600	3000	0	120	600	3000
Adrenal, medulla:								
No. examined	27	7	3	27	21	12	13	34
Not remarkable	17	4	0	20	11	9	12	25
- Ben. phaechromocytoma	8	3	3	4	1	1	0	0
- Mal. phaechromocytoma	3	0	0	0	0	2	0	3

Parameters	♂ (ppm)				♀ (ppm)			
	0	120	600	3000	0	120	600	3000
Thyroid:								
No. examined Not remarkable	28 17	0 -	0 -	27 13	21 14	0 -	0 -	34 25
- Mal. C cell carc. - Hyperplasia, follicular focal - Ben. follicular aden. - Mal. follicular carc. - Ben. C cell aden. - Mal. carc., ultimobranchial	2 2 3 3 3 0	- - - - - -	- - - - - -	2 0 3 1 4 0	1 1 1 0 4 1	- - - - - -	- - - - - -	3 1 2 0 4 0
Liver:								
No. examined Not remarkable	28 11	33 15	28 6	27 12	21 4	23 11	23 11	34 12
- Mal. hepatocellular carc. - Ben. hepatocellular carc.	3 0	0 0	0 0	0 1	1 0	0 0	0 0	1 0
Kidney:								
No. examined Not remarkable	28 3	33 2	28 3	27 5	21 0	23 3	23 3	34 3
- Ben. tubule cell adenoma	1	0	0	0	0	0	0	0
Pancreas:								
No. examined Not remarkable	28 23	0 -	0 -	27 21	21 17	0 -	0 -	34 31
- Ben. Islet cell aden. - Mal. islet cell carc.	3 1	- -	- -	2 0	2 1	- -	- -	1 0
Rectum:								
No. examined Not remarkable	28 25	0 -	0 -	27 27	21 18	1 0	0 -	34 28
- Mal. leiomyosarcoma	0	-	-	0	0	1	-	0
Lymph, mesenteric:								
No. examined Not remarkable	28 27	0 -	2 0	27 26	21 21	1 0	1 0	34 34
- Mal. hemangiosarcoma - Mal. carc.	0 0 0	- - -	- - -	1 0 0	0 0 0	0 0 1	0 1 0	0 0 0

Parameters	♂ (ppm)				♀ (ppm)			
	0	120	600	3000	0	120	600	3000
- Ben. haemangioma								
Testes:								
No. examined Not remarkable	28 18	9 1	8 0	27 19	- -	- -	- -	- -
- Ben. Interstitial cell tumor	4	5	6	4	-	-	-	-
Ovary:								
No. examined Not remarkable	- -	- -	- -	- -	21 7	5 1	2 1	34 9
- Ben. sertoliform tubular aden. - Mal. cystadenocarc.	- -	- -	- -	- -	2 0	0 0	0 0	3 1
Uterus:								
No. examined Not remarkable	- -	- -	- -	- -	21 17	3 0	6 1	34 29
- Ben. Endometrial stromal polyp	-	-	-	-	1	2	1	1
- Mal. squamous cell carc.	-	-	-	-	0	0	0	1
Uterus, cervix:								
No. examined Not remarkable	- -	- -	- -	- -	21 20	0 -	1 0	34 34
- Ben. granular cell tumor	-	-	-	-	1	-	0	0
Vagina:								
No. examined Not remarkable	- -	- -	- -	- -	21 21	1 0	0 -	34 33
- Ben. endo. stromal polyp	-	-	-	-	0	0	-	1
- Ben. leiomyoma	-	-	-	-	0	1	-	0
Mammary gland:								
No. examined Not remarkable	3 2	5 4	4 3	4 4	21 8	13 1	19 6	33 14
- Ben. fibroaden. - Mal. carc. - Ben. aden.	1 - -	0 - -	0 - -	0 - -	7 6 0	7 3 0	8 5 1	11 5 2
Bone, sternum:								
No. examined Not remarkable	28 28	0 -	0 -	27 27	21 21	0 -	0 -	33 32
- Mal. osteosarcoma	0	-	-	0	0	-	-	1

Parameters	♂ (ppm)				♀ (ppm)			
	0	120	600	3000	0	120	600	3000
Skin:								
No. examined Not remarkable	24 1	26 1	25 0	20 0	8 3	8 1	7 5	6 3
- Ben. keratoacanthoma	3	6	3	1	1	1	0	1
- Ben. squamous cell papilloma	0	0	0	0	0	1	0	0
- Ben. sebaceous gland aden.	0	0	0	1	0	0	0	0
Haematoneoplasia:								
No. examined Not remarkable	28 28	1 0	0 -	27 27	21 21	0 -	1 0	34 34
- Mal. lymphoma, histoiocytic	0	1	-	0	0	-	0	0
- Mal. lymphoma, lymphocytic	0	0	-	0	0	-	1	0

Mal.: malignant
Ben.: benign
Abs: absolute
Carc.: carcinoma
Aden.: adenoma
Endo.: endometrial

Acceptability

The study was performed in accordance with OECD guideline 453, with the exception that haematology and clinical biochemistry measurements should have been performed in 20 animals/sex/dose instead of 10 animals/sex/dose. However, the study is considered acceptable for evaluation. Historical control data for alkaline phosphatase activity (range males 45 - 114, females 16 – 69, n=70) and on liver histopathology were presented by the notifier separately (time period 1986-1996, Sprague Dawley rats).

Conclusions

The NOAEL is set at 600 ppm (equal to 27.2 mg/kg bw/day in males and 34.4 mg/kg bw/day in females), based on changes in clinical biochemistry and increased liver weights and histopathological changes in the liver. Carcinogenicity is not demonstrated.

RMS conclusion during renewal: Agreed with the original conclusion, NOAEL is 600 ppm (27.2 mg/kg bw/day) based on bw changes, liver weight changes, changes in clincal chemistry and pahtological changes in the liver found at the top dose. No indications for carcinogenicity were found in this study.

3.9.1.2 CA 5.5.1/02 (1991b): Oral (dietary) carcinogenicity study in the mouse

Report

CA 5.5.1/02 (1991b)

Oncogenicity study in mice with S-31183

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-11-0084

Report

Amendment to the final report: Oncogenicity study in mice with S-31183 (1994). Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-41-0117

Report

Supplemental data and review of oncogenicity study with S-31183 (Sumilarv) in mice (1994). Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-41-0116

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment; additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

reference	:	CA 5.5.1/02, 1991b[1]	exposure	:	78-weeks, diet
type of study	:	carcinogenicity study	doses	:	0, 120, 600 and 3000 mg/kg food[2]
year of execution	:	1988 – 1990	Vehicle	:	None
test substance	:	Pyriproxyfen (S-31183), batch no. PYG 87074, purity 95.3%	GLP statement	:	Yes
route	:	oral	Guideline	:	OECD guideline 451
species	:	mouse, ICR	Acceptability	:	acceptable
group size	:	60/sex/group	**NOAEL**syst	:	<16.4 mg/kg bw/day
			NOAELcarc	:	107.3 mg/kg bw/day (precautionary)

1 In an addendum supplemental data and an additional review were provided (NNT-41-0117,1994). In an amendment, correction pages on the cause of death were provided (NNT-11-0084, 1994).

2 equal to 0, 16.4, 81.3 and 422.5 mg/kg bw/day in males and 0, 21.1, 107.3 and 532.8 mg/kg bw/day in females

Study design

The study was performed in accordance with OECD guideline 451. In addition, clinical haematology evaluations were performed on 10 animals/sex/dose in week 52 and 78. All surviving animals in week 78 and the animals used for the haematology evaluations in week 52 were sacrificed.

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PYG-87074

Purity: 95.3%

CAS No.: 95737-68-1

Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Mouse
Strain:	Crl:CD-1(ICR)BR
Age:	7 weeks
Weight at dosing:	♂: 27.7 – 35.6 g; ♀: 19.8 – 28.5 g
Source:	Charles River Laboratories, Inc. (New York, USA)
Acclimation period:	3 weeks

B. Study Design and Methods

1.In life dates:	12 July 1988 to 15 January 1990
2.Animal assignment and treatment:	Animals were randomly assigned to 4 groups, 60/sex/dose: control, low dose of 120 ppm, mid-dose of 600 ppm and a high group of 3000 ppm. Fresh dietary mixtures were prepared and fed to the mice every week.
3.Statistics	Absolute body weight, food consumption, body weight gain, organ weights were compared statistically to the data from the same sex of each treated group. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Tests for homogeneity of variance and ANOVA were evaluated at the 5% one-tailed probability level. Group comparisons were evaluated at the 5% two-tailed probability level.
C. Methods	
1.Observations	The mice were observed twice daily for mortality and moribundity. Careful cageside observations were performed once daily for indications of toxic effects. Hand-on physical examinations were performed once each week.
2.Body weights	Body weights were measured and recorded prio to treatment, weekly for weeks 1 – 16 and once every 4 weeks thereafter.
3.Food consumption	Food consumption was measured and recorded weekly for weeks 1-16 and once every 4 weeks thereafter.
4.Ophthalmoscopic examination	Prior to treatment an indirect ophthalmoscopic examination was performed on each animal.

5.Clinical pathology Ten mice/sex/group were designated for the interim kill (week 52) and the first 10 surviving mice/sex/group during week 78 were fasted overnight for clinical sampling (haematology). At the end of the study period, necropsies were performed on all animals. Organ weights were measured and histopathology was studied.

6.Terminal studies

Results

Analytical chemistry: Stability of the test material in the diet was demonstrated for 14 days. Homogeneous mixes were shown at all dietary levels. Verification of dietary levels revealed values within ± 10% of target concentration and were considered acceptable for this study.

Observations: At 3000 ppm, a statistically significant reduced survival rate was noted in males and females; at 600 ppm the survival rate was statistically significant reduced in males. Systemic amyloidosis was identified as the primary cause of death. Most animals died in the second part of the study. Survival rates were derived from survival analyses and ranged from 18 to 57% for the males and 36 to 61% in females. Statistical analyses of survival revealed that the mid- and high-dose males and high-dose females had a significantly lower survival rate than the respective control groups. Probabilities for trend and group comparisons are presented in the following table:

Table B.6.5-7: Survival rates and probabilities for trend/group comparisons

Dose (ppm)	% survival		Comparison	Probabilities	
	Male	female		Male	Female
0	57	61	Trend	0.0002	0.0021
120	45	56	0 ppm vs. 120 ppm	0.0541	0.4547
600	28	45	0 ppm vs. 600 ppm	0.0023	0.0918
3000	18	36	0 ppm vs. 3000 ppm	0.0001	0.0061

A slight increased incidence in reduced motor activity and hunched posture were noted among males and females given 3000 ppm.

Table B.6.5-8: Survival and clinical signs

	0 ppm		120 ppm		600 ppm		3000 ppm	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Survival	31/50	33/50	24/50	32/49	15/50[1]	26/50	12/50[1]	20/50[1]

	0 ppm		120 ppm		600 ppm		3000 ppm	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Clinical signs								
- hunched posture	12/50	13/50	11/50	20/49	15/50	22/50	19/50	27/50
- reduced motor activity	3/50	2/50	6/50	5/49	7/50	8/50	11/50	8/50

Body weights: Mean absolute body weights were slightly reduced throughout the study period for males given 3000 ppm (<10%). Body weight gain was statistically significant reduced in males from the start of the study until week 24, and was normal over the study periods 0-52 weeks and 0-78 weeks. A slightly reduced body weight gain was noted in females over the study period (0-76 weeks, 89% of control).

Table B.6.5-9: Mean body weights and body weight gains (in grams)

	Males				Females			
Dose (ppm)	**Week 13**	**Week 24**	**Week 52**	**Week 76**	**Week 13**	**Week 24**	**Week 52**	**Week 76**
Mean body weight								
0	39.4	41.3	42.7	42.3	29.3	31.1	34.3	35.8
120	39.2	40.4	41.7	42.6	29.7	317	35.0	36.5
600	39.7	41.6	43.4	44.8*	28.9	31.4	34.8	35.8
3000	37.5*	38.8*	40.9*	41.2	29.3	30.9	34.5	33.9*
Body weight gain								
	Wk 0-13	**Wk 0-24**	**Wk 0-52**	**Wk 0-76**	**Wk 0-13**	**Wk 0-24**	**Wk 0-52**	**Wk 0-76**
0	7.9	9.8	11.2	10.9	5.6	7.4	10.6	12.2
120	8.1	9.4	10.8	11.7	6.0	8.0	11.3	13.0
600	8.3	10.2	11.9	13.0*	5.6	8.1	11.4	12.4
3000	6.6*	7.9*	10.1	10.1	5.9	7.5	11.1	10.8

*Significantly different from control value ($p \leq 0.05$)

Food consumption: No consistent treatment-related changes in food consumption were noted throughout the study.

Haematology and clinical chemistry: A statistically significant decrease in haemoglobin (93% of controls) was noted in females at 3000 ppm and in MCV (95% of controls) in males at 3000 ppm. Both changes were only noted in week 52. A statistically significant decrease in leucocyte count and corrected leucocyte count was noted in males given 600 ppm in week 78. In the absence of a dose-response this finding was not considered toxicologically relevant.

Table B.6.5-10: Hameatological findings

Parameter	0 ppm		120 ppm		600 ppm		3000 ppm	
	m	f	m	f	m	f	m	f
Haemoglobin								
-week 52	14.1	15.3	15.1	15.5	14.5	15.2	14.5	14.2*
-week 78	14.8	14.7	13.7	13.7	15.0	14.2	13.6	13.6
MCV								
-week 52	48.6	50.1	48.2	49.3	47.5	49.8	46.0	48.2
-week 78	46.9	50.0	48.0	48.6	46.4	48.7	47.2	47.7

*Significantly different from control value (p≤0.05)

Organ weights: At interim sacrifice, absolute liver weight was increased in females at 600 (115% of controls) and 3000 ppm (131% of controls). Relative liver weights were increased at interim sacrifice in females at 3000 ppm (123% of controls). In addition, at interim sacrifice, absolute spleen weights were increased in females at 3000 ppm (150% of controls).

At terminal sacrifice relative liver weights were slightly increased at 3000 ppm in males and females (both 107% of controls). A slight increase in relative liver weight was also noted in males at 600 ppm (106% of controls). Absolute kidney weights were decreased in males at 3000 ppm at terminal sacrifice (86% of controls).

Table B.6.5-11: Organ weights

Parameter	0 ppm		120 ppm		600 ppm		3000 ppm	
	m	f	m	f	m	f	m	f
Absolute organ weights (g)								
Liver								
-interim	1.93	1.50	1.82	1.61	2.00	1.72	1.97	1.97*
-final	1.99	1.82	1.80	1.78	2.00	1.98	2.02	1.80
Spleen								
-interim	0.15	0.12	0.12	0.13	0.16	0.13	0.13	0.18*
-final	0.14	0.16	0.20	0.14	0.12	0.18	0.12	0.15
Kidney								
-interim	0.77	0.50	0.71	0.49	0.69	0.50	0.67	0.49
-final	0.79	0.58	0.75	0.57	0.80	0.62	0.68*	0.52
Testis								
-interim	0.25	-	0.24	-	0.24	-	0.27	-
-final	0.21	-	0.22	-	0.21	-	0.21	-
Ovaries								
-interim	-	0.031	-	0.037	-	0.092	-	0.065
-final	-	0.131	-	0.506	-	0.177	-	0.077
Organ-to-body-weight ratios (g)								
Liver								
-interim	5.122	5.232	4.855	5.191	5.343	5.499	5.619	6.441*
-final	5.283	5.943	5.210	5.734	5.507	6.294	5.662	6.372
Spleen								

-interim	0.389	0.438	0.318	0.414	0.428	0.403	0.371	0.574
-final	0.373	0.529	0.584	0.463	0.325	0.583	0.323	0.531
Kidney								
-interim	2.039	1.738	1.899	1.587	1.823	1.600	1.910	1.608
-final	2.110	1.905	2.158	1.851	2.217	1.965	1.911	1.863
Testis								
-interim	0.663	-	0.635	-	0.643	-	0.761	-
-final	0.576	-	0.623	-	0.579	-	0.596	-
Ovaries								
-interim	-	0.1053	-	0.1189	-	0.2899	-	0.2097
-final	-	0.4292	-	1.6273	-	0.5673	-	0.2799

*Significantly different from control value ($p \leq 0.05$)

Pathology: At post-mortem necropsy, an increased incidence in granular/pitted/rough kidneys was noted in males and females at 3000 ppm, mainly among the unscheduled deaths.

Histopathological changes included a treatment-related increase in incidence and severity of systemic amyloidosis. Amyloidosis was noted several organs as the adrenal cortex, thyroid, heart, spleen, kidneys, liver, stomach, ovary, testes, etc. An increase in severity of amyloidosis was noted in males at 600 and 3000 ppm and females at 3000 ppm, in unscheduled deaths and animals surviving until interim or terminal sacrifice. Furthermore, histopathological examination of the kidneys revealed an increased incidence of mineralization of the renal tubules (unscheduled deaths and terminal sacrifice), chronic progressive nephropathy (interim and terminal sacrifice) and segmental cortical atrophy (interim and terminal sacrifice) at 3000 ppm in males and/or females.

Table B.6.5-12: Amyloidosis findings

Organ	**Males**				**Females**			
	0	**120**	**600**	**1200**	**0**	**120**	**600**	**3000**
UNSCHEDULED DEATHS								
Thyroid (total finding/total nr examined; %)	**19/22 (86%)**	**23/28 (82%)**	**30/37 (81%)**	**37/42 (88%)**	**14/20 (70%)**	**19/23 (83%)**	**20/29 (69%)**	**32/32 (100%)**
Minimal	0	1	0	0	1	0	0	0
Slight	10	10	6	2	4	10	13	7
Moderate	8	5	15	16	6	5	7	20
Moderate-severe	1	7	8	17	3	4	0	5
Severe	0	0	1	2	0	0	0	0
Heart (total finding/total nr examined; %)	**19/22 (86%)**	**21/28 (75%)**	**29/37 (78%)**	**37/42 (88%)**	**14/20 (70%)**	**15/23 (65%)**	**19/29 (66%)**	**31/32 (97%)**
Minimal	0	1	0	0	1	1	3	3
Slight	11	12	17	8	7	4	10	17
Moderate	6	5	11	25	6	6	3	10
Moderate-severe	2	3	1	4	0	4	3	1
Severe	0	0	0	0	0	0	0	0

Kidneys (total finding/total nr examined; %)	**19/22 (86%)**	**24/28 (86%)**	**32/37 (86%)**	**38/42 (90%)**	**16/20 (80%)**	**21/23 (91%)**	**23/29 (79%)**	**32/32 (100%)**
Minimal	0	0	0	0	0	1	3	0
Slight	0	2	1	2	1	1	1	0
Moderate	1	2	2	5	2	1	0	1
Moderate-severe	17	18	22	17	2	11	6	4
Severe	1	2	7	14	11	7	13	27
Liver (total finding/total nr examined; %)	**19/22 (86%)**	**20/28 (71%)**	**32/37 (86%)**	**35/42 (83%)**	**15/20 (75%)**	**16/23 (70%)**	**20/29 (69%)**	**32/32 (100%)**
Minimal	0	0	3	0	1	0	2	0
Slight	11	12	20	11	10	12	12	10
Moderate	8	8	8	22	4	3	6	20
Moderate-severe	0	0	1	1	0	1	0	2
Severe	0	0	0	1	0	0	0	0
Stomach (total finding/total nr examined; %)	**8/22 (36%)**	**9/28 (32%)**	**24/37 (65%)**	**32/42 (76%)**	**6/20 (30%)**	**8/23 (35%)**	**9/29 (31%)**	**25/32 (78%)**
Minimal	1	1	1	0	2	1	1	3
Slight	4	5	11	4	4	2	6	6
Moderate	3	3	11	20	0	2	2	11
Moderate-severe	0	0	1	7	0	3	0	5
Severe	0	0	0	1	0	0	0	0
INTERIM SACRIFICE								
Adreral cortex (total finding/total nr examined; %)	**6/9 (67%)**	**-**	**-**	**5/9 (56%)**	**4/10 (40%)**	**-**	**-**	**0/0 (0%)**
Minimal	1	-	-	0	0	-	-	0
Slight	0	-	-	1	2	-	-	0
Moderate	3	-	-	1	2	-	-	0
Moderate-severe	2	-	-	3	0	-	-	0
Thyroid (total finding/total nr examined; %)	**4/9 (44%)**			**4/9 (44%)**	**1/10 (10%)**			**6/10 (60%)**
Minimal	3	-	-	0	1	-	-	0
Slight	1	-	-	3	0	-	-	3
Moderate	0	-	-	1	0	-	-	3
Heart (total finding/total nr examined; %)	**6/9 (67%)**			**5/9 (56%)**	**2/10 (20%)**			**6/10 (60%)**
Minimal	3	-	-	1	0	-	-	1
Slight	3	-	-	4	2	-	-	4
Moderate	0	-	-	0	0	-	-	1
Liver (total finding/total nr examined; %)	**5/9 (56%)**	**6/10 (60%)**	**7/9 (78%)**	**3/9 (33%)**	**3/10 (30%)**	**2/10 (20%)**	**2/9 (22%)**	**8/10 (80%)**
Minimal	1	0	1	0	1	1	0	0
Slight	4	6	6	3	2	1	2	7

Moderate	0	0	0	0	0	0	0	1
Kidney (total finding/total nr examined; %)	**7/9 (78%)**	**8/10 (80%)**	**7/9 (78 %)**	**5/9 (56%)**	**4/10 (40%)**	**4/10 (40%)**	**5/9 (56%)**	**8/10 (80%)**
Minimal	0	1	0	0	1	2	2	0
Slight	3	6	5	5	3	1	3	3
Moderate	4	1	1	0	0	1	0	3
Moderate-severe	0	0	1	0	0	0	0	2
Stomach (total finding/total nr examined; %)	**0/9 (0%)**	**-**	**-**	**2/9 (22%)**	**0/10 (0%)**	**-**	**-**	**2/10 (20%)**
Minimal	0	-	-	1	0	-	-	0
Slight	0	-	-	1	0	-	-	0
Moderate	0	-	-	0	0	-	-	2
TERMINAL SACRIFICE								
Adrenal cortex (total finding/total nr examined; %)	**16/28 (57%)**			**1/9 (11%)**	**10/30 (33%)**			**5/18 (28%)**
Minimal	0	-	-	0	1	-	-	0
Slight	1	-	-	0	2	-	-	1
Moderate	3	-	-	0	5	-	-	1
Moderate-severe	12	-	-	0	2	-	-	3
Severe	0	-	-	1	0	-	-	0
Thyroid (total finding/total nr examined; %)	**15/28 (54%)**			**1/9 (11%)**	**9/30 (30%)**			**5/8 (63%)**
Slight	7	-	-	0	4	-	-	1
Moderate	7	-	-	0	3	-	-	2
Moderate-severe	1	-	-	1	1	-	-	2
Severe	0	-	-	0	1	-	-	0
Heart (total finding/total nr examined; %)	**11/28 (39%)**			**1/9 (11%)**	**6/30 (20%)**			**3/8 (38%)**
Slight	9	-	-	0	4	-	-	3
Moderate	2	-	-	0	2	-	-	0
Moderate-severe	0	-	-	1	0	-	-	0
Liver (total finding/total nr examined; %)	**16/28 (57%)**	**7/22 (32%)**	**6/14 (43 %)**	**1/9 (11%)**	**9/30 (30%)**	**9/27 (33%)**	**8/22 (36%)**	**4/18 (22%)**
Minimal	0	1	0	0	1	0	1	0
Slight	12	6	6	0	7	6	7	2
Moderate	4	0	0	1	1	3	0	2
Kidney (total finding/total nr examined; %)	**22/28 (79%)**	**13/22 (59%)**	**9/14 (64 %)**	**3/9 (33%)**	**15/30 (50%)**	**14/27 (52%)**	**12/22 (55%)**	**8/18 (44%)**
Minimal	5	5	2	2	4	3	2	1
Slight	2	1	2	0	1	1	0	2
Moderate	0	3	0	1	3	3	2	3
Moderate-severe	15	4	5	0	5	1	8	0
Severe	0	0	0	0	2	6	0	2

Table B.6.5-13: Pathology findings

	0 ppm		120 ppm		600 ppm		3000 ppm	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Pathology								
Macroscopy								
Gran/pitted/rough kidneys	2/23	3/20	3/28	3/23	0/37	2/29	12/42	24/32
Unscheduled deaths:	0/9	0/10	0/10	0/10	1/9	0/9	0/9	3/10
Interim sacrifice:	1/28	0/30	0/22	0/27	0/14	0/22	0/9	5/18
Terminal sacrifice:								
microscopy								
non-neoplastic lesions								
UNSCHEDULED DEATHS	3/23	1/19	3/28	3/23	3/36	3/29	14/42	29/32
kidneys:								
- mineralization tubules								
INTERIM								
kidneys:								
- chr. progr. nephropathy	4/9	3/10	2/10	3/10	2/9	3/9	6/9	9/10
- seg. cortical atrophy	2/9	0/10	1/10	1/10	1/9	2/9	4/9	6/9
TERMINAL								
Kidneys:	11/28	7/30	10/22	10/27	6/14	6/22	8/9	10/18
- chr. progr. nephropathy	12/28	2/30	8/22	1/27	5/14	1/22	1/9	16/18
- mineralization tubules	0/28	2/30	1/22	3/27	1/14	4/22	1/9	6/18
- seg. cortical atrophy								
Neoplastic findings								
UNSCHEDULED DEATHS								
No. examined	23	19	27	20	37	29	42	32
Adrenal cortex								
Benign adenoma	0	0	0	0	0	0	0	0
Thyroid								
Malign C-cell carcin	0	0	0	0	0	0	0	1 (3%)
Heart								
Malign haemangiosarc	0	0	0	0	1 (3%)	0	0	0
Liver								
Benign hepatocell adenoma	1 (4%)	1 (5%)	2 (7%)	0	2 (5%)	1 (3%)	2 (5%)	0
Malign hepatocell carcin	1 (4%)	0	0	1 (5%)	0	0	0	0

	0 ppm		120 ppm		600 ppm		3000 ppm	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Malign haemangiosarc	0	0	0	1 (5%)	0	0	1 (2%)	2 (6%)
INTERIM								
No. examined	9	10	10	10	9	9	9	10
Adrenal cortex								
Benign adenoma	0	0	0	0	0	0	0	0
Thyroid								
Malign C-cell carcin	0	0	0	0	0	0	0	0
Heart								
Malign haemangiosarc	0	0	0	0	0	0	0	0
Liver								
Benign hepatocell adenoma	0	0	0	0	2 (22%)	0	1 (11%)	0
Malign hepatocell carcin	0	0	0	0	0	0	0	0
Malign haemangiosarc	0	0	0	0	0	1 (11%)	0	0
TERMINAL SACRIFICE								
Adrenal, cortex								
No examined	28	30	0	0	0	0	9	18
Benign adenoma	2	0	0	0	0	0	0	0
Thyroid								
Malign C-cell carcin.	0	0	-	0	-	-	0	0
Heart								
Malign haemangiosarc.	0	0	-	-	-	-	0	0
Liver								
Benign hepatocel aden	2	0	1	0	4	1	2	0
Malign hepatocel carcin	1	0	0	0	0	0	0	0
Malign haemangiosarc	0	0	1	0	0	0	0	1
TOTAL								
No. examined	60	60	60	60	60	60	60	60
Adrenal cortex								
Benign adenoma	2	0	0	0	0	0	0	0
Thyroid								
Malign C-cell carcin	0	0	0	0	0	0	0	1
Heart								
Malign haemangiosarc	0	0	0	0	1	0	0	0
Liver								

	0 ppm		120 ppm		600 ppm		3000 ppm	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Benign hepatocell adenoma	3	1	3	0	8	2	5	0
Malign hepatocell carcin	2	0	0	1	0	0	0	0
Malign haemangiosarc	0	0	1	1	0	1	1	3

Acceptability

Survival was less than 50% in males and females at 3000 mg/kg food and males at 600 mg/kg food. As the difference in survival became apparent from approximately week 60 onwards and since no treatment-related increase in neoplastic les.ions was observed in the surviving animals, the study is considered acceptable. Because there were no indications for a carcinogenic effect of the test substance and since the test substance has shown to be non-genotoxic (see B.6.4), no additional carcinogenicity data are considered necessary. Historical control data on liver histopathology were submitted by the notifier separately (time period 1986-1996, CD-1 mice).

Conclusions

Based on the reduced survival rate, increased liver weights, increased severity of systemic amyloidosis and histopathological changes in kidneys, the NOAEL is set at 120 ppm (equal to 16.4 mg/kg bw/day in males and 21.1 mg/kg bw/day in females). There was no evidence that pyriproxyfen has carcinogenic potential.

RMS conclusion: Agreed with the original conclusion.

During pesticide peer review meeting 190 (28 Jan – 2 Feb 2019), the finding of liver haemangiosarcoma was discussed. This finding was observed in females at all doses (1, 1, and 3 in the low, mid and high dose, respectively). Considering the low number of incidences, the majority of experts agreed that they represent only an equivocal evidence of carcinogenic potential, not sufficient to propose classification. However, the majority of experts agreed to set a carcinogenic NOAEL of 600 ppm (equivalent to 107.3 mg/kg bw/day) as a precautionary approach.

In addition, following the pesticide peer review meeting, the systemic NOAEL was changed into a systemic LOAEL of 16.4 mg/kg bw/day (120 ppm) based on decreased survival rate observed at all dose levels.

Following the outcome of the expert meeting, the applicant provided historical control data for the liver haemangiosarcoma finding in female mice and a statement regarding this finding. RMS has included this information below.

Information and statement as provided by the applicant:

Haemangiosarcoma is a spontaneous malignant tumour occurring in aging rodents. The tumour arises from the anarchical proliferation of vascular endothelial cells but can also arise from transformation of tissue-resident endothelial cell populations, from circulating progenitors, adult stem cells recruited from bone marrow, or possibly also from extramedullary sites of haematopoiesis such as the liver and spleen. Haemangiosarcoma is extremely rare in humans with less than 0.001% affected [2], while in various strains of mice (and rats) the spontaneous incidence ranges from 2 to 5% [3], being higher in B6C3F1, CD-1, and BALB/c strains than CBA/J. In CD-1 mice, the spontaneous incidence of haemangiosarcoma is reported to be about 3.1% on average, observed mainly in liver, spleen, subcutaneous tissue, bone marrow, and skeletal muscles [4]. There are only two examples of haemangiosarcoma induction by a chemical stimulus that occur in both humans and rodents (i.e., mouse and rat). Both examples are genotoxic carcinogens (vinyl halides and Thorotrast), and both produce primarily liver haemangiosarcoma ([5], [6]).

The incidence of liver haemangiosarcoma in females (3/60, 5%) in diets containing pyriproxyfen at 3000 ppm [1] was marginally above the conducting laboratory's [Hazelton Laboratories America, Virginia] historical control range, did not achieve statistical significance and was consistent with the incidence reported at other laboratories (refer to Table). A low incidence of this lesion was also observed in males, however the single incidence occurred in the low dose group, was not dose related and consistent with the concurrent control. In all cases, this lesion was associated with animals on the border of middle aged and senescence, with the lesion observed from week 53 onwards. The accepted view is that middle age mice are on the border of old age, due to the speed at which they age.

Table 1:
Overview of 18 month oncogenicity study in CD-1 mice treated orally (*via* diet) with pyriproxyfen: selected liver histopathology data (incidence)

1) Pyriproxyfen study report NNT-11-0084 on CD-1 mice									
Parameters		♂ (ppm)				♀ (ppm)			
		0	120	600	3000	0	120	600	3000
Test article intake (mg/kg bw/d)		0	16.4	81.3	422.5	0	21.1	107.3	532.8
Ter. bwts (g):		36.3	35.9	38.1	35.8	30.1	29.9	30.6	28.8
Histopathology, liver									
Haemangio-sarcoma	Ud	1/23	0/28	0/37	0/42	0/20	1/23	0/29	2/32
	Int.	0/9	0/10	0/9	0/9	0/10	0/10	1/9	0/10
	Ter	0/28	1/22	0/14	0/9	0/30	0/27	0/22	1/18
	Total	1/60 [1.7%]	1/60 [1.7%]	0/60	0/60	0/60	1/60 [1.7%]	1/60 [1.7%]	3/60 [5%]
2) Liver haemangiosarcoma available data on CD-1 mice									
Hazelton Laboratories America (Virginia) HCD [7]		Total: 9/1148 (0.8%) Range: 0/75 – 2/50 (0 – 4%)							
Cohen *et al* (2009) [3]		Mean: 3.1%							
CiToxLAB HCD [8]		Total: 14/712 (2.0%)				Total: 11/712 (1.4%)			
HCD from JSTP [11]		Range 0.0-9.65 (3.2%)				Range 0.0-7.7% (1.3%)			

Charles River HCD [9]	n = 52 studies Total: 48/2941 (1.63%) Range: 1/90 - 6/70 (1.11 – 8.57%)	n = 54 studies Total: 25/3110 (0.80%) Range: 1/70 – 4/65 (1.43 – 6.15%)

Ter. bwt.: terminal body weights
Ud.: unscheduled deaths
Int.: interim sacrifice
Ter.: terminal sacrifice
HCD: historical control data
JSTP: Japanese society of toxicological pathology

Overall, as there were no pre-neoplastic lesions or benign tumours in females the increase above concurrent and historical controls was marginal and restricted to a single sex. The weight of evidence suggests that in females, liver haemangiosarcomas are not related to treatment, with the incidence observed comparable with that in other laboratories and consistent with the published literature.

Discussion and conclusion

There is no convincing evidence in the mouse oncogenicity study that pyriproxyfen causes liver haemangiosarcomas in female mice when the study data are considered in the context of the historical control incidence and the plethora of public domain data.

In conclusion, there is insufficient evidence in the mouse oncogenicity study for a treatment-related carcinogenic effect of pyriproxyfen on the liver based on the following considerations:

- *study incidence (5%) marginally exceeds the laboratory's limited concurrent historical control range (4%)*
- *no effect was observed in males*
- *pattern of occurrence is consistent with published data on spontaneous liver haemangiosacrcoma incidences in the same strain (up to 7.7%)*
- *evidence of mechanism of action attributed to chemically induced liver haemangiosarcoma is limited in the public domain, occurring in both rats and mice. No such effects were observed in the rat carcinogenicity study*
- *the development of liver haemangiosarcomas was exclusively associated with animals on the border of middle aged and senescence, with the latency period not being decreased and consistent with published data on spontaneous tumour incidences.*

References:

1. *CA 5.5.1/02 (1991) Oncogenicity study in mice with S-31183. Hazleton Laboratories America, Inc. Virginia, United States of America. Laboratory report No.: 343-215. Sumitomo Chemical Co. Ltd, Unpublished report no.: NNT-11-0084*
2. *Pognan, F., Mahl, J.A., Ledieu, D., Raccuglia, M., Theil, D., Voytek, S.B., Devine, P.J., Kubek-Luck, K., Claudio, N., Cordier, A., Heier, A., Kolly, C., Hartmann, A., Chilbout, S-D., Bouchard, P. & Trendelenburg, C. (2018). Induction of haemangiosarcoma in mice after chronic treatment with S1P-modulator siponmiod and its lack of relevance to rat and human. Arch. of Toxicol.* ***92*** *pp 1877-1891*
3. *Cohen SM, Storer RD, Criswell KA et al (2009) Hemangiosarcoma in rodents: mode-of-action evaluation and human relevance. Toxicol Sci.* ***111(1)*** *pp 4–18.*
4. *RITA (2016) RITA: registry of industrial toxicology animal-data: database for historical data of tumors and pre-neoplastic lesions. https://reni.item.fraunhofer.de/reni/public/rita/*
5. *Boivin-Angèle, S., Lefrançois, L., Froment, O., Spiethoff, A., Bogdanffy, M. S., Wegener, K., Wesch, H., Barbin, A., Bancel, B., Trépo, C., (2000). Ras gene mutations in vinyl chloride-induced liver tumours arecarcinogen-specific but vary with cell type and species. Int. J. Cancer* ***85*** *pp 223 - 227*
6. *Lipshutz, G. S., Brennan, T. V., Warren, R. S. (2002). Thorotrast-induced liver neoplasia: A collective review. J. Am. Coll. Surg.* ***195*** *pp 713 - –718. Cited in [**Error! Bookmark not defined.**]*

7. *Incidence of liver haemangiosarcoma in the CD-1 mouse (June 1985 – June 1990) from Hazleton Laboratories, Vienna, Unites States of America. Covance Laboratories Inc.*
8. *Le Bigot, J-F., Thirlon-Delalande, C., Palate, B. & Foster, R. CiToxLAB. Lifetime carcinogenicity studies in the CD-1 mouse: historical data for survival and neoplasms. CiToxLAB in France, BP 563 – 27005 Evreux cedex, France. https://www.citoxlab.com/wp-content/uploads/2014/07/Lifetime-carcinogenicity-studies-in-the-CD-1-mouse-historical-data-for-survival-and-neoplasms.pdf*
9. *Spontaneous neoplastic lesions in the Crl:CD-1 (ICR) mouse in control groups from 18 month to 2 year studies (March 2005). Charles River Laboratories. https://www.criver.com/sites/default/files/resources/SpontaneousNeoplasticLesionsintheCrlCD-1ICRMouseinControlGroupsfrom18Monthto2YearStudies%E2%80%94March2005.pdf*
10. *Historical control data: CD-1 mouse dietary survival (1991 – 1996) [page 3] from Hazleton Laboratories, Vienna, Unites States of America. Covance Laboratories Inc.*
11. *Incidence of spontaneous tumors in control ICR (CD-1) mice (78W) [page 731-735], Toxicological histopathology, JSTP, Nishimura –syoten, Tokyo, Japan*

RMS reply:

The HCD provided from the conducting laboratory originate from a total of 31 studies (78-week studies in CD-1 mice) performed between June 1985 and June 1990. Wihtin these studies a total of 1148 livers was examined and a total of 9 incidences of haemangiosarcoma were reported. The range for this finding in the HCD was 0 – 4.0%, with a mean value of 0.8%.

RMS does not consider pyriproxyfen to have carcinogenic potential which is substantiated by the HCD and statement as provided by the applicant. However, the carcinogenic NOAEL was agreed upon during the expert meeting during the renewal process of this active substance.

3.9.2 Human data

No human data on the carcinogenicity of pyriproxyfen are available.

3.9.3 In vitro data

For an overview of mutagenicity studies, lease refer to section 3.8.

3.9.4 Other data

No other data on the carcinogenicity of pyriproxyfen are available.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 CA 5.6.1/01 (1991): Two generation reproductive toxicity study in the rat

Report

CA 5.6.1/01 (1991)

A dietary 2-generation (1 litter) reproduction study of S-31183 in the rat

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-11-0087

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, additional information on the materials and methods and results were added. Agreed with the original conclusions.

Characteristics

reference	:	CA 5.6.1/01, 1991	exposure	:	Diet continuously throughout the study period
type of study	:	2-generation reproduction study	doses	:	0, 200, 1000 and 5000 mg/kg food[1]
year of execution	:	1989-1990	vehicle	:	None
test substance	:	S-31183 (Pyriproxyfen), batch no. PYG-87074, purity 95.3%	GLP statement	:	Yes
route	:	Oral	guideline	:	EPA 40 CFR 158.135, No. 83-4. OECD 416
species	:	Rat, Sprague Dawley Crl:CD®(SD)BR	acceptability	:	Acceptable
group size	:	26/sex/dose	**NOAEL**par	:	13.3 mg/kg bw/day
			NOAELdev	:	66.7 mg/kg bw/day
			NOAEL repro	:	≥ 333.3 mg/kg bw/day

1 equivalent to 13.3, 66.7 and 333.3 mg/kg bw/day for males and females.

Study design

The study was performed in accordance with EPA 40 CFR 158.34, Pesticide Assessment Guideline No. 83-4. Dose levels were selected on the bases of a dose range finding study (which was not available for evaluation). In this range finding study the dose levels were 0, 1000, 3000, 6000 and 10000 mg/kg in the diet. Rats were fed diets for 4 weeks before mating and during the period of mating, gestation, parturition, lactation and until weaning. Parental males and females exposed to 6000 or 10000 mg/kg food and females exposed to 3000 mg/kg food showed a decrease in body weight (gain) and food consumption. No changes were detected in parental reproductive parameters and pup viability parameters in the treatment groups when compared to the control group.

Pup weights were noted as decreased in the 6000 and 10000 mg/kg dose groups by day 4 post-partum and also in the 3000 mg/kg dose group from day 7 post-partum onwards.

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Lot/Batch No.:	PYG-87074
Purity:	95.3%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2.Vehicle and/or positive control:	Diet / none
3.Test animals:	
Species:	Rat
Strain:	Crl:CD® (SD) BR
Age:	F_0: 44 days
Weight at dosing:	F_0: ♂: 205 – 250 g; ♀: 157 – 195 g
Source:	Charles River Breeding Laboratories, Kingston, New York, USA
Acclimation period:	15 days

B. Study Design and Methods:

1.In life dates:	4 October 1989 to 25 June 1990
2.Mating procedure:	Following 70 days of treatment for the F_0 generation and following 77-90 days treatment for the F_1 generation (98-111 days of age), one female was placed with one male of the same dose group for a maximum of 21 days. Females were examined daily for evidence of mating by examination of the vaginal lavage for spermatozoa (the presence of copulatory plugs were also noted). The day of positive identification of spermatozoa was termed day 0 of gestation. Following confirmation of mating, females were housed individually.
3.Study schedule:	The day that parturition was completed was designated as day 0 *post-partum* (*pp*). On day 4 *pp* the size of the litter was reduced, by random pup selection, to a maximum of 8, preferably 4 male and 4 female. Culled pups were discarded. On day 21 *pp*, F_1 weanlings separated from their dams and housed up to 4/cage. At least 1 animal of each sex/litter were selected randomly

	individual cages as to provide 26 males and 26 females/group for mating of the F_2 generation. An additional 10 animals/sex/group from at least 10 litters were selected randomly for necropsy examination. On day 21 *pp*, F_2 pups were separated from their dams and 10 animals/sex/group were randomly selected from at least 10 litters for necropsy examination. Approximately 3 weeks post mating parental F_0 and F_1 males were killed and subjected to necropsy. F_0 and F_1 dams were killed on day 21, 22 or 23 *pp* and given a macroscopic examination.
4.Animal assignment and treatment:	The conduct of the study followed OECD 416 (1981). Four groups of 26 male and 26 female SD rats forming the F_0 generation received the test article by dietary administration at concentrations of 0, 200, 1000, 5000 ppm (equivalent to 0, 13.3, 66.7, 333.3 mg/kg bw/day, respectively for both sexes) for 10 weeks prior to and throughout mating. Following weaning of the F_1 generation, 26 animals/sex/group were selected to form the F_1 adult generation. These animals were treated with pyriproxyfen comparable to that of the F_0 generation. F_2 pups were killed following weaning.
5.Diet preparation and analysis:	Test diets were prepared weekly by the addition of the test article to basal diet to prepare a premix. Samples of diet taken in weeks 1, 4, 8, 16, 24 and 32 were analysed for the concentration of test article. Samples were taken from the top, middle and bottom of each diet mixture for analysis of homogeneity. Analysis of stability under the conditions of storage use (up to 14 days) was performed on a concentration range of 100 to 20000 ppm in an earlier range-finding study (data not reported).
6.Statistics:	Parental data: ANOVA was used to analyse numerical data. Where the F value was significant ($p \leq 0.05$) comparisons between test and control groups were made using Dunnett's test. Mating data were analysed using the Kruskal-Wallis test. Where the H value was found to be significant ($p \leq 0.05$), differences between test and control groups were analysed using the Mann-Whitney U test. Maternal performance: no. of live pups at birth, implant scars and post-implantation were evaluated using the Kruskal-Wallis and Mann-Whitney U tests. The incidence of dead pups at birth, gestation index was analysed using Fisher's exact probability test (with cumulative

probabilities, where appropriate) or the chi-square (with Yate's correction).

Litter data: viability, survival and lactation indices were evaluated using the Kruskal-Wallis and Mann-Whitney U tests. Litter mean pup body weight, and group litter mean were evaluated using an ANOVA. Where the F value was significant ($p \leq 0.05$) comparisons between test and control groups were made using Dunnett's test.

C. Methods:

1. Parental observations: Parental animals of both sexes were observed for clinical signs and mortality twice daily (once on holidays). During weighing, each animal was examined for the presence of abnormalities.

From day 20 of gestation, observations increased thrice daily for signs of parturition

2. Body weights: All animals were weighed weekly except for mated females which were weighted on days 0, 6, 12, 18 and 21 of gestation and on days 0, 4, 7, 14 and 21 *pp*. All animals were weighed on their day of necropsy

3. Food consumption: Determined weekly during the treatment periods for both males and females. For females during gestation, food consumption was determined from days 0-6, 6-12, 12-18, 18-21 and from day 0-4, 4-7, 7-10, 10-14, 14-17, 17-19, 19-21 *pp*.

4. Reproductive performance: Oestrus cycle: Vaginal smears were taken prior to mating.

Mating index: Copulation was confirmed by the presence of vaginal plugs or sperm in vaginal smears. Mating indices were calculated for each group of animals.

Fertility index: pregnancy was confirmed by the occurrence of parturition or by the presence of implantation sites in the uterine horns.

Conception index: determined by the occurrence of pregnant females *vs*. the number of females mated.

Gestation index: parturitions resulting in birth of one or more live pups were considered to be normal.

Duration of gestation: represented as the days from detection of copulation to completion of parturition.

Number of implantation sites: the number of implantation sites in the uterus was counted for each female.

5. Offspring observations: Clinical signs of toxicity: daily during the lactation period.

Sex ratio: sexed externally on day 4 *pp*.

	Viability index: the number of pups surviving in each litter were counted on days 0, 4, 7, 14 and 21 *pp*.
6.Bodyweights:	Recorded at birth, day 4, 7, 14 and 21 *pp*.
7.Necropsy	Paternal: any animal found dead or killed for humane reasons was subjected to a detailed external and internal gross examination. Approximately 3 weeks after the end of the F0 and F1 generations' mating phases, the males were killed and were subjected to necroscopy. Females whiched failed to mate were killed 26 or 27 days after the end of the mating period and were given a detailed macrocopic examination. Females which mated but did not litter were sacrificed on day 26, 27 or 28 post coitum and were given a macroscopic examination. Offspring: All pups born dead or dying before day 7 *pp* were subject to detailed necropsy. Ten weanling/sex/group of the F_1 and F_2 generations were randomly selected from 10 separate litters on day 21, 22 or 23 *pp* and subject to detailed necropsy.
8.Organ weights and tissue preservation:	F_1 paternal organ weights: brain, epididymides, kidneys, liver, mammary gland, ovaries, pituitary, prostate, seminal vesicles, testes, uterus, vagina, any abnormalities. F_1 parental histopathology: epididymides, liver, kidney, ovaries, prostate, seminal vesicles, testes, uterus, vagina, any abnormalities from control and high dose animals. Liver and any abnormalities were examined for all low and mid dose group parental animals. Offspring histopathology: any abnormalities.

Results

Formulation analysis:

Homogeneity and verification of dietary levels confirmed that mean values were generally within the range of ±10% of nominal concentration confirming accurate formulation.

Parental animals

Clinical signs and mortality:

F0: One male in the 200 ppm group was sacrified in poor health on day 98 of treatment and one female of the 5000 ppm group (and her litter) were sacrificed on day 1 *pp*. due to her moribund condition. No other animals

died or were sacrificed in poor condition during the study. There were no treatment-related clinical findings. Incidental clinical findings included instances of cranial/periorbital staining and of scabs in the cervical region.
F1: One male of the control group was found dead on day 98 of treatment. Two males in the 200 ppm group were sacrificed in poor condition on days 114 and 63 of treatment, respectively. Incidental clinical findings included instances of prominent circular striations on tails, which was observed in males and/or females of all treatment groups.

Body weight:
F0: Overall body weight gains for males during the premating phase (weeks 0-10) were significantly reduced in the 5000 ppm group (-8.5% compared to control). Body weights for weeks 10-11 were significantly reduced at 5000 ppm level (-6% compared to control). Both these findings were considered to be treatment related.
In females, body weight gains in the 5000 ppm group were significantly reduced during weeks 1 and 2 (-20% compared to control), resulting in significantly reduced body weights for the duration of the premating period. Such variation is common in the time just prior to mating and appears to result from placing males and females in adjacent cages. During gestation in general body weight gains were unaffected. During lactation, body weight gains were significantly increased in the 5000 ppm group, resulting in body weight values that were comparable to the control by day 21 *pp*. Body weights of the 200 and 1000 ppm F_0 and F_1 adults were unaffected by treatment.

F1: Overall body weights for males during the premating phase (weeks 0-12) were significantly reduced in the high dose group only. In high dose group females, whilst body weight gains were not statistically significant, values however were lower than the respective control group during the premating phase (weeks 0-12). During gestation and lactation body weights were significantly lower for the high dose group on all occasions, except for day 21 *pp*. During lactation, body weight gains in the 1000 and 5000 ppm groups tended to be higher than control values resulting in overall body weight gains (day 0-21 *pp*) which were statistically significantly increased in both groups. For the high dose group this was deemed to be a treatment related effects as a similar effect was observed in F_0 females. Body weights of the 200 and 1000 ppm F_0 and F_1 adults were unaffected by treatment.

Table B.6.6.1-1 Body weight gains (g)

Duration	Males				Females			
	0 ppm	200 ppm	1000 ppm	5000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
F0 adults								

Wk 0-10 pre-mating	316.6	314.6	314.7	289.7*	144.9	141.9	140.8	113.9**
Day 0-21 gestation	-	-	-	-	165.6	159.0	173.4	155.8
Day 0-21 lactation	-	-	-	-	2.9	17.3	11.9	34.6**
F1 adults								
Pre-mating wk 0-12	498.0	500.6	501.0	453.9*	258.5	266.2	257.5	239.6
Pre-mating wk 0-18	580.3	582.5	578.9	524.5*	-	-	-	-
Day 0-21 gestation	-	-	-	-	171.9	155.7	154.1	162.1
Day 0-21 lactation	-	-	-	-	-0.7	10.8	21.5*	37.2**

*Statistically different from control (p≤0.05), **Statistically different from control (p≤0.01).

Food consumption:

F0: In high dose group males significantly lower food consumption was observed during the last week of acclimatisation and the first and sixth weeks of treatment. Although not reaching statistical significance for subsequent weeks, values were slightly lower than controls.

For females, food consumption was significantly lower than control values in the 5000 ppm group during weeks 8 and 9 of the premating phase and between days 0-6 and 6-12 of gestation.

F1: For high dose group male's food consumption was reduced on all occasions, reaching statistical significance during weeks 1, 2, 4, 7, 10 and 12-18. For high dose group females, food consumption was significantly reduced during the first week of the premating phase and over days 6 to 12 of gestation.

Food consumption of the 200 and 1000 ppm F_0 and F_1 adults were unaffected by treatment.

Table B.6.6.1-2 Food consumption (g/animal)

Duration	**Males**				**Females**			
	0 ppm	**200 ppm**	**1000 ppm**	**5000 ppm**	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**
F0 adults								
Week 5-6 pre-mating	227.8	226.0	219.7	213.1*	155.5	157.4	157.7	148.8
Week 7-8 pre-mating	223.5	218.6	222.1	214.1	160.4	157.7	157.8	146.7**
Week 8-9 pre-mating	220.7	218.8	224.4	216.4	159.3	157.0	156.0	145.0**
Day 0-6 gestation	-	-	-	-	159.1	154.5	157.2	142.7*

Day 6-12 gestation	-	-	-	-	180.2	184.0	176.9	163.1**
F1 adults								
Week 0-1 pre-mating	118.7	118.9	118.4	99.6**	110.7	113.8	115.8	98.9*
Week 1-2 pre-mating	162.6	164.2	164.3	142.9**	145.3	145.5	146.4	136.7
Week 3-4 pre-mating	216.8	216.4	214.3	196.9**	156.2	154.5	157.7	153.7
Week 6-7 pre-mating	238.3	220.5*	234.7	220.1*	158.9	161.3	159.7	151.9
Week 9-10 pre-mating	233.1	231.2	236.8	217.7*	156.7	156.6	159.8	152.6
Week 11-12 pre-mating	234.3	228.3	228.4	215.3**	154.0	160.2	157.8	144.4
Week 15-16 pre-mating	228.2	221.9	224.3	210.2**	-	-	-	-
Week 16-17 pre-mating	232.5	228.3	223.8	216.2*	-	-	-	-
Week 17-18 pre-mating	225.3	227.7	224.4	208.1*	-	-	-	-
Day 6-12 gestation	-	-	-	-	171.5	163.5	162.0	150.0**
Day 18-21 gestation	-	-	-	-	80.9	71.4**	71.3**	70.9*

*p≤0.05, **p≤0.01

Reproductive performance

The oestrus cycle was unaffected by treatment with pyriproxifen. The mating and fertility indices and the conception rate were unaffected by treatment. The mean day of mating for the control and treated groups was not significantly different.

F0: The gestation index was 100% in all groups. The gestation length and observations of the dams at parturition were unaffected. The number of live pups at birth, implant site scars, post-implantation losses and sex-ratios revealed no significant differences between treated groups and the controls. There were no pups observed at birth with major malformations. The number of dead pups at birth in the control groups were high relative to the historical control values (0.05 - 0.38, mean no. of dead pups per litter from 3 studies, performed between Oct 1988 and Feb 1990). This resulted in significant lower values in the total number of dead pups at birth in the 1000 ppm group. The number of litters with dead pups at birth in the 5000 ppm group was also significantly low. These differenes are not considered to be treatment-related, but due to the high number of dead pups in the control group.

F1: The gestation index was at least 95.2% in all groups. Consideration of the number of live and dead pups at birth, implant site scars and post-implantation losses revealed no significant differences from control values. The sex ratios were also unaffected by treatment.

Table B.6.6.1-3 Reproductive performance

	F0 generation				F1 generation			
Parameter	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**
Mating index (%)	100.0	92.3	88.5	96.2	88.5	88.5	100.0	92.3
Fertility index (%)	84.6	84.6	76.9	84.6	76.9	73.1	80.8	80.8
Conception rate (%)	84.6	91.7	87.0	88.0	87.0	82.6	80.8	87.5
Gestation index (%)	100	100	100	100	100	100	95.2	100
Length of gestation (days)	21.9	21.7	21.7	21.7	21.8	21.8	21.9	21.6
Sex ratio (% male)	51.0	53.2	49.9	52.5	51.7	49.9	49.7	54.2
No. of live pups at birth / litter	14.9	14.4	15.8	14.7	15.4	14.7	14.4	15.3
Dead pups at birth								
-litters affected	7	5	2	1*	5	3	6	6
-pups affected	13	6	4*	6	5	4	7	11
Post-implantation loss (%)	10.4	9.5	7.8	11.4	9.3	12.5	16.0	9.3

*Significantly different from control ($p \leq 0.05$)

Organ weights:

F1 adults: Absolute and relative liver weights in the males and females of the 5000 ppm group were significantly increased compared to controls. Relative liver weights were significantly increased in males of the 1000 ppm group. In males, relative kidney weights were significantly increased in the 1000 and 5000 ppm groups. For males in the 5000 ppm group, statistically significant increases in relative brain, left testis and left epididymides weights were not attributed to treatment when considered together with the absolute wegith and relative-to-brain weight values and in light of the fact that only one of the paired organ was statistically affected. Also in the 5000 ppm group a statistically significant decrease in absolute prostate weight was not attributed to treatment since relative to brain and relative to body weight values were similar to control values.

Table B.6.6.1-4 Absolute and relative organ weights

Organ	**Males**				**Females**			
	0 ppm	**200 ppm**	**1000 ppm**	**5000 ppm**	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**
Absolute organ weights (g)								

Liver	19.195	18.820	20.753	21.412*	16.814	16.576	18.313	20.371**
Kidney								
-left	2.197	2.139	2.343	2.123	1.336	1.393	1.408	1.332
-right	2.235	2.190	2.401	2.170	1.397	1.448	1.443	1.384
Gonad								
-left	1.916	1.957	2.050	1.979	-	-	-	-
-right	2.009	1.948	1.995	1.926	-	-	-	-
Prostate	1.392	1.455	1.369	1.190*	-	-	-	-
Epididymis								
-left	0.744	0.750	0.761	0.738	-	-	-	-
-right	0.777	0.764	0.759	0.735	-	-	-	-
Relative organ weights (relative to body weight)								
Liver	3.055	3.046	3.358**	3.905**	4.738	4.605	5.128	6.032**
Kidney								
-left	0.351	0.347	0.380*	0.388**	0.377	0.386	0.394	0.394
-right	0.357	0.356	0.390**	0.397**	0.394	0.402	0.404	0.409
Gonad								
-left	0.306	0.319	0.334	0.363**	-	-	-	-
-right	0.324	0.318	0.327	0.354	-	-	-	-
Prostate	0.224	0.237	0.223	0.217	-	-	-	-
Epididymis								
-left	0.119	0.123	0.124	0.135**	-	-	-	-
-right	0.126	0.126	0.124	0.135	-	-	-	-

*Statistically different from control (p≤0.05), ** Statistically different from control (p≤0.01).

Pathology:

F0: There were no gross or histopathological findings which could be attributed to treatment.

F1: There were no gross or histopathological findings which were clearly attributed to treatment. Multiple kidney findings which were indicative of chronic interstitial nephritis were seen more frequently in males than females. The overall incidence and severity of these findings was greated amoung males in the 5000 ppm group, however, the 200 and 100 ppm groups were unaffected. Amoung the 5000 ppm group males, a higher incidence of focal clear cells in the liver, a common finding, was noted. The localised nature of this finding suggests it is not the cause of the increased liver weights.

Table B.6.6.1-5 Gross pathology and histopathology results

Organ	**Males**				**Females**			
	0 ppm	**200 ppm**	**1000 ppm**	**5000 ppm**	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**
F0 generation								

Kidney								
-cyst	0/26	1/26	0/26	2/26	0/26	0/26	0/26	0/26
-dilatation	2/26	1/26	3/26	1/26	0/26	0/26	0/26	0/26
-hydronephrosis	2/26	1/26	3/26	1/26	0/26	0/26	0/26	0/26
Liver								
-area pale	1/26	2/26	2/26	2/26	0/26	0/26	0/26	1/26
-necrosis	0/26	0/26	0/26	0/26	0/26	0/26	1/26	2/26
F1 generation								
Kidney								
-dilatation	2/26	4/26	3/26	6/26	1/26	1/26	0/26	4/26
-chronic interstitial nephritis	7/26	3/26	7/26	15/26	0/26	0/26	0/26	0/26
-hydronephrosis	5/26	10/26	8/26	7/26	1/26	3/26	1/26	4/26
Liver								
-area pale	3/26	3/26	5/26	10/26	2/26	1/26	3/26	1/26
-focal clear cells	2/26	2/9	4/8	9/26	1/26	1/7	2/8	0/26

Offspring

Clinical signs:

F1 pups: Occasional incidental findings seen in the control and/or treated groups included bruises to the muzzle/facial region. There were no clinical observations related to treatment.

F2 pups: Occasional findings were incidental and were not considered to be treatment-related.

Body weight:

F1 pups: Pup weights at birth were not significantly different from control values. On days 14 and 21 *pp.* female and total (male+female) weights were significanlty reduced in the 5000 ppm group (-16%). Male pup weights were significanly reduced on day 21 *pp.* in the 5000 ppm group (-12%).

F2 pups: Pup weights at birth were not significantly different from control values. From day 14 *pp.* male, female and total litter pup weights were significantly reduced in the 5000 ppm group (-13% and -11%, respectively).

Table B.6.6.1-6 Pup body weights post partum (g)

Day post partum (pp.)	**Males**				**Females**			
	0 ppm	**200 ppm**	**1000 ppm**	**5000 ppm**	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**
F1 pups								

Day 0 *pp.*	6.6	6.7	6.6	6.5	6.3	6.3	6.3	6.2
Day 7 *pp.*	15.8	17.0	17.1	15.2	15.5	16.0	16.1	14.4
Day 14 *pp.*	30.6	31.6	33.1	27.9	30.3	30.3	31.3	26.8*
Day 21 *pp.*	51.0	54.6	54.2	44.7**	51.0	52.3	51.7	42.9**
F2 pups								
Day 0 *pp.*	6.4	6.5	6.6	6.4	6.1	6.1	6.2	6.1
Day 7 *pp.*	16.9	16.0	17.0	15.8	16.0	15.4	15.8	15.2
Day 14 *pp.*	34.5	32.1	34.2	31.0*	32.7	31.4	32.5	30.1*
Day 21 *pp.*	57.0	52.7	55.5	49.7**	54.0	51.4	52.8	48.3**

*Statistically different from control ($p \leq 0.05$), ** Statistically different from control ($p \leq 0.01$).

Pathology:

F1 pups: Among incidental findings for pups found dead between days 0 and 7 *pp.* were instances of cannibalism, subcutaneous oedema to the head and body and reduced or absent renal papillae. Amoung pups found dead or sacrificed between days 8 and 23 *pp.* there were few findings. One pup in the control group and one in the 1000 ppm group showed findings of cannibalism.

F2 pups: Occasional incidental findings for pups found dead between day 0 and 7 *pp.* included cannibalism, subcutaneous oedema to the head and body and reduced renal papillae. Among pups sacrificed between day 8 and 23 *pp.* pathological findings were incidental and included a low incidence of cysts or pelvic dilatation in the kidneys of some male pups of the 1000 and 5000 ppm groups, findings commonly observed in rat pups. These findings were not attributed to treatment.

Acceptability

The study was performed according the EPA guideline 40 CFR 158.135, Pesticide Assessment Guideline, No 83-4. Compared to the OECD 416 of 2001, the following analyses were not performed: sperm parameters, developmental and functional observations of pups, organ weights of F_0 animals, weighing of the adrenals, coagulating gland, ovaries, spleen, thyroids, uterus and vagina of F_1 animals, and histopathology of the cervix and coagulating gland (F_0 and F_1 animals). However, results from reproductive indices did not suggest any adverse effects.

In addition, histopathology was not performed on the livers of all animals of the 200 and 1000 ppm groups. Based on this consideration and in the absence of clinical biochemistry, effects on liver weights were considered adverse for the establishment of the NOAEL.

Conclusions

Parental F_0 and F_1 animals showed no treatment-related changes in clinical signs, mortality rate, reproductive parameters, or oestrus cycle. Parental F_0 and F_1 animals receiving 5000 ppm showed decreased body weights

during pre-mating, gestation and lactation phases. F_1 parental males at 5000 ppm showed significantly lower food consumption values in most weeks of the pre-mating phase. F_1 parental males and females at 5000 ppm showed significantly increased absolute (112% and 121% of control, respectively) and relative (128% and 127% of control, respectively) liver weights, and F_1 parental males at 1000 ppm showed significantly increased liver weights relative to body weight only (110% of control). Increased kidney weights relative to body weight were observed in F_1 parental males at 1000 and 5000 ppm (109 and 111% of control, respectively). Histopathological examination revealed higher incidences of focal clear cells in the liver and of chronic interstitial nephritis in the kidneys in F_1 parental males at 5000 ppm than in F_1 male controls. There were no treatment-related findings among F_0 and F_1 parental animals exposed to 200 ppm.

No treatment-related changes were detected in litter size, viability/survival/lactation indices, sex ratio and gross pathology for F_1 and F_2 pups. Although no differences in birth weight were noted, body weight development of male and female F_1 and F_2 pups during the lactation phase was retarded at 5000 ppm.

Based on increased relative liver weights in F_1 males at 1000 ppm, the NOAEL for parental toxicity was set at 200 ppm (equivalent to 13.3 mg/kg bw/day). The NOAEL for reproductive toxicity was 5000 ppm (equivalent to 333.3 mg/kg bw/day). The NOAEL for developmental toxicity was 1000 ppm (equivalent to 66.7 mg/kg bw/day), based on reduced F_1 and F_2 pup body weight development at 5000 ppm.

3.10.1.2 CA 5.6.1/02 (1988a): Gavage reproductive toxicity study in the rat

Report

CA 5.6.1/02 (1988a)

Study by oral administration of S-31183 to rats prior to and in the early stages of pregnancy

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-81-0036

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, additional information on the materials and methods and results sections were added. NOAEL for development is changed to 1000 mg/kg bw/day, since no dose-related effect on foetal body weight was found and no morphological anomalies or variations were found related to treatment. The reproductive NOAEL was set is 500 mg/kg bw/day based on decreased number of corpora lutea, decreased number of live foetuses and increased placental weight seen at the high dose.

Characteristics

reference	:	CA 5.6.1/02, 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	:	developemental 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
			NOAELrepr	:	500 mg/kg bw/day
			NOAELdev	:	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

Formulation analysis:

No formulation analysis was undertaken. Doses were prepared in advance and used within 7 days (based on results of stability data).

Parental

Mortality and clinical signs:

One male animal in the 500 mg/kg bw/day group died due to an intubation error on day 84 of administration. Two female rats in the top dose group died on days 5 and 7 of administration. At necropsy of these females, congestion and enlargement of the liver, atrophy of the thymus, enlargement of the adrenal glands and miliary ulcerations of the gastric mucosa were observed.

Toxic signs related to treatment included soft stools or diarrhea, erythema and swelling of the periproctal region and salivation.

Table B.6.6.1-7 Mortality and clinical signs

Dose (mg/kg bw/day)	0		100		300		500		1000	
Finding	**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	0/24	0/24	0/24	0/24	0/24	9/24	20/23	22/24	24/24	24/24
-erythema and swelling of periproctal region	0/24	0/24	0/24	0/24	0/24	1/24				
-excessive salivation	0/24	0/24	18/24	1/24	21/24	5/24	22/23	9/24	24/24	23/24
-excessive lacrimation	0/24	0/24	0/24	0/24	0/24	0/24	24/24	0/24	24/24	8/24
-hypoactivity	0/24	0/24	0/24	0/24	0/24	0/24	4	0/24	4	0/24
-wasting			0/24		0/24		0/24	0/24	3/24	4/24
							0/24		0/24	6/24
							0/24		0/24	

Body weight:

Body weight in the 1000 mg/kg bw/day group was decreased on day 3 of administration for both male and female rats. In males, in the 300 and 500 mg/kg bw/day groups, significantly lower body weights from day

45 and day 28 of dosing, respectively were observed. In the low dose group males (100 mg/kg bw/day) body weight and body weight gain were comparable to the control group.
In the 500 mg/kg bw/day females, significantly lower body weight values than the control group were found during the pre-mating and gestation periods. In the 300 mg/kg bw/day females, body weight tended to be slightly depressed during the pre-mating period. During gestation, body weight was somewhat lower and significant differences were sporadically observed. In the 100 mg/kg bw/day females, body weight during the pre-mating period was slightly lower than that of the controls, but this difference was not statistically significant.

Table B.6.6.1-8 Body weight (g)

	Dose (mg/kg bw/day)				
Days of treatment	**0**	**100**	**300**	**500**	**1000**
Males					
Day 3	191.2	194.6	193.6	191.9	169.9**
Day 7	221.5	225.3	223.3	223.2	200.5**
Day 21	307.7	311.4	305.4	297.7	272.5**
Day 28	342.1	344.6	334.1	323.0*	297.8**
Day 45	403.8	400.8	383.0*	363.6**	334.6**
Day 83	490.2	479.8	461.9*	429.4**	389.3**
Females					
Day 3	214.2	212.7	211.7	208.2*	196.8**
Day 7	228.2	224.7	223.7	219.5**	208.9**
Day 1 of pregnancy	256.0	244.6*	248.4	246.8	244.2*
Day 3 of pregnancy	264.3	255.7	255.7	254.2*	251.6*
Day 7 of pregnancy	278.9	265.8**	268.2*	264.9**	263.3**
Day 21 of pregnancy	396.8	384.2	389.4	380.4*	382.8*

*Statistically different from control ($p \leq 0.05$), ** Statistically different from control ($p \leq 0.01$).

Food and water consumption:

In the high dose group, food consumption was significantly decreased. During the gestation period, food consumption was significantly higher in the high dose females compared to the control group. In female rats, food consumption was significantly dereased in the 500 mg/kg bw/day group at the start of administration but was comparable to the control group thereafter. In females in the 300 and 100 mg/kg bw/day groups, no differences in food consumption were observed compared to the control group.
Water consumption was increased in each dose group, in the 500 and 1000 mg/kg bw/day groups water consumption was 1.5 to 2 fold greater than the control group. In the 100 mg/kg bw/day group, increases were already significant, with increases up to 122% of the control group.

Table B.6.6.1-9 Water consumption

	Dose (mg/kg bw/day)				
Days of treatment	**0**	**100**	**300**	**500**	**1000**
Males					
3	22.4	24.0*	25.4**	27.7**	35.8**
7	23.9	26.2*	27.5**	31.0**	39.4**
14	24.0	26.8*	30.2**	32.6**	43.7**
21	24.7	28.0*	28.7**	37.3**	46.7**
28	24.4	27.5	28.8*	36.0**	46.3**
35	23.6	26.9*	27.9**	36.2**	48.0**
42	22.4	27.1**	30.0**	38.0**	43.6**
52	23.3	25.7	30.8**	36.4**	46.6**
62	21.8	26.6*	30.8**	37.5**	47.3**
Females					
3	19.0	20.7	22.8	34.5**	38.5**
7	20.5	19.5	22.5	32.9**	46.8**
13	21.3	19.6	23.8	37.0**	50.5**
Day 1 of pregnancy	19.7	18.2	21.0	30.9**	44.4**
Day 3 of pregnancy	24.4	25.1	29.9**	36.7**	45.6**
Day 7 of pregnancy	24.0	22.9	27.7*	37.7**	47.9**
Day 16 of pregnancy	35.4	33.1	32.7	36.9	38.9
Day 21 of pregnancy	33.9	34.8	30.2*	32.5	33.7

*Statistically different from control ($p \leq 0.05$), ** Statistically different from control ($p \leq 0.01$).

Organ weights:

In males, dose-dependent increases or tendencies in absolute and relative weights of the liver, kidneys and adrenal glands were found in the 100 mg/kg bw/day group and higher doses.

For the liver, absolute weight increased by 23%, 24% and 27% for 300, 500 and 1000 mg/kg bw/day, respectively; relative liver weight increased significantly by 8%, 31%, 41% and 61% for the 100, 300, 500 and 1000 mg/kg bw/day groups, respectively.

For the kidneys, absolute weight increased by 9%, 6% and 8% for 300, 500 and 1000 mg/kg bw/day groups, respectively. Relative kidney weight increased significantly by 10%, 16%, 22% and 38% for the 100, 300, 500 and 1000 mg/kg bw/day groups, respectively.

For the adrenal glands, absolute weight increased by 8%, 28%, 36% and 62% for the 100, 300, 500 and 1000 mg/kg bw/day groups, respectively. Relative adrenal weight increased significanty by 13%, 37%, 56% and 108% for the 100, 300, 500 and 1000 mg/kg bw/day groups, respectively.

In addition, dose-dependent decrease or decreasing trend in the absolute and relative weight of the thymus was observed from the 300 mg/kg bw/day group upwards. Decreases in absolute thymus weight were -11%, -22% and -42% for the 300, 500 and 1000 mg/kg bw/day groups, respectively.

In females, in all pyriproxifen treated groups the relative weight of the kidneys were increased (+8%, +5%, +8%, +12% for the 100, 300, 500 and 1000 mg/kg bw/day, respectively) and the absolute kidney weight was increased in the 1000 mg/kg bw/day group (+8%).

An increase or trend in the absolute and relative weights of the adrenal glands, thymus and spleen was observed in the high dose females. For the adrenal glands, absolute weight was increased by 10% and the relative weight was increased by 14% in the 1000 mg/kg bw/day group. For the thymus, no significant changes in absolute weight were observed; the relative thymus weight was only significantly increased in the high dose (+13%). For the spleen, no significant changes in absolute weight were observed; the relative spleen weight was only significantly increased in the high dose (+10%).

Table B.6.6.1-10 Absolute organ weights (g)

Organ	Males					Females				
	0	**100**	**300**	**500**	**1000**	**0**	**100**	**300**	**500**	**1000**
Liver	16.911	17.842	20.827**	20.896**	21.426**	14.266	13.721	14.169	14.076	14.315
Kidney	2.434	2.589	2.641*	2.592	2.639*	1.555	1.616	1.600	1.613	1.683**
Adrenal	0.050	0.054*	0.064**	0.068**	0.081**	0.067	0.067	0.067	0.066	0.074**
Thymus	0.348	0.335	0.309*	0.271**	0.202**	0.297	0.292	0.293	0.305	0.323
Spleen	0.811	0.775	0.774	0.698**	0.631**	0.606	0.593	0.609	0.608	0.641
Testis	5.479	5.485	5.402	5.226*	5.069**	-	-	-	-	-

*Statistically different from control (p≤0.05), ** Statistically different from control (p≤0.01).

For kidney, adrenal and testis total weight is given.

Pathology:

For males, in the 300 mg/kg bw/day group and higher doses, the liver, kidneys and adrenal glands were enlarged. The liver showed dark red colouration and the surface of the kidneys was pitted. The severity of these changes was dose-dependent. Histopathological changes in the kidneys consisted of regenerative changes of the tubules, dilation of the tubules and accumulation of neutrophiles or degenerated cells within the dilated and regenerated tubules.

In female rats, no specific anomalies were found at necropsy in all groups including the control group.

Table B.6.6.1-11 Pathological findings

Finding	Males					Females				
	0	100	300	500	1000	0	100	300	500	1000
Liver										
-enlargement	0/24	0/24	18/24	21/23	22/24	0/24	0/24	0/24	0/24	1/24
-dark red colour	0/24	0/24	21/24	23/23	24/24	0/24	0/24	0/24	0/24	0/24
Kidney										
-enlargement	0/24	0/24	1/24	6/23	16/24	0/24	0/24	0/24	0/24	0/24
-pitted surface	0/24	0/24	8/24	22/23	22/24	0/24	0/24	0/24	0/24	0/24
Thymus, atrophy	0/24	0/24	1/24	1/23	12/24	0/24	0/24	0/24	0/24	1/24
Situs transversus	0/24	1/24	0/24	0/23	0/24	0/24	0/24	0/24	0/24	0/24

Reproductive and foetal observations

The number of days required for confirmed copulation was comparable for all groups. The copulation and delivery rates were comparable between the treated and control groups.

The number of corpora lutea in the high dose group was significantly lower than the control group. The number of implantations and the implantation rates were also slightly lower than in the control group. Significantly higher placental weight was found for foetuses in the high dose group.

As for external anomalies in foetuses, club foot was found in one foetus of the control group and complications of club foot, club hand, vestigial tail and anal atresia in one foetus in the 500 mg/kg bw/day group. Visceral anomalies were observed sporadically in one to five foetuses in all groups including the control group. The numbers of foetuses with the visceral anomalies ranged from two cases (2.5%) to seven cases (6.3%) in the dosage groups compared to ten cases (9.5%) in the control group. There was no increased in incidence.

As for skeletal anomalies, multiple vertrebral malformations were observed in one foetus with external anomalies in the 500 mg/kg bw/day group. Skeletal variations were observed, but the incidence ranged from 2.5 to 6.9% in the dosage groups compared to 4.8% in the control group, so there were no pyriproxifen related increases in incidence.The number of ossificated phalanges of the forelimbs was higher in the dosage groups than in the control group, and there were no decreases in the number of ossificated bones suggesting retarded ossification.

Table B.6.6.1-12: Reproductive and foetal findings

	Dose (mg/kg bw/day)

Finding	0	100	300	500	1000
Reproductive findings					
1st mating					
-copulated/mated (%)	91.7	100.0	95.8	100.0	95.5
-pregnant/copulated (%)	90.9	100.0	95.7	95.8	85.7
2nd mating					
-copulated/mated (%)	100.0	-	100.0	-	66.7(m) / 100.0(f)
-pregnant/copulated (%)	100.0	-	100.0	-	100.0
No. of dams	22	24	23	23	19
No. of corpora lutea	15.8	15.4	15.9	15.3	14.2**
Implantation rate	93.6	87.1**	95.2	91.8**	94.9
Rate of resorbed or dead foetuses	3.7	5.8	4.6	6.6	6.3
No. of live foetuses					
-total	314	302	331	301	240
-mean	14.3	12.6*	14.4	13.1	12.6*
Sex ratio	0.47	0.50	0.49	0.52	0.50
Body weight live foetuses (g)					
-male	4.82	5.11**	4.94	5.04**	5.11**
-female	4.58	4.87**	4.72*	4.76**	4.86**
Placental weight (g)					
-male	0.40	0.42	0.41	0.41	0.44**
-female	0.40	0.41	0.41	0.41	0.44**
Visceral findings in foetuses					
No. of foetuses examined (litters)	105(22)	100(24)	111(23)	102(23)	80(19)
No of litters with abnormal foetuses	9	3*	6	4	2*
No. of foetuses with abnormality (%)	10(9.5)	3(3.)	7(6.3)	4(3.9)	2(2.5)
Skeletal variations in foetuses					
No of foetuses examined (liters)	209(22)	202(24)	220(23)	199(23)	160(19)
No. of foetuses with skeletal variation (%)	10(4.8)	13(6.4)	11(5.0)	5(2.5)	11(6.9)
Cervical rib	1	2	0	0	2
Shortening 13th rib	0	1	0	0	0
Opening foramen transversium of the 7th cervical vertebrae	0	6*	3	0	3

No of ossificated phalanges of the forelimbs -proximal	6.7	7.4*	7.2	7.4**	7.6**
No. of foetuses with poorly ossified (%) -sternebrae -hyoid bone -cervical vertebral arch	2(1.0) 0(0.0) 0(0.0)	3(1.5) 0(0.0) 0(0.0)	2(0.9) 0(0.0) 0(0.0)	2(1.0) 0(0.0) 0(0.0)	1(0.6) 0(0.0) 0(0.0)

*Statistically different from control ($p \leq 0.05$), ** Statistically different from control ($p \leq 0.01$).

Acceptability

There is no OECD guideline with an equivalent study design. However, the study is considered acceptable since it adds useful additional information. Appendices I to III were not included in the study report. Therefore, results could not be compared with historical control data.

Conclusions

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 and placental weights were significantly higher in dams at 1000 mg/kg bw/day. 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. Based on lower numbers of corpora lutea and live foetuses and increased placental weight at 1000 mg/kg bw/day, the NOAEL for developmental toxicity was considered to be 500 mg/kg bw/day.

RMS conclusion during renewal:

The lower number of corpora lutea and live foetuses and the increased placental weight at 1000 mg/kg bw/day should be taken into account for setting the reproductive NOAEL not for the developmental NOAEL. Therefore, the reproductive NOAEL is set at 500 mg/kg bw/day.

Since the body weight of foetuses showed no dose-related effect and no morphological anomalies or variations were found related to treatment, the developmental NOAEL is set at 1000 mg/kg bw/day.

3.10.1.3 CA 5.6.2/01 (1988b): Perinatal and postnatal toxicity study in the rat

Report

CA 5.6.2/01 (1988b)

Perinatal and postnatal study of S-31183 orally administered to rats

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0030

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results section were added. Agreed with the original conclusions.

Characteristics

reference	:	CA 5.6.2/01, 1988b	exposure	:	Days 17 of gestation until day 20 postpartum, gavage (5 ml/kg bw/day)
type of study	:	Peri- and postnatal toxicity study	doses	:	0, 30, 100, 300 and 500 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	:	23-24/females/dose	**NOAEL**mat	:	100 mg/kg bw/day
			NOAELdev	:	30 mg/kg bw/day

Study design

S-31183 was daily administered by oral gavage to pregnant rats from day 17 of gestation to day 20 postpartum at dose levels of 0, 30, 100, 300 or 500 mg/kg bw/day. The effects on dams and the F_1-offspring were examined.

During pregnancy and lactation clinical signs, body weights, food consumption and water consumption were recorded. The dams were allowed to deliver and the gestation length was calculated. After birth the F_1-offspring was examined for the numbers of live born and stillborn pups, sex, external anomalies and pup viability. In addition, pups were regularly weighed and the development was examined by functional, emotional, learning and reproductive ability tests. After culling of each litter (8 pups litter, 4/sex), pups not selected for further investigations were prepared for skeletal examinations. At weaning (day 21 postpartum) all dams and F_1-offspring (except 2/sex/litter selected for learning tests) were necropsied and subjected to macroscopic examinations. The heart, lungs, liver, spleen, kidneys, adrenal glands (F_0), testes including epididymis (F_1), thymus (F_0), ovaries and brain (F_1 selected for learning tests) were removed, weighed and fixed for possible microscopic examinations. F_1-offspring necropsied at weaning were also prepared and examined for skeletal 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 17 of gestation until day 7 postpartum. In the 1000 mg/kg group all rats died and toxic signs consisted of soft stool or diarrhea, hypoactivity, rough hair, hypothermia, wasting and decreased body weight. At necropsy these animals showed congestion of the liver, atrophy of the spleen and thymus, enlarged adrenals and hemorrhage of the gastric mucosa (fundus glands). In the 500 mg/kg group no deaths occurred and animals showed soft stool or diarrhea, erythema and swelling of the periproctal region, reduced food consumption and slightly increased water consumption after parturition. Necropsy findings in surviving animals of the 500 and 250 mg/kg dose groups were characterized by atrophy of the spleen and thymus accompanied with weight loss of these organs. In all treated groups, male and female offspring revealed low body weights during the first week postpartum.

Materials and methods

A. Materials:

1. Test Material:	S-31183 (pyriproxyfen)
Lot/Batch No.:	PTG-86011
Purity:	97.2%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2. Test animals:	
Species:	Rats
Strain:	Slc:SD (SPF)
Age:	9 weeks
Source:	Shizuoka Laboratory Animal Center
Acclimation period	2 weeks

3.In life dates:	5 January 1987 to 14 May 1987
4.Animal assignment and treatment:	Prior to mating, females in good general condition were randomly divided into five groups: dour dosage groups and a control group, consisting of 32 animals each. When the rats were 11 weeks old, each pair of male and female rats cohabited overnight and copulation was confirmed the next morning (presence vaginal plug, sperm appearing in vaginal smear). Dose levels were determined based on the results of a preliminary study. The test substance was diluted in corn oil at concentrations to make a constant administration volume of 5 ml/kg bw for each dose group. The test solution was administered by using a stomach catheter once daily from day 17 of gestation to day 20 postpartum.
5.Statistics	Values were statistically analysed by Student's t-test or Welch's test. Values expressed as frequency were analysed by the x^2 test and Wilcoxon's rank sum test.
B. Methods	
1.Observations:	Clinical signs and mortality of the animals were checked twice daily throughout the test period.
2.Body weights and food consumption:	Body weight and food and water consumption were measured during the gestation period on days 0 (day of confirmed copulation), 4, 7, 14 and 17 of gestation and daily thereafter, and on days 0 (day of delivery), 4, 7, 14 and 21 postpartum during the lactation period.
3. Necropsy	At weaning (day 21 after delivery) every dam was sacrificed and necropsied and the major organs were macroscopically examined. The heart, lungs, liver, spleen, kidneys, adrenal glands, thymus and ovaries were weighed and fixed. The birth rate was calculated from the number of implantations and number of liveborn pups.
4.Observation of delivery and newborn:	All dams were allowed to deliver naturally and the gestation period was calculated. The number of liveborn and stillborn offspring were determined, the pups were sexed and examined for external anomalies. On day 4 postpartum, the size of each litter was adjusted to 8 and number of males/females per litter was adjusted. The extra pups were sacrificed and examined for skeletal anomalies. Body weight of offspring was measured at birth, and on days 4, 7, 14 and 21 postpartum during the lactation period. After weaning, one male and

	one female from each litter were weighed every seven days until 11 weeks old and thereafter these animals were used in reproductive performance tests. After weaning at 3 weeks of age, offspring were necropsied, except for two of each sex per litter which were examined for learning ability, and major organs were weighed and fixed. The offspring used for learning ability examination, were necropsied at 8 weeks of age.
5.Functional, emotional, learning and reproductive ability tests:	*Sensory function test*: on day 20 postpartum, all ofspring were examined for visual, algetic, auditory and other sensory functions. *Emotional and motor coordination tests*: The emotional ability was examined with the open field est at 4 weeks of age. Motor coordination was tested with the rotarod performance test at 5 weeks of age. *Learning ability test*: Offspring tested for emotional and motility were also examined for learning ability at 6 weeks of age using the water filled multiple T-maze. *Reproductive ability test:* One male and female per litter were raised until 11 weeks and were then paired. Males that copulated successfully were sacrificed and necropsied. Copulated females were weighed every three days and necropsied on day 21 of gestation.

Results

Dams

Mortalities and clinical signs: Two animals in the high dose group died on day 5 of administration. Another animal in the high dose group died on day 5 of administration due to an error in administration.
Clinical signs, i.e. soft stools or diarrhoea, erythema and swelling of the periproctal region, hypoactivity, rough hair, hypothermia, lacrimation and (transient) salivation were observed in the high dose group.

Table B.6.6.2-13 Mortality and clinical signs in dams

Finding	Dose (mg/kg bw/day)				
	0	**30**	**100**	**300**	**500**
Mortality	0/23	0/23	0/23	0/24	3/24[b]
Clinical signs					
-soft stools or diarrhea	0	0	0	3	22
-erythema and swelling of the periproctal region	0 0 0	0 0 0	0 0 0	0 0 0	8 4 3

Finding	Dose (mg/kg bw/day)				
	0	30	100	300	500
-hypoactivity	0	0	0	0	4
-rough hair	0	0	0	0	3
-hypothermia	0	0	1	2	4
-lacrimation					
-salivation (transient)					

Body weight: Body weight in the high dose group decreased after the first administration and a significant decrease was observed from day 20 of gestation to day 0 postpartum compared to the control group. Weight gain was significantly lower from day 19 of gestation throughout the gestation period. However, body weight gain was significantly higher from day 4 postpartum throughout the lactation period and the inhibited weight gain during the gestation period was reversed gradually and mean body weight of the group was similar to that of the control on day 21 postpartum. In the 300 mg/kg bw/day group, body weight also tended to be depressed although not significant. Body weight gain in this group was significantly decreased from day 19 to 21 of gestation; weight gain in the late lactation period was higher in this group compared to the control.

Table B.6.6.2-14 Body weight and weight gain of dams during pregnancy and after delivery

Parameter	Dose (mg/kg bw/day)				
	0	30	100	300	500
Body weight (g)					
Day 7 pregnancy	268.8	272.1	275.4	273.4	273.9
Day 14 pregnancy	300.0	301.3	305.6	304.7	302.2
Day 21 pregnancy	361.7	356.2	361.7	361.0	340.4**
Day 21 pregnancy	377.0	369.1	375.6	373.7	343.4**
Day 0 after delivery	294.7	297.7	298.6	293.6	279.1*
Day 7 after delivery	300.2	302.8	300.3	296.7	292.9
Day 21 after delivery	297.7	301.8	301.4	303.2	301.1
Body weight gain (g)					
Day 18 pregnancy	9.3	8.8	8.3	7.9	7.6
Day 19 pregnancy	23.2	21.6	20.7	18.1	11.9**
Day 21 pregnancy	52.3	46.2	46.2	43.5	19.0**
Day 22 pregnancy	60.6	51.9	49.4	36.9	-5.5**
Day 4 after delivery	-0.4	-3.0	-2.9	-1.6	5.1*

Day 7 after delivery	5.5	4.8	1.7	4.7	13.5*
Day 14 after delivery	17.1	15.4	11.7	18.4	27.1**
Day 21 after delivery	3.0	3.7	2.8	11.1	21.8**

*significantly different from control ($p \leq 0.05$), **significantly different from control ($p \leq 0.01$)

Food and water consumption: Food consumption in the high dose group was significantly lower than that of the control from day 19 of the gestation period (40-60% decrease compared to control). Food consumption in the 300 mg/kg bw/day group was also slightly, albeit significantly lower (13-20% decrease compared to control). Water consumption was significanlty increased in the 300 and 500 mg/kg bw/day groups (15-20% and 25-60% increase compared to control, respectively).

Necropsy findings and organ weights: In the high dose group necropsy findings showed enlargement of the liver, atrophy of the spleen, enlargement of the adrenals, atrophy of the thymus, congestion of the liver and ulceration of the gastric mucosa in the region of the fundus glands. None of these macroscopic anomalies were observed in the other dose groups.

Both absolute and relative weights of the liver were significanlty increased in the 300 (9% and 7%)and 500 mg/kg bw/day groups (18% and 17%). Also the relative weight of the heart in the 300 mg/kg bw/day group was significantly lower, but the change was slight and a dose-dependent relationship was not observed.

Table B.6.6.2-15 Necropsy findings including organ weights

Parameter	**Dose (mg/kg bw/day)**				
	0	**30**	**100**	**300**	**500**
Necropsy findings					
No. of dams examined	23	23	23	24	23
Liver enlargement	0	0	0	0	1
Liver congestion	0	0	0	0	2[a]
Spleen atrophy	0	0	0	0	4[a]
Adrenal enlargement	1	0	0	0	5[a]
Thymus atrophy	0	0	0	0	4[a]
Ulcer stomach	0	0	0	0	2[a]
Organ weights (g)					
Liver -absolute -relative	 12.381 4.157	 12.862 4.258	 12.610 4.185	 13.480** 4.449**	 14.658** 4.873**
Heart					

-absolute -relative	0.940 0.316	0.928 0.308	0.932 0.309	0.917 0.302**	0.945 0.314
Thymus -absolute -relative	 0.229 0.077	 0.231 0.077	 0.237 0.79	 0.222 0.073	 0.232 0.077
Adrenal, absolute	0.073	0.070	0.071	0.071	0.076
Ovary, absolute	0.096	0.093	0.097	0.093	0.091

aNumber includes the dams that died during the study

*significantly different from control (p≤0.05), **significantly different from control (p≤0.01)

Reproductive and offspring findings

Delivery: All dams delivered normally with the exeption of one animal in the control group and 3 animals in the high dose group. Gestation period was shorter in the 300 mg/kg bw/day group, although only slightly and no dose-response relationship was observed. There were 26 stillbirths in the high dose group, the majority delivered by the two dams with abnormal delivery. Birth rate in the high dose group was lower, although not statistically significant. Pup body weight at delivery was lower in the two high dose groups compared to the control.

Examination for external anomalies revealed a conjoined twin in a stillborn in the control group and a case of craniorachischisis in a stillborn in the 30 mg/kg bw/day group.

Table B.6.6.2-16 Delivery results

Parameter	**Dose (mg/kg bw/day)**				
	0	**30**	**100**	**300**	**500**
Pregnant animals	23	23	23	24	21
No of females with live newborn	22	23	23	24	19
Delivering rate	95.7	100.0	100.0	100.0	90.5
Birth rate	86.2	86.6	87.1	93.3	77.2
Gestation period (days)	21.8	21.8	21.6	21.3**	21.5
Body weight live newborns (g), male/female	5.9 / 5.6	5.9 / 5.6	5.8 / 5.6	5.3** / 5.1**	5.0** / 4.7**
Survival rate (%), male / female	98.8 / 96.2	97.6 / 91.0	98.4 / 98.3	93.7 / 97.1	87.4 / 79.1*
Weanling rate (%), male / female	97.4 / 98.9	97.4 / 97.0	97.8 / 97.8	93.5 / 93.8	74.4* / 78.1*

*significantly different from control (p≤0.05), **significantly different from control (p≤0.01)

Survival rate on day 4 was somewhat low in the high dose group. Weanling rate from birth to day 21 postpartum was significantly lower in the high dose group.

Body weight: Body weight at birth was decreased in the two high dose groups. Thereafter, weight gain in these groups was inhibited throughout the lactation period.

Table B.6.6.2-17 Body weight results

Day	Males					Females				
	0	30	100	300	500	0	30	100	300	500
Day 4	8.0	7.9	8.0	7.1**	6.6**	7.7	7.3	7.6	6.9**	6.2**
Day 7	12.4	11.6	12.0	10.6**	10.3**	12.0	10.9*	11.4	10.4**	9.4**
Day 14	25.4	24.4	24.3	21.9**	21.1**	24.8	23.0*	23.3	21.5**	19.0**
Day 21	41.1	39.3	38.5	34.3**	32.7**	39.3	37.2	37.0	34.2**	31.8**
Day 28	70.8	68.3	66.9	61.5**	60.5**	66.1	62.4	62.0	57.8**	57.2**
Day 56	266.2	258.6	254.7	242.7*	240.8*	180.7	173.9	171.6	171.0*	173.1
Day 63	310.2	304.1	299.1	290.3	285.5	198.7	193.1	189.6	189.2	192.4

*significantly different from control (p≤0.05), **significantly different from control (p≤0.01).

Postnatal development: Retardation of auricular separation, emergence of abdominal hair, opening of eyelids and a trend of retardation of eruption of lower incisors was observed in the 300 and 500 mg/kg bw/day groups. Descent of the testes in the 300 mg/kg bw/day group and opening of the vagina in the 500 mg/kg bw/day group were significantly delayed compared to the control group.

Table B.6.6.2-18 Postnatal development

Finding	Dose (mg/kg bw/day)				
	0	30	100	300	500
No. of offspring examined (litters)	302 (22)	266 (23)	275 (23)	336 (24)	229 (19)
Separation auricle	3.1	3.3	3.2	3.9**	3.6**
Emergence hair	10.2	11.0**	10.5	11.3**	11.5**
Opening eyelids	16.1	16.3	16.0	16.8**	16.6*
Eruption lower incisor	12.1	11.9	11.4*	12.8*	12.5
Descent testis	23.0	24.1	23.8	24.9**	24.6
Opening vagina	35.6	36.8	36.9	36.9	37.3*

*significantly different from control (p≤0.05), **significantly different from control (p≤0.01).

External, visceral and skeletal examinations: No external anomalies were observed in any group. At necropsy performed at 3 weeks of age, the incidence of visceral anomalies was 10.0, 1.2, 17.2 and 28.6% in the 30, 100, 300 and 500 mg/kg bw/day groups respectively compared to 0% in the control group. Necropsy

at 8 weeks of age showed no treatment-related differences. No treatment-related differences were observed regarding organ weights.

At 3 weeks of age no skeletal anomalies were found in any offspring. Variations were observed, however, they were observed in every group including the control. The incidence of these variations was not increased by administration of the test compound.

Table B.6.6.2-19 Visceral findings and organ weights

Finding	Dose (mg/kg bw/day)				
	0	**30**	**100**	**300**	**500**
Visceral examinations					
At 3 weeks of age					
No. of offspring examined	85	80	82	87	49
Dilatation renal pelvis	0	3	1	12**	9**
Hyperemia and/or inflammatory cell infiltration inpropria of urinary bladder	0	3	0	5	6**
At 8 weeks of age					
No. of offspring examined	44	43	44	45	26
Dilatation renal pelvis	2	1	0	3	2
Small testis and/or seminal vesicle	0	4	2	1	0
Enlarged testis	1	2	2	3	0
At end fertility test					
No. of offspring examined	44	44	46	45	26
Dilatation renal pelvis	0	1	1	2	0
Small testis and/or seminal vesicle	2	3	1	3	2
Enlarged testis	1	0	0	0	0
Skeletal examinations					
No. of offspring examined (litters)	85	89	82	87	49
Cervical rib	8	2	4	7	6
Splitting 1st lumbar transverse process	4	2	4	2	4
Hypoplasia 13th rib	0	1	0	0	0
Hypoplasia 2nd cervical vertebral body	3	0	1	2	2
Accessory sternebrae	1	0	0	0	0

*significantly different from control ($p \leq 0.05$), **significantly different from control ($p \leq 0.01$)

Emotionality, motor coordination and learning abilities: In the open field test, significantly high values were found for the frequency of ambulation of males in the 100 mg/kg bw/day group and higher doses and the frequency of rearing of males in the 100 mg/kg bw/day group. Statistically significant decreases were observed in the frequency of male preening in the 30 and 300 mg/kg bw/day groups and defecation of males in the 100 mg/kg bw/day group. In the rotarod performance test, no differences were observed. In the T-maze test, no differences were found in time required to reach the goal.

Table B.6.6.2-20 Results for emotionality, motor coordination and learning abilities

Parameter	Dose (mg/kg bw/day)				
	0	**30**	**100**	**300**	**500**
Locomotor and emotionality in open field test					
No of offspring examined, male / female	22/22	22/21	23/21	22/23	13/13
Ambulation, male / female	52.0 / 59.6	59.5 / 60.7	68.3* / 66.6	78.2** / 69.8	71.3* / 56.8
Rearing, male / female	8.3 / 9.4	9.9 / 8.5	12.4* / 10.5	9.5 / 9.2	8.7 / 6.6
Preening, male / female	1.5 / 0.8	0.7* / 0.7	1.0 / 1.0	0.5* / 0.6	0.6 / 1.2
Defecation, male / female	1.5 / 0.5	1.0 / 0.2	0.5* / 0.7	1.0 / 0.2	0.5 / 0.6
Motor coordination by rotarod					
Median of falls, male / female	2.0 / 4.0	2.5 / 4.0	4.0 / 4.0	3.0 / 4.0	2.0 / 6.0
Learning ability in T-maze test[a]					
Time 1st day, male/female	70.1 / 50.3	66.4 / 48.6	75.7 / 52.1	66.2 / 51.3	69.0 / 56.1
Time 2nd day, male/female	254.3 / 180.9	222.6 / 186.7	236.0 / 217.1*	214.5* / 199.6	231.5 / 199.5
Time 3rd day, male/female	108.1 / 76.9	101.9 / 88.5	107.5 / 76.0	93.6 / 83.3	106.7 / 81.3

[a]Time during 1st day represent harmonic mean of total elapsed time (sec) during 5 trials in a straight channel; time during 2nd and 3rd day represents harmonic mean of total elapsed time (sec) during 5 trials in a maze.
*significantly different from control ($p \leq 0.05$), **significantly different from control ($p \leq 0.01$).

Reproductive ability test: There were no significant differences in copulation or fertility rates. Body weight during the gestation period was similar to that of the control group. There were no differences in number of corpora lutea, however in the high dose group the number of implantations was slightly but significantly lower. No differences were observed in sex ratios or weight of live foetuses.

Table B.6.6.2-21 Reproductive ability findings

Parameter	Dose (mg/kg bw/day)				
	0	**30**	**100**	**300**	**500**
No. mated	22	22	23	22	14
No. copulated	21	19	22	18	13
No. pregnant	21	17	18	14	12
Body weight during pregnancy					
Day 7	261.1	246.8	247.9*	253.2	254.6
Day 14	291.3	277.8	277.3	283.3	283.5
Day 21	364.7	339.1*	347.8	350.7	340.2*
No. corpora lutea	13.6	11.9**	13.4	12.9	13.1
No. of implantations	12.3	10.2**	11.9	10.7	9.2*
Sex ratio	0.49	0.50	0.54	0.46	0.51
Weight of live foetuses (g), male / female	4.92 / 4.68	4.81 / 4.59	4.86 / 4.63	4.84 / 4.66	4.89 / 4.66

*significantly different from control ($p \leq 0.05$), **significantly different from control ($p \leq 0.01$).

Acceptability

The study is considered acceptable as it produces useful additional information. However, there is no OECD guideline describing a similar design and conduct of this study.

Conclusions

Maternal toxicity was evident at 500 mg/kg bw/day as indicated by the death of 3 females (one of which was considered to be due to an intubation error), decreased body weights and food consumption, increased water consumption and clinical signs, i.e. soft stools or diarrhea, erythema and swelling of the periproctal region, hypoactivity, rough hair, hypothermia, lacrimation and (transient) salivation. At necropsy, treatment-related findings consisted of thymus atrophy, liver congestion, liver enlargement, spleen atrophy, adrenal enlargement, kidney congestion, hemorrhage of the mucous membrane and ulceration in the stomach and significantly increased liver weights.

Signs of maternal toxicity at 300 mg/kg bw/day included soft stool or diarrhea and (transient) salivation, reduced body weights and food consumption, increased water consumption and significantly increased liver weights. No toxicological significant changes were observed in the 100 and 30 mg/kg dose groups.

Treatment of dams at 500 mg/kg bw/day resulted in reduced values of the number of females with live newborns and the delivering rate, due to increased mortality of dams.
Developmental effects included an increased number of stillborns at 500 mg/kg bw/day (although this finding was not significant in statistical terms), significantly reduced body weights of live newborns at 300 and 500 mg/kg bw/day, significantly reduced offspring survival rates on day 4 and at weaning at 500 mg/kg bw/day, and significantly retarded offspring growth up to day 77 postpartum at 300 and 500 mg/kg bw/day. There were no biologically significant deviations in physical and sensory development, locomotor activity, motor coordination, learning ability and reproductive performance of the offspring. External, visceral and skeletal examinations of the offspring revealed no treatment-related finding
At necropsy of the offspring after 3 weeks postpartum, an increased incidence of dilatation of the renal pelvis, hyperemia and/or inflammatory cell infiltration in the propria of the urinary bladder was noted in the 500 and 300 mg/kg bw/day dose groups, but no such effects were seen in offspring examined after 8 weeks postpartum.

No toxicologically significant changes were observed in dams and offspring of the 100 and 30 mg/kg bw/day dose groups. Accordingly, the NOAEL for maternal and developmental toxicity was set at 100 mg/kg bw/day.
RMS conclusion: Agreed with the original conclusion.
Following pesticide peer review meeting 190 (28 Jan – 1 Feb 2019), the experts considered that the statistically significant changes in ambulation in males from 100 mg/kg bw/day onwards should be considered as an adverse finding. Therefore, the experts agreed to lower the developmental NOAEL to 30 mg/kg bw/day based on increased ambulation in male pups (locomotor and emotionality in the open field test).

3.10.1.4 CA 5.6.2/02 (1988c): Oral (gavage) developmental toxicity study in the rat

Report

CA 5.6.2/02 (1988c)

Study by administration of S-31183 during the period of foetal organogenesis in rats.

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0029

Report

Study by administration of S-31183 during the period of foetal organogenesis in rats: Addendum to the final report (1989). Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-91-0061

Previous evaluation	In DAR (2005)

Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results section were added. Agreed with the original conclusion.

Characteristics

reference	:	CA 5.6.2/02, 1988c	exposure	:	Days 7-17 of gestation, gavage (5 ml/kg)
type of study	:	developmental 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 in the study report, 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

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 F_1-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 F_1-offspring were necropsied and subjected to macroscopic examinations. The heart, lungs, liver, spleen, kidneys, adrenal glands (F_0), testes including epididymis (F_1), thymus (F_0), ovaries and brain (F_1 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

Maternal effects

Mortality and clinical signs:

Twelve animals died among the 42 animals in the 1000 mg/kg bw/day group. Three animals died on day 4 of administration, two animals died on day 5 of administration, two died on day 6, four died on day 7 and one died on day 9 of administration.

Clinical signs included soft stools or diarrhoea, erythema and swelling of the periproctal region, hypoactivity, wasting, bloody dirtiness around the nose, blanching of the auricle and extremities and hypothermia.

Table B.6.6.2-22 Mortality and clinical signs in dams

	Dose (mg/kg bw/day)			
Finding	**0**	**100**	**300**	**1000**
No of dams examined	36	36	36	42
Mortality	0	0	1[a]	12
Clinical signs				
-diarrhea				42
-erythema and swelling of periproctal region			1[j]	19 10
-hypoactivity			1[j]	9
-wasting				4
-rough hair				2
-lacrimation			1[j]	9
-hypothermia				
-blanching of auricle and extremity			1[j]	3 6
-bloody dirtiness around nose				

[a]The animal was sacrificed, because there was no delivery at day 25 of pregnancy.

Body weight:

Body weight in the high dose group was decreased from the next day of the start of administration (day 8 of gestation). Although the mean body weight in this group changed to increase from day 4 of administration (day 11 of gestation), it nevertheless remained significantly lower than the control group throughout the gestation and lactation periods. Body weight in the 300 mg/kg bw/day group was slightly but significantly lower through gestation period after day 13 up to day 20 and throughout most the lactation period. Body weight in the 100 mg/kg bw/day group was comparable to that of the controls throughout the gestation and lactation periods.

Table B.6.6.2-23 Body weight dams

Parameter	Dose (mg/kg bw/day)			
	0	**100**	**300**	**1000**
Day of pregnancy				
3	251.7	248.8	248.0	253.9
5	251.7	248.8	248.0	253.9
7	265.0	263.3	260.4	266.7
9	271.2	269.2	265.4	258.2**
13	291.4	287.2	281.4*	258.8**
14	295.4	290.2	284.5*	266.2**
17	319.8	314.1	307.7*	288.6**
20	355.9	351.0	342.5*	326.3
21	370.1	366.6	357.7	341.0**
Day after delivery				
0	298.5	283.2	280.0*	267.8**
4	294.6	285.6	282.6	276.1*
7	303.2	294.1	287.4*	284.1*
14	320.7	310.4	302.9*	299.4**
21	300.8	295.0	286.5	285.3*

*Statistically different from control (p≤0.05), ** Statistically different from control (p≤0.01).

Table B.6.6.2-24 Body weight gain dams

Parameter	Dose (mg/kg bw/day)			
	0	**100**	**300**	**1000**
Day of pregnancy				
7	0.0	0.0	0.0	0.0
8	1.3	1.4	1.0	-0.8**
9	6.2	5.9	5.0	-8.5**

10	11.8	10.4	9.8*	-12.3**
14	30.3	26.9**	24.1**	1.1**
21	105.1	103.2	97.3	76.1**
22	110.2	110.2	83.9	92.3
Day after delivery				
4	-3.9	2.4	2.7*	8.3**
7	4.7	10.7	7.4	16.3**
14	22.2	27.0	23.0	31.6
21	2.3	11.6	6.7	17.5**

*Statistically different from control (p≤0.05), ** Statistically different from control (p≤0.01).

Food and water consumption

Food consumption in the high dose group was significantly lower than controls during the administration period (up to 60% decrease). Thereafter, however, slightly but significanlty higher values were found during the gestation period (11-19% increase) and at the early stage of the lactation period (12-50% increase, days 1-7 after delivery). Although food consumption was significantly decreased in the mid-dose group throughout the administration period, no effect was observed thereafter.

Water consumption in the mid-dose group and high-dose group was increased significantly during the administration period and throughout the gestation period. In the mid-dose group the increases ranged between 14 and 30 % compared to the control group; in the high-dose group the increase was 26 - 55% compared to the control.

Necropsy

In the dead animals, congestion of the liver, atrophy of the spleen, enlargement of the adrenals and involution of the thymus were found in all animals, while haemorrhage in the gastric mucosa was observed in half, and haemorrhagic ulceration was also observed in some of the animals (3 out of 12 animals).

In necropsy of the live animals, enlargement of the adrenals was found in the high dose group. No macroscopic changes were observed in the other dose groups.

Table B.6.6.2-25 Autopsy findings in sacrificed animals

Parameter	**Dose (mg/kg bw/day)**			
	0	**100**	**300**	**1000**
Sacrificed on day 21 of pregnancy				
No. of dams examined	23	23	23	20
Thymus atrophy	0	0	0	13
Spleen atrophy	0	0	0	1

Adrenal enlargement	0	0	0	15
Kidney enlargement	0	0	0	1
Sacrificed on day 21 postpartum				
Adrenal enlargement	0	0	1	1

Organ weights

At cesarean section, mean weight of the thymus was decreased, while the weights of the kidneys and adrenal glands were increased in the high-dose group. Low absolute weight of the heart and high relative weight of the liver were also observed in this group. In the mid-dose group, slightly higher relative weights were found in the liver (126% of control) and kidneys (122% of control).

At weaning of pups, no significant changes were observed in any groups and the control, except the significantly lower absolute weight of the spleen in the high-dose group.

Table B.6.6.2-26 Absolute organ weights in sacrificed animals (g)

Organ	**Dose (mg/kg bw/day)**			
	0	**100**	**300**	**1000**
Sacrificed on day 21 of pregnancy				
Thymus	0.260	0.252	0.243	0.146**
Heart	0.807	0.812	0.785	0.737**
Liver	12.665	12.852	13.002	12.883
Kidney (total)	1.451	1.449	1.509	1.552*
Adrenal	0.063	0.064	0.063	0.082**
Sacrificed on day 21 postpartum				
Thymus	0.230	0.222	0.235	0.230
Heart	0.929	0.889	0.895	0.891
Liver	11.752	11.644	11.167	11.637
Kidney (total)	1.850	1.793	1.770	1.848
Adrenal	0.069	0.069	0.067	0.068
Spleen	0.562	0.532	0.549	0.507**
Ovary (total)	0.087	0.093	0.091	0.090

*Statistically different from control ($p \leq 0.05$), ** Statistically different from control ($p \leq 0.01$).

Reproductive observations:

There were no differences in the numbers of corpora lutea or implantations, or the implantation rates in the treated groups compared to the control. There were no differences in the weight of live foetuses or placental weight or sex ratio in the treated groups compared to the control.

With the exception of one animal in the mid-dose group, all pregnant animals delivered normally and the gestation period in the treated groups was comparable to the control group. There were no stillbirths in any of the groups and the number of implantations, birth rate, number of live newborns and body weight of the live offspring at birth in the treated groups was comparable to those in the control group.

The survival rates of the offspring in the treated groups were comparable to the control group. In addition, no reduction in weanling rates was observed in the treated groups.There were no deaths of offspring in any group after weaning.

Table B.6.6.2-27 Reproductive findings

Parameters	Dose level (mg/kg bw/day)			
	0	100	300	1000
Litter effects				
- No. of dams examined	23	23	23	20
- Mean no. of *corpora lutea*	15.1	15.1	15.5	15.2
- Mean no. of implantations	13.8	14.0	14.1	13.8
- Implantation rate	93.0	92.2	90.5	91.9
- Rate of resorbed / dead fetuses				
- Total (%)	4.7	7.0	7.7	14.6
- Early (%)	4.4	6.7	7.7	14.6
- Late (%)	0.3	0.3	0.0	0.0
- Total no. of live foetuses (♂/♀)	303 (149/154)	299 (155/144)	299 (158/141)	232 (116/116)
- Sex ratio	0.49	0.52	0.54	0.50
- Live foetal weight (g) (♂/♀)	4.95 / 4.67	4.99 / 4.75	5.01 / 4.77	5.02 / 4.70
-Placental weight (g) (♂/♀)	0.41/0.41	0.42/0.41	0.40/0.39	0.38/0.37

* $p \leq 0.05$; **$p \leq 0.01$

Foetal observations:

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. In the study report historical control data are mentioned for this finding: data from 20 conducted tests (performed between 1983 and 1985) at this test facility using the same strain of rats the incidence of this finding was 0 – 3.2%. The incidences in this study with pyriproxyfen were 1.5% at 100 mg/kg bw/day (and thus within the HCD), 5% at 300 mg/kg bw/day and 14% at 1000 mg/kg bw/day.

No treatment related effects in skeletal abnormalities or visceral findings were observed.

Table B.6.6.2-28 Skeletal findings (variations) in foetuses

Parameters	Dose level (mg/kg bw/day)			
	0	100	300	1000
Examination of foetuses				
- No. of foetuses examined (litters)	202 (23)	200(23)	200 (23)	154 (18)[a]
Skeletal findings (variation)				
No of foetuses with skeletal variation (%)	14 (6.9)	14 (7.0)	15 (7.5)	37 (24.0)**
-Cervical rib	6	3	2	1
-Lumbar rib	7	6	4	11
-Shortening of 13th rib	1	1	1	1
-7 lumbar vertebrae	1	1	0	1
-Opening of foramen transversarium of the 7th cervical vertebrae	0	3	10*	22**

* $p \leq 0.05$; **$p \leq 0.01$

[a]Two dams in the high dose group had total litter resorption, therefore results from 18 litters are given.

Table B.6.6.2-29 Reproductive ability offspring

Parameters	Dose level (mg/kg bw/day)			
	0	100	300	1000
1st mating				
Copulated / Mated (%)	76.9	91.7	100.0	100.0
Pregnant / copulated (%)	80.0	81.8	90.9	100.0
No of dams sacrificed	8	9	10	10
No of corpora lutea	13.4	14.7	13.3	12.9
Implantation rate	84.4	88.7	91.9	88.0
No of live foetuses, total (M/F)	88 (40/48)	108 (50/58)	116 (52/64)	110 (55/55)
Sex ratio	0.52	0.46	0.44	0.54
Boody weight live foetuses (M/F)	5.00/4.66	4.80/4.54	5.00/4.69	5.06/4.74
No of foetuses with external abnormalities	0	0	0	0

* $p \leq 0.05$; **$p \leq 0.01$

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. 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 and kidney 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 a statistically significant increase of early implantation loss. 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.

RMS conclusion: Agreed with the original conclusions.

Following pesticide peer review meeting 190 (held 28 Jan – 1 Feb 2019), the experts considered that based on the litter incidence of opening of the foramen transversarium of the 7th cervical vertebra (0, 3, 5, 11), a developmental LAOEL of 100 mg/kg bw/day should be set.

3.10.1.5 CA 5.6.2/03 (1988): Oral (gavage) developmental toxicity study in the rabbit

Report

CA 5.6.2/03 (1988)

Study of S-31183 by oral administration during the period of foetal organogenesis in rabbits

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0033

Report

Addendum to the final report: Study of S-31183 by oral administration during the period of foetal organogenesis in rabbits (1994). Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-40-0109

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results sections were added. Agreed with the original conclusions.

Characteristics

reference	:	CA 5.6.2/03, 1988	exposure	:	Days 6-18 of gestation, gavage
type of study	:	developmental study	doses	:	0, 100, 300 and 1000 mg/kg bw/day (1, 0.09, 0.26 and 0.87 ml/kg, respectively)
year of execution	:	1986-1987	vehicle	:	Heated (50 °C), adjusted to ambient temperature and dosed as such Vehicle control: sterile distilled water
test substance	:	S-31183 (Pyriproxyfen), batch no. PTG-86011, purity 97.2%	GLP statement	:	Yes
route	:	Oral	guideline	:	Not indicated, see study design
species	:	Rabbit JW-NIBS	acceptability	:	Acceptable
group size	:	15-18 females/dose	**NOAEL**mat	:	100 mg/kg bw/day
			NOAELdev	:	100 mg/kg bw/day

Study design

S-31183 was daily administered by oral gavage at dose levels of 0, 100, 300 or 1000 mg/kg bw/day, to groups of 12-14 pregnant rabbits from day 6 to day 18 of gestation inclusive. On day 28 of gestation all rabbits were subjected to a cesarean section and macroscopic examination of the organs in the thoracic and abdominal cavities. The numbers of corpora lutea, implantations, live fetuses and dead embryo's and fetuses were recorded. All live foetuses were weighed and sexed and examined for external, visceral and skeletal abnormalities.

Dose levels were selected on the basis of a dose range finding study (ref. 1, Kannan et al 1988, Sumitomo Chemicals Study Number 375), which was not available for evaluation. In this preliminary study, dose levels of 100, 300 and 1000 mg/kg bw/day were administered to non-pregnant rabbits (no numbers) for 14 consecutive days. Decreased body weights were noted in the 300 and 1000 mg/kg dose groups. In addition, one animal of the 1000 mg/kg dose group did not eat and showed traces of haemorrhage in the stomach and a thinned caecal wall with changed appearance of its contents.

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Lot/Batch No.:	PTG-86011
Purity:	97.2%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Rabbit
Strain:	JW-NIBS
Age:	5 - 6months
Source:	Nihon Seibutsu Kagaku Research Institute
Acclimation period	2 weeks
3.In life dates:	5 November 1986 to 9 February 1987
4.Animal assignment and treatment:	Dose levels were determined at 0, 100, 300 and 1000 mg/kg bw/day based on a preliminary study in rabbits. Each animal was assigned to one of the groups according to body weight as measured on day 0 of gestation, so as to be no difference in body weight between groups. The day of confirmed copulation was regarded as day 0 of gestation. The test substance was given via gavage once a day from day 6 to day 18 of gestation.
5.Statistics	Body weight and body weight gain of dams, food consumption, number of corpora lutea/implantations/live foetuses, body weight of live foetuses, number of ossified vertebrae and phalanges were analysed by t-test. The rank sum test was used to statistically analyse the implantation rate, % of post-implantation loss, sex ratio, incidences of anomalies and variations in foeuses and incidence unossified sternebrae/metacarpal bone/5th middle phalanx/talus.
B. Methods	
1.Observations:	Dams were monitored daily throughout the gestation period to record changes in general signs and mortality.
2.Body weights:	Dams were weighed on days 0, 6, 9, 12, 15, 18, 22, 25 and 28 of gestation.
3.Food consumption:	Food consumption was measured on the days of body weight measurement, except day 0 of gestation.

4.Autopsy and cesaerian section:	Dams were sacrificed on day 28 of gestation. Cesarean section was performed and autopsy was performed on the dams to examine organs in the thoracic and abdominal cavities. The numbers of corpora lutea, implantations, live foetuses, dead embryos and foetuses were recorded.
5.Examination live foetuses:	Live foetuses were examined for external anomalies, including the oral cavity, and weighed individually. All foetuses were examined for visceral and skeletal anomalies and were sexed.

Results

Maternal findings

Clinical signs and mortality:

Abortion or premature delivery were observed in one dam of the control group, three of the mid-dose group, and six of the high-dose group. In the high-dose group, one dam died during administration period and two were sacrificed because of debility.

No toxic signs were observed in the low dose group. Toxic signs were seen only in animals which aborted, prematurely delivered, died or were sacrificed in the mid- and high-dose groups. These signs included soft stool, emaciation, lusterless fur, decrease in spontaneous activity and bradypnea or deep breathing. In addition, in the high dose group, diarrhoea, mucous stool and ananastasia were also noted.

Table B.6.6.2-30 Maternal findings

	Dose (mg/kg bw/day)			
Finding	**0**	**100**	**300**	**1000**
No of copulated females	15	17	15	18
Non-pregnant females	0	4	1	5
No of dams examined	14	12	14	13
Mortality	0/14	0/12	0/14	3/13[b]
Non-pregnant animals	0	4	1	5
No of dams aborted or premature delivery	1	0	3	6[e]
Clinical signs -soft stool or	1	1	1	9

Finding	Dose (mg/kg bw/day)			
	0	100	300	1000
diarrhea	0	0	0	1
-mucous stool	0	0	1	2
-ananastasia	0	0	3	10
-emaciation	0	0	3	7
-lusterless fur	0	0	2	7
-decrease in spontaneous activity -bradypnea or deep breathing	0	0	2	7

Body weight:

In the high-dose group, body weight and body weight gain were markedly decreased from day 9 to 25 of gestation. In the mid-dose group, the mean body weight gain was tended to decrease from day 15 to day 25 of gestation.

Table B.6.6.2-31 Maternal body weight and weight gain

Parameter	Dose (mg/kg bw/day)			
	0	100	300	1000
Body weight (kg)				
Day 6	3.11	3.01	3.11	3.04
Day 9	3.12	3.01	3.11	2.99
Day 12	3.15	3.03	3.13	2.92**
Day 15	3.21	3.11	3.17	2.86**
Day 18	3.21	3.11	3.13	2.77**
Day 22	3.26	3.16	3.18	2.71**
Day 25	3.29	3.19	3.17	2.86*
Day 28	3.28	3.23	3.31	3.15
Body weight gain (kg)				
Day 9	0.02	0.00	-0.01	-0.05*
Day 12	0.04	0.02	0.01	-0.12**
Day 15	0.10	0.10	0.05	-0.19**
Day 18	0.11	0.10	0.02	-0.29**
Day 22	0.15	0.15	0.05	-0.30**
Day 25	0.18	0.18	0.06	-0.12*
Day 28	0.19	0.22	0.17	0.16

*Statistically different from control ($p \leq 0.05$), **Statistically different from control ($p \leq 0.01$).

Food consuption:

Food consumption was markedly reduced from day 9 to day 25 of gestation in the high-dose group and tended to be low from day 15 to 25 of gestation in the mid-dose group.

Table B.6.6.2-32 Mean food consumption during the pregnant period (day of pregnancy) in grams

Parameter	Dose (mg/kg bw/day)			
	0	100	300	1000
Day 6	168	159	167	158
Day 9	165	151	148	112**
Day 12	154	141	143	77**
Day 15	141	129	116	44**
Day 18	145	138	119	41**
Day 22	144	139	118	52**
Day 25	121	122	103	78
Day 28	112	118	114	116

*Statistically different from control ($p \leq 0.05$), **Statistically different from control ($p \leq 0.01$).

Autopsy findings:

In cesarean section in the last stage of pregnancy, trace amounts of haemorrhage throughout the lungs was observed in two dam each of the control and three drug-treated group, discolouration in one of the control group, subcutaneous haemorrhage in the right uterine wall in one of the mid-dose group, and white spots scattering on placenta in one of the control group and one of the mid-dose group. White spots on placenta was also found in one dam of the high-dose group which prematurely delivered.

Litter data

Live foetuses were obtained from 13 dams in the control group, 12 in the low-dose group, 11 in the mid-dose group and 4 in the high-dose group.

There were no significant differences in numbers of corpora lutea and implantations, percentage of post-implantation loss, number of live foetuses, sex ratio or body weight of live foetuses.

Table B.6.6.2-33 Litter findings

Parameter	Dose (mg/kg bw/day)			
	0	100	300	1000
No of dams examined	13	12	11	4
Live foetuses	94	91	89	26
Litter Size	7.2	7.6	8.1	6.5

Parameter	Dose (mg/kg bw/day)			
	0	100	300	1000
No of corpora lutea	8.9	8.8	9.7	8.0
No of implantations (mean)	8.0	8.1	8.6	7.0
Post implantation loss[a]	9.6	6.2	6.3	7.1
Sex ratio	0.36	0.36	0.37	0.38
Foetal body weight (g)				
-male	34.73	36.31	38.28	36.55
-female	37.11	35.28	36.42	38.41

[a] (no. of post implantation loss / no. of implantations) x 100

External examination live foetuses:

No foetuses had any external anomalies in the mid- or high-dose group. One of the control group (1.1%) had multiple malformations consisting of cranioschisis, cleft palate, manus valga and umbilical hernia. One foetus of the low-dose group (1.1%) showed articular flexion contracture in foreleg.

Skeletal examination live foetuses:

No skeletal malformations were observed, except defect of 3rd distal phalanx of hinder leg in one foetus of the mid-dose group.

There were no significant differences in the incidences of anomalies among the groups.

The number of ossified middle phalanges of forelegs of the low-dose group was significantly higher than that of the control group. There were no significant differences in other indicators of ossification among the groups.

Visceral examination live foetuses:

Two foetuses (2.2%) had visceral malformation and one (1.1%) had slight anomaly in the mid-dose group. No other foetuses of each group had any anomalies.

There were no significant differences and no certain tendencies in the incidences of visceral anomalies between the control and treated groups.

Table B.6.6.2-34 Skeletal and visceral findings

Parameter	Dose (mg/kg bw/day)			
	0	100	300	1000
Skeletal examination				
No. of examined foetuses	93	90	89	26
Defect 3rd distal phalanx hinder leg	0	0	1 (1.1)	0
Fusion cervical vertebrae	12 (12.9)	9 (10.0)	0 (0.0)	2 (7.7)
Assymetric sternebrae	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Hypoplasia 3rd distal phalanx foreleg	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Hypoplasia 2nd distal phalanx hinder leg	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
13 ribs	7 (7.5)	3 (3.3)	5 (5.6)	2 (7.7)
No. of ossified middle phalanges	3.5	3.8*	3.7	3.8
Visceral examination				
No. of foetuses examined	93	90	89	26
Cystic lung	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Hypoplasia left atrial auricle	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Persistent truncus arterious	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Ventricular septal defect	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Defect gallbladder	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Persistent left azygos vein	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Abnormal location posterior vena cava	17 (18.3)	14 (15.6)	14 (15.7)	9 (34.6)
Abnormal location right subclavian artery	5 (5.4)	0 (0.0)	5 (5.6)	0 (0.0)
Bifurcation vermiform appendix	0 (0.0)	2 (2.2)	2 (2.2)	1 (3.8)

Numbers in table are given as: no. of foetuses with the malformation (incidence).

*statistically different from control ($p \leq 0.05$).

Acceptability

The study design was in accordance with the requirements of the OECD 414 Guideline. However, the number of dams evaluated in each dose group was not in agreement with the numbers required according the OECD 414 of 2001 (i.e., at least 16 dams/group). Moreover, the number of dams remaining in the top dose group was insufficient for useful evaluations. Since no teratogenic effects were noted in the 300 mg/kg bw/day group, the

study is considered acceptable. Other differences noted between this study and the OECD 414 included: gravid uterus not weighed.

Conclusions

Maternal toxicity at 1000 mg/kg bw/day was indicated by the death of 3 dams, increased number of dams with abortion or premature delivery (6 dams), markedly decreased body weights and food consumption and higher incidences of severe clinical signs and macroscopic findings.

No deaths occurred among dams receiving 300 mg/kg bw/day. In this group lower incidences of premature delivery or abortion (3 dams), clinical signs and macroscopic findings were noted when compared to the high dose group and decreased tendencies of body weights and food consumption. In the 100 mg/kg dose group no adverse effects of treatment were detected.

There were no statistically significant effects on the mean number of corpora lutea. Examination of the uterus contents on day 28 of gestation did not reveal any changes in the litter response, number of implantations, number of post implantation loss, foetal body weight, foetal sex, foetal morphology and skeletal ossification of foetuses that could be attributed to treatment with S-31183.

Based on abortion or premature delivery, the NOAEL for maternal toxicity was established as being 100 mg/kg bw/day. The number of dams remaining in the top dose group was insufficient for useful evaluations. However, since no developmental effects were noted at 300 mg/kg bw/day, the NOAEL for developmental effects was established at 300 mg/kg bw/day.

RMS conclusion: Agreed with the original conclusion.

Following pesticide peer review meeting 190 (28 Jan – 2 Feb 2019), the experts agreed to lower the developmental NOAEL to 100 mg/kg bw/day, considering multiple visceral malformations in 1 animal and single visceral malformation in 2 animals was observed at 300 mg/kg bw/day and considering that the high dose of 1000 mg/kg bw/day could not be assessed as an insufficient number of dams remained at this high dose level.

3.10.1.6 CA 5.8.3/01 (2012a): Pubertal development and thyroid fuction assay in male rats

Report

CA 5.8.3/01 (2012a)

A pubertal development and thyroid function assay of pyriproxyfen T.G. administered orally in intact juvenile/peripubertal male rats

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNT-0210

Submitted for the purpose of renewal

Guidelines:	OPPTS 890.1500 (2009)
GLP:	Yes (certified laboratory)
Acceptability:	The study is considered to be acceptable

Materials and Methods

A. Materials:

1. Test Material:	Pyriproxyfen T.G.
Description:	White solid
Lot/Batch No.:	080506G
Purity:	99.5%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2. Vehicle and/or positive control:	Corn oil / n.a
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Age:	♂: PND 23 at time of dosing
Weight at dosing:	♂: 55.9-71.8g (PND 23).
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	From PND 0 to PND 21

B. Study Design and Methods:

1. In life dates:	11 October 2011 to 12 March 2012
2. Animal assignment and treatment:	Time mated female rats were received from the supplier on gestation days 7 and were used to obtain the juvenile males for this study. Maternal data were recorded but not presented in the report. Pups were weaned until PND 21 and monitored for physical health and growth. Litters were culled to 8 males and 8 females on PND 4 to reduce variability. Pups were weighed and physically examined on PND 21 prior to assignment to 3 groups of 15 males/group using a computerised method based on stratification of bodyweights in a block design; littermates were not placed in the same group.

	Dose levels of pyriproxyfen were 0, 500 or 1000 mg/kg bw/d administered daily by oral gavage from PND 23 to PND 53 or 54. The dose levels were selected based on the results of previous studies.
3.Homogeneity and achieved concentration analysis of the dose:	Dosing solutions were prepared weekly but were not analysed for achieved concentration or homogeneity. Stability data was also available to confirm suitable stability of the dosing formulations.
4.Statistics:	All analyses were conducted using appropriate computing device or programs. Each endpoint was tested for homogeneity and the data transformed as appropriate if it was significant. The data were subjected to a non-parametric test if the test was still significant following transformation. Analysis of variance was conducted and a 2-sided Dunnett's test or a non-parametric Kruskal-Wallis test followed by Dunn's test was used to compare the treated groups with the vehicle control group. In addition, organ weights and balanopreputial separation data were subject to analysis of covariance using the bodyweight at PND 21 as a covariate. Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests. Gross pathology findings and histopathology findings presenting as a dichotomous response were analysed with pairwise Fisher's exact tests. Significance was judged at 5% for all tests.
C. Methods:	
1.Observations:	All animals were observed twice a day for mortality and moribundity and daily for individual clinical signs. They were also observed for signs of toxicity at approximately 1 hour post dose
2.Body weights:	All animals were weighed daily from PND 23 to termination
3.Food consumption:	Not measured.
4.Balanopreputial separation:	All animals were observed daily from PND 30 until complete balanopreputial separation (PPS) was attained and the age and bodyweight were recorded.
5.Sacrifice and pathology:	At termination on PND 53 or 54 all animals were sacrificed by decapitation and blood was collected for examination. All animals were given a gross pathological examination and any macroscopic lesions were preserved for possible subsequent histopathological examination (see below).

6.Blood analysis:	Hormone analysis: T_4, TSH and testosterone Biochemistry: Creatinine and urea nitrogen.
7.Organ weights:	Adrenal glands, epididymides, kidneys, levator ani plus bulbocavernosus muscle complex (LABC), liver, pituitary gland, prostate (ventral and dorsolateral), testes, thyroid, seminal vesicles (including fluids and coagulating gland).
8.Histopathology:	Epididymis (right), kidneys, liver, thyroid (at least 2 sections), testis.

Results and Discussion

Homogeneity and achieved concentration analysis:

Homogeneity, resuspension homogeneity, and stability (for 8 hours of room temperature storage and for 8 days of refrigerated storage) of the test substance in formulation at a concentration range of 10-500 mg/mL were established in a previous study (B.6.7.1 – study 1). Therefore, homogeneity, resuspension homogeneity, and stability analyses were not conducted in this study.

The analysed dosing formulations were within range for suspensions (85% to 115%).

Mortality and clinical signs:

All animals survived until the scheduled sacrifice. A test article-related clinical finding of clear material around the mouth was noted for 14 and 15 males in the 500 and 1000 mg/kg bw/d groups, respectively, during PND 31 through euthanasia at approximately 1 hour following dose administration. There were no other test article-related clinical findings noted at either dose level; clinical findings in the test article-treated groups occurred infrequently and/or in a manner that was not dose-related.

Body weight:

Significantly ($p \leq 0.05$) lower mean body weight gain (9% decrease) was noted in the 1000 mg/kg bw/d group compared to the control group when the entire treatment period (PND 23-53) was evaluated as a result of the slightly lower mean body weight gains noted intermittently throughout the treatment period. As a result, mean body weights in the 1000 mg/kg bw/ d group were lower (up to 11%) than the control group throughout treatment; the differences were significant ($p \leq 0.05$) during PND 31-53.

Balanopreputial separation:

An indirect test article-related delay in the mean age of attainment of complete balanopreputial separation was noted for the 1000 mg/kg bw/day group (47.5 days) when compared to the control group (45.6 days). The difference observed however did not achieve statistical significance. When a second analysis was performed, adjusting the day of attainment for those males with 3 or more consecutive days of incomplete separation (persistent threads), a delay in the mean age of attainment of balanopreputial separation continued to be noted (44.5 days) group when compared with the control group (42.9 days). Although the differences were not statistically significant when evaluated using an ANOVA or ANCOVA, there was a dose-related increasing linear trend using both the ANOVA ($p \leq 0.05$) and ANCOVA ($p \leq 0.05$) models. The mean body

weight at the age of attainment of balanopreputial separation in this group (231.9 g) was comparable to the control group (237.1 g), and hence this delay was considered to be secondary to the effects on body weight gain in this dose group and not a direct toxic effect of the test article. Seven males each in the control, 500, and 1000 mg/kg bw/day groups showed incomplete separation for greater than 3 days.

The mean age of attainment of complete balanopreputial separation (46.5 days) and mean body weight at the age of attainment (239.2 g) were unaffected by test article administration in the 500 mg/kg bw/day group. None of the differences from the control group were statistically significant (refer to Table B.6.8.3-4).

Table B.6.8.3-4:
Summary of balanopreputial separation from the *in vivo* intact juvenile/peripubertalassay in male rats

Parameter	♂ (mg/kg bw/d)		
	0	500	1000
Mean ±SD age at PPS (days)			
PND ANOVA (PND) ANCOVA (PND)	45.6 ±2.56 45.6 45.6	46.5 ±1.73 $46.6^{NS, -}$ $46.6^{NS,-}$	47.5 ±3.50 $47.4^{NS,NS}$ $47.4^{NS,NS}$
Mean ±SD age at PPS (incomplete)			
PND ANOVA (PND) ANCOVA (PND)	42.9 ±1.94 43.0 43.0	43.9 ±2.34 $43.9^{NS,-}$ $43.9^{NS,-}$	44.5 ±1.77 $44.5^{NS,*}$ $44.5^{NS,*}$

PND: post-natal day
PPS: balanopreputial separation
ANOVA: analysis of variance
ANCOVA: analysis of covariance

Subscript values refer to statistical analysis conducted by the Dunnett's test and Trend test analysis
NS: not significant
* $p \leq 0.05$

Gross pathology:

Two 1000 mg/kg bw/day group males had small testes and epididymides, and for one of these males, small coagulating glands, seminal vesicles, and prostate gland were also noted. These findings showed up as incidences of 1 or 2 of 15 males in the high-dose group for these male reproductive tissues; these same findings were not observed in the control or 500 mg/kg bw/day groups. These findings correlated with lower organ weights for the male reproductive tissues. There were no other macroscopic observations considered to be test article-related. Other noted gross observations in the kidneys, eyes, lung, and skin exhibited low incidences, also occurred in the control group, and/or were considered a common rat background finding unrelated to test article administration.

Hormone analysis:

There were test article-related, dose-related lower mean serum testosterone levels in the 500 (42%) and 1000 (57%) mg/kg bw/d groups. The values were significant at $p \leq 0.01$ in the 500 mg/kg bw/day group and at $p \leq 0.001$ in the 1000 mg/kg/day group. Mean serum thyroxine (Total T_4) and thyroid stimulating hormone (TSH) levels were unaltered by test article administration.

Biochemistry:

There were test article-related, slightly higher mean serum urea nitrogen levels noted in the 500 (22%) and 1000 (42%) mg/kg bw/day groups; a dose-related response was present. These were significant at $p \leq 0.01$ for the 500 mg/kg bw/day group and at $p \leq 0.001$ for the 1000 mg/kg bw/day group. These higher values may be correlated with renal tubular degeneration and dilatation noted microscopically. The mean creatinine levels were also higher for the 500 (100%) and 1000 (100%) mg/kg bw/day groups, but these elevations were slight and fell within the laboratory's historical control reference ranges. The value was significant at $p \leq 0.05$ in the 1000 mg/kg bw/day group.

Table B.6.8.3-5:
Summary of selected blood chemistry parameters from the *in vivo* intact juvenile/peripubertalassay in male rats

Parameter	♂ (mg/kg bw/d)			Laboratory historical control range[a]
	0	500	1000	
- Total T_4 (µg/gL)	6.00	6.37	5.35	5.29 - 6.82
- TSH (ng/mL)	11.26	10.98	12.68	8.28 - 15.13
- Testost. (ng/mL)	2.640	1.540**	1.131***	1.62 – 4.195
- Creat. (mg/dL):	0.1	0.2	0.2*	0.1 – 0.3
- Urea (mg/dL)	13.9	16.9**	19.8***	11.3 – 16.6

*$p \leq 0.05$; **$p \leq 0.01$; *** $p \leq 0.001$
TSH: thyroid stimulating hormone
Testost.: testosterone
Creat.: creatinine
[a]HCD for T_4, TSH and testosterone are based on studies performed between Aug 2010 and Jan 2012 with a total number of 150 animals. HCD for creatinine and urea are based on 15 studies conducted between Dec 2005 and Aug 2009.

Organ weights:

Test article-related organ weight changes were noted in the liver, kidneys, testes, epididymides, prostate gland, seminal vesicles/coagulating glands, and LABC.

Liver: Mean absolute liver and liver to body weight ratios were statistically significant higher for the 500 (14% and 15%, respectively) and 1000 mg/kg bw/d (20% and 29%, respectively) groups. These higher liver weights correlated with higher incidences of microscopic hepatocellular hypertrophy in the 500 and 1000 mg/kg/d group animals.

Kidney: Mean absolute kidney and kidney to body weight ratios were statistically significant higher for the 1000 mg/kg bw/d group males (8% and 14%, respectively), and these differences correlated with increased microscopic incidences of kidney tubular degeneration and dilatation.

Testis and epididymides: For the 1000 mg/kg bw/d group animals, mean left and right absolute testis weights were lower (14% and 19%, respectively) and mean left and right absolute epididymis weights were lower (15% and 10%, respectively). The right testis value was statistically significant ($p \leq 0.05$) in the 1000 mg/kg bw/day group.

The left and right epididymis values were statistically significant ($p \leq 0.01$ and $p \leq 0.05$, respectively) in the 1000 mg/kg bw/d group. These lower mean testicular and epididymal weights were due primarily to abnormally low tissue weights for 2 individual males, which microscopically exhibited testicular hypospermatogenesis or necrosis, and epididymal hypospermia. However, if the testis and epididymis weights for these 2 males are excluded from the group, the mean right and left testes and epididymal weights for the 1000 mg/kg bw/d group males remain lower than the corresponding means for the control and 500 mg/kg bw/d male groups. Additionally, there was an individual control group male with a high right testis weight (2.56 g), which correlated with an enlarged right testis grossly and microscopic seminiferous tubule dilatation and sperm granuloma.

Prostate: Mean absolute dorsolateral and ventral prostate weights were statistically significant lower for the 500 (15% and 25%, respectively) and 1000 mg/kg bw/d (17% and 33%, respectively) group males.

Seminal vesicle / coagulating gland: Mean absolute seminal vesicle/coagulating gland weights, with and without fluid, were lower for the 500 (16% and 13%, respectively) and 1000 mg/kg bw/d (24% and 21%, respectively) group animals. The seminal vesicle/coagulating gland weight without fluid value was statistically significant in the 1000 mg/kg bw/d group ($p \leq 0.01$) and the seminal vesicle/coagulating gland weight with fluid value was statistically significant in the 1000 mg/kg bw/d group ($p \leq 0.01$).

LABC: The mean absolute LABC weights were statistically significant lower for the 1000 (20%) mg/kg bw/d group animals.

There were no other test article-related effects on organ weights.

Table B.6.8.3-6:
Summary of selected organ weights from the *in vivo* intact juvenile/peripubertalassay in male rats

Parameter	♂ (mg/kg bw/d)			Historical control data[a]
	0	500	1000	
Liver abs (u/a) rel	13.51 / 13.52 4.55	15.42* / 15.41* 5.25***	16.23** / 16.25*** 5.86***	6.4 – 15.1
Kidney abs (u/a) rel	2.03 / 2.03 0.69	2.05/2.05 0.70	2.19* / 2.19* 0.79***	1.74 – 3.14
Testis abs (L, u) abs (R, u/a)	1.3768 1.4625 / 1.4654	1.4047 1.3969 / 1.3941	1.1182 1.1900* / 1.929*	1.18 – 1.65
Epidid. abs (L, u/a) abs (R, u/a)	0.208 / 0.2082 0.2079 / 0.2082	0.201 / 0.2008 0.2128 / 0.2125	0.1772** / 0.1775* 0.1871* / 0.1874	0.200 – 0.350
V.prostate abs (u/a)	0.2759 / 0.2768	0.2067** / 0.2058**	0.1845*** / 0.1854***	0.12 – 0.70
D. prostate abs (u/a)	0.1202 / 0.1205	0.1023* / 0.1020*	0.1001* / 0.1004*	
SV/CG w/o abs (u/a)	0.4330 / 0.4336	0.3756 / 0.3750	0.3430** / 0.3436**	-
SV.CG w abs (u/a)	0.7047 / 0.7046	0.5935 / 0.5918	0.5354** / 0.5350**	-
LABC abs (u/a)	0.5397 / 0.5412	0.4961 / 0.4946	0.4341** / 0.4356**	-

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$
abs: absolute
rel: relative
L, R: left, right
u/a: unadjusted / adjusted
Epidid: epididymides
V.prost: ventral prostate
[a]HCD are based on 16 studies performed between Aug 2005 and Jul 2010.
D.prost: dorsolateral prostate
SV/CG w/o: seminal vesicle / coagulating gland without fluid
SV/CG w: seminal vesicle / coagulating gland with fluid
LABC: levator ani plus bulbocavernosus muscle complex

Histopathology:

Test article-related microscopic findings were seen in the liver, kidney and thyroid gland

Liver: Minimal or mild hepatocellular hypertrophy was diagnosed in 13/15 males from the 500 mg/kg bw/d group, and mild to moderate hepatocellular hypertrophy was seen in 14/15 males from the 1000 mg/kg bw/d group. The hypertrophy, hepatocellular, diffuse values in the 500 and 1000 mg/kg bw/d groups were significant at $p \leq 0.05$.

Kidney: There were dose-related effects of tubular degeneration and/or tubular dilatation in the kidneys. Additionally, pelvic dilatation was increased in the 1000 mg/kg/d group animals. Multifocal degenerative tubules were characterised by cellular basophilia with occasional karyorrhexis of the tubular epithelium and sloughing of cells into the lumen.

Thyroid gland: The incidence of thyroid findings for the follicular cell height and colloid area was based on the 5-point grading scale.

Thyroid colloid area was slightly reduced in 3/15 and 10/15 males from the 500 and 1000 mg/kg bw/d groups, respectively. This was characterised microscopically as predominantly small follicles with subjectively estimated 25% to 33% being collapsed with no visible colloid or decreased amount of colloid (Grade 3). One control group male also had reduced (Grade 2) colloid area. Thyroid follicular cell height was higher (Grade 2) in 3/15 and 8/15 males from the 500 and 1000 mg/kg bw/d groups, respectively. One control group animal also had slightly elevated follicular cell height (Grade 2). Thyroid follicular cell height was graded as normal (Grade 1) in all remaining study animals. The colloid values in the 1000 mg/kg bw/d group were significant at $p \leq 0.01$ and the follicular cell height values in the 1000 mg/kg bw/d group were significant at $p \leq 0.05$.

Testis: The right testis of male one male in the 1000 mg/kg bw/d group exhibited diffuse severe necrosis of seminiferous tubules, characterised microscopically as eosinophilic coagulation of germinal and supporting cells within the tubules. Some degenerative tubules were evident around the periphery, but condensed interstitial Leydig cells and the vasculature appeared normal. The right testis of another male in the 1000 mg/kg bw/d group showed moderate hypospermatogenesis, characterised microscopically as multiple seminiferous tubules lacking germinal epithelia, containing only Sertoli cells. The severe hypospermia in the right epididymis of both animals was secondary to the lack of, or reduced spermatogenesis, in the right testis. Both of these animals exhibited bilateral small testes at necropsy. The severe testicular necrosis of the one male was locally extensive coagulative necrosis without specificity to the type of necrotic cells. It was suggestive of local circulatory disturbance such as ischemia. On the other hand, the other male showed moderate hypospermatogenesis. However, hypospermatogenesis is sporadically seen as a background change in rat testes. The testicular changes observed in these 2 males were different types. Similar changes or suspected changes were not observed in any other 1000 mg/kg bw/day males. The right testes of 13/15 of the 1000 mg/kg bw/day males were completely normal microscopically. Therefore, the right testes changes in male previous mentioned two males in the top dose group were not considered to be test article-related.

A control group male had an enlarged right testis (unilateral), which microscopically exhibited dilated seminiferous tubules and a sperm granuloma, and a small right epididymis, which showed hypospermia. These findings were suggestive of rete testis blockage, which was probably a developmental alteration.

Table B.6.8.3-7:
Summary of selected histopathology findings from the *in vivo* intact juvenile/peripubertalassay in male rats

Parameter	♂ (mg/kg bw/d)		
	0	500	1000
- Liver: hypertrophy	0/15 [-,-,-]	13*/15 [9,4,-]	14*/15 [-,11,3]
- Kidney: degen. tubules	0/15 [-,-,-]	3/15 [2,1,-]	14*/15 [3,11,-]
- Kidney: dil. tubules	0/15 [-,-,-]	5*/15 [5,-,-]	15*/15 [4,9,2]
- Thyroid: colloid area	15 [0,1,0,14,0]	15 [0,0,3,12,0]	15 [0,0,10,5,0]
- Thyroid: follicular height	15 [14,1,0,0,0]	15 [12,3,0,0,0]	15 [7,8,0,0,0]

* $p \leq 0.05$
Degen.: degeneration
Dil.: dilatation
Values in parenthesis refer histopathology grading [minimal, mild, moderate]. However for the thyroid the values in parenthesis refer to the grading used on a scale of 1 to 5 (1=shortest/smallest, 5=tallest/largest)

Discussion:

Test article-related lower mean body weight gains, in comparison to the control group, were noted when the entire treatment period (PND 23-53) was evaluated in males administered 1000 mg/kg bw/day and resulted in lower (up to 11.15%) mean body weights during PND 31-53. Mean body weight and body weight gain in the 500 mg/kg/day group were unaffected by test article administration.

An indirect test article-related delay in the mean age at attainment of balanopreputial separation was noted for the 1000 mg/kg bw/day group. The mean body weight at the age of attainment of balanopreputial separation in this group was comparable to the control group, and hence this delay was considered to be secondary to the effects on body weight gain in this dose group and not a direct effect to the endocrine system of the test article. The mean age of attainment of complete balanopreputial separation and mean body weight at the age of attainment were unaffected by test article administration in the 500 mg/kg bw/day group.

Test article-related organ weight changes included: higher liver weights at 500 and 1000 mg/kg bw/day, higher kidney weights at 1000 mg/kg bw/day, and lower dorsolateral prostate, ventral prostate, and seminal vesicle/coagulating gland at 500 and 1000 mg/kg bw/day, and lower testis, epididymis and levator ani plus bulbocavernosus muscle weights at 1000 mg/kg bw/day. Testosterone levels were reduced in the 500 and 1000 mg/kg bw/day group animals. It was considered that lower reproductive organ weights were related to lower testosterone levels. No clear test article-related histopathological findings were observed in the 500 and 1000 mg/kg/day group animals in testis and epididymis.

Hepatocellular hypertrophy and increased hepatic mitotic figures were evident in the liver at ≥500 mg/kg bw/day.

The histopathological findings suggest increased metabolism of testosterone by the liver, resulting in a pseudo-anti-androgenic effect on the male reproductive organ weights. As the major route of metabolism of

pyriproxyfen is hydroxylation *via* hepatic microsomal enzyme induction this has a direct effect on the metabolism of testosterone. The results of *in vitro* androgen receptor binding assay (B.6.8.3 – study 2), *in vitro* steroidogenesis assay (B.6.8.3 – study 4), and *in vivo* Hershberger assay (B.6.8.3 – study 6) did not indicate any anti-androgenic effects or the inhibition of testosterone production. Furthermore, if a true anti-androgenic effect were observed there would have been an increase in testosterone levels; however, a decrease in testosterone levels were observed in this study.

Slight effects on the thyroid gland, including reduced colloid area and increased follicular cell height, were noted in the 500 and 1000 mg/kg/day group animals. However, the effects on the thyroid gland were considered secondary to the hepatic microsomal enzyme induction of the test substance. Since these effects were very slight, it was not considered that the test substance gave any impairment to the thyroid function.

Deficiencies:

None.

Conclusion:

In this male pubertal assay conducted to the US EPA guideline, the administration of pyriproxyfen caused effects on body weight, liver, and kidney as evidence of systemic toxicity, with no evidence of any direct test article-related androgenic or anti-androgenic effects, nor was there any evidence of direct test article-related effects on pubertal development or thyroid function in the juvenile/peripubertal male rats at dosage levels of 500 and 1000 mg/kg bw/day (deemed to be a suitable maximum dose for this assay type).

RMS NL conclusion: A non-significant delay in complete balanopreputial separation was seen in the high dose group, however, this seems to be an indirect effect related to lower body weight gain in this group compared to the control. Significant decreases in the weight of epididymides, prostate, testis (right) and seminal vesicles was observed. However, no treatment-related histopathological changes were observed in testes, epididymides or thyroid. Based on these findings, pyriproxyfen is considered to be negative for apical endpoints.

Concerning the hormonal acitvity, the only result observed was a significant reduction in serum testosterone, in both pyriproxyfen doses tested. The explanation given in the study report that this is likely linked to increased metabolism of testosterone by the liver resulting in a pseudo-anti-androgenic efffect on male reproductive organs is accepted by the RMS.

Overall, it is considerd that pyriproxyfen did not cause direct effects on pubertal development or thyroid function in this study.

3.10.1.7 CA 5.8.3/02 (2012b): Pubertal development and thyroid function in female rats

Report

CA 5.8.3/02 (2012b)

A pubertal development and thyroid function assay of pyriproxyfen T.G. administered orally in intact juvenile/peripubertal female rats

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNT-0211

Submitted for the purpose of renewal

Guidelines:	OPPTS 890.1450 (2009)
GLP:	Yes (certified laboratory)
Acceptability:	The study is considered to be acceptable

Materials and Methods

A. Materials:

1.Test Material:	Pyriproxyfen T.G.
Description:	White solid
Lot/Batch No.:	080506G
Purity:	99.5%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2.Vehicle and/or positive control:	Corn oil / n.a
3.Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Age:	♀: PND 22 at time of dosing
Weight at dosing:	♀: 50.7 – 64.1g (PND 22).
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	From PND 0 to PND 21

B. Study Design and Methods:

1.In life dates:	11 October 2011 to 10 March 2012
2.Animal assignment and treatment:	Time mated female rats were received from the supplier on gestation days 7 and were used to obtain the juvenile females for this study. Maternal data were recorded but not presented in the report. Pups were weaned until PND 21 and monitored for physical health and growth. Litters were culled to 8 males and 8 females on PND 4 to reduce variability. Pups were weighed and physically examined on PND 21 prior to assignment to 3 groups of 15 females/group using a computerised

	method based on stratification of bodyweights in a block design; littermates were not placed in the same group. Dose levels of pyriproxyfen were 0, 500 or 1000 mg/kg bw/d administered daily by oral gavage from PND 22 to PND 42 or 43. The dose levels were selected based on the results of previous studies.
3.Homogeneity and achieved concentration analysis of the dose:	Dosing solutions were prepared weekly and analysed for achieved concentration. Stability data was also available to confirm suitable stability of the dosing formulations.
4.Statistics:	All analyses were conducted using appropriate computing device or programs. Each endpoint was tested for homogeneity and the data transformed as appropriate if it was significant. The data were subjected to a non-parametric test if the test was still significant following transformation. Analysis of variance was conducted and a 2-sided Dunnett's test or a non-parametric Kruskal-Wallis test followed by Dunn's test was used to compare the treated groups with the vehicle control group. In addition, organ weights and balanopreputial separation data were subject to analysis of covariance using the bodyweight at PND 21 as a covariate. Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests. Gross pathology findings and histopathology findings presenting as a dichotomous response were analysed with pairwise Fisher's exact tests. Significance was judged at 5% for all tests.
C. Methods:	
1.Observations:	All animals were observed twice a day for mortality and moribundity and daily for individual clinical signs. They were also observed for signs of toxicity at approximately 1 hour post dose.
2.Body weights:	All animals were weighed daily from PND 22 to termination.
3.Food consumption:	Not measured.
4.Vaginal opening:	Each female was observed for vaginal perforation beginning on PND 22. The age and bodyweight were also recorded.
5. Oestrus cyclicity:	Vaginal lavages were performed daily beginning on the day vaginal opening was observed and the slides were evaluated microscopically to determine the stage of the oestrous cycle of each female until necropsy. The age of first vaginal oestrus was recorded. Standard definitions

	were used for determination of regular cycling, irregular cycling, non-cycling, and insufficient data in the female pubertal assay. The data were used to calculate the percent cycling and percent cycling regularly.
6.Sacrifice and pathology:	At termination on PND 42 or 43 all animals were sacrificed by decapitation and blood was collected for examination. All animals were given a gross pathological examination and any macroscopic lesions were preserved for possible subsequent histopathological examination (see below).
7.Blood analysis:	Hormone analysis: T_4 and TSH. Biochemistry: Creatinine and urea nitrogen.
8.Organ weights:	Adrenal glands, kidneys, liver, pituitary gland, ovaries, thyroid.
8.Histopathology:	Kidneys, liver, ovaries, uterus and thyroid.

Results and Discussion

Homogeneity and achieved concentration analysis:

Homogeneity, resuspension homogeneity, and stability (for 8 hours of room temperature storage and for 8 days of refrigerated storage) of the test substance in formulation at a concentration range of 10-500 mg/mL were established in a previous study (B.6.7.1 – study 1). Therefore, homogeneity, resuspension homogeneity, and stability analyses were not conducted in this study

The analyzed dosing formulations were within range for suspensions (85% to 115%).

Mortality and clinical signs:

One animal from the low and high dose groups were found dead on PND 34.

Surviving animals: A test article-related clinical finding of clear material around the mouth of females in the 500 and 1000 mg/kg bw/d groups, from PND 25 through to termination.

Unscheduled deaths: Limited to clear material on the ventral abdominal and urogenital areas and around the mouth. Due to the limited frequency of mortality in this study (no more than 1 animal per group), these deaths were not attributed to the test article.

Body weight:

Mean body weight gains for females in the high dose group were generally lower than the control group during PND 22-33. As a result, mean cumulative body weight gain (PND 22-42) in this group was significantly ($p \leq 0.05$) lower than the control group (6.7% decrease compared to control) and mean body weights were significantly ($p \leq 0.05$) lower (up to 12.51%) *vs*. the control group during PND 23-42.

Vaginal opening:

A delay in the mean age of attainment was noted in the high dose group. When analysed by day of complete opening, the difference from the control group was not statistically significant and the mean control group

value (36.3 days) was above the maximum acceptable performance criteria value (35.62 days). However, when a separate analysis was conducted for females that showed 3 or more days of incomplete opening, the age of attainment in this dose group (36.1 days) was significantly ($p \leq 0.009$) higher than the control group (33.7 days). The large difference between control group means (36.3 days and 33.7 days) for the 2 analyses was attributed to 6 females in this group that showed 4 to 11 days of incomplete opening prior to complete opening. This delay in attainment of vaginal opening noted in the high dose group was considered secondary to the body weight effects noted for females in this group. Although the mean body weights at the age of attainment in the 500 and 1000 mg/kg bw/day groups were lower (but not achieving statistical significance) than the control group. The control group mean body weight (141.6g) was above the maximum acceptable performance criteria value (131.44g) and did not take into account the females with 3 or more days of incomplete opening (*i.e.*, body weights at the time of complete opening were used). When the body weights on the first day of incomplete opening were used for these 6 females, the control group mean was 124.3 g, which was similar to the 500 and 1000 mg/kg/day group values (131.3 g and 130.8 g, respectively) and was within the acceptable performance criteria range. Furthermore, there was no effect of treatment on age at first oestrus, oestrous cycle length, or the number or percentage of females cycling regularly.

The mean age of attainment of vaginal patency and body weight at attainment in the 500 mg/kg bw/day group were unaffected by test article administration and were not statistically significant compared to the control group (refer to Table B.6.8.3-13).

Oestrous cycles:

There were no treatment related effects on oestrus cycling.

Table B.6.8.3-13:
Summary of vaginal opening and oestrus cycling data the *in vivo* intact juvenile/peripubertalassay in female rats

Parameter	♀ (mg/kg bw/d)		
	0	500	1000
Vaginal opening			
- Age (PND) (u/a): - Age (PND) incomplete (u/a): - Body weight at VO (g) (u/a):	36.3 / 34.7 33.7 / 32.6 141.6 / 131.1	35.3 / 33.8 34.9 / 33.8 131.1 / 121.5	37.1 / 36.0 36.1* / 35.2* 130.8 / 123.7
Oestrous cyclicity			
- Mean age at 1st vaginal oestrous (PND): - Mean cycle length (days): - % cycling: - % regularly cycling:	37.7 4.7 100 83.3	37.0 4.7 100 70	38.4 3.8 92.9 60

* $p \leq 0.05$
PND: post-natal day
VO: vaginal opening
u/a: unadjusted / adjusted

Hormone analysis:

The mean TSH level in the 1000 mg/kg bw/d group was higher than the control group, but did not reach statistical significance. The higher value was partially attributed to a high individual value for a single female (26.90 ng/mL), the results correlated with an increased follicular cell height (Grade 2) for this individual animal. These results were considered secondary to hepatic microsomal enzyme induction.

The mean Total T_4 level in the low dose group was significantly ($p \leq 0.05$) higher than the control group; however, this result did not occur in a dose-related manner, and therefore was not deemed to be test article related.

Biochemistry:

Increases in mean urea nitrogen levels were noted in a dose-related manner for females in the low and high dose groups, with the changes observed in the high dose group reaching statistical significance ($p \leq 0.001$). The results likely correlating with the microscopic renal tubular degeneration and dilatation and therefore were considered to be test article-related.

Table B.6.8.3-14:
Summary of selected blood chemistry parameters from the *in vivo* intact juvenile/peripubertalassay in female rats

Parameter	♀ (mg/kg bw/d)			Laboratory historical control range[a]
	0	500	1000	
- Total T_4 (µg/gL)	4.83	5.74*	5.07	3.9 – 4.64[a]
- TSH (ng/mL)	9.05	9.7	11.54	3.74 – 8.79[a]
- Urea (mg/dL)	14.9	16.3	19.0***	12.9 – 17.8[b]

* $p \leq 0.05$; *** $p \leq 0.001$
TSH: thyroid stimulating hormone
[a]HCD from studies performed between Aug 2010 and Mar 2012, based on data from 177 animals.
[b]HCD from 14 studies performed between Dec 2005 and Aug 2009

Organ weights:

Test article-related organ weight changes were noted in the liver, kidneys, thyroid and ovaries.

Liver: Mean absolute liver weights and relative liver weight ratios were higher in the 500 and 1000 mg/kg/day groups, achieving statistical significance ($p \leq 0.05$) in the low (relative weight) and high (absolute and relative weights) groups. These higher liver weights correlated with higher incidences of microscopic hepatocellular hypertrophy in the 500 and 1000 mg/kg/day group animals.

Kidney: Mean absolute kidney weight and kidney to body weight ratio were higher in the 1000 mg/kg bw/day group. The difference in relative weight in the 1000 mg/kg/day group was significant ($p \leq 0.01$). These higher kidney weights correlated with higher incidences of microscopic kidney tubular degeneration and dilatation in the 1000 mg/kg bw/day group animal.

Thyroid: Mean absolute thyroid gland weights were slightly higher in the 1000 mg/kg/day group animals. However, the difference from the control group was not statistically significant and there was no clear microscopic correlation as why these altered thyroid gland weights as colloid area was slightly lower in 4

animals from each of the 500 and 1000 mg/kg bw/day groups, with follicular cell height increased in only one 1000 mg/kg bw/day group animal. Colloid area was also slightly lower in 2 control group animals. These differences in organ weights were considered a secondary effect of microsomal hepatic enzyme induction.
Ovaries: Mean absolute ovary weights were significantly lower ($p \leq 0.05$) in the high dose group. There were no microscopic findings which correlated with the lower ovarian weights.

Table B.6.8.3-15:
Summary of selected organ weights from the *in vivo* intact juvenile/peripubertalassay in female rats

Parameter		♂ (mg/kg bw/d)			Historical control data
		0	500	1000	
- Liver:	abs (u/a)	7.8 / 7.8	8.4 / 8.4*	9.2*** / 9.2***	4.6 – 10.6
	rel	4.6	5.0**	5.6***	2.957 – 5.513
- Kidney:	abs (u,a)	1.3 / 1.3	1.4 / 1.4	1.4 / 1.4	1.22 – 2.39
	rel	0.8	0.8	0.9**	0.739 – 1.380
- Thyroids	abs (u/a)	0.0120 / 0.0121	0.0112 / 0.0112	0.0129 / 0.0128	0.0073 – 0.0187
- Ovaries:	abs (u/a)	0.0737 / 0.0737		0.0670* / 0.0670*	0.0794 – 0.1479
- Uterus.:	blot (u/a)	0.0800 / 0.0799	0.2846 / 0.2845	0.2470 / 0.2475	0.23 – 0.76
	wet (u/a)	0.2818 / 0.2828	0.3601 / 0.3594	0.2895 / 0.2930	
		0.3341 / 0.3346			

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$
abs: absolute
rel: relative
HCD from 15 studies performed between Aug 2005 and Apr 2010.
u/a: unadjusted / adjusted
blot: blotted

Gross pathology:

At scheduled necropsy no test article related effects were observed. Of the two unscheduled deaths that occur, no abnormal internal findings were observed, these deaths were therefore considered unrelated to test article administration.

Histopathology:

Test article-related microscopic findings were seen in the liver, kidney and thyroid gland.
Liver: Minimal or mild hepatocellular hypertrophy was diagnosed in 3/14 animals from the low and 14/14 animals from the high dose group. The incidence of this finding in the high dose group was significant ($p \leq 0.05$). Microscopically, this was characterised by slightly enlarged hepatocytes with abundant cytoplasm extending from the centrilobular to periportal regions. The hepatocellular cytoplasm exhibited paler eosinophilia with less basophilic stippling and less clear spaces. Additionally, hepatocellular mitotic figures were increased in incidence and severity for both test article treated groups. The incidences of these findings were significant ($p \leq 0.001$). Thus, both an increase in hepatocyte size (hypertrophy) and hepatocyte number correlated with the higher liver weights.

Kidney: There were dose-related effects of tubular degeneration and/or tubular dilatation in the kidneys, affecting most of the low dose group animals and all of the high dose group animals. Degenerative tubules were multifocal and characterised by cellular basophilia with occasional karyorrhexis of the tubular epithelium and sloughing of cells into the lumen. Affected tubules were sometimes slightly dilated, but tubular dilatation was also distinct as multifocal expanded tubular lumina, which were often bulbous near the cortico-medullary junction (outer medulla). Occasionally cellular debris was evident in the dilated tubules. The incidences of degenerative tubules and tubular dilatation in the 500 and 1000 mg/kg bw/day groups were significant ($p \leq 0.05$). The incidences of basophilic tubules in the 1000 mg/kg bw/day group and mononuclear infiltrate in the 500 and 1000 mg/kg bw/day groups were significantly ($p \leq 0.05$) lower than the control group; however, decreased incidences of these findings were not considered toxicologically relevant. Additionally, the incidences of basophilic tubules in the 1000 mg/kg bw/day group and mononuclear infiltrate in the 500 and 1000 mg/kg bw/day groups were masked by the incidences of degenerative tubules.
Thyroid gland: Thyroid colloid area was slightly decreased in 4/13 and 4/14 animals from the 500 and 1000 mg/kg bw/day groups, respectively. This was characterised microscopically as predominantly small follicles with 25% to 33% being collapsed with no visible colloid or decreased amount of colloid (Grade 3). Two control group animals also had slightly decreased (Grade 3) colloid area. One 1000 mg/kg bw/day group animal had slightly increased follicular cell height (Grade 2) and this same animal had the highest single serum TSH value (26.90 ng/mL). Follicular cell height was graded as normal (Grade 1) in all remaining study animals. Although these histopathologic thyroid gland changes were slight and low in incidence, there was a slight cumulative thyroid gland effect, including slightly higher weights and higher TSH levels. These changes were considered secondary to hepatic microsomal enzyme induction.

Table B.6.8.3-16:
Summary of selected organ weights from the *in vivo* intact juvenile/peripubertalassay in female rats

Parameter	♀ (mg/kg bw/d)		
	0	500	1000
- Liver: hypertrophy	0/15 [-,-,-,-]	3/14 [3,0,0,0]	14/14 [8,6,0,0]
- Liver: mitotic figures	7/15 [5,2,-,-]	13/14 [5,2,3,3]	12/14 [1,2,4,5]
- Kidney: degen. tubules	0/15 [-,-,-,-]	5/14 [4,1,0,0]	13/14 [10,3,0,0]
- Kidney: dil. tubules	0/15 [-,-,-,-]	4/14 [4,0,0,0]	14/14 [6,8,0,0]
- Thyroid: colloid area	15 [0,0,2,13,0]	13 [0,0,4,9,0]	14 [0,0,4,10,0]
- Thyroid: follicular height	15 [15,0,0,0,0]	13 [13,0,0,0,0]	14 [13,1,0,0,0]

Degen.: degeneration
Dil.: dilatation
Values in parenthesis refer histopathology grading [minimal, mild, moderate, severe]. However for the thyroid the values in parenthesis refer to the grading used on a scale of 1 to 5 (1=shortest/smallest, 5=tallest/largest)

Discussion:

A delay in attainment of vaginal opening was noted for females in the high dose group. However, this delay was considered secondary to the test article-related effect on body weights for these females and not a direct effect of the test article to the endocrine system. No effects on the age of attainment of vaginal opening or body weight at attainment were noted in the low dose group. Age at first oestrus, oestrous cycle length, and the number and percentage of females cycling normally were unaffected by test article administration in either of the dose groups. There were no microscopic findings which correlated with the lower ovarian weights. In addition, as fertility and reproductive performances were unaffected in the previous rat two-generation reproductive study it was considered that the lower ovarian weights observed were not the result of any effects on the endocrine system.

Test article related liver and kidney effects were apparent. Liver effect included higher absolute and relative liver weights in both treatment groups, corresponding to microscopic findings of hepatocellular hypertrophy and increased hepatic mitotic figures in these groups. Kidney effects included increases in absolute and relative kidney weights in the high dose group, corresponding to microscopic findings of tubular degeneration and dilatation in both treatment groups. In addition, mean urea nitrogen levels were increased in the both the low and high dose groups. The major route of metabolism of pyriproxyfen is hydroxylation *via* hepatic microsomal enzyme induction. Thus, the liver effects of increased liver weights, hepatocellular hypertrophy and increased hepatic mitotic figures were adaptive metabolic effects of pyriproxyfen exposure. Evidence of extensive renal toxicity was observed in 1000 mg/kg bw/day group, suggesting that a dose level of 1000 mg/kg bw/day was considered to exceed the MTD. In addition, the dose levels selected in this assay were based on the results of a 7-day dose range-finding toxicity study, which were relatively high compared to the maximum doses selected for previous general toxicity studies. The dose level of 500 mg/kg bw/day was suitable for the evaluation of the effects to the endocrine system.

Slight effects on the thyroid were observed (slightly higher thyroid gland weights and TSH levels as well as reduced colloid area and increased follicular cell height in high dose animals). These effects were considered secondary to the hepatic enzyme induction capability of the test article and therefore the thyroid function was not considered to be impaired by administration of the test article.

Deficiencies:

None.

Conclusion

In this female pubertal assay conducted to the US EPA guideline, the administration of pyriproxyfen caused effects on body weight, liver, and kidney as evidence of systemic toxicity, with no evidence of any direct test article-related oestrogenic or anti-oestrogenic effects, nor was there any evidence of direct test article-related effects on pubertal development or thyroid function in the juvenile/peripubertal female rat up to a dose level of 1000 mg/kg bw/day (a dose level which was deemed to exceed the maximum tolerated

3.10.2 Human data

No human data are available.

3.10.3 Other data (e.g. studies on mechanism of action)

No other relevant data are available.

3.11 Specific target organ toxicity-single exposure

3.11.1 Animal data

3.11.1.1 CA 5.7.1/01 (2010): Range-finding acute neurotoxicity study in the rat

Report

CA 5.7.1/01 (2010)

An oral (gavage) dose range-finding acute neurotoxicity study of pyriproxyfen T.G. in rats

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNT-0181

Submitted for the purpose of renewal

Guidelines:	Not relevant as this was a range-finding study
GLP	Yes (certified laboratory)

Materials and Methods

A. Materials:

1. Test Material:	Pyriproxyfen T.G.
Description:	White solid
Lot/Batch No.:	080506G
Purity:	99.5%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2. Vehicle and/or positive control:	Corn oil / n.a.
3. Test animals:	
Species:	Rat
Strain:	Crl:CD (Sprague Dawley)
Age:	6 weeks
Weight at dosing:	♂: 163-195g; ♀: 159-182g

Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	At least 13 days

B. Study Design and Methods:

1.In life dates:	11 May 2010 to 27 May 2010
2.Animal assignment and treatment:	Animals selected for the study were randomised by computer into four groups of 3 animals/sex. The test article was formulated in corn oil, with all animals receiving a single oral gavage dose at 0, 300, 1000 or 2000 mg/kg bw, using a dose volume of 5 mL/kg. All animals were euthanized and discarded without macroscopic examination on study day 1.
3 Homogeneity and achieved concentration analysis of the dose:	Stability analysis was undertaken, along with achieved concentration and homogeneity analysis of the dosing solutions.
4.Statistics:	Body weight and post-dosing continuous detailed clinical observation data were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p \leq 0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Detailed clinical observation parameters that yielded scalar or descriptive data were analysed using Fisher's Exact.

C. Methods:

1.Observations:	Mortality and moribundity: observed twice daily, once in the morning and once in the afternoon. Clinical observations: recorded for all animals at approximately 1 hour intervals for the first 8 hours following dose administration on study day 0. Detailed clinical observations: ease of removal from cage, lacrimation/chromodacryorrhea, piloerection, palpebral closure, eye prominence, red/crusty deposits, mobility, convulsions/tremors, grooming, bizarre/stereotypic behaviour, ease of handling animal in hand, salivation, fur appearance, respiratory rate/character, mucous membranes/eye/skin colour, muscle tone, gait, arousal, urination/defecation, backing.

2.Body weights:	Recorded on study day 0 prior to test article formulation administration. Body weights were recorded for the purpose of dose calculations only.
3.Food consumption:	Not undertaken.
4.Functional observation battery (FOB):	Not undertaken.
5.Motor activity:	Not undertaken as this was a range finding study.
6.Gross pathology:	Not undertaken as this was a range finding study.
7.Neurohistopathology:	Not undertaken as this was a range finding study.
8.Organ weights:	Not undertaken as this was a range finding study.

Results and Discussion

Homogeneity and achieved concentration analysis:

The analysed dosing formulations were considered homogenous (relative SD <10%), and were stable for 8 hours (stored 4 hours at room temperature followed by 4 hours in an approximately 40°C water bath) and 8 days (stored refrigerated). The test article was not detected in the vehicle formulation that was administered to the control group. Achieved concentrations were within the range ±3% of nominal concentration.

Mortality and clinical signs: All animals survived until the scheduled sacrifice. One male in the 2000 mg/kg bw group had hair loss on the forelimbs, no clinical findings were noted for animals in the 300, 1000, and 2000 mg/kg bw groups prior to test article administration.

No test article-related effects were noted. Slightly soiled fur was noted for one high dose male and female at approximately 6, 7, and/or 8 hours following dose administration. In the absence of other effects on detailed clinical observations, the occurrence of slightly soiled fur in a single male and female was not considered to be related to test article administration.

Conclusions

Under the conditions of this study 2000 mg/kg bw was tolerated and deemed to be a suitable maximum dose for the definitive acute oral neurotoxicity study (Study 2 in this section). The time of peak effect was determined to be 8 hours post dosing.

3.11.1.2 CA 5.7.1/02 (2011a): Acute neurotoxicity study in the rat

Report

CA 5.7.1/02 (2011a)

An oral (gavage) acute neurotoxicity study of pyriproxyfen T.G. in rats

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNT-0194

Submitted for the purpose of renewal

Guidelines:	OECD 424 (1997)
GLP:	Yes (certified laboratory)
Acceptability:	The study is considered to be acceptable

Materials and Methods

A. Materials:

1. Test Material:	Pyriproxyfen T.G.
Description:	White solid
Lot/Batch No.:	080506G
Purity:	99.5%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2. Vehicle and/or positive control:	Corn oil / n.a.
3. Test animals:	
Species:	Rat, Crl:CD(SD)
Strain:	Sprague Dawley
Age:	6 weeks
Weight at dosing:	♂: 138 – 227 g; ♀: 135 – 185 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	At least 13 days

B. Study Design and Methods:

1. In life dates:	15 June 2010 to 7 September July 2010
2. Animal assignment and treatment:	Animals selected for the study were randomised by computer into four groups of 12 animals/sex. The test article was formulated in corn oil, with all animals receiving a single oral gavage dose at 0, 300, 1000 or 2000 mg/kg bw, using a dose volume of 5 mL/kg. All animals were euthanized on study 15.
3 Homogeneity and achieved concentration analysis of the dose:	Homogeneity and stability analysis were confirmed under B.6.7.1 study 1, therefore only analysis for achieved concentration was conducted.

4.Statistics:

Body weight and post-dosing continuous detailed clinical observation data were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p \leq 0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Detailed clinical observation parameters that yielded scalar or descriptive data were analysed using Fisher's Exact.

FOB parameters that yielded scalar or descriptive data and non-graded histopathologic findings were analysed using Fisher's Exact Test.

Repeated measures analysis of variance (RANOVA) statistical analyses for total and ambulatory locomotor activity counts recorded during pre-test and after dosing were conducted as follows. Each analysis endpoint was analysed, by sex and session, with a RANOVA. Factors in the model included treatment group (TRT), time interval (TIME), and the interaction of time interval and treatment group (TRT*TIME). The random effect of animal was included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion (AIC).

The monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dose levels. The linear dose by time interaction was evaluated and, if significant at the $p \leq 0.05$ level, trend tests on treatment means were performed $p \leq 0.05$ level for each time interval. If the linear dose by time interaction was not significant, the trend test was conducted across the pooled time intervals for the entire session only.

Non-monotonic dose responses were evaluated whenever no significant linear trends were detected but TRT and/or TRT*TIME interaction was significant $p \leq 0.01$. Within the framework of the RANOVA, pairwise comparisons were made for each individual test article-treated group with the control group through linear contrasts. If TRT*TIME was significant, the comparisons were conducted for each time interval. If only the TRT effect was significant, the comparisons were conducted across the pooled time intervals for the entire session. These non-monotonic dose-response comparisons were conducted at the $p \leq 0.01$ level.

C. Methods:

1.Observations:	Mortality and moribundity: observed twice daily, once in the morning and once in the afternoon Clinical observations: recorded for all animals daily. These observations included (but not limited to) changes in the appearance of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system function, somatomotor activity, and behaviour patterns. Detailed physical examinations: conducted approximately 24 h post dosing to assess the potential of the test article to cause delayed effects. Animals were removed from their home cages and placed in a standard arena for observation of changes in gait, posture, or clonic or tonic movements. Stereotypies (*e.g.*, excessive grooming, repetitive circling), bizarre behaviour (*e.g.*, self-mutilation, walking backwards), and permanent or semi-permanent signs, such as skin lesions and hair loss were also recorded.
2.Body weights:	Recorded weekly, starting 1 week prior to dose administration.
3.Food consumption:	Not recorded.
4.Functional observation battery (FOB):	Recorded for all animals prior to the initiation of dose administration, at the time of peak effect (8 hours post-dosing) on study day 0, and on study days 7 and 14. Observations included: Home cage observations: posture, convulsions/tremors, faeces consistency, biting, palpebral (eyelid) closure. Handling observations: ease of removal from cage, lacrimation/chromodacryorrhea, piloerection, palpebral closure, eye prominence, red/crusty deposits, ease of handling animal in hand, salivation, fur appearance, respiratory rate/character, mucous membranes/eye/skin colour, muscle tone. Open field observations (evaluated over a 2 minute period): mobility, rearing, convulsions/tremors, grooming, bizarre/stereotypic behaviour, time to first step (seconds), gait, arousal, urination/defecation, gait score, backing. Sensory observations: approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch response, eye-blink response, hindlimb extension, olfactory orientation.

	Neuromuscular observations: hind-limb extensor strength, hind-limb foot splay, grip strength-hind and forelimb, rotarod performance. Physiological observations: catalepsy, body temperature, body weight.
5.Locomotor activity:	Recorded for all animals prior to the initiation of dose administration, at the time of peak effect (8 hours post-dosing) on study day 0, and on study days 7 and 14. Locomotor activity assessment were recorded after completion of FOB. Each animal was tested separately. Data were collected in 5-minute epochs, and the test session duration was 60 minutes. These data were compiled as six 10-minute subintervals for tabulation. Total motor activity: defined as a combination of fine motor skills (*i.e.*, grooming, interruption of 1 photobeam). Ambulatory motor activity: interruption of 2 or more consecutive photobeams.
6.Gross pathology:	The central and peripheral nervous system tissues were dissected and preserved. Any observable gross changes and abnormal coloration or lesions of the brain and spinal cord were recorded.
7.Neurohisto-pathology:	The following nerve tissues were prepared for a microscopic Neuropathologic examination from 6 randomly selected animals/sex in the control and 2000 mg/kg bw groups: Brain (olfactory bulbs, cerebral cortex (2 levels), hippocampus/dentate gyrus, basal ganglia, thalamus, hypothalamus, midbrain, cerebellum, pons, medulla oblongata), spinal cord (cervical swellings C_3-C_7, lumbar swellings T_{13}-L_4), trigeminal ganglia/nerves, lumbar dorsal root ganglia at T_{13}-L_4, lumbar dorsal root fibres at T_{13}-L_4, lumbar ventral root fibres at T13-L4, cervical dorsal root ganglia at C_3-C_7, cervical dorsal root fibres at C_3-C_7, cervical ventral root fibres at C_3-C_7, cervical spinal nerve, lumbar spinal nerve), sciatic nerves (mid-thigh region – transverse and longitudinal [t+l] sections), sciatic nerves (at sciatic notch, t+l sections), sural nerves (t+l sections), tibial nerves (t+l sections), peroneal nerves (t+l sections),optic nerves, eyes, skeletal muscle (gastrocnemius), other sites (if deemed necessary)
8.Organ weights:	Fixed brain weight and brain dimensions (length [excluding olfactory bulbs] and width) were recorded.

Results and Discussion

Homogeneity and achieved concentration analysis:

The test article was not detected in the vehicle formulation that was administered to the control group. Achieved concentrations were within the range ±2% of nominal concentration.

Mortality and clinical signs: No mortalities occurred and no test-article related effects were observed during clinical observations. Detailed physical examinations showed unkempt appearance for 1/12 male and 6/12 female rats in the 2000 mg/kg bw group, approximately 24 hours following dose administration.

Body weight: No test-article related effects were observed on body weight or body weight gain.

Functional observation battery:

Home cage observations:

Although significantly (p<0.05) fewer females in the 1000 and 2000 mg/kg bw groups were sitting or standing normally in the home cage, compared with the control group on study day 0, this was considered normal for the following reasons:

- significantly (p≤0.05) more females in these groups (7 and 9 females, respectively) were asleep (lying on the side or curled up), which is normal behaviour for animals in the home cage and was generally consistent with control group female responses (7 females) during the pre-test evaluation; on study day 0,
- significantly (p≤0.05) fewer females with wide open eyelids were noted in the 2000 mg/kg bw group due to significantly (p≤0.05) more females in this group with completely shut eyelids (8/12 females), which corresponded to the number of females that were asleep;

On study day 14, significantly (p≤0.05) fewer females with wide open eyelids were noted in the 300 mg/kg bw group when compared to the control group due to more females (not statistically significant) in this group with completely shut eyelids (6/12 females).

Handling observations: No test article related effects were observed.

Open field observations:

No test article related effects were observed. On study day 0, increased defecation was noted for the 300 mg/kg bw group males when compared to the control group. Although this difference was significant (p<0.05), it was not observed in a dose-responsive manner. Therefore, increased defecation was not considered to be test article-related.

Sensory, neuromuscular and physical observations: No test article related effects were observed.

Motor activity:

Pre-dose: There were no biologically important differences observed in the motor activity assessments undertaken in either males or females assigned to the test article treated groups pre-dose.

8 hours post dose:

Total locomotor activity: Compared to the control group, mean overall motor activity counts were 20.9% and 21.4% lower (total) for the 1000 and 2000 mg/kg bw group males, respectively, reaching significance

($p \leq 0.05$) for the 2000 mg/kg bw group males. Total motor activity counts were unaffected by test article administration for males at a dose level of 300 mg/kg bw and for females at 300, 1000, and 2000 mg/kg bw (refer to Table B.6.7.1-1).

Ambulatory locomotor counts:

Test article-related, lower ambulatory counts were noted for the 1000 and 2000 mg/kg bw group males compared to the control group on study day 0. This was primarily due to reductions (up to 50%, although generally not statistically significant) in activity during the first few sub-intervals of the session (0-10 and 11-20 minutes). Compared to the control group, mean overall ambulatory counts were 20.0% and 27.4% for the 1000 and 2000 mg/kg group males, respectively, and were significant ($p \leq 0.05$) for the 2000 mg/kg bw group males (refer to Table B.6.7.1-1).

Although significant ($p \leq 0.05$) decreases in ambulatory counts were noted on study day 0 for females in the 1000 and 2000 mg/kg bw during the 0-10 minute interval, no significant changes were noted in these groups during the other sub-intervals on day 0 or any other given day that motor counts were recorded. The differences were not dose-related and/or the values were similar to the laboratory's acute locomotor activity historical control data. Therefore, these differences were considered sporadic and were not attributed to the test article (refer to Table B.6.7.1-2).

No remarkable shifts in the pattern of habituation occurred in any of the test article-treated groups when the animals were evaluated on study days 0.

Days 7 to 14: By study day 7 and continuing to study day 14, mean total and ambulatory activity counts for the 1000 and 2000 mg/kg group males were unaffected by test article administration.

No remarkable shifts in the pattern of habituation occurred in any of the test article-treated groups when the animals were evaluated on study days 7, and 14.

Table B.6.7.1-1:
Overview of motor counts in male rats

Parameters	♂ (mg/kg bw)				Historical control range[a]
	0	300	1000	2000	
Day 0 – Total locomotor activity counts					
- 0-10 mins	1142	1080	932	945	1064 – 1190 mean: 1134
- 11-20 mins	528	500	349	331	444 – 721 mean: 562
- 21-30 mins	152	146	80	146	102 – 426 mean: 243
- 31-40 mins	88	83	87	90	58 – 188 mean: 128
- 41-50 mins	102	157	98	62	51 – 161 mean: 101
- 51-60 mins	143	166	159	120	51 – 240 mean: 155
- Cumulative	2155	2133	1704	1694*	2109 – 2638 mean: 2323
Day 0 – Ambulatory locomotor activity counts					
- 0-10 mins	341	318	267	248*	290 – 348 mean: 321
- 11-20 mins	88	63	55	44	57 – 152 mean: 95
- 21-30 mins	5	2	13	11	2 – 74 mean: 27
- 31-40 mins	1	2	1	11	0 – 20 mean: 8

Parameters	♂ (mg/kg bw)				Historical control range[a]
	0	300	1000	2000	
- 41-50 mins	4	8	6	0	1 – 13 mean: 5
- 51-60 mins	7	7	14	10	0 – 43 mean: 15
- Cumulative	445	401	356	323*	418 – 582 mean: 471

* $p \leq 0.05$

a laboratory historic control data (Rat CRL:CD(SD) . Range: min.- max. (refer to KCA 5.7.1/02, Appendix K) collected 2007 - 2010

Table B.6.7.1-2:
Overview of motor counts in female rats

Parameters	♀ (mg/kg bw)				Historical control range[a]
	0	300	1000	2000	
Day 0 – Total locomotor activity counts					
- 0-10 mins	1408	1378	1146	1204	1260 – 1420 mean: 1321
- 11-20 mins	639	640	584	90	511 – 692 mean: 596
- 21-30 mins	202	216	299	295	206 –349 mean: 284
- 31-40 mins	227	146	145	216	38 –317 mean: 188
- 41-50 mins	247	63	78	186	102 – 233 mean: 183
- 51-60 mins	170	126	167	217	63 – 281 mean: 179
- Cumulative	2892	2569	2420	2708	2480 – 3016 mean: 2752
Day 0 – Ambulatory locomotor activity counts					
- 0-10 mins	459	451	357*	382*	378 –524 mean: 438
- 11-20 mins	142	150	127	156	93 –173 mean: 129
- 21-30 mins	24	29	68	66	27 – 73 mean: 49
- 31-40 mins	58	4	21	51	1 – 57 mean: 26
- 41-50 mins	56	1	4	45	6 – 49 mean: 23
- 51-60 mins	22	1	27	46	1 – 50 mean: 23
- Cumulative	761	635	604	746	604 – 835 mean: 688

* $p \leq 0.05$

a laboratory historic control data (Rat CRL:CD(SD) . Range: min.- max. (refer to KCA 5.7.1/02, Appendix K) collected 2007 - 2010

Gross pathology: No test article related effects were observed.

Neurohistopathology: No test article-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined from 6 animals/sex in the 2000 mg/kg bw group. Axonal degeneration of the peripheral nerves was noted for 1 male (sciatic nerve) and 1 female (sciatic nerve) in the control group and 2 males (sciatic nerves) and 2 females (tibial, sciatic and/or sural nerves) in the 2000 mg/kg bw group. The changes in each animal were of similar nature and severity and characterised by 1 fragmented axon within a distended digestion chamber. A maximum of 1 degenerated axon was present in each section of nerve examined. The change was graded and in all instances was minimal, and was considered a spontaneous finding unrelated to administration of the test article (refer to Table B.6.7.1-3).

Organ weights: Brain weights and measurements were unaffected by test article administration. There were no statistically significant differences between the control and test article-treated groups (refer to Table B.6.7.1-3).

Table B.6.7.1-3:
Overview of clinical signs, brain weights and neurohistopathology findings

Parameters	♂ (mg/kg bw)				♀ (mg/kg bw)			
	0	300	1000	2000	0	300	1000	2000
Clinical signs and terminal body weights (in grams)								
- unkempt appearance	0/0	0/0	0/0	1/12	0/0	0/0	0/0	6/12
Ter. body wts (g)	302	297	288	296	199	198	200	191
Brain parameters (n=12)								
- Weight (abs): - Length (mm): - Width (mm):	2.13 21.4 15.8	2.13 21.4 15.7	2.16 21.5 15.7	2.10 21.3 15.6	1.99 21.1 15.2	1.99 20.9 15.3	1.98 20.9 15.1	1.97 20.7 15.2
Neurohistopathology, axonal degeneration (incidence/total examined [severity: minimal, mild, moderate, severe])								
- Sciatic nerve	1/6 [1,0,0,0]	-	-	2/6 [2,0,0,0]	1/6 [1,0,0,0]	-	-	1/6 [1,0,0,0]
- Tibial nerve	0/6 [0,0,0,0]	-	-	0/6 [0,0,0,0]	0/6 [0,0,0,0]	-	-	1/6 [1,0,0,0]
- Sural nerve	0/6 [0,0,0,0]	-	-	0/6 [0,0,0,0]	0/6 [0,0,0,0]	-	-	1/6 [1,0,0,0]

* $p \leq 0.05$ abs: absolute
Ter: terminal

. Deficiencies:

None.

. Discussion:

Effects following a single dose of pyriproxyfen were observed in males only at 1000 mg/kg bw and both in males and females at 2000 mg/kg bw. Unkempt appearance was noted in 6/12 females and 1/12 males in the 2000 mg/kg bw at the detailed physical examination approximately 24 hours following dose administration. In addition, decreased overall total and ambulatory counts were noted in the 1000 and 2000 mg/kg bw group males at the time of peak effect on study day 0. Locomotor activity in these groups on study day 0 was up to 21.4% (total) and 27.4% (ambulatory) lower than the control group when the overall session was evaluated, primarily due to lower activity during the first few sub-intervals (0-10 and 11-20 minutes). There were no effects on the pattern of habituation at any dose level when evaluated on study days 0, 7, and 14. It is considered that the decreased motor activity noted in the 1000 and 2000 mg/kg bw group males was a result of systemic toxicity rather than typical neurotoxicity.

No test article-related effects were apparent in brain weights or brain dimensions for perfused animals. In addition, no test article-related neuropathological lesions were observed upon microscopic examination of central and peripheral nervous system tissues from 6 animals/sex in the 2000 mg/kg bw group. From the weight of evidence it is concluded that pyriproxyfen is devoid of acute neurotoxic potential.

Conclusions

Unkempt appearance was noted in the 2000 mg/kg bw group males and females at the detailed physical examination approximately 24 hours following dose administration. No test article related difference in either neurobehavioral evaluations (FOB), neurohistopathology, brain weights or brain dimensions were observed in either sex. Whilst decreased overall locomotor activity (total and ambulatory counts) was noted for males in the 1000 and 2000 mg/kg bw groups at the time of peak effect on study day 0 this was deemed to be a result of systemic toxicity rather than typical neurotoxicity. From the weight of evidence it is concluded that pyriproxyfen is devoid of acute neurotoxic potential. Based on the results of this study, the NOAEL for general toxicity following a single oral dose *via* gavage is considered to be 300 mg/kg bw for males and 1000 mg/kg bw for females.

The NOAEL for neurotoxicity is considered to be >2000 mg/kg bw for both sexes.

RMS NL conclusion:

Total locomotor activity counts and ambulatory locomotor activity counts were decreased in males at 1000 and 2000 mg/kg bw. These findings reached significance only at the top-dose level of 2000 mg/kg bw, however, the activity counts (total and ambulatory) for both these dose groups were outside the historical control data. Therefore, RMS considers this effect for the setting of a neurotoxic NOAEL.

In females, ambulatory locomotor activity counts were significantly decreased at 1000 and 2000 mg/kg bw only at 0-10 mins, not thereafter. In addition, no dose response was seen for this finding with the count found at 2000 mg/kg bw being within the HCD. Therefore, in females this is not regarded as a treatment-related adverse effect.

The NOAEL for systemic toxicity is 1000 mg/kg bw for both males and females based on the clinical finding of unkempt appearance.

The NOAEL for neurotoxicity is considered to be 300 mg/kg bw for males based on the significant decrease in total and ambulatory motor activity counts at 2000 mg/kg bw and the decrease outside of historical control data seen at 1000 mg/kg bw. For females, the NOAEL for neurotoxicity is considered to be the highest dose tested of 2000 mg/kg bw.

3.11.2 Human data

No human data on the specific organ toxicity (single exposure) of pyriproxyfen are available.

3.11.3 Other data

With the exception of the acute toxicity studies summarised in Sections 3.1, 3.2 and 3.3, no other data on the specific organ toxicity (single exposure) of pyriproxyfen are available.

3.12 Specific target organ toxicity-repeated exposure

3.12.1 Animal data

3.12.1.1 CA 5.3.1/01 (1998a): 28-day oral toxicity study in the rat

Report

CA 5.3.1/01 (1998a)

One-month oral toxicity study of S-31183 in rats

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0038

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, however, additional information on the materials and methods section and the results were added. Agreed with the original conclusion, NOAEL is 28.8 mg/kg bw/day.

Characteristics

Reference	:	CA 5.3.1/01, 1988a	exposure	:	28 days, diet
Type of study	:	Subacute oral toxicity study	dose	:	0, 300, 1000, 3000 and 10000 mg/kg food[1]
year of execution	:	1986 - 1988	vehicle	:	none
test substance	:	S-31183 (Pyriproxyfen), lot no PTG-86011, purity 97.2%	GLP statement	:	yes
route	:	oral	guideline	:	predominantly in accordance with OECD 407 (1995)
species	:	rat, Sprague-Dawley, Crj:CD (SD)	acceptability	:	Acceptable
group size	:	12/sex/dose	**NOAEL**	:	29 mg/kg bw/day

1 Equal to 0, 29.3, 97.6, 286 and 913 mg/kg bw/day in males and 0, 28.8, 95.8, 286 and 869 mg/kg bw/day.

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PTG-86011

Purity: 97.2%

CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study

2.Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Age:	4 weeks
Weight at dosing:	♂: 146 – 164 g; ♀: 114 – 135 g
Source:	Charles River Japan Inc.
Acclimation period:	8 days

B. Study Design and Methods

1.In life dates:	21 May 1986 to 19 June 1986
2.Animal assignment and treatment:	The conduct of the study generally followed the current OECD 407, with the exceptions listed below. Four groups of 12 male and 12 female Crj:CD(SD) rats received the test article by dietary administration at concentrations of 0, 300, 1000, 3000, 10000 ppm for 28 days, equivalent to 0/0, 29.3/28.8, 97.6/95.8, 286/286, 913/869 mg/kg bw/day for males/females, respectively. Body weight gain, water and food consumption were measured at regular intervals. Control and high dose animals were subjected to ophthalmoscopy, with all animals subjected to haematology, clinical chemistry, urinalysis, organ weights, and macropathology. Histopathological examinations were carried out on a limited set of organs/tissues from the control and high dose group and on macroscopically abnormal tissue from any animal on study. All other animals had liver, kidney and lung examined.
3.Statistics	Data for body weight, food and consumption, organ weight, haematology and blood biochemistry were subjected to analysis of variance in one-way classifications. When a significant result was found (5% probability), the least significant difference method was used as a test for significant difference compared to control values. The data for urinalysis were subjected to Kruskal-Wallis analysis. When a significant result was found at 5% probability, Scheffe type rank sum test was used to determine significant difference from the control values.

C. methods

1.Observations	All animals were observed once daily for general behaviour and toxic signs throughout the treatment period.
2.Body weights	Body weights were measured at the start of treatment and on days 2, 7, 14, 21 and 28 of treatment.
3.Food consumption	Cumulative food and water consumption for each cage for 2 consecutive days were measured weekly throughout the treatment period.
4.Urinalysis	On days 22 to 26 of treatment, fresh urine was collected from each animal and parameters were measured. On day 26 or 27, all animals of the control, 3000 ppm and 10000 ppm groups were individually housed in metabolic cages and urine was collected for 3 hours for testing appearance, urine volume and microscopic examination of sediment.
5.ophthalmoscopic examination	On day 23 of treatment, ophthalmoscopic examination was conducted on each animal of the control and 10000 ppm group.
6.Haematology	At sacrifice, blood samples were collected from all animals and analysed.
7.Blood chemistry	From the blood sample collected, serum and plasma were obtained and analysed for blood chemistry parameters.
8.Pathology	At sacrifice, all animals were examined grossly for abnormalities and organs were collected. Selected organs were weighed and fixed for histopathological examination.

Results

Formulation analysis: Homogeneity and verification of dietary levels confirmed that mean values were within the range ±4% of nominal concentration confirming accurate formulation.

Observations: No mortality occurred. In the first week of study, loss of hair was seen in 6/12 males and 4/12 females at 10000 ppm. An increased incidence in soft stool was seen in males and females at 10000 ppm, throughout the study period.

Body weight: Body weight and body weight gain was significantly reduced in males at 3000 ppm (-5%) and 10000 ppm (-7%) and females at 10000 ppm (-11%).

Table B.6.3.1-1 Mean body weights during the treatment period (in grams)

Group / days of treatment	0	2	7	14	21	28
Males						
0	155	172	212	282	305	345
300 ppm	155	172	212	285	311	353

1000 ppm	154	172	210	263	305	348
3000 ppm	154	171	209	255	298	327*
10000 ppm	155	159**	188**	248**	289**	320**
Females						
0	124	135	155	179	201	218
300 ppm	125	135	154	175	194	208
1000 ppm	125	136	156	178	197	208
3000 ppm	124	134	153	175	195	210
10000 ppm	123	123**	146	165**	185**	194**

*Significantly different from control ($p<0.05$), ** significantly different from control ($p<0.01$)

Food and water consumption: Food consumption was significantly reduced in males (33%) and females (36%) at 10000 ppm in the first week of the study.

In the 10000 ppm group, significantly higher water consumption was noted in males during the first 3 weeks of treatment (up to 31% increase) and in females during the first 2 weeks of treatment (up to 23% increase).

Ophthalmoscopy: No treatment related findings.

Urinalysis: Significantly higher values of urinary pH were noted in males at 1000 ppm and a significantly lower number of positive observations in ketone bodies was noted in females of the 10000 ppm group. In macroscopic examination of sediment, slightly higher incidence of leukocytes was noted in females of the 10000 ppm group, however, there was no difference from the control in the 3000 ppm group. The incidence of yellow urine increased in both sexes of the 10000 ppm group.

Haematology: Significantly lower values for erythrocyte count, haemoglobin concentration and haematocrit were observed in males of all treatment groups compared to the control. However, these changes were not considered to be treatment related at 3000 ppm and below, due to the lack of a clear dose response. Although significant lower mean value for MCHC was noted in females at the top dose, this change was considered to be of little toxicological significance and unrelated to treatment due to the absence of any difference from control values for haemoglobin concentration or haematocrit value. Significant reduction of prothrombin time was noted at 3000 ppm and above. However, this change was not considered to be related to treatment because the difference was very marginal.

Table B.6.3.1-2: Haematological findings

Dose (ppm)	**0**		**300**		**1000**		**3000**		**10000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
RBC	795	752	755**	751	747**	759	761*	739	732**	737
HGB	15.0	14.7	14.4**	14.8	14.2**	14.8	14.3**	14.5	13.8**	14.2
HCT	45.2	41.7	43.1**	41.3	42.4**	42.3	43.2*	41.7	41.9**	41.2
MCHC	33.1	35.2	33.3	35.3	33.8	35.0	33.2	34.8	33.0	34.5**
Prothrombin time (sec)	16.7	14.9	15.8	14.8	15.5*	14.8	16.8	14.3**	17.6	14.3**

*Significantly different from control ($p<0.05$), ** significantly different from control ($p<0.01$)

Clinical chemistry:

Significantly higher α2-fraction values and significantly lower β-fraction values were observed in males of the 10000 ppm group and females of the 10000 ppm group, respectively. These changes were marginal but they were considered to be related to treatment. Significant differences found in other globulin fraction ratios were secondary changes caused by higher albumin values and unrelated to treatment.

Table B.6.3.1-2: Blood biochemistry findings

Dose (ppm)	0		300		1000		3000		10000	
	m	f	m	f	m	f	m	f	m	f
Cholesterol	62	64	72	63	73*	71	85**	73	132**	112**
Glucose	132	108	124	109	139	110	146*	117	148*	115
Triglyceride	32	2	26	2	24	3	19	3	19	11**
Urea nitrogen	15	23	15	28	16	27	17*	26	17*	32**
Calcium	9.3	9.2	9.6*	9.3	9.5	9.5*	9.4	9.8**	9.9**	10.2**
Total protein	5.5	5.6	5.4	5.6	5.4	5.7	5.8	5.8**	5.9**	8.1**
Phospholipid	95	114	108*	112	106	121	123**	123	182**	178**
GOT	151	144	131	141	143	131	139	123*	114**	99**
CGT	0	2	0	2	0	3	0	2	2**	5**
Potassium	5.2	4.8	5.4	4.8	5.1	4.8	5.0	4.7	4.7*	4.7
Cholinesterase	898	2070	726	2517	807	2325	833*	2871**	884**	2072
Albumin (g/dL)	3.3	3.5	3.3	3.5	3.4*	3.8	3.4**	3.8**	3.7**	4.1**
Globulin-α2 (%)	0.30	0.28	0.31	0.30	0.32	0.30	0.31	0.26	0.38**	0.31
Globulin-β (%)	0.81	0.81	0.83	0.80	0.80	0.81	0.76	0.76	0.78	0.66**
A/G ratio	1.17	1.41	1.22	1.43	1.33*	1.44	1.37**	1.80**	1.38**	1.72**

*Significantly different from control ($p<0.05$), ** significantly different from control ($p<0.01$)

Organ weights:

The organ weight findings are show in the following table.

Table B.6.3.1-3: Absolute and relative organ weights

Dose (ppm)	0		300		1000		3000		10000	
	m	f	m	f	m	f	m	f	m	f
Heart, absolute	1.11	0.78	1.22*	0.74	1.12	0.74	1.12	0.72	1.06	0.68**
relative to bw	0.35	0.37	0.38	0.37	0.35	0.37	0.37	0.38	0.36	0.37
Kidney, absolute	2.51	1.66	2.87	1.63	2.54	1.66	2.52	1.69	2.80	1.67
relative to bw	0.79	0.81	0.82	0.82	0.79	0.83	0.82	0.84	0.87**	0.91**

Liver, absolute	9.85	5.98	10.34	5.84	10.25	5.82	11.32**	8.66*	14.78**	8.42**
relative to bw	3.08	2.90	3.18	2.92	3.20	2.80	3.88**	3.30**	4.92**	4.68**
Lung, absolute	1.21	0.90	1.21	0.86	1.12*	0.88	1.12*	0.87	1.07**	0.80**
relative to bw	0.38	0.44	0.37	0.43	0.35	0.44	0.37	0.43	0.38	0.43
Ovary, absolute	-	86	-	91	-	82	-	87	-	72**
relative to bw	-	42.0	-	45.2	-	40.9	-	43.1	-	39.2
Pituitary, absolute	17	17	18*	17	14**	18	14**	17	13**	15
relative to bw	5.5	8.4	4.8*	8.3	4.5**	8.2	4.7**	8.4	4.4**	8.3
Prostate, absolute	0.49	-	0.47	-	0.47	-	0.44	-	0.40	-
relative to bw	0.15	-	0.15	-	0.15	-	0.14	-	0.13	-
Testes, absolute	3.00	-	2.89	-	2.88	-	2.83	-	2.83	-
relative to bw	0.94	-	0.89	-	0.91	-	0.82	-	0.85	-
Thymus, absolute	0.88	0.45	0.61	0.47	0.88	0.48	0.54	0.47	0.59	0.48
relative to bw	0.21	0.22	0.19	0.23	0.20	0.24	0.18	0.23	0.20	0.25
Thyroid, absolute	22	17	19*	19	18**	18	20	17	22	18
relative to bw	8.9	8.4	5.7**	9.8*	5.7**	7.8	6.4	8.5	7.3	8.8

*Significantly different from control ($p<0.05$), ** significantly different from control ($p<0.01$)

Absolute and relative weights of the liver were significantly higher in both sexes at 3000 ppm and higher compared to the control. Absolute liver weight was increased by +15% and +50% (males); +45% and +41%(females) for the 3000 and 10000 ppm doses, respectively. Relative liver weight was increased by +26% and +60% (males); +14% and +61% (females) for the 3000 and 10000 ppm doses, respectively. Relative weights of the kidney were significantly higher in both sexes of the 10000 ppm group compared to the control (+10% for the males, +12% for the females).

Changes in absolute weight of the lung, heart and ovaries were observed, however, the relative weights of these organs were not statistically different from the control values and no histopathological findings were observed in these organs. Therefore, these changes are considered to be marginal.

Absolute and relative weights of the pituitary were significantly lower in males at 300 ppm and above whhen compared to the control. However, these values were between the control values and the background data

(11±1.4 mg, 10±2.1 mg). Therefore, the study director considers this lower pituitary weight to be within the range of physiologial variations. Additionally, there was no histopathological finding related to low pituitary weight.

Pathology

Gross pathology examinations showed enlargement and white foci of the liver in both sexes at 10000 ppm. Other findings such as white contents in the urinary bladder of males, fluid in the uterus, red thymus, brown foci of the lung and diaphragmatic hernia of the liver were observed sporadically without dose dependency. Histopathological examination showed periportal and/or midzonal hepatocellular hypertrophy in all males and one female of the 10000 ppm group. This finding was not observed in any of the lower dose levels. Although focal necrosis on the liver was found in one male and one female of the 10000 ppm group, it was not considered to be related to treatment due to the low incidence.

Table B.6.3.1-4: Pathological findings

Dose (ppm)	**0**		**300**		**1000**		**3000**		**10000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Gross pathology										
Liver:										
-enlargement	0	0	0	0	0	0	0	0	10	9
-white foci	0	0	0	0	0	0	0	0	10	3
-diaphr. hernia	0	1	0	0	0	0	0	0	0	1
Urinary bladder, white contents	4	0	3	0	0	0	1	0	6	0
Uterus, fluid	-	2	-	1	-	1	-	1	-	3
Thymus, red	0	0	0	1	0	0	0	0	0	1
Lung, brown foci	0	0	1	0	2	0	0	1	0	1
Histopathology										
Liver, hypertrophy	0	0	0	0	0	0	0	0	12*	1
Liver, necrosis	0	0	0	0	0	0	0	0	1	1

*Significantly different from control ($p<0.01$)

Acceptability

No arena observations, sensory reactivity to auditory, visual and proprioceptive stimuli, assessment of grip strength and motor activity assessment were performed. However, considering the overall targets of the test substance, the study is considered acceptable.

Conclusions

Based on changes in biochemistry indicative of liver injury at 1000 ppm and above, the NOAEL is set at 300 ppm (equal to 29.3 mg/kg bw/day for males and 28.8 mg/kg bw/day for females).

RMS conclusion during renewal: The target organ in this study was the liver, with increases in liver weight, pathological and histopathological findings, and changes in biochemistry indicative of liver injury (increased γ-glutamyl transpeptidase, triglycerides and cholesterol). RMS agrees with the previously defined NOAEL of 300 ppm (equal to 29.3 mg/kg bw/day for males and 28.8 mg/kg bw/day for females).

3.12.1.2 CA 5.3.1/02 (1987): 28-day oral toxicity study in the dog

Report

CA 5.3.1/02 (1987)

Four-week oral toxicity study of S-31183 in dogs. Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-70-0013

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results was added. Agreed with original conclusion.

Characteristics

Reference	:	CA 5.3.1/02, 1987	exposure	:	28 days, gelatine capsule
Type of study	:	28-day oral toxicity study	dose	:	0, 100, 300 and 1000 mg/kg bw/d
year of execution	:	1986	vehicle	:	gelatin capsule
test substance	:	S-31183 tech. (pyriproxyfen), Lot no. PTG-86011, purity 97.2%	GLP statement	:	yes
route	:	oral	guideline	:	no guideline available; predominantly in accordance with OECD 409 (1998)
species	:	dog, Beagle	acceptability	:	acceptable
group size	:	2/sex/dose	**NOAEL**	:	-

Study design

There is no guideline available for a subacute study in dogs (28 days). However, the study was generally in compliance with OECD 409 (1998) without functional observations. Haematology and clinical biochemistry were performed at the start and after 2 and 4 weeks of exposure. Urinalysis and faecal examination were performed at the start and after 4 weeks of exposure. Dosing was based on an acute oral study in dogs (not available to reviewer).

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Lot/Batch No.:	PTG-86011
Purity:	97.2%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Dog
Strain:	Beagle
Age:	6 months
Weight at dosing:	♂: 8.5 – 9.7 kg; ♀: 7.4 – 9.4 kg
Source:	White Eagle Laboratories Inc. (USA)
Acclimation period:	Not stated

B. Study Design and Methods

1.In life dates:	25 February 1986 to 26 March 1986
2.Animal assignment and treatment:	Three dose levels were assigned (100, 300 and 1000 mg/kg bw/day), orally via gelatin capsules. Each group consisted of two males and two females.
3.Statistics	Not performed
C. methods	
1.Observations	On weekdays, animals were observed for clinical signs before and after treatment at intervals of 2-3 hours until 5pm. On the weekend they were observed at least three times a day.
2.Body weights	Body weights were measured twice weekly before drug administration at one week prior to the start and up to the end of treatment.
3.Food consumption	For seven days before the start of and up to the end of treatment, residual food was weighed every morning to be able to calculate food consumption.
4.Urinalysis	Urinalysis was performed prior to, and at four weeks after administration.
5.Fecal examination	Before the initiation of and at week 4 of treatment, faeces were examined for wet weight and occult blood.

6.Haematology	Before and at weeks 2 and 4 of the initiation of treatment, blood was collected prior to drug administration.
7.Blood chemistry	Before and at weeks 2 and 4 of the initiation of treatment, blood was collected and serum was separated to determine blood biochemistry parameters.
8.Pathology	All animals were subjected to pathological examination at the end of the treatment period. At sacrifice, all animals were examined grossly for abnormalities and organs were collected. Selected organs were weighed and fixed for histopathological examination.

Results

Observations: No mortalities occurred. In all dose groups including the control group, vomiting was sporadically observed, however, its frequency was not dose-related and no significant differences were seen. Soft faeces, diarrheal or mucosal faeces were sporadically seen in al dose group, however, there was no dose-relation.

Food consumption: No treatment-related findings.

Body weight: No treatment-related findings were observed.

Haematology: No treatment-related findings.

Clinical chemistry: No treatment-related findings.

Urinalysis: No treatment-related findings.

Organ weights: Absolute and relative liver weight was increased in all dose groups, however, no dose-relationship was found.

Gross pathology: Several changes were observed, however, these were observed only in one animal and the changes concerned are frequently noticed in beagle dogs.

Histopathology: In the liver, enlargement of hepatocytes centring on the lobule was observed in the high dose group, although their degree of change was very slight. In addition, circumscribed cellular infiltration was seen in the medium and high dose groups.

Table B.6.3.1-5: Histopathological findings

Dose (mg/kg bw/d)	**0**		**100**		**300**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
microscopy - liver, centrilob.	0/2	0/2	0/2	0/2	0/2	0/2	2/2	2/2	

Dose (mg/kg bw/d)	0		100		300		1000		dr
	m	f	m	f	m	f	m	f	
hypertrophy									

Acceptability

The study is considered acceptable as a pilot study for subchronic studies. In view of the limited number of animals per sex and group, derivation of a NOAEL is not considered appropriate.

Conclusions

Histopathological examination of the liver showed slight centrilobular hypertrophy to be present in all animals at 1000 mg/kg bw. The study can be considered to be indicative of effects in the liver.

RMS conclusion during renewal: Study is still considered acceptable as a pilot study and is indicative of liver effects in the dog. No NOAEL can be derived.

3.12.1.3 CA 5.8.2/01 (2011): 28-day immunotoxicity study in the mouse

Report

CA 5.8.2/01 (2011)

Pyriproxyfen: 4 week dietary immunotoxicity study in the female CD-1 mouse

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-0204

Submitted for the purpose of renewal

Guidelines: US EPA OCSPP (OPPTS) 870.7800

GLP: Yes (certified laboratory)

Acceptability: The study is considered to be acceptable

Materials and Methods

A. Materials:

1. Test Material: Pyriproxyfen

Description: White solid

Lot/Batch No.: 080506G

Purity: 99.5%

CAS No.: 95737-68-1

Stability of test compound:	Confirmed stable for the duration of the study
2. Vehicle and/or positive control:	Basal diet / cyclophosphamide
3. Test animals:	
Species:	Mouse
Strain:	Crl:CD1™ (ICR)
Age:	6 - 7 weeks (ages at the start of treatment)
Weight at dosing:	♀: 21.1-27.7g
Source:	Charles River (UK) Ltd
Acclimation period:	7 days

B. Study Design and Methods:

1. In life dates:	14 April 2011 to 9 November 2011
2. Animal assignment and treatment:	Animals selected for the study were randomised by computer into four groups of 10 animals with variations in bodyweight of animals not exceeding ±20% and the group mean values were not statistically significantly different from each other. The test article was mixed in with the basal diet and fed to animals at dose levels of 0, 1000, 2000, 5000 ppm (equivalent to 0, 228, 449, 1139 mg/kg bw/day) over a period of 28 consecutive days. The overall achieved dose for animals given 5000 ppm exceeded the limit dose (1000 mg/kg bw/day) defined in the OPPTS 870.7800 guideline. A further eight females received cyclophosphamide (a positive control) at 20 mg/kg bw/day, administered by oral gavage on days 22 to 26. On day 25 (four days prior to necropsy), all animals received a single intravenous dose (0.2 mL by bolus injection) of sheep red blood cells (2 x 10^9 cells/mL) in 0.9% saline. On study day 29 all animals were killed.
3. Homogeneity and achieved concentration analysis of the dose:	Stability analysis was undertaken, along with achieved concentration and homogeneity analysis of the prepared diet.
4. Statistics:	For test article treatment group, statistical analysis was undertaking using appropriate parametric (Barlett's test, Williams' test) or non-parametric (Shirley's test) tests. For positive control group, Bartlett's test was applied for comparison with the control group, if this test was

not significant at the 1% level, Student-t-test was applied. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, Wilcoxon rank sum test was applied.

C. Methods:

1.Observations: Recorded for all animals daily. A more detailed weekly physical examination was performed on each animal to monitor general health.

2.Body weights: Recorded on the day that treatment commenced (day 1), twice weekly throughout the treatment period and before necropsy.

3.Food consumption: Recorded weekly.

4.Water consumption: Recorded weekly.

5.Opthalmoscopic examination: Not conducted.

6.Immunotoxicity assessment: The spleen from each animal was used as the source of splenocytes for conducting immunotoxicology investigations. The whole spleen was transferred to individual containers of Hank's Balanced Salt Solution (HBSS) and held on ice (water) until processed for analysis.
Splenocyte suspensions were prepared by mechanical dissociation and used for the plaque forming cell (PFC) assays. Duplicate tests/animal were evaluated.
After preparation of the spleen cell suspensions an assessment of cellular viability was performed during the cell counting stage using a Trypan blue dye exclusion method.
PFC assay: The adaptive or acquired immune response of the animals was assessed using a modification of the Jerne Plaque Forming Cell assay (PFC assay). Animals were sensitised with a suspension of sheep red blood cells. This foreign antigenic preparation elicits a T-lymphocyte-dependent antibody response (TDAR) in the animals, which was measured by challenged leukocytes from the spleen in an *ex vivo* assay where sheep red blood cells are present in an agar matrix. This resulted in the formation of antibody-dependent lytic plaques, which were counted and indicated the activity of the immune response.

- Following preparation of a single spleenic cell solution, ACK lysing buffer was used to remove any red blood cells and then the cell suspension filtered through a 70 μm cell strainer and re-suspended in RPMI media.

- Viable and dead cells were counted and diluted to an appropriate concentration for the assay (1 *x* 10^6 cells/mL and 2 *x* 10^6 cells/mL). A 100 µL aliquot of the appropriate spleen cell suspension was then mixed with a 0.5% agar matrix in Earle's Balanced Salt Solution containing DEAE dextran at 0.05%, a suspension of the SRBCs (1.67% v/v) and guinea pig serum complement (5% v/v). This mixture was poured into petri dishes, allowed to set and incubated for up to 3 hours.
- Individual B lymphocytes secreting antibodies to SRBCs formed small foci where antibody dependent, complement mediated lysis occurred. After checking that the plaques had developed sufficiently the plates were fixed with 0.25% glutaraldehyde in phosphate buffered saline and then stored at 4°C until being read. The plates were coded and randomised before being read.
- In addition to the control and treatment groups a positive control group was included. These animals received the immunosuppressant cyclophosphamide.
- The number of lytic plaques for each animal was determined by microscopic examination and group mean responses calculated.

7.Gross pathology: All animals were subject to a detailed necropsy. All external features and orifices were examined visually. The cranial roof was removed to allow observation of the brain, pituitary gland and cranial nerves. After ventral mid-line incision, the neck and associated tissues and the thoracic, abdominal and pelvic cavities and their viscera were exposed and examined in situ. Any abnormal position, morphology or interaction was recorded.

Samples (or the whole) of the lymph nodes (mandibular, mesenteric and left axillary), adrenals, liver and thymus from all animals were preserved in 10% neutral buffered formalin.

8.Organ weights: Liver, spleen, thymus and adrenals taken from each animal were dissected free of adjacent fat and other contiguous tissue and the weights recorded.

Results and Discussion

Homogeneity and achieved concentration analysis:

Analysed diet admix formulations used for test article administration met the acceptance criteria for test article concentration acceptability (±10% of nominal). No test article was detected in the analysed basal diet provided to the control group.

Observations:

All animals survived to the scheduled necropsy on study day 29. There were no test article-related clinical findings noted at any concentration.

Body weight:

Lower bodyweight gain compared to the controls, was observed in animals 1000 or 5000 ppm, with the reduction being approximately 30% different from controls.

The bodyweight gains of animals receiving 1000 or 2000 ppm were considered unaffected.

The overall group mean weight gains at 1000 ppm were approximately 29% lower than the controls but this difference, which was not statistically significant, was attributed to low weight gains in three animals (# 14, 16, 17). The mean reduction of weight gain at 1000 ppm, in the absence of any similar finding at 2000 ppm, was therefore attributed to normal variation and not related to treatment with pyriproxyfen.

Food and water consumption:

There were no test article-related effects on food consumption.

A slight but statistically significant increase of water consumption, compared to the pre-treatment and control values, was observed in animals receiving 2000 or 5000 ppm.

Water consumption in animals receiving 1000 ppm was similar to the control (refer to Table B.6.8.2-1).

Immunotoxicity assessment:

There were no statistically significant changes in the numbers of cells/spleen, PFC/10^6 viable cells or PFC/spleen for CD-1 mice in any of the dosed groups when compared to the control.

Treatment with cyclophosphamide at 20 mg/kg bw/day seven to three days prior to termination resulted in a significant reduction of the PFC response. The numbers of cells/spleen, PFC/10^6 viable cells and PFC/spleen (all $p \leq 0.001$) were all significantly reduced when compared to the control (refer to Table B.6.8.2-1), thus demonstrating the sensitivity and specificity of the assay.

Table B.6.8.2-1
Overview of the 28 day immunotoxicity study in mice orally (*via* diet) with pyriproxyfen: selected parameters

Parameters	♂ (ppm)				CPA (20 mg/kg bw/d)
	0	1000	2000	5000	
Water cons. (mL/animal/d)	7	7	10**	10**	
Ter. body wt (g)	27.6	27.0	28.7	27.4	
Organ weights (g)					
- Liver: rel abs	5.65 1.556	6.21* 1.676	6.56** 1.875**	7.12** 1.951**	

Parameters	♂ (ppm)				CPA (20 mg/kg bw/d)
	0	1000	2000	5000	
Immunotoxicology findings					
Viable cells/spleen (10^7)	7.62	7.85	6.87	6.46	5.13***
PFC/10^6 cells	1404.3	1473.5	1361.5	1247.0	177.8***
PFC/spleen	105177	116551	90289	80491	9474***

* $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$ Rel: relative
Abs: absolute
Ter: terminal

Gross pathology:

There were no test article-related effects on macroscopic findings noted at any concentration.

Organ weights:

After four weeks of treatment there was a dose related increase of absolute and bodyweight relative liver weight and, with the exception of absolute liver weights at 1000 ppm, these differences from controls were statistically significant ($p \leq 0.05$ of $p \leq 0.01$). The statistically significant increase in relative liver weight at 1000 ppm was considered not to be adverse as the increase in liver weight was <10%, without any associated macropathology

Deficiencies:

None

Conclusions

Under the conditions of this study, the NOAEL for general toxicity following 28 days of continuous dietary is considered to be 1000 ppm (equivalent to 228 mg/kg bw/day) based on increase in water consumption and relative and absolute liver weights.

No test article related effects on the immune function, as assessed by the measurement of antigen-specific, T-cell dependent antibody formation. The NOAEL for immunotoxicity was therefore greater than 5000 ppm in females (equivalent to 1139 mg/kg/day, a dose which exceeds the maximum recommended dose (1000 mg/kg bw/day) for repeat dose toxicity studies).

3.12.1.4 CA.5.3.2/01 (1989): 90-day oral toxicity study in the rat

Report

CA 5.3.2/01 (1989)

Sub-chronic toxicity study with S-31183 in rats.

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-91-0045

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

Reference	:	CA 5.3.2/01, 1989	exposure	:	13 weeks, diet
Type of study	:	13-week oral toxicity study	dose	:	0, 400, 2000, 5000 and 10000 mg/kg food/day (nominal)[1]
year of execution	:	1987/88	vehicle	:	none
test substance	:	S-31183 tech. (pyriproxyfen), Lot no. PYG-87074, purity 95.3%	GLP statement	:	yes
route	:	oral	guideline	:	predominantly in accordance with OECD 408 (1998)
species	:	rat, Crl:CD® BR	acceptability	:	acceptable
group size	:	10/sex/dose	**NOAEL**	:	23.5 mg/kg bw/day for males and 27.7 mg/kg bw/day for females

[1]equal to 0, 23.5, 118, 309 and 642 mg/kg bw/d for males and 0, 27.7, 141, 356 and 784 mg/kg bw/d for females

Study design

The study was generally in compliance with OECD 408 (1998). However, functional observations were not performed. Also the weight of epididymides, thymus, spleen, brain, heart, ovaries and uterus was not determined, neither was the reticulocyte count measured.

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PYG-87074

Purity: 95.3%

CAS No.: 95737-68-1

Stability of test compound: Confirmed stable for the duration of the study

2. Test animals:

Species: Rat

Strain: Crl:CD®BR

Age: 6 weeks

Weight at dosing: ♂: 189.8 – 241.1 g; ♀: 151.2 – 197.2 g

Source:	Charles River Laboratories Inc. (Raleigh, USA)
Acclimation period:	14 days

B. Study Design and Methods

1.In life dates:	13 October 1987 to 14 January 1988
2.Animal assignment and treatment:	Animals were assigned to different groups, compromising the following dose levels: 0, 400, 2000, 5000 and 10000 ppm. Dietary mixtures containing the test substance were prepared every week.
3.Statistics	Body weight, food and water consumption, clinical pathology data, organ weight data were compared statistically. If variances of untransformed data were heterogeneous in the ANOVA program, analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed routinely at the 5% two-tailed probability level.

C. methods

1.Observations	Rats were observed for mortality and moribundity twice daily. Observation for toxic effects was performed once daily and a physical examination was conducted each week.
2.Body weights	Body weights were measured and recorded prior to treatment and weekly for weeks 1-13.
3.Food and water consumption	Food consumption was measured and recorded weekly for weeks 1-13. Water consumption was measured and recorded twice weekly at 4- and 3-day intervals to yield a composite weekly water consumption value for weeks 1-13.
4.Ophthalmoscopic examination	Prior to treatment and at week 13, an indirect ophthalmoscopic examination was performed on all animals.
5.Urinalysis	Following 13 weeks of treatment, all animals were placed in urine collection racks. Urine samples were collected during the overnight fast in individual urine collection cages.
6.Haematology and clinical chemistry	After 13 weeks of treatment, samples for haematology and serum chemistry were obtained via orbital sinus puncture.
7.Pathology	Necropsies were performed on all animals. Following 13 weeks of treatment, all surviving animals were weighed, anesthetised and

exsanguinated. Organ weights were determined and tissues were preserved for histopathology.

Results

Formulation analysis: The test material was stable for 14 days and was homogeneous in the diet. Concentration analyses yielded acceptable overall mean values; however, there was a fair amount of sample variation during weeks 4-7 of the study. This resulted in a modification of the mixing procedure and there was no unacceptable sample variation after that time.

Compound intake: The average compound consumption values for weeks 1013 are presented as follows:

Table B.6.3.2-1: Mean test compound intake (mg/kg bw/day)

	Males	Females
400 ppm	23.49	27.68
2000 ppm	117.79	141.28
5000 ppm	309.05	356.30
10000 ppm	641.81	783.96

Observations: One female in the 2000 ppm dose group died during week 11. This death was considered accidental. Clinical signs that were seen during the study (dyspnoea, alopecia, and lacrimation) are commonly seen in rats and occurred sporadically across all dose groups. Therefore, they are not considered treatment-related.

Body weight: Throughout the study, mean body weight values were generally decreased with increasing dose. At the end of the study, there were statistically significant negative trends for males and females, and the mean values for the 5000 and 10000 ppm groups were significantly lower than the control values. Body weight gain was significantly reduced at 5000 and 10000 ppm in both sexes (88-92% of control) with a dose-related trend.

Table B.6.3.2-2 Mean body weights

Dose (ppm)	Males				Females			
	Week 1	Week 6	Week 9	Week 13	Week 1	Week 6	Week 9	Week 13
0	276.8	454.2	508.0	555.1	201.8	275.0	289.4	304.0
400	278.6	438.1	487.3	520.3	198.3	272.1	292.4	305.4
2000	274.6	441.8	489.9	529.5	198.2	256.6	271.4	285.6
5000	270.6	430.8	478.0	508.6*	195.0	255.1	268.6	276.6*
10000	243.6	411.0	450.4	487.2*	189.5	248.5	261.4	268.5*

*Significantly different from control, p≤0.05

Food and water consumption: No treatment-related effects on food or water consumption were observed.

Ophthalmoscopy: There were no ocular lesions observed in study animals prior to compound administration or after 13 weeks of treatment.

Haematology: The analysis of haematology parameters showed decreased erythrocyte counts, haemoglobin and haematocrit values in males at 2000 ppm (93-94% of control), and in males and females at 5000 and 10000 ppm (91-97% of control), with no dose-related trend. The decrease in females at 5000 ppm was attributed to lower values in one animal. Mean cell volume (MCV) was significantly decreased in females at 10000 ppm (97% of control), while mean cell haemoglobin (MCH) was significantly increased in males at 5000 and 10000 ppm (103-104% of control); no significant deviations were noted in mean cell haemoglobin concentration (MCHC).

Table B.6.3.2-3: Haematological findings

Dose (ppm)	0		400		2000		5000		10000	
	m	f	m	f	m	f	m	f	m	f
RBC	9.5	8.71	9.32	8.52	8.79*	8.93	8.69*	8.18*	8.92*	8.30
Haemoglobin	17.2	17.0	17.2	16.6	16.1*	17.1	16.4*	15.9*	16.7	15.8*
Haematocrit	48.0	47.7	48.5	46.5	45.0*	47.4	45.3*	44.0*	45.9*	43.9*
MCH	18.1	19.5	18.5	19.5	18.3	19.1	18.9*	19.4	18.7*	19.1
Mean cell volume	50.6	54.8	52.1	54.6	51.2	53.1*	52.2	53.9	51.5	52.9*

*Significantly different from control, p≤0.05

Clinical chemistry: Blood urea nitrogen and creatinine values were significantly increased in females at 10000 ppm (114 and 117% of control, resp.). Total cholesterol and phospholipid values were increased in males at 2000, 5000 and 10000 ppm (cholesterol: 148-206% of control; phospholipid: 133-178% of control) and in females at 5000 and 10000 ppm (cholesterol: 130-177% of control; phospholipid: 112-155% of control), with a dose-related trend. Triglycerides were decreased at 5000 and 10000 ppm in males (66-59% of control). Total protein and albumin values were significantly increased at 10000 ppm in males (109 and 114% of control, resp.) and females (111 and 119% of control, resp.). Calcium was significantly increased at 10000 ppm in females (106% of control), which was probably related to higher albumin values. Gamma-glutamyltransferase (γ-GT) activity was significantly increased at 10000 ppm in both sexes (100 and 300% of control, resp.). The changes observed in cholesterol and phospholipid values point to perturbations in liver lipid metabolism at 2000 ppm and above, while higher total protein, albumin, urea and creatinine values point to dehydration at 10000 ppm. The higher γ-GT values noted at 10000 ppm would be consistent with hepatobiliary effects (bile duct hyperplasia was noted in one female at this exposure level).

Table B.6.3.2-4: Clinical chemistry findings

Dose (ppm)	0		400		2000		5000		10000	
	m	f	m	f	m	f	m	f	m	f
BUN	13	14	13	14	13	14	13	16	14	16*
Creatinine	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.7*
Cholesterol	62	77	72	81	92*	81	121*	108*	128*	136*

Total protein	6.9	7.1	6.7	7.1	6.8	6.9	7.1	7.2	7.5*	7.9*
Albumin	4.9	5.3	4.8	5.4	4.9	5.3	5.1	5.7	5.6*	6.3*
Calcium	10.2	10.3	10.3	10.3	10.1	10.2	10.3	10.5	10.7	10.9*
Phospholipids	95	129	102	133	126*	124	167*	145	169*	200*
Γ-GT	0	1	0	1	0	1	1	1	1*	3*

*Significantly different from control, p≤0.05

Urinalysis: No treatment-related changes in urinalysis parameters were noted.

Organ weights: Absolute and relative liver weights were increased at 2000 ppm in males (114% of control) and significantly increased at 5000 and 10000 ppm in males (131-143% and 121-166% of control, resp.) and females (118-139% and 132-162% of control, resp.). Relative kidney weight was significantly increased at 5000 ppm in males (118% of control) and at 10000 ppm in males (118% of control) and females (114% of control), which was attributed to lower terminal body weights. Similarly, the significantly increased relative adrenal weight (130% of control) in males at 10000 ppm was attributed to lower terminal body weights.

Table B.6.3.2-5: Organ weight findings

Dose (ppm)	**0**		**400**		**2000**		**5000**		**10000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Absolute organ weights										
Adrenal	0.056	0.071	0.060	0.071	0.053	0.073	0.060	0.070	0.063	0.067
Kidney	3.40	1.92	3.17	1.94	3.59	1.88	3.67	1.85	3.47	1.88
Liver	12.36	7.09	12.05	6.94	14.10	7.24	16.25*	8.38*	17.66*	9.89*
Testis	3.34	-	3.37	-	3.39	-	3.50	-	3.37	-
Relative organ weights (organ/bw ratio)										
Adrenal	0.0108	0.0248	0.0122	0.0245	0.0106	0.0271	0.0126	0.0272	0.0140*	0.0272
Kidney	0.645	0.670	0.642	0.674	0.718	0.703	0.764*	0.715	0.763*	0.762*
Liver	2.335	2.463	2.434	2.401	2.816*	2.708	3.385*	3.246*	3.880*	3.991*
Testis	0.637	-	0.690	-	0.679	-	0.728	-	0.749	-

*Significantly different from control, p≤0.05

Gross pathology and histopathology: Macroscopic examinations revealed an enlarged liver in 2 males at 10000 ppm. Also noted in the livers was dark discolouration or mottling of the papillary processes in 2 males at 10000 ppm; in both cases there was histological evidence of moderate to severe midzonal necrosis in these areas. Microscopic examinations showed that livers of male and female rats at 2000 ppm and above exhibited a slight increase in cytoplasmic content reflected in a visibly reduced nucleus cytoplasmic ratio and diminution of sinusoidal spaces; these microscopic changes corresponded with the increased liver weights at the three higher exposure levels. Bile duct hyperplasia was noted in one female at 10000 ppm.

Acceptability

The study is considered acceptable.

Conclusions

The study results indicate that liver and red blood cells are potential targets of pyriproxyfen in rats. The NOAEL is set at 400 ppm (equal to 23.5 mg/kg bw for males and 27.7 mg/kg bw for females) based on increased liver weight and histopathological changes in the liver.

3.12.1.5 CA 5.7.1/03 (2011b): 90-day oral neurotoxicity study in the rat

Report

CA 5.7.1/03 (2011b)

A 90-day oral dietary neurotoxicity study of pyriproxyfen T.G. in rats

Sumitomo Chemical Co. Ltd, Unpublished report no.: NNT-0202

Submitted for the purpose of renewal

Guidelines	OECD 424 (1997)
GLP	Yes (certified laboratory)
Acceptability	The study is considered to be acceptable

Materials and Methods

A. Materials:

1. Test Material:	Pyriproxyfen T.G.
Description:	White solid
Lot/Batch No.:	080506G
Purity:	99.5%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2. Vehicle and/or positive control:	Basal diet / n.a.
3. Test animals:	
Species:	Rat, Crl:CD(SD)
Strain:	Sprague Dawley
Age:	6 weeks
Weight at dosing:	♂: 155 – 208 g; ♀: 134 – 167 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	At least 10 days

B. Study Design and Methods:

1.In life dates:	5 October 2010 to 14 March 2011
2.Animal assignment and treatment:	Animals selected for the study were randomised by computer into four groups of 12 animals/sex. The test article was mixed in with the basal diet and fed to animals at dose levels of 0, 1500, 5000, 15000 ppm for 90 d (equivalent to 0/0, 108/120, 359/407, 1111/1212 mg/kg bw/day for ♂/♀, respectively). All animals were anesthetised and perfused *in situ* during study week 13
3.Homogeneity and achieved concentration analysis of the dose:	Stability analysis was confirmed under a previous study and confirmed that test diet formulations for a period of 14 days under normal laboratory conditions at a concentration range of 500 - 20000 ppm. Therefore, stability analysis was not conducted as part of the current study. Samples were collected monthly from each diet formulation, including the control diet formulation, for concentration verification and homogeneity.
4.Statistics:	Body weight and post-dosing continuous detailed clinical observation data were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p \leq 0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Detailed clinical observation parameters that yielded scalar or descriptive data were analysed using Fisher's Exact. FOB parameters that yielded scalar or descriptive data and non-graded histopathologic findings were analysed using Fisher's Exact Test. Repeated measures analysis of variance (RANOVA) statistical analyses for total and ambulatory locomotor activity counts recorded during pre-test and after dosing were conducted as follows. Each analysis endpoint was analysed, by sex and session, with a RANOVA. Factors in the model included treatment group (TRT), time interval (TIME), and the interaction of time interval and treatment group (TRT*TIME). The random effect of animal was included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion (AIC). The monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dose levels. The linear dose by time interaction was evaluated and, if significant at

	the $p \leq 0.05$ level, trend tests on treatment means were performed $p \leq 0.05$ level for each time interval. If the linear dose by time interaction was not significant, the trend test was conducted across the pooled time intervals for the entire session only. Non-monotonic dose responses were evaluated whenever no significant linear trends were detected but TRT and/or TRT*TIME interaction was significant $p \leq 0.01$. Within the framework of the RANOVA, pairwise comparisons were made for each individual test article-treated group with the control group through linear contrasts. If TRT*TIME was significant, the comparisons were conducted for each time interval. If only the TRT effect was significant, the comparisons were conducted across the pooled time intervals for the entire session. These non-monotonic dose-response comparisons were conducted at the $p \leq 0.01$ level.
C. Methods:	
1.Observations:	Mortality and moribundity: observed twice daily, once in the morning and once in the afternoon Clinical observations: Detailed physical examinatios were conducted weekly, beginning one week prior to test diet administration and continuing until the scheduled necropsy. These observations included (but not limited to) changes in the appearance of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system function, somatomotor activity, and behaviour patterns.
2.Body weights:	Recorded weekly, starting 1 week prior to dose administration.
3.Food consumption:	Recorded weekly, starting 1 week prior to dose administration.
4.Opthalmoscopic examination:	Conducted on all animals prior to the initiation of test diet administration and near the end of the treatment period.
5.Functional observation battery (FOB):	Recorded for all animals prior to the initiation of the test diet and on study weeks 1, 3 7 and 12. Observations included: Home cage observations: posture, convulsions/tremors, faeces consistency, biting, palpebral (eyelid) closure. Handling observations: ease of removal from cage, lacrimation/chromodacryorrhea, piloerection, palpebral closure, eye prominence, red/crusty deposits, ease of handling animal in hand,

	salivation, fur appearance, respiratory rate/character, mucous membranes/eye/skin colour, muscle tone. Open field observations (evaluated over a 2 minute period): mobility, rearing, convulsions/tremors, grooming, bizarre/stereotypic behaviour, time to first step (seconds), gait, arousal, urination/defecation, gait score, backing. Sensory observations: approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch response, eye-blink response, hindlimb extension, olfactory orientation. Neuromuscular observations: hind-limb extensor strength, hind-limb foot splay, grip strength-hind and forelimb, rotarod performance. Physiological observations: catalepsy, body temperature, body weight.
6.Motor activity:	Recorded for all animals prior to the initiation of the test diet and on study weeks 1, 3 7 and 12. Locomotor activity assessment were recorded after completion of FOB. Each animal was tested separately. Data were collected in 5-minute epochs, and the test session duration was 60 minutes. These data were compiled as six 10-minute subintervals for tabulation. Total motor activity: defined as a combination of fine motor skills (*i.e.* grooming, interruption of 1 photobeam). Ambulatory motor activity: interruption of 2 or more consecutive photobeams.
7.Gross pathology:	The central and peripheral nervous system tissues were dissected and preserved. Any observable gross changes and abnormal colouration or lesions of the brain and spinal cord were recorded.
8.Neurohisto-pathology:	The following nerve tissues were prepared for a microscopic neuropathologic examination from 6 randomly selected animals/sex in the control and 2000 mg/kg bw groups: Brain (olfactory bulbs, cerebral cortex (2 levels), hippocampus/dentate gyrus, basal ganglia, thalamus, hypothalamus, midbrain, cerebellum, pons, medulla oblongata), spinal cord (cervical swellings C_3-C_7, lumbar swellings T_{13}-L_4), trigeminal ganglia/nerves, lumbar dorsal root ganglia at T_{13}-L_4, lumbar dorsal root fibres at T_{13}-L_4, lumbar ventral root fibres at T13-L4, cervical dorsal root ganglia at C_3-C_7, cervical dorsal root fibres at C_3-C_7, cervical ventral root fibres at C_3-C_7, cervical spinal

	nerve, lumbar spinal nerve), sciatic nerves (mid-thigh region – transverse and longitudinal [t+l] sections), sciatic nerves (at sciatic notch, t+l sections), sural nerves (t+l sections), tibial nerves (t+l sections), peroneal nerves (t+l sections),optic nerves, eyes, skeletal muscle (gastrocnemius), other sites (if deemed necessary)
9.Organ weights:	Fixed brain weight and brain dimensions (length [excluding olfactory bulbs] and width) were recorded.

Results and Discussion

Homogeneity and achieved concentration analysis:

Analysed diet admix formulations used for test article administration met the acceptance criteria for test article concentration acceptability (±10% of nominal). No test article was detected in the analysed basal diet provided to the control group.

Mortality and clinical signs:

All animals survived to the scheduled necropsy on study day 91. There were no test article-related clinical findings noted at any concentration.

Ophthalmoscopy:

No ophthalmic lesions indicative of toxicity were observed in any of the test article-related exposed groups.

Body weight:

In the 15000 ppm group, significantly ($p \leq 0.01$) lower mean body weight gains were noted during study days 0-14 and 0-7 for the males and females, respectively. Mean body weight gains for males and females in this group generally remained lower (occasionally significant; $p \leq 0.01$) than the control group throughout the remainder of the study and resulted in significantly ($p \leq 0.01$) lower mean cumulative body weight gains (study days 0-91). As a result, mean body weights for the 15000 ppm group males and females were 12.1% to 16.4% and 8.1% to 10.8% lower, respectively, than the control group during study days 7-91 and 35-91, respectively; significance ($p \leq 0.05$ or $p \leq 0.01$) was achieved beginning on study days 7 and 35, respectively, and continued to be observed throughout the remainder of the study. The effects on body weight were considered to be adverse.

There were no test article-related effects on mean body weights and body weight gains in the 1500 and 5000 ppm group males and females (refer to Table B.6.7.1-4).

Food consumption:

Test article-related, lower mean food consumption (g/animal/day) was noted in the 15000 ppm group males and females throughout the treatment period (study days 0-91); differences were generally significant ($p \leq 0.05$ or $p \leq 0.01$) for males and occasionally significant ($p \leq 0.05$ or $p \leq p \leq 0.01$) for females. However, when evaluated on a g/kg/day basis, mean food consumption in the 15000 ppm group was lower for the males during study days 0-7, but thereafter was often similar to or higher than that in the control group as a result

of the test article-related decreased mean body weights noted in this group throughout the study for both sexes (refer to Table B.6.7.1-4).

No test article-related effects on mean food consumption were noted in the 1500 and 5000 ppm group males or females.

Table B.6.7.1-4:
Overview of body weight and food consumption data

Parameters	♂ (ppm)				♀ (ppm)			
	0	1500	5000	15000	0	1500	5000	15000
Mean achieved test article intake (mg/kg bw/day)								
Day 0-90	0	108	359	1111	0	120	407	1121
Body weight (g)								
- Day 0	178	176	177	175	151	153	152	154
- Day 91	549	564	551	470**	278	294	281	251**
Body weight gain (g)								
- Day 0 - 7	53	56	54	29**	18	23	13	7**
- Day 7 - 14	55	55	56	37**	22	25	24	19
- Day 35 - 42	33	34	30	24**	9	10	10	8
- Day 42 -49	25	22	22	17**	8	4	5	28
- Day 63 - 70	26	23	22	18**	8	6	8	5
- Day 0 - 91	371	388	374	295**	126	141	129	98**
Food consumption (g/animal/day)								
- Day 0 - 7	25	24	24	21**	19	19	18	17
- Day 7 - 14	27	27	27	22**	19	20	19	18*
- Day 14 - 21	28	29	29	25**	19	20	19	18
- Day 21 - 28	28	30	29	26*	20	20	19	18
- Day 35 - 42	28	28	28	25**	19	20	19	17**
- Day 42 - 49	28	28	28	24**	18	20*	18	16*

* $p \leq 0.05$, $p \leq 0.01$

Functional observational battery:

Home cage observations:

No test article related effects were observed. The only significant ($p \leq 0.05$) differences compared to the control group were noted during study week 1 and consisted of a higher number of 1500 ppm group males that were asleep, lying on their side or curled up, lower numbers of 1500 and 5000 ppm group males with wide open eyelids, and lower numbers of 1500 and 15000 ppm group males with faecal pellets (with a corresponding increase in the number of males with no faecal pellets). These transient findings are not considered abnormal in the home cage and there was no dose-response relationship. Therefore, no relationship to the test article was apparent.

Handling observations:

No test article related effects were observed.

Open field observations:

No test article related effects were observed.

An increased number of urine pools were noted for the 5000 ppm group males when compared to the control group during study week 7. Whilst this increase was significant ($p \leq 0.05$), as it did not occur in a dose-related manner and did not persist to the study week 12 evaluations, it was not considered test article-related.

Sensory observations:

No test article related effects were observed.

Neuromuscular observations:

No test article related effects were observed.

Shorter mean rotarod times (62.8 seconds) were noted during study week 1 for the 1500 ppm group males and the mean forelimb grip strength (658.3 g) during study week 3 for the 5000 ppm group males was 26.4% lower than the control group; the differences were significant ($p \leq 0.05$). However, the mean control group values for rotarod times (111.6 seconds) and forelimb grip strength (893.9 g) were higher than the mean rotarod times and forelimb grip strength values (87.3 seconds ± 13.55 seconds and 748.0 g ± 172.93 g, respectively) in the laboratory's historical control range for the respective intervals and as these reductions did not occur in a dose-related manner, they were not considered test article-related.

Other differences between the test article-exposed and control groups were not statistically significant and did not occur in an exposure-related manner.

Physiological observations:

Mean body weights for the 15000 ppm group were significantly ($p \leq 0.01$) lower than the control group during the study week 1, 3, 7, 12 evaluations for the males and the study week 7 and 12 evaluations for the females. The effects on mean body weights during the physiological evaluations correlated with the test article-related effects during the weekly body weight evaluations.

Physiological parameters were unaffected by test diet consumption for the 1500 and 5000 ppm group males and females. There were no statistically significant differences between the control and test article-exposed groups (by sex) at the pre-test and study week 1, 3, 7, and 12 evaluations.

Locomotor activity:

Locomotor activity patterns (mean ambulatory and total motor activity counts) were unaffected by test diet consumption. Habituation was present for males and females in all test article-exposed groups at the study week 1, 3, 7, and 12 evaluations. Statistically significant differences from the control group were as follows.

Males:

In dose groups 5000 and 15000 ppm statistically significantly ($p \leq 0.01$) higher mean cumulative total counts (3168 and 2772 counts, respectively) were noted at the study week 12 evaluation compared to the concurrent control group (2267 counts); however, the values were within the laboratory's historical control range (2357-3885 counts). The increased mean cumulative total counts observed in these groups were attributed to the

slightly lower number of total counts observed in the control group. Mean cumulative total counts in the control group were lower during study week 12 compared to study weeks 1, 3, and 7 (3069-3599 counts) while the mean cumulative counts in the 15000 ppm group were generally similar throughout the study. Therefore, the higher mean cumulative total counts observed for the 5000 and 15000 ppm group males during study week 12 were not considered related to test article exposure (refer to Table B.6.7.1-5).

Females:

Statistically significant lower ($p \leq 0.05$) mean cumulative total counts (2909 and 3066 counts) were noted in the 5000 and 15000 ppm group respectively, during the study week 7 evaluation compared to the concurrent control group (4003 counts) and the minimum mean value in the laboratory's historical control range (3196 counts). However, the mean cumulative total counts in the control group were higher during study week 7 compared to study weeks 3 and 12 (3321 counts and 3215 counts, respectively) while the mean cumulative counts in the 5000 and 15000 ppm groups were generally similar throughout the study. It did not occur in a clear dose-related manner. These reductions were due to the slightly better habituation noted for females in the 5000 and 15000 ppm groups compared to the control group and as there was no effect during the study week 12 evaluation, they were not considered related to test article exposure (refer to Table B.6.7.1-6). Higher ($p \leq 0.01$) mean ambulatory counts were noted during study week 3 for the 1500 ppm group females during the 21-30 minute interval. This sporadic increase did not occur in a dose-related manner and therefore, was not attributed to the test article.

Table B.6.7.1-5:
Overview of motor counts in male rats

Parameters	♂ (ppm)				Historical control range[a]
	0	1500	5000	15000	
Total locomotor activity counts: cumulative					
- Pre-test	3089	2864	2749	2877	2043 - 3659 mean: 2830
- Wk 1	3599	3849	4311	3597	2515 - 3947 mean: 3277
- Wk 3	3069	3541	3345	3280	2495 - 4718 mean: 3384
- Wk 7	3333	3026	3627	3210	2734 - 3638 mean: 3178
- Wk 12	2267	2497	3168**	2772**	2357 - 3885 mean: 3166

* $p \leq 0.05$

a. laboratory historic control data (Rat CRL:CD(SD) . Range: min.- max. (refer to KCA 5.7.1/03, Appendix L) collected 2007 - 2010

Table B.6.7.1-6:
Overview of motor counts in female rats

Parameters	♀ (ppm)				Historical control range[a]
	0	1500	5000	15000	
Total locomotor activity counts: cumulative					
- Pre-test	3193	2922	2557	3136	2458 - 4187 mean: 3092
- Wk 1	3726	4225	3484	3863	2856 - 4126 mean: 3576
- Wk 3	3321	3491	2786	3635	2818 - 4837 mean: 3880

- Wk 7 - Wk 12	4003 3215	3503 3211	2909 2772	3066* 3085	3196 - 3871 mean: 3471 2480 - 4417 mean: 3519
Wk 3 – Ambulatory locomotor activity counts					
- 0-10 mins - 11-20 mins - 21-30 mins - 31-40 mins - 41-50 mins - 51-60 mins - Cumulative	351 166 69 67 63 31 747	379 101 181* 141 79 60 940	301 81 95 72 36 66 651	361 151 141 84 115 68 920	361 - 639 mean: 468 128 - 288 mean: 209 53 - 154 mean: 117 11 - 163 mean: 87 4 - 167 mean: 75 0 - 131 mean: 71 730 - 1424 mean: 1027

* $p \leq 0.05$

a. laboratory historic control data (Rat CRL:CD(SD) . Range: min.- max. (refer to KCA 5.7.1/03, Appendix L) collected 2007 - 2010

Gross pathology:

No test article related effects were observed. However, a 1-mm diameter depressed area in the right cerebral cortex of one female in the15000 ppm group was noted. However, this finding was not considered related to test article administration, because no macroscopic or microscopic findings were noted in the brain of any other animal examined in this dose group.

Neurohistopatholgy:

There were no test article-related histologic changes. There were instances of axonal degeneration in the peripheral nerves and in the spinal nerve roots. This axonal degeneration was of minimal severity, typically with only a single 'digestion chamber' and consistent with incidental alterations. Minimal axonal degeneration in the peripheral nerves and spinal nerve roots is a common background lesion (Eisenbrandt *et al.*[1]).

Organ weights:

Brain weights and measurements were unaffected by test article administration. Of note, the mean brain length of high dose females was 21.2 mm, 2% lower (statistically significant at $p \leq 0.01$) than the control group but the value was within the laboratory's historical control range (mean ±2SD; 20.22-21.98mm). These changes were therefore considered spurious and unrelated to test article administration.

Table B.6.7.1-7:
Overview of brain weights and neurohistopathology findings

Parameters	♂ (ppm)				♀ (ppm)			
	0	1500	5000	15000	0	1500	5000	15000
Ter. body wt (g)	552	566	553	473**	278	291	281	254*
Brain parameters (n=12)								

1 Eisenbrandt, D.L.; Mattsson, J.L.; Albee, R.R.; Spencer P.J.; Johnson, K.A. (1990). Spontaneous lesions in subchronic neurotoxicity testing of rats. *Toxicologic Pathology* **18**, pp 154-164.

Parameters	♂ (ppm)				♀ (ppm)			
	0	1500	5000	15000	0	1500	5000	15000
- Weight (abs): - Length (mm): - Width (mm):	2.37 22.85 15.4	2.37 22.4 15.3	2.36 22.4 15.4	2.33 22.4 15.3	2.11 21.7 14.8	2.09 21.5 14.9	2.13 21.7 14.9	2.07 21.2** 14.8
Neurohistopathology, axonal degeneration (incidence/total examined [severity: minimal, mild, moderate, severe])								
- Cerv. vent. fib.	0/6 [0,0,0,0]	-	-	1/6 [1,0,0,0]	0/6 [0,0,0,0]	-	-	0/6 [0,0,0,0]
- Lumbar dor. fib	0/6 [0,0,0,0] 1/6 [1,0,0,0]	-	-	1/6 [1,0,0,0] 1/6 [1,0,0,0]	0/6 [0,0,0,0] 2/6 [2,0,0,0]	-	-	2/6 [2,0,0,0] 1/6 [1,0,0,0]
- Lumbar ven. fib	0/6 [0,0,0,0]	-	-	1/6 [1,0,0,0]	1/6 [1,0,0,0]	-	-	0/6 [0,0,0,0]
- Peroneal nerve	3/6 [3,0,0,0]	-	-	1/6 [1,0,0,0]	2/6 [2,0,0,0]	-	-	1/6 [1,0,0,0]
- Sciatic nerve	0/6 [0,0,0,0]	-	-	1/6 [1,0,0,0]	1/5 [1,0,0,0]	-	-	0/6 [0,0,0,0]
- Spinal n. lum	0/6 [0,0,0,0]	-	-	1/6 [1,0,0,0]	0/6 [0,0,0,0]	-	-	0/6 [0,0,0,0]
- Sural nerve								

* $p \leq 0.05$; ** $p \leq 0.01$
Ter: terminal
Cerv. vent. Fib.: cervical ventral fibre
Lumbar dor. fib.: lumbar dorsal fibre
Lumbar ven. fib.: lumbar ventral fibre

Deficiencies:

None

Conclusions

Based on the result of this study, the NOAEL for general toxicity following 90 days of continuous dietary exposure is considered to be 5000 ppm (equivalent to 359 / 407 mg/kg bw/day for males / females), based on lower mean body weights and body weight gains.

No test article related differences in either neurobehavioral evaluations (FOB, motor activity) or neurohistopathology were observed in either sex. Therefore the NOAEL for neurotoxicity is considered to be >15000 ppm for both sexes (equivalent to 1111 / 1212 mg/kg bw/day for males / females, a dose which exceeds the maximum recommended dose (1000 mg/kg bw/day) for repeat dose toxicity studies).

3.12.1.6 CA 5.3.2/02 (1988b): 6-month oral toxicity study in the rat

Report

CA 5.3.2/02 (1988b)

Six month chronic oral toxicity study of S-31183 in rats

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0039

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

Reference	:	CA 5.3.2/02, 1988b	exposure	:	26 weeks, diet
Type of study	:	6-month oral toxicity study	dose	:	0, 80, 400, 2000 and 10000 ppm (nominal)[1]
year of execution	:	1986/88	vehicle	:	none
test substance	:	S-31183 tech. (pyriproxyfen), Lot no. PTG-86011, purity 97.2%	GLP statement	:	yes
route	:	oral	guideline	:	predominantly in accordance with OECD 452 (1998)
species	:	rat, Crj:CD (SD)	acceptability	:	acceptable
group size	:	21/sex/dose	**NOAEL**	:	24.0 mg/kg bw/day for males and 27.5 mg/kg bw/day for females

[1]equal to 0, 4.80, 24.0, 121 and 682 mg/kg bw/d for males and 0, 5.36, 27.5, 136 and 688 mg/kg bw/d for females

Study design

The study was generally in compliance with OECD 452 (1981). However, haematology and clinical biochemistry were only performed at sacrifice and urinalysis at week 26. Reticulocyte counts were not performed.

The dose levels were based on the results of the 13-week rat study. Serum activities of aspartate and alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase, creatinine phosphokinase and gamma-glutamyl transpeptidase were measured. Histopathology was performed on all organs from the control group and at 10000 mg/kg food, and on liver, kidney and lung from the other dose groups.

Materials and methods

A. Materials:

1.Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PTG-86011

Purity: 97.2%

CAS No.: 95737-68-1

Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Rat
Strain:	Crj:CD (SD)
Age:	5 weeks
Weight at dosing:	♂: 165 – 189 g; ♀: 133 – 161 g
Source:	Charles River Japan, Inc.
Acclimation period:	9 days

B. Study Design and Methods

1.In life dates:	20 October 1986 to 24 April 1987
2.Animal assignment and treatment:	Rats were selected and assigned to groups according to randomization by body weight stratification. After randomization and prior to treatment, animals were further observed for 3 days to assess body weight across groups. The animals were assigned to five groups, each consisting of 21 animals, at dose levels of 0, 80, 400, 2000 and 10000 ppm. The dietary mixture was prepared once biweekly after confirmation of stability.
3.Statistics	Parametric analysis of data on body weight, food consumption, water intake, organ weight, urinalysis, haematology, and clinical chemistry was performed using one-way analysis of variance. If the difference was significant at the 5% level, the LSD method was used to test the significant difference from the control group. The urinalysis data were tested by the Kruskal-Wallis test and if the difference was significant at the 5% level, the Scheffe-type rank sum test was used.
C. methods	
1.Observations	Animals were observed for mortality and moribundity and for any toxic symptoms or changes in behaviour once daily.
2.Body weights	Body weight was measured on days 1 and 3 of treatment, subsequently once weekly until week 13, and every four weeks from weeks 14 of treatment.
3.Food and water consumption	The food consumption and water intake over two consecutive days were measured by cage once weekly until week 13 of treatment and once every four weeks from week 14.

4.Ophthalmoscopic examination	At week 26 of treatment, ophthalmoscopic examinations were performed before and after mydriasis in all animals.
5.Urinalysis	At week 26 of treatment, urine samples were collected from all animals for urinalysis.
6.Haematology and clinical chemistry	At the end of treatment, all animals were fasted over 16 hours and blood samples were collected for haematological analysis. A portion of these samples were used to determine clinical chemistry parameters.
7.Pathology	All animals were necropsied and examined grossly for abnormal changes. Organs were weighed and fixed for histopathological examination.

Results

Clinical observations: No mortality was observed upon dietary exposure of rats to 80, 400, 2000 or 10000 ppm of pyriproxyfen technical. Soft faeces (yellowish white) were observed in animals at 10000 ppm; the incidence was highest in the initial three weeks of treatment and gradually decreased thereafter; the sign was no longer seen after 13 weeks.

Food and water consumption: No treatment-related effects on food or water consumption were observed.

Body weight: Body weights were significantly lower at 10000 ppm in both sexes throughout the study (-13% in males and females), resulting in marked decreases in body weight gain.

Table B.6.3.2-5: Body weight and body weight gain

Dose (ppm)	Males				Females			
	Week 2	Week 17	Week 52	Week 182	Week 2	Week 17	Week 52	Week 182
Body weight (g)								
0	191	304	444	583	152	203	268	328
80	192	302	437	576	155	212*	279	349*
400	192	303	438	581	154	206	261	320
2000	192	305	439	578	153	204	262	329
10000	179**	281**	393**	505**	145**	194**	246**	287**
Body weight gain (g)								
0	15	47	19	0	9	24	7	-1
80	15	48	18	3	11	26	8	6**
400	14	47	18	0	10	22	4	1
2000	15	48	18	2	9	22	8	4*
10000	4**	44	13**	0	0**	19**	6	2

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01.

Ophthalmoscopy: No treatment related findings.

Haematology: Erythrocyte counts, haemoglobin and haematocrit were significantly decreased in males at 2000 ppm and in males and females at 10000 ppm (93-97% of control). Mean cell haemoglobin concentration (MCHC) was significantly decreased in females at 2000 and 10000 ppm (99 and 98% of control, resp.), while mean cell haemoglobin (MCH) was significantly increased in males at 10000 ppm (102% of control). Platelet count was significantly decreased at 10000 ppm in females (90% of control).

Table B.6.3.2-6: Haematological findings

	0 ppm		80 ppm		400 ppm		2000 ppm		10000 ppm	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
RBC	835	729	845	737	830	711	807**	738	786**	685**
Haemoglobin	15.0	14.3	15.1	14.3	15.1	13.8*	14.6**	14.4	14.5**	13.3**
Haematocrit	43.6	40.8	43.9	40.8	43.8	39.7	42.4*	41.6	42.3*	38.5**
MCH	18.0	19.7	17.9	19.4	18.2	19.5	18.0	19.6	18.4*	19.4
MCHC	34.5	35.2	34.5	35.0	34.5	34.8	34.4	34.7*	34.4	34.5**
Platelet count	103.3	97.7	104.8	98.1	101.0	98.9	101.7	96.9	96.4	87.7**

*Significantly different from control, $p \leq 0.05$; **Significantly different from control, $p \leq 0.01$.

Clinical chemistry: Serum total protein and albumin values were significantly increased at 10000 ppm in both sexes (104-106% of control), probably a result of dehydration. The significantly increased serum calcium levels observed in both sexes at 10000 ppm (105% of control) were probably related to higher serum albumin levels at the highest exposure level. Serum protein electrophoresis indicated decreased serum β globulin levels in males at 2000 ppm (92% of control) and in males and females at 10000 ppm (86-92% of control), which could possibly reflect perturbations in β-lipoprotein synthesis by the liver, increased serum α_2 globulin levels in males and females at 10000 ppm (which in conjunction with proteinuria would be consistent with injury to the glomerular membrane), and increased serum α_1 globulin levels in females at 10000 ppm. Serum potassium and chloride levels were significantly lower in males at 10000 ppm, while serum sodium levels were significantly higher in females at 2000 and 10000 ppm. The hypernatraemia is consistent with dehydration while the hypokalaemia in conjunction with a higher urinary potassium output suggests that the kidneys were unable to conserve potassium adequately. The significantly increased blood urea nitrogen level in males at 10000 ppm is consistent with dehydration (which reduces renal blood flow and glomerular filtration rate). Serum glucose was decreased at 10000 ppm in both sexes (86-88% of control), a deviation frequently noted in animals that fail to thrive and gain weight. Serum cholesterol and phospholipid values were significantly increased at 2000 and 10000 ppm in males (cholesterol: 138-195% of control; phospholipids: 130-180% of control), with a dose-related trend, and at 10000 ppm in females (148% and 127% of control for cholesterol and phospholipid, respectively). Triglycerides were significantly decreased at 10000 ppm in males (54% of control). Gamma-glutamyl peptidase was significantly increased at 10000 ppm in males (600% of control), which in conjunction with the presence of bilirubin in the urine points to hepatobiliary effects and cholestasis. Serum cholinesterase activity was significantly reduced in females at 10000 ppm, which could reflect reduced

cholinesterase synthesis by the liver. Serum glutamic oxaloacetic acid transaminase (aspartate aminotransferase) activity was significantly reduced in males and females at 10000 ppm, while serum glutamic pyruvic transaminase (alanine aminotransferase) activity was significantly reduced in females at 2000 and 10000 ppm. The decreased transaminase activities were possibly caused by decreased hepatocellular production or release of the enzymes. Other significant deviations that were sporadically observed were not dose-related.

Table B.6.3.2-7: Clinical chemistry findings

	0 ppm		80 ppm		400 ppm		2000 ppm		10000 ppm	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Total protein	5.7	8.7	5.8	8.7	5.7	6.5	5.6	6.4*	5.9**	7.1**
Albumin (absolute)	3.3	4.1	3.3	4.0	3.3	4.0*	3.4	4.0*	3.8**	4.5**
A/G ratio	1.08	1.74	1.09	1.59*	1.14	1.86	1.20**	1.87	1.28**	1.80
Globulin α1	21.1	11.3	20.6	11.2	19.7*	10.5	19.1**	10.5	18.7**	12.5*
Globulin α2	5.1	4.3	5.4	4.6	5.0	4.7	5.2	4.5	5.8**	4.9
Globulin β	16.0	13.7	16.0	14.5	16.0	14.5	15.0**	14.1	14.1**	11.3**
BUN	16	23	15	24	15	22	16	24	18**	28**
Cholesterol	60	83	52	92	87	92	83**	91	117**	123**
Triglycerides	28	22	32	25	39*	22	28	12	15**	13
Phospholipids	80	154	75	161	90	157	104**	143	144**	185**
Cholinesterase	690	4481	680	4397	753	4235	670	4432	748	2862**
Calcium	9.3	9.8	9.2	9.7	9.4	9.7	9.4	9.7	9.8**	10.3**
γ-GT	0	1	1	1	1	1	1	1	6**	2
Potassium	5.1	4.4	5.0	4.3	4.9*	4.4	5.0	4.3	4.8**	4.1
Sodium	142	140	142	141	142	140	142	141**	143	142**
Chloride	108	107	108	107	108	106	109	107	107**	107
Glucose	154	133	156	138	165	136	142	130	132**	117**
AST	146	242	141	275	157	327	150	146	104**	104**
ALT	58	106	57	122	77	137	48	54*	40	41**

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01.

Urinalysis: In both sexes in the 10000 ppm group, the number of animals positive for urinary protein increased significantly. In females in this group, the number of animals positive for bilirubin increased significantly and that of animals positive for ketone bodies decreased significantly. The tendency of an increase in number of bilirubin-positive was also observed in the 10000 ppm male group. In both sexes in the 10000 ppm group, an increase in number of yellowness or brownish-yellowness in appearance was observed, indicating a slight darkening of urine colour. In the 10000 ppm group, the number of females with elevated urine specific gravity increased. The increases in urinary potassium excretion and sodium excretion were observed in both sexes in the 10000 ppm group. No treatment-related changes were found in the urinary sediment, volume or osmotic pressure.

Organ weights: Absolute liver weight was significantly increased at 10000 ppm in males and females (134% and 128 % of control, respectively), and relative liver weight was significantly increased at in males at 2000 ppm (109% of control) and in males and females at 10000 ppm (156 and 148% of control, resp.). The microscopic correlate of the increased liver weights at 10000 ppm was hepatocellular hypertrophy, but no microscopic correlate was seen at 2000 ppm. Absolute kidney weight was increased at 10000 ppm in males (107% of control) and relative kidney weight was significantly increased at 10000 ppm in both sexes (125 and 116% of control, resp.).

Table B.6.3.2-8: Organ weight findings

Organ	0 ppm		80 ppm		400 ppm		2000 ppm		10000 ppm	
	m	f	m	f	m	f	m	f	m	f
Absolute organ weights (g)										
Liver	12.78	7.59	12.67	8.05	13.23	7.50	13.89	7.90	17.18**	9.75**
Kidney	3.41	2.13	3.63	2.22	3.56	2.12	3.61	2.10	3.64	2.14
Adrenal	52	72	54	75	51	71	53	71	58	64**
Pituitary	15	31	14	28	15	29	16	26*	15	24**
Thyroid	23	19	23	19	22	19	22	21	23	23**
Thymus	0.17	0.16	0.20	0.19	0.19	0.16	0.16	0.19	0.13*	0.16
Lung	1.51	1.13	1.45	1.20*	1.49	1.10	1.49	1.17	1.39**	1.05**
Heart	1.58	1.03	1.57	1.09*	1.58	1.01	1.67	1.02	1.44**	0.96**
Brain	2.22	2.02	2.16	2.06	2.16	2.01	2.17	2.03	2.16	2.01
Spleen	0.78	0.53	0.78	0.55	0.79	0.54	0.80	0.51	0.75	0.44**
Testis	3.52	-	3.49	-	3.48	-	3.44	-	3.62	-
Ovary	-	60	-	65	-	69	-	67	-	57
Relative organ weights (organ/bw ratio)										
Liver	2.29	2.43	2.29	2.43	2.37	2.49	2.50**	2.53	3.58**	3.80**
Kidney	0.61	0.68	0.68	0.67	0.64	0.70	0.65	0.67	0.76**	0.79**
Adrenal	9.4	23.0	9.8	23.0	9.3	23.5	9.7	22.8	12.0**	23.6
Pituitary	2.6	9.8	2.6	8.5	2.6	9.5	2.8*	8.3	3.1*	8.9
Thyroid	4.1	6.1	4.1	5.7	3.9	6.4	4.0	6.7	4.9*	8.5**
Thymus	0.03	0.05	0.04	0.06	0.03	0.05	0.03	0.06	0.03	0.06
Lung	0.27	0.36	0.26	0.36	0.27	0.37	0.27	0.37	0.29*	0.39**
Heart	0.28	0.33	0.29	0.33	0.29	0.33	0.31	0.33	0.30	0.35**
Brain	0.40	0.65	0.39	0.63	0.39	0.67	0.40	0.65	0.45**	0.74**
Spleen	0.14	0.17	0.14	0.17	0.14	0.18	0.14	0.18	0.16**	0.18
Testis	0.63	-	0.64	-	0.63	-	0.63	-	0.76**	-
Ovary	-	19.6	-	19.7	-	22.8	-	21.5	-	21.0

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01.

Pathology: Macroscopy revealed blackish brown colouration of the liver in most males at 10000 ppm (20/21). This finding was not observed in males in any other dose group or in females. Microscopic examination

showed hepatocellular hypertrophy to be present in all animals (males and females) of the 10000 ppm group; this hypertrophy was not observed in any of the other dose groups.

Acceptability

The study is considered acceptable.

Conclusions

Based on increased liver weights, higher serum cholesterol and phospholipid levels and reduced serum β globulin levels at 2000 ppm and above, the NOAEL is set at 400 ppm (= 24 mg/kg bw/day for males and 27.5 mg/kg bw/day for females).

3.12.1.7 CA 5.3.2/03 (1988c): 90-day oral toxicity study in the dog

Report

CA 5.3.2/03 (1988)
Three-month oral toxicity study of S-31183 in dogs
Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0037

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

Reference	:	CA 5.3.2/03, 1988	exposure	:	90 days, gelatin capsule
Type of study	:	90 day oral toxicity study	dose	:	0, 100, 300 and 1000 mg/kg bw/d
year of execution	:	1986	vehicle	:	gelatin capsule
test substance	:	S-31183 tech. (pyriproxyfen), Lot no. PTG-86011, purity 97.2%	GLP statement	:	yes
route	:	oral	guideline	:	predominantly in accordance with OECD 409 (1998)
species	:	dog, Beagle	acceptability	:	acceptable
group size	:	4/sex/dose	**NOAEL**	:	100 mg/kg bw/day

Study design

The study was generally in compliance with OECD 409 (1998) without functional observations.

Materials and methods

A. Materials:

1.Test Material:	S-31183 (pyriproxyfen)
Lot/Batch No.:	PTG-86011
Purity:	97.2%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Dog
Strain:	Beagle
Age:	6 months
Weight at dosing:	♂: 7.7 – 11.1 kg; ♀: 7.1 – 11.1 kg
Source:	White Eagle Laboratories Inc. (USA)
Acclimation period:	Not given

B. Study Design and Methods

1.In life dates:	28 August 1986 to 29 November 1986
2.Animal assignment and treatment:	The animals were assigned to three dose groups (100, 300 and 1000 mg/kg bw/day) and a control group. The test substance was administered once a day, orally via gelatin capsules. Dose levels were based on a preliminary study (28-day in dogs).
3.Statistics	Data of all examination items, excluding the results of observations and semi-quantitative analyses, were analysed for significant differences between the control and each dose group by ASSIT method (Yamazaki et al, 1981, J. Takeda Res. Lab. 40(3/4), 163-187).
C. methods	
1.Observations	Clinical signs were observed before administration and every two or three hours after administration to about 5 pm.
2.Body weights	Body weight was measured once a week for two weeks preceding the beginning of administration and during the administration period.

3.Food consumption	The amount of leftover food was measured daily in the morning two weeks preceding the beginning of administration and during the administration period.
4.Ophthalmoscopic examination	Ophthalmoscopic examination was performed at weeks 0, 6 and 12 of administration.
5.ECG	Electrocardiogram was recorded on weeks 0, 5 and 12 of administration.
6.Haematology and clinical chemistry	Blood samples were drawn on week 0, 4, 8 and 12 of administration. These blood samples were used to determine haematological and clinical chemistry parameters.
7.Urinalysis	Urinalysis was performed on weeks 0, 6 and 11 of administration.
8.Liver and kidney function test	To examine liver function, BSP retention test was performed on weeks 0, 6 and 13. To examine renal function, PAH retention test was performed on weeks 0, 5 and 11.
9.Pathology	Pathological examination was performed on all animals at the end of the administration period. Animals were macroscopically examined and organs were weighed and analysed for histopathology.

Results

Clinical observations: No mortality was observed upon capsule exposure of dogs to 100, 300 or 1000 mg/kg bw/day of pyriproxyfen technical. The incidence of soft faeces and diarrhoea was increased in 3 females at 1000 mg/kg bw.

Food consumption: No treatment-related effects on food consumption were observed.

Body weight: No treatment-related effects on body weight were observed.

Table B.6.3.2-9: Body weight

Dose (mg/kg bw/day)	Males				Females			
	Week 1	Week 4	Week 9	Week 13	Week 1	Week 4	Week 9	Week 13
0	10.0	10.4	11.0	11.4	9.4	9.8	10.6	11.0
100	10.2	10.5	11.1	11.4	9.5	9.9	10.3	10.6
300	10.5	10.9	11.5	12.0	9.7	10.0	10.4	10.7
1000	10.4	10.8	11.6	12.0	9.2	9.7	10.1	10.5

Ophthalmoscopy: Ophthalmoscopy revealed no treatment-related findings.

ECG: Electrocardiography revealed no treatment-related findings.

Haematology: No statistically significant changes were observed in haematology parameters; however, platelet count was increased, when compared to the pre-test value, in females at 1000 mg/kg bw during the whole exposure period (increase max. 30%, not significant).

Clinical chemistry: Only few statistically significant changes were observed in biochemical parameters that were considered not to be toxicologically relevant. However, alkaline phosphatase and lactate dehydrogenase activities were increased, during the exposure period, when compared to pre-test values, in males at 1000 mg/kg bw/day (alkaline phosphatase: 114-119% of pre-test value; lactate dehydrogenase: 114-128% of pre-test value). Similarly, serum cholesterol (112-123%, 113-126% and 120-131% of pre-test value for the 100, 300 and 1000 mg/kg bw/day groups, respectively; range given for measurements at 4, 8 and 12 weeks) and phospholipids levels (105-116%, 102-112% and 113-123% of pre-test value for the 100, 300 and 1000 mg/kg bw/day groups, respectively; range given for measurements at 4, 8 and 12 weeks) were generally higher during the exposure period than at pre-test in all groups of treated males, while values in control males during the exposure period were decreased relative to pre-test values.

Table B.6.3.2-10: Clinical chemistry findings

Parameters		♂ (mg/kg bw/d)				♀ (mg/kg bw/d)			
	wk	0	100	300	1000	0	100	300	1000
Total bilirubin (mg/dL)	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	8	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	12	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
GOT (U/I)	0	34	33	32	35	28	31	30	32
	4	38	34	32	34	28	31	31	31
	8	34	39	31	36	26	32	33	32
	12	38	34	32	38	29	33	32	33
GPT (U/I)	0	47	44	44	39	42	48	41	52
	4	51	44	42	32**	48	43	40	49
	8	50	51	43	38	42	48	48	51
	12	55	51	51	43	50	49	46	58
ALP (I/U)	0	137	144	146	161	166	153	143	140
	4	126	132	138	165	157	125	136	162
	8	117	112	137	192	147	116	121	159
	12	86	88	101	184	128	105	98	128
CPK (I/U)	0	161	197	273	180	175	142	156	189
	4	132	163	134	150	131	111	133	151
	8	92	132	132	129	98	95	143	95
	12	107	149	142	158	134	133	123	141
γGT	0	3	3	2	2	2	3	2	3

Parameters		♂ (mg/kg bw/d)				♀ (mg/kg bw/d)			
	wk	0	100	300	1000	0	100	300	1000
(U/I)	4	3	3	3	3	2	3	3	3
	8	3	3	2	3	2	2	3	2
	12	3	2	2	3	1	3	3	2
LDH (mg/dL)	0	128	142	143	147	145	101	96	158
	4	108	126	135	158	128	115	153	184
	8	92	133	167	188	83	96	141	136
	12	92	139	147	168	119	139	144	200
Total cholesterol (mg/dL)	0	152	176	145	144	145	150	140	154
	4	129	197	164	173	141	148	163	191
	8	132	207	182	188	160	171	192	211
	12	130	216	187	178	161	179	196	203
Phospholipids (mg/dL)	0	302	331	289	288	298	301	294	307
	4	270	347	297	322	285	283	313	348
	8	273	385	3214	347	323	326	354	384
	12	280	382	323	336	338	343	358	388

** statistically different from control ($p<0.01$)

Urinalysis and faecal examination: Urinalysis and faecal examination showed no treatment-related findings.

Liver and renal function: No treatment-related effects were found.

Organ weights: Absolute liver weights were significantly increased in males at 300 and 1000 mg/kg bw/day (130-126% of control), relative liver weights were significantly increased in males at 300 mg/kg bw/day (124% of control, resp.) and increased in males at 1000 mg/kg bw/day (121% of control). Absolute and relative liver weight were increased in females at 1000 mg/kg bw/day (111 and 116% of control, resp.).

Table B.6.3.2-11: Absolute and relative organ weights

	Dose (mg/kg bw/day)							
	0		**100**		**300**		**1000**	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Absolute organ weights (g)								
Thyroid	0.83	0.85	0.98	0.81	0.94	1.04	0.92	1.03
Thymus	13.0	10.8	12.2	15.2	13.4	19.4	12.0	13.3
Liver	304	299	338	291	395*	334	384*	333
Spleen	27.0	27.8	25.1	25.8	30.5	23.1	25.6	25.3
Kidneys	51.8	40.8	51.0	41.8	52.5	43.4	49.3	39.5
Adrenals	1.14	1.24	1.08	1.27	1.08	1.35	1.30	1.08
Testes	21.3	-	18.4	-	19.1	-	19.1	-
Prostate	4.7	-	4.7	-	8.1	-	3.7	-
Ovaries	-	0.74	-	0.72	-	0.83	-	0.71

Uterus	-	2.4	-	3.5**	-	2.5	-	2.5
Relative organ weights (organ/bw ratio)								
Thyroid	0.0075	0.0078	0.0089	0.0078	0.0079	0.0099	0.0078	0.0095
Thymus	0.116	0.100	0.109	0.144	0.115	0.179	0.102	0.125
Liver	2.71	2.75	3.01	2.78	3.35*	3.17	3.27	3.18
Spleen	0.242	0.256	0.228	0.245	0.258	0.220	0.215	0.248
Kidneys	0.458	0.375	0.458	0.399	0.444	0.415	0.416	0.385
Adrenals	0.0104	0.0113	0.0095	0.0122	0.0091	0.0131	0.0111	0.0106
Testes	0.189	-	0.185	-	0.161	-	0.162	-
Prostate	0.042	-	0.042	-	0.052	-	0.031	-
Ovaries	-	0.0067	-	0.0069	-	0.0079	-	0.0070
Uterus	-	0.022	-	0.035	-	0.027	-	0.026

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01.

Pathology: The liver was found to be enlarged in 2 males and 1 female at 1000 mg/kg bw/day. Histopathological examination of the liver showed hepatocellular hypertrophy to be present in all animals at 1000 mg/kg bw/day and in 3 out of 4 females at 300 mg/kg bw/day. Eosinophilic bodies in the liver were observed in 2 males and 2 females at 1000 mg/kg bw/day. Electron microscopy of the control group and the 1000 mg/kg bw/day group revealed increased SER in all animals at 1000 mg/kg bw/day accompanied by slight dilatation in 1 male and all females. These changes are consistent with microsomal enzyme induction.

Acceptability

The study is considered acceptable.

Conclusions

Alkaline phosphatase and lactate dehydrogenase activities were increased, during the exposure period, when compared to pre-test values, in males at 1000 mg/kg bw/day. Similarly, serum cholesterol and phospholipids levels were generally higher during the exposure period than at pre-test in all groups of treated males, while values in control males during the exposure period were decreased relative to pre-test values.

Absolute liver weights were significantly increased in males at 300 and 1000 mg/kg bw/day, relative liver weights were significantly increased in males at 300 mg/kg bw/day. Absolute and relative liver weight were increased in females at 1000 mg/kg bw/day . The liver was found to be enlarged in 2 males and 1 female at 1000 mg/kg bw/day. Histopathological examination of the liver showed hepatocellular hypertrophy to be present in all animals at 1000 mg/kg bw/day and in 3 females at 300 mg/kg bw/day. Eosinophilic bodies in the liver were observed in 2 males and 2 females at 1000 mg/kg bw/day. Electron microscopy of the control group and the 1000 mg/kg bw/day group revealed increased SER in all animals at 1000 mg/kg bw/day accompanied by slight dilatation in 1 male and all females. These changes are consistent with microsomal enzyme induction. Changes in cholesterol and phospholipids at 100 mg/kg bw/day were not accompanied by changes in liver

weight or histopathological changes in the liver. Therefore, the NOAEL is established at 100 mg/kg bw/day, based on the increased liver weight and histopathological changes in the liver at 300 mg/kg bw/day.
RMS conclusion: RMS agrees with the original conclusion.

Following the commenting round, the applicant has provided an additional statement regarding the microsomal enzyme indiction, liver weight and histological changes:
Generally, proliferation (including dilatation) of smooth endoplasmic reticulum (SER), which is typically characterized by "increased SER", is a common adaptive change that occurs in the liver of animals treated with microsomal enzyme inducers. It is well known that the effects on the liver including increased liver weight and hepatocellular hypertrophy are observed when microsomal enzyme induction occur (Amacher et al (2001)), which parallels with the rat.
In this study, 300 mg/kg/day and above groups animals showed increased SER, along with associated effects on the liver including increased liver weight and hepatocellular hypertrophy, and therefore these changes are considered to indicate microsomal enzyme induction caused by treatment with Pyriproxyfen.

Additional remark RMS NL:
The statement of the applicant contains the following reference:
Amacher, D.E., Schomaker, S.J., Burkhardt, J.E., 2001: The relationship among microsomal enzyme induction, liver weight and histological change in beagle dog toxicology studies. Food and Chemical Toxicology 39 (2001) 817-825.
Abstract
The present study represents a retrospective analysis of hepatic microsomal enzyme induction data collected over a period of years for the beagle dog. Comparisons were completed for up to six enzyme activities and P450 content versus histopathological examination of the liver for hepatic changes and serum chemistry data analysis for markers indicative of hepatic injury. In addition, qualitative comparisons were made for these compounds to data reported in the rat by the same authors. In this analysis of canine study data for nine different compounds comprising five different pharmacological classes, significant elevations in several microsomal enzyme activities were observed under study conditions that did not result in liver weight increases, histological changes or serum chemistry changes that would be indicative of hepatocellular or hepatobiliary damage. Despite some species differences in cytochome P450 homologues, for this compound set, there was clearly a general association between the response in dog liver and that of the rat liver. Compounds that elicited significant increases in more than one canine P450 endpoints were also likely to produce an inductive response in rat liver; however, the magnitude of the response and the P450 endpoint involved were not always identical. We conclude that hepatic drug-metabolizing enzyme induction in the beagle dog liver is typically a benign adaptive response, which parallels that reported previously in the rat.

3.12.1.8 CA.5.3.2/04 (1991): 1-year toxicity oral study in the dog

Report

CA 5.3.2/04 (1991)

S-31183: Toxicity study by oral (capsule) administration to Beagle dogs for 52 weeks

Amended final report. Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-11-0081

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

Reference	:	CA 5.3.2/04, 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.

Materials and methods

A. Materials:

1.Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PYG-87074

Purity: 95.3%

CAS No.: 95737-68-1

Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Dog
Strain:	Beagle
Age:	16 – 20 weeks
Weight at dosing:	7.0 – 9.2 kg
Source:	Consort Limited (Herefordshire, UK)
Acclimation period:	7 weeks

B. Study Design and Methods

1.In life dates:	4 August 1988 to 7 August 1989.
2.Animal assignment and treatment:	Animals were randomly assigned to different treatment groups (4 animals/sex/dose), with the following dose levels: 0, 30, 100, 300 or 1000 mg/kg bw/day. The formulation was given orally once each day seven days per week via gelatin capsules. All capsules were prepared at least 24 hours before administration but within the stability period of 4 days.
3.Statistics	The significance of inter-group differences in body weight change, myeloid to erythroid ratio, blood composition and urinary volume, pH, SG and electrolytes was assessed by Students t-test using a pooled error variance. Inter-group differences in organ weights were assessed by Dunnett's test and differences in histopathology were assessed using Fisher's Exact Probability test.
C. methods	
1.Observations	Dogs were inspected regularly throughout the working day for evidence of reaction to treatment or ill-health. In addition, individual daily observations of all animals were recorded before and shortly after each dose. A more detailed weekly examination was performed on each animal. In addition, each animal was subjected to a rigorous veterinary examination before commencement of treatment and after 12, 26, 38 and 51 weeks of treatment.
2.Body weights	Each animal was weighed at weekly intervals throughout the acclimatisation and treatment period.
3.Food consumption	The weight of the food refused by each animal and an estimate of the amount spilled was recorded each day. From these records, the

	consumption per animal was calculated for the final two weeks of acclimatisation and for each complete week of the treatment period.
4.Ophthalmoscopic examination	Before commencement of the study and after 26 and 51 weeks of treatment both eyes of all dogs were examined.
5.Haematology and clinical chemistry	Before commencement of the study and after 12, 24, 37 and 50 weeks of treatment, blood samples were withdrawn for haematological examination and clinical chemistry. In addition, several days before the terminal sacrifice, bone marrow samples were obtained by biopsy from the iliac crest for haematology examination of the bone marrow.
6.Urinalysis	Before commencement of the study and after 11, 23, 35 and 49 weeks of treatment, each animal was placed in an individual metabolism cage and an overnight urine sample was collected for urinalysis.
7.Pathology	All animals were subjected to a detailed necropsy. Selected organs were weighed and examined for histopathology.

Results

Observations: Two males at 1000 mg/kg bw/day 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 diarrhoea was noted at 1000 mg/kg bw/day in both sexes.

Food consumption: A small reduction of food consumption was observed in males at 1000 mg/kg bw/day during the whole exposure period.

Body weight: Body weight gains were significantly reduced in males at 300 mg/kg bw/day and in females at 300 and 1000 mg/kg bw/day after 13 weeks of treatment. At termination, there was a significant weight gain deficit in males and females at 300 mg/kg bw/day and in females at 1000 mg/kg bw/day.

Table B.6.3.2-12: Body weight gains

Dose (mg/kg bw/day)	Males					Females				
	D0-91	D91-182	D182-273	D273-364	D0-364	D0-91	D91-182	D182-273	D273-364	D0-364
0	2.8	0.4	0.6	0.1	3.9	1.8	0.8	0.3	0.7	3.5
30	2.5	0.5	0.7	0.2	3.9	1.9	0.6	0.5	0.5	3.4
100	2.0	0.1	0.5	0.1	2.7	1.9	0.2	0.7	0.2*	3.0

300	1.5*	0.1	0.5	0.0	2.0**	1.5	-0.1*	0.6	0.2*	2.2*
1000	0.3**	0.3	1.1	0.6*	3.6	0.9*	0.2	0.9*	0.1*	2.0*

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01.
D = days of treatment

Ophthalmoscopy: Ophthalmoscopy revealed no treatment-related findings.

Haematology: Slightly low haematocrit, haemoglobin and erythrocyte counts were apparent in males at ≥300 mg/kg bw/day (87-89% of control) after 12 and 24 weeks of treatment and in females at ≥100 mg/kg bw/day (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/day (105-106% of control) in the first six months of treatment and in females at ≥100 mg/kg bw/day (103-107% of control) throughout treatment, suggesting that the anaemia was macrocytic. Platelet count was significantly increased at ≥300 mg/kg bw/day in males (138-221% of control) and at 1000 mg/kg bw/day in females (123-147% of control). Prothrombin time was significantly increased at ≥300 mg/kg bw/day in males (106-164% of control) and at 1000 mg/kg bw/day 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.

Table B.6.3.2-13: Haematological findings

Parameter	0		30		100		300		1000	
	m	f	m	f	m	f	m	f	m	f
Week 12										
RBC	6.47	6.80	6.02	6.35	6.21	5.85**	5.63**	5.66**	5.75*	6.11*
MCV	65	65	67	66	68	69***	68**	69***	69**	69***
Platelet count	190	228	228	213	210	211	255	239	399***	315**
Prothrombin time	7.7	8.2	7.9	8.4	7.6	8.0	8.5	8.1	9.7***	8.9*
Week 24										
RBC	6.59	6.41	6.20	6.29	6.15	6.43	5.61**	5.88	5.97	6.05
MCV	66	67	69	69	68	70*	70*	70*	70*	71***
Platelet count	181	234	240	225	232	227	267*	243	400***	288*
Prothrombin time	8.1	8.1	7.8	8.4	8.1	8.2	8.6*	8.4	9.2***	8.6
Week 50										
RBC	6.83	6.51	6.52	6.25	6.59	6.49	6.18	6.25	6.99	6.30
MCV	65	65	66	67*	66	67*	68	66	68	69***
Platelet count	168	200	196	207	212	200	232*	212	286**	269**
Prothrombin time	7.4	7.4	7.2	7.6	7.3	7.4	7.6	7.4	12.1***	8.6*

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01; *** Significantly different from control, p≤0.001.

Clinical chemistry: Alanine aminotransferase activity was significantly and markedly increased during the whole exposure period at 1000 mg/kg bw/day 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/day in males (135-247% of control) after 12, 24 and 37 weeks. Alkaline phosphatase activity was markedly increased at ≥300 mg/kg bw/day in males (227-496% of control) and females (221-356% of control) throughout treatment. Total bilirubin was significantly increased at 1000 mg/kg bw/day 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/day 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/day 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/day, probably a result of frequent vomiting.

Table B.6.3.2-14: Clinical chemistry findings at week 50

	0		30		100		300		1000	
Parameter	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
AP	55	72	59	75	114	68	151*	94	273***	109
ALT	44	28	45	49	38	27	76	38	255**	121*
AST	33	16	30	49*	17*	23	38	23	45	30
Total bilirubin	0.2	0.3	0.2	0.2	0.2	0.2	0.3	0.3	0.5**	0.3
Cholesterol	103	222	154	168*	265***	172*	251**	179	103	173*
Triglycerides	33	47	33	54	59**	48	62**	59	49	58
Glucose	102	110	107	109	99	100*	105	100*	78*	101
Chloride	119	114	118	117*	116*	115	117	115	118	115

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01; *** Significantly different from control, p≤0.001.

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

Organ weights: Absolute liver weights were significantly increased in males at ≥ 100 mg/kg bw/day (147-191% of control) and in females at ≥ 300 mg/kg bw/day (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/day (157-148% of control). Absolute thyroid weights were significantly increased in females at ≥ 300 mg/kg bw/day (163-182% of control) and relative thyroid weights were significantly increased in females at ≥ 100 mg/kg bw/day (175-200% of control).

Table B.6.3.2-15: Organ weight findings

Parameter	0		30		100		300		1000	
	m	f	m	f	m	f	m	f	m	f
Absolute organ weights (g)										
Kidneys	60	56	66	59	68	58	73	65	78	63
Liver	365	389	476	416	538*	486	613**	561*	697*	542*
Ovaries	-	1.73	-	1.32	-	1.48	-	1.74	-	0.98
Prostate	13.03	-	9.55	-	7.29	-	7.73	-	10.16	-
Testes	19.6	-	21.8	-	17.9	-	21.7	-	18.2	-
Thymus	11.1	11.1	13.2	14.1	12.6	13.5	8.7	10.8	9.8	12.4
Uterus	-	12.6	-	7.3	-	10.7	-	13.2	-	5.6
Thyroid	0.76	0.57	0.81	0.76	0.89	0.85	0.86	1.04**	0.88	0.93*
Relative organ weights (organ weight/body weight, in g)										
Kidneys	0.4	0.4	0.4	0.4	0.5	0.4	0.6*	0.5*	0.5	0.5*
Liver	2.4	2.8	3.1*	2.9	3.8**	3.8	4.6**	4.4**	4.8**	4.3**
Ovaries	-	0.012	-	0.009	-	0.012	-	0.014	-	0.008
Prostate	0.083	-	0.063	-	0.053	-	0.057	-	0.070	-
Testes	0.13	-	0.14	-	0.13	-	0.16	-	0.13	-
Thymus	0.07	0.08	0.09	0.10	0.09	0.10	0.07	0.09	0.07	0.10
Uterus	-	0.09	-	0.05	-	0.09	-	0.10	-	0.04
Thyroid	0.005	0.004	0.005	0.005	0.006	0.007*	0.006	0.008**	0.006	0.007**

*Significantly different from control, p≤0.05; **Significantly different from control, p≤0.01.

Pathology: 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/day, an irregular surface of the liver of three animals at 1000 mg/kg bw/day, 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/day. Histopathological examination revealed significant liver damage in all but one of the animals at 1000 mg/kg bw/day, 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.

Table B.6.3.2-16 Gross pathology and histopathology results

Dose (mg/kg bw)	0		30		100		300		1000	
	m	f	m	f	m	f	m	f	m	f
Pathology										

Dose (mg/kg bw)	0		30		100		300		1000	
	m	f	m	f	m	f	m	f	m	f
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

Acceptability

The study is considered acceptable.

Conclusions

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.

RMS conclusion during renewal: In males, liver weight was increased significantly from 30 mg/kg bw/day and higher. In addition, cholesterol was elevated at all dose levels, reaching significance from 100 mg/kg bw/day onwards. However, also for cholesterol the level measured at 30 mg/kg bw/day was already 50% higher than the control values. Therefore, for males, the originally proposed NOAEL of <30 mg/kg bw/day is still considered acceptable.

In females, liver weights were increased reaching significance ≥300 mg/kg bw/day, however, already at 100 mg/kg bw/day the liver weight was >20% higher than control values. Therefore, the originally proposed NOAEL of 30 mg/kg bw/day for females is still considered acceptable.

3.12.1.9 CA 5.3.2/05 (1993): 1-year oral toxicity study in the dog

Report

CA 5.3.2/05 (1993) S-31183

Toxicity study by oral (capsule) administration to Beagle dogs for 52 weeks (additional investigation)

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-31-0102

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

Reference	:	CA 5.3.2/05, 1993	exposure	:	52 weeks, capsule
Type of study	:	52-week oral toxicity study	dose	:	0, 3 and 10 mg/kg bw/day
year of execution	:	1991/92	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**	:	10 mg/kg bw/day for males and females

Study design

The study was generally in compliance with OECD 452 (1981). Electrocardiography was not performed. This study is a follow-up of a 52-week study with higher dose levels (Chapman, 1991; see study 4).

Materials and methods

A. Materials:

1.Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PYG-87074

Purity:	95.3%
CAS No.:	95737-68-1
Stability of test compound:	Confirmed stable for the duration of the study
2.Test animals:	
Species:	Dog
Strain:	Beagle
Age:	19 - 24 weeks
Weight at dosing:	7.0 to 9.0 kg (males); 6.1 to 8.9 kg (females)
Source:	Consort Limited (Herefordshire, UK)
Acclimation period:	4 weeks

B. Study Design and Methods

1.In life dates:	23 September 1991 to 24 September 1992
2.Animal assignment and treatment:	Animals were randomly assigned to the different treatment groups (4/sex/dose), consisting of the following doses: 0, 3 and 10 mg/kg bw/day. The test substance was administered via gelatin capsules; the capsules were administered within 5 days of preparation. The test substance was administered orally once each day, seven days a week.
3.Statistics	The significance of inter-group differences in bw gain, haematology, blood chemistry and urinalysis was assessed by Student's t-test using a pooled error variance. For organ weights, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant, a Behren's Fisher test was used to perform pairwise comparisons, otherwise, a Dunnett's test was used. Inter-group differences in ophthalmoscopy, macroscopic pathology and histopathology were assessed using Fisher's Exact test. Because of the small number of animals in each group, the results of these tests cannot be considered definitive and are used merely as a guide in the interpretation of the results.
C. methods	
1.Observations	Dogs were inspected regularly throughout the working day for evidence of reaction to treatment or ill-health. In addition, individual daily observations of all animals were recorded before and shortly after each dose. A more detailed weekly examination was performed on each animal. In addition, each animal was subjected to a rigorous

	veterinary examination before commencement of treatment and after 12, 26, 38 and 51 weeks of treatment.
2.Body weights	Each animal was weighed at weekly intervals throughout the acclimatisation and treatment period.
3.Food consumption	The weight of the food refused by each animal and an estimate of the amount spilled was recorded each day. From these records, the consumption per animal was calculated for the final two weeks of acclimatisation and for each complete week of the treatment period.
4.Ophthalmoscopic examination	Before commencement of the study and after 26 and 51 weeks of treatment both eyes of all dogs were examined.
5.Haematology and clinical chemistry	Before commencement of the study and after 12, 24, 37 and 50 weeks of treatment, blood samples were withdrawn for haematological examination and clinical chemistry. In addition, several days before the terminal sacrifice, bone marrow samples were obtained by biopsy from the iliac crest for haematology examination of the bone marrow.
6.Urinalysis	Before commencement of the study and after 11, 23, 35 and 49 weeks of treatment, each animal was placed in an individual metabolism cage and an overnight urine sample was collected for urinalysis.
7.Pathology	All animals were subjected to a detailed necropsy. Selected organs were weighed and examined for histopathology.

Results

Observations: No mortality and clinical signs were observed.

Food consumption: Throughout the treatment period, food consumption was similar in control and treated animals.

Body weight: There were no inter-group differences in body weight gain over the treatment period that were attributed to the administration of pyriproxifen. The weight gains of females receiving 3 mg/kg bw/day were slightly lower than those of the controls (-20%). This was considered to be due mainly to one animal with a lower weight gain than expected. Because there was no similar change among females receiving 10 mg/kg bw/day, this was considered to be a chance event.

Ophthalmoscopy: No treatment-related findings were observed.

Haematology: Platelet counts were significantly increased at 10 mg/kg bw/day in males after 36 weeks (126% of control) and in females after 36 and 50 weeks (144-125% of control), and at 3 mg/kg bw/day in males after 24, 36 and 50 weeks (126-140% of control). Prothrombin time was slightly but significantly increased in females at 3 mg/kg bw/day after 24 and 50 weeks (108% of control) and in females at 10 mg/kg bw/day after

50 weeks (110% of control). In the absence of consistent dose-time-effect relationships and since effects were not observed at higher dose levels (Chapman, 1991), these deviations were considered to be incidental.

Table B.6.3.2-17 Haematological findings

Parameters		♂ (mg/kg bw/d)			♀ (mg/kg bw/d)		
	wk	0	3	10	0	3	10
PCV (%)	12	41 ±2	41 ±2	40 ±1	43 ±4	43 ±3	42 ±3
	24	43 ±2	45 ±3	44 ±1	46 ±3	47 ±2	47 ±2
	36	43 ±3	45 ±4	44 ±2	46 ±4	47 ±5	47 ±3
	50	49 ±2	49 ±3	48 ±3	46 ±3	52 ±3*	49 ±2
Hb (g%)	12	13.2 ±0.9	13.4 ±0.9	13.0 ±0.4	13.9 ±1.2	14.1 ±1.3	13.7 ±0.8
	24	14.0 ±0.7	14.5 ±1.1	14.2 ±0.2	15.0 ±1.2	15.2 ±0.8	15.1 ±0.9
	36	14.6 ±0.8	15.2 ±1.3	14.9 ±0.6	15.6 ±1.4	15.7 ±1.6	15.4 ±0.8
	50	16.6 ±1.0	16.1 ±1.3	16.0 ±1.1	15.3 ±1.2	17.3 ±0.9*	16.1 ±0.6
RBC (mL/cmm)	12	5.93 ±0.43	6.00 ±0.34	5.73 ±0.18	6.18 ±0.62	6.09 ±0.54	6.09 ±0.47
	24	6.38 ±0.38	6.61 ±0.50	6.35 ±0.13	6.79 ±0.53	6.71 ±0.21	6.84 ±0.45
	36	6.43 ±0.49	6.64 ±0.58	6.39 ±0.23	6.75 ±0.64	6.54 ±0.56	6.72 ±0.48
	50	7.43 ±0.44	7.31 ±0.56	7.13 ±0.43	6.89 ±0.56	7.55 ±0.34	7.24 ±0.36
MCHC (%)	12	33 ±1	33 ±1	33 ±1	33 ±1	33 ±1	33 ±0
	24	33 ±0	33 ±1	33 ±1	33 ±1	33 ±1	33 ±1
	36	34 ±1	34 ±0	34 ±1	34 ±1	33 ±1	33 ±1
	50	34 ±1	33 ±1*	33 ±1*	34 ±1	34 ±1	33 ±0
MCV (cμ)	12	69 ±2	69 ±2	70 ±1	69 ±2	71 ±1	69 ±2
	24	67 ±2	68 ±2	69 ±1	68 ±1	70 ±2	68 ±2
	36	68 ±2	67 ±1	68 ±1	69 ±1	71 ±1**	69 ±1
	50	66 ±1	66 ±2	68 ±1*	66 ±1	68 ±3	67 ±1
MCH (ρg)	12	22 ±0	23 ±1	23 ±1*	22 ±1	23 ±1*	23 ±1
	24	22 ±1	22 ±1	22 ±1	22 ±0	23 ±1*	22 ±0
	36	23 ±1	23 ±1	23 ±0	23 ±0	24 ±0	23 ±0
	50	22 ±0	22 ±1	23 ±1	22 ±1	23 ±1	22 ±1
Total WBC (1000/cmm)	12	18.6 ±5.4	15.1 ±3.2	18.4 ±5.5	14.9 ±4.6	11.2 ±1.6	13.5 ±0.7
	24	19.6 ±7.4	15.4 ±3.3	15.1 ±3.7	15.8 ±4.6	12.1 ±2.3	13.5 ±1.5
	36	17.3 ±6.2	14.2 ±2.4	14.8 ±3.3	13.4 ±3.2	12.1 ±3.1	14.1 ±2.7
	50	13.7 ±1.8	12.4 ±1.3	12.8 ±2.1	12.1 ±2.9	10.3 ±1.6	12.6 ±1.7
Platelets (10^3/mm^3)	12	341 ±57	409 ±53	417 ±92	321 ±28	306 ±41	380 ±44
	24	367 ±33	461 ±55*	456 ±77	384 ±54	352 ±42	440 ±39
	36	329 ±36	462 ±51 **	415 ±41 *	332 ±37	367 ±45	478 ±77 **
	50	326 ±33	414 ±46 *	404 ±66	344 ±57	351 ±47	430 ±23 *
PT (sec)	12	7.2 ±0.3	7.1 ±0.4	7.0 ±0.4	7.4 ±0.1	7.6 ±0.2	7.4 ±0.3

Parameters		♂ (mg/kg bw/d)			♀ (mg/kg bw/d)		
	wk	0	3	10	0	3	10
	24	7.3 ±0.4	7.4 ±0.6	6.9 ±0.6	7.2 ±0.1	7.8 ±0.3 **	7.5 ±0.2
	36	7.1 ±0.2	7.0 ±0.3	6.9 ±0.4	7.1 ±0.1	7.3 ±0.2	7.1 ±0.2
	50	6.4 ±0.3	6.4 ±0.4	6.4 ±0.3	6.3 ±0.0	6.8 ±0.2*	6.9 ±0.4 *

* $p \leq 0.05$; ** $p \leq 0.01$
PCV: packed cell volume
Hb: haemoglobin
RBC: red blood cell
MCHC: mean cell Hb concentration
MCV: mean cell volume
MCH: mean cell Hb
WBC: white cell count
PT: prothrombin time

Clinical chemistry: No treatment-related findings were observed.

Table B.6.3.2-18 Clinical chemistry findings

Parameters		♂ (mg/kg bw/d)			♀ (mg/kg bw/d)		
	wk	0	3	10	0	3	10
ALP (iu/L)	12	73 ±11	59 ±7*	60 ±7	78 ±12	68 ±9	64 ±5*
	24	50 ±8	37 ±9	41 ±8	62 ±9	54 ±14	45 ±10
	36	52 ±12	43 ±7	42 ±11	62 ±14	62 ±21	46 ±6
	50	43 ±8	37 ±13	37 ±7	84 ±38	53 ±26	39 ±9*
ALT (iu/L)	12	23 ±5	28 ±3	22 ±4	28 ±3	39 ±4**	28 ±3
	24	29 ±10	31 ±1	32 ±18	27 ±3	42 ±5**	31 ±5
	36	32 ±8	33 ±2	32 ±8	32 ±2	39 ±4	31 ±6
	50	41 ±9	38 ±4	39 ±16	34 ±2	47 ±3***	30 ±5
AST (iu/L)	12	31 ±2	31 ±3	32 ±7	37 ±5	40 ±7	33 ±5
	24	41 ±5	40 ±7	40 ±10	43 ±9	37 ±2	39 ±3
	36	43 ±5	36 ±4	46 ±5	48 ±16	42 ±13	43 ±8
	50	46 ±7	41 ±6	50 ±10	41 ±5	45 ±11	41 ±7
CPK (iu/L)	12	93 ±21	89 ±13	99 ±44	146 ±18	300 ±266	177 ±70
	24	183 ±23	149 ±26	154 ±49	174 ±62	141 ±24	137 ±7
	36	208 ±87	137 ±13	156 ±35	135 ±11	164 ±61	156 ±37
	50	193 ±54	137 ±31	200 ±57	154 ±17	156 ±44	145 ±11
Urea (mg%)	12	24 ±5	26 ±3	26 ±1	26 ±3	27 ±4	26 ±3
	24	25 ±8	28 ±5	25 ±4	28 ±3	31 ±2	31 ±6
	36	23 ±5	22 ±6	25 ±1	23 ±2	27 ±5	23 ±6
	50	34 ±8	30 ±5	33 ±3	28 ±2	33 ±5*	28 ±2
Creatinine (mg%)	12	0.8 ±0.1	0.7 ±0.1	0.7 ±0.1	0.8 ±0.1	0.7 ±0.1	0.7 ±0.1
	24	0.7 ±0.2	0.7 ±0.1	0.7 ±0.1	0.8 ±0.1	0.8 ±0.2	0.7 ±0.1
	36	0.9 ±0.1	1.0 ±0.1	0.9 ±0.1	0.9 ±0.1	1.0 ±0.2	0.8 ±0.1
	50	1.0 ±0.1	0.9 ±0.2	0.9 ±0.1	0.9 ±0.1	0.9 ±0.2	0.8 ±0.1
Glucose	12	106 ±2	100 ±5*	104 ±2	104 ±6	97 ±6	101 ±6

Parameters		♂ (mg/kg bw/d)			♀ (mg/kg bw/d)		
	wk	0	3	10	0	3	10
(mg%)	24	89 ±7	91 ±8	96 ±7	91 ±11	97 ±7	94 ±9
	36	96 ±4	98 ±3	99 ±8	99 ±8	100 ±6	101 ±4
	50	99 ±7	98 ±6	104 ±9	107 ±4	106 ±3	104 ±4
Total bilirubin (mg%)	12	0.2 ±0.1	0.2 ±0.0	0.2 ±0.1	0.2 ±0.0	0.2 ±0.1	0.3 ±0.1
	24	0.2 ±0.1	0.1 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1	0.2 ±0.1
	36	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.1	0.1 ±0.0	0.1 ±0.0
	50	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.2 ±0.1
Triglycerides (mg%)	12	25 ±3	29 ±3	30 ±3*	26 ±10	23 ±5	26 ±2
	24	27 ±5	32 ±6	35 ±6	34 ±9	30 ±6	27 ±9
	36	24 ±4	28 ±8	34 ±4*	30 ±8	31 ±4	27 ±9
	50	32 ±8	36 ±3	35 ±7	41 ±15	34 ±6	30 ±6
Total cholesterol (mg%)	12	145 ±21	130 ±17	141 ±141	120 ±23	125 ±21	124±24
	24	137 ±18	143 ±27	153 ±12	124 ±27	122 ±30	123 ±32
	36	133 ±25	156 ±21	149 ±23	117 ±26	156 ±50	138 ±25
	50	140 ±16	142 ±22	157 ±24	175 ±41	151 ±4	139 ±28
Total plasma proteins (g%)	12	5.5 ±0.3	5.4 ±0.2	5.5 ±0.2	5.3 ±0.1	5.2 ±0.3	5.3 ±0.2
	24	5.6 ±0.2	5.6 ±0.4	5.4 ±0.4	5.5 ±0.2	5.4 ±0.4	5.5 ±0.3
	36	5.8 ±0.2	6.0 ±0.2	5.6 ±0.2	5.7 ±0.2	5.7 ±0.6	5.6 ±0.1
	50	6.4 ±0.3	6.3 ±0.3	6.1 ±0.1	6.5 ±0.3	6.2 ±0.2	6.2 ±0.3
A/G ratio (-:1)	12	0.9 ±0.1	1.1 ±0.1	1.0 ±0.3	1.3 ±0.1	1.2 ±0.2	1.3 ±0.1
	24	1.0 ±0.1	1.0 ±0.1	1.0 ±0.1	1.2 ±0.2	1.2 ±0.1	1.3 ±0.1
	36	2.9 ±0.1	2.8 ±0.2	2.6 ±0.3	2.9 ±0.3	2.8 ±0.8	3.0 ±0.1
	50	1.0 ±0.1	1.1 ±0.1	1.1 ±0.1*	1.1 ±0.2	1.2 ±0.2	1.3 ±0.1
Na (mmol/L)	12	146 ±1	145 ±1	145 ±2	147 ±1	147 ±1	147 ±2
	24	146 ±1	146 ±1	146 ±1	147 ±0	148 ±1	147 ±1
	36	144 ±1	146 ±1*	145 ±1	147 ±1	146 ±2	146 ±2
	50	148 ±1	147 ±1	148 ±3	149 ±1	147 ±2	147 ±1
K (mmol/L)	12	4.6 ±0.2	4.3 ±0.3	4.5 ±0.3	4.3 ±0.2	4.2 ±0.2	4.2 ±0.2
	24	4.5 ±0.3	4.5 ±0.3	4.5 ±0.3	4.3 ±0.1	4.1 ±0.2	4.3 ±0.2
	36	4.2 ±0.2	4.3 ±0.1	4.2 ±0.2	4.1 ±0.1	4.2 ±0.4	4.3 ±0.3
	50	4.1 ±0.3	4.3 ±0.2	4.6 ±0.6	3.7 ±0.2	3.8 ±0.1	3.9 ±0.3
Cl (mmol/L)	12	117 ±1	116 ±2	118 ±2	116 ±1	117 ±3	116 ±1
	24	117 ±1	116 ±2	118 ±1	116 ±2	116 ±1	117 ±1
	36	118 ±2	117 ±2	119 ±2	119 ±1	119 ±4	117 ±1
	50	117 ±1	117 ±2	119 ±2	117 ±1	116 ±3	116 ±1
Ca (mmol/L)	12	2.6 ±0.0	2.7 ±0.1	2.6 ±0.1	2.7 ±0.0	2.6 ±0.1	2.6 ±0.0
	24	2.6 ±0.1	2.6 ±0.1	2.5 ±0.0	2.6 ±0.1	2.5 ±0.1	2.6 ±0.1

Parameters		♂ (mg/kg bw/d)			♀ (mg/kg bw/d)		
	wk	0	3	10	0	3	10
	36	2.5 ±0.1	2.5 ±0.0	2.5 ±0.0	2.5 ±0.1	2.5 ±0.1	2.5 ±0.1
	50	2.6 ±0.0	2.6 ±0.1	2.6 ±0.1	2.6 ±0.1	2.5 ±0.1	2.5 ±0.0
P (mmol/L)	12	2.0 ±0.3	2.0 ±0.2	2.1 ±0.1	2.0 ±0.2	2.0 ±0.2	1.9 ±0.2
	24	1.8 ±0.3	1.7 ±0.3	1.7 ±0.1	1.6 ±0.2	1.7 ±0.3	1.6 ±0.2
	36	1.5 ±0.2	1.4 ±0.2	1.5 ±0.2	1.4 ±0.1	1.4 ±0.5	1.4 ±0.1
	50	1.3 ±0.2	1.3 ±0.2	1.3 ±0.2	1.2 ±0.2	1.3 ±0.5	1.1 ±0.1

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$
ALP: alkaline phosphatase
ALT: alanine aminotransferase
AST: aspartate aminotransferase
CPK: creatine phosphokinase
T. bili.: total bilirubin
Trigly.: triglyceride
T. chol.: total cholesterol
A/G: albumin : globulin ratio
Na: sodium
K: potassium
Cl: chloride
Ca: calcium
P: phosphorus

Urinalysis: No treatment-related findings were observed.

Organ weights: Analysis of organ weights revealed no significant differences between control and treated animals.

Pathology: Macroscopic and histopathological examination revealed no treatment-related findings.

Acceptability

The study is considered acceptable.

Conclusions

No treatment-related findings were observed for organ weights, macroscopy and histopathology.

The NOAEL is set at 10 mg/kg bw/day for males and females.

3.12.1.10 CA 5.3.3/01 (1993): 21-day dermal toxicity study in the rat

Report

CA 5.3.3/01 (1993)

21-day dermal toxicity study in rats with S-31183

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-31-0094

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods was added. Agreed with the original conclusion.

Characteristics

Reference	:	CA 5.3.3/01, 1993	exposure	:	21-days, 6 hours/day, 5 x 5 cm, dorsal-thoracic-lumbar region, semi-occlusive
type of study	:	subacute dermal toxicity	dose	:	0, 100, 300 and 1000 mg/kg bw/day
year of execution	:	1992	vehicle	:	Corn-oil
test substance	:	Pyriproxyfen (S-31183), batch no. 007024, purity 97.2%	GLP statement	:	Yes
Route	:	Dermal	guideline	:	In accordance with OECD 410 (1981)
Species	:	Rats, Sprague-Dawley, Crl:CD®BR	acceptability	:	Acceptable
group size	:	5/sex/dose	**NOAEL**	:	1000 mg/kg bw/day

Study design

The study was performed in accordance with OECD 410 (1981).

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: 007024

Purity: 97.2%

CAS No.: 95737-68-1

Stability of test compound: Confirmed stable for the duration of the study

2. Test animals:

Species: Rat

Strain: Sprague-Dawley, Crl: CD®BR

Age: 42 days

Weight at dosing: ♂: 204 – 263 g; ♀: 170 – 208 g

Source: Charles River Laboratories Inc.

Acclimation period: 14 days

B. Study Design and Methods

1. In life dates: 20 July 1992 to 10 August 1992.

2. Animal assignment and treatment: A total of 40 rats (20/sex) were assigned to study using a computerized weight randomization program. Animals were assigned to groups as follows: 5/sex/dose for dose levels 0, 100, 300 and 1000 mg/kg

	bw/day. The test compound was mixed with the vehicle (corn oil) and transferred to vials for dosing at a factor of 2.0 mL/kg bw. Approximately one week prior to initiation of dosing, the hair on the entire trunk of each rat was clipped and this clipping was repeated one day prior to the first dermal application and repeated throughout the study when necessary. The control of rest material solutions were applied to the treatment site (5 x 5 cm in the dorsal-thoracic-lumbar region) of each animal. The test site was then covered with gauze which was held in place with sterile surgical tape. At the end of the daily exposure period, the dressing was removed and the site washed with distilled water and wiped with facial tissues.
3.Statistics	Bw (gain), food consumption, clinical pathology data, organ weight data were compared statistically. If variances of untransformed data were heterogeneous, a series of transformations was performed in effort to achieve variance homogeneity. When the series of transformations was not successful, analyses were performed on rank-transformed data.
C. methods	
1.Observations	The rats were observed for mortality and moribundity twice daily. A thorough physical examination was conducted at each weighing interval. Cage side observations were performed once daily after the body wrap was removed. Dermal responses were graded daily, immediately prior to dosing, using the criteria of the modified Draize scale.
2.Body weights	Body weights were measured prior to initiation of dosing, weekly and at termination.
3.Food consumption	Food consumption was measured and recorded weekly.
4.Clinical pathology	At termination, all animals were fasted overnight prior to clinical sampling. Samples for haematology and serum chemistry were obtained.
5.Pathology	After fasting overnight, animals were weighed on the day of scheduled necropsy. Selected organs were weighed and examined macro- and microscopically.

Results

Observations: All animals survived to the terminal sacrifice. No treatment-related clinical signs were observed.
Body weights: Throughout the study, there were no significant differences between the control and dose groups for body weight or body weight gain.
Food consumption: No treatment-related findings.
Haematology: Evaluation of the haematology data revealed statistically significant findings: mean cell volumes in Group 2 males were significantly decreased and mean cell haemoglobin values in group 4 females were significantly increased. The differential leukocyte counts and cellular morphology were comparable between the control and pyriproxifen-groups.
Clinical chemistry: Aspartate aminotransferase values for group 2 males were significantly decreased. In the absence of a dose-response relationship, the decreased enzyme activity was considered a spurious finding of no biological consequence.
Organ weights: Mean absolute and relative organ weights were similar between the control and pyriproxifen-groups.
Pathology: During gross pathological examination, no treatment-related findings were observed. In the control and 1000 mg/kg bw/day groups, there was no microscopic evidence of any dermal toxicity in the treated skin. In two group 1 females, there was histologic evidence of slight inflammation on the surface of the skin and in the dermis. These lesions were from the area of contact with the surgical tape. As these sores were observed grossly in several groups and on the perimeter of the treatment site, these lesions could not be attributed to administration of the test substance.

Acceptability
The study is considered acceptable.

Conclusions
Dermal exposure to test substance concentrations of 0, 100, 300 and 1000 mg/kg bw/day did not result in local skin effects. There were no indications of systemic toxicity at any dose: no treatment-related effects on mortality, clinical observations, clinical biochemistry, organ weights and histopathology (kidneys, liver) were noted. A statistically significant increase in mean cell haemoglobin was noted in females at 1000 mg/kg bw/day. However, as this change was rather slight (104% of control) and occurred in the absence of further indications of anaemia, the observed effect was considered incidental and not an adverse effect.
In the absence of local and systemic effects, the NOAEL for both local and systemic effects is set at 1000 mg/kg bw/day.

3.12.1.11 CA 5.3.3/02 (1988): 28-day inhalation toxicity study in the rat

Report
CA 5.3.3/02 (1988)

Sub-acute inhalation toxicity study of S-31183 in rats

Sumitomo Chemical Co. Ltd, Unpublished Report No.: NNT-80-0031

Previous evaluation	In DAR (2005)
Evaluation RMS	No remarks on original assessment, some additional information on the materials and methods and results were added. Agreed with the original conclusion.

Characteristics

Reference	:	CA 5.3.3/02, 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.

Materials and methods

A. Materials:

1. Test Material: S-31183 (pyriproxyfen)

Lot/Batch No.: PTG-86011

Purity: 97.0%

CAS No.: 95737-68-1

Stability of test compound: Confirmed stable for the duration of the study

2. Test animals:

Species: Rat

Strain: Sprague-Dawley

Age: 5 weeks

Weight at dosing: ♂: 204 – 263 g; ♀: 170 – 208 g

Source: Clea Japan Inc.

Acclimation period: 8-9 days

B. Study Design and Methods

1.In life dates:	31 March 1987 to 29 April 1987
2.Animal assignment and treatment:	Rats were randomly grouped, 10/sex/dose to doses of 0, 269, 482 and 1000 mg/m^3. The test material diluted in corn oil was continuously fed into a glass atomizer at a certain rate and injected to produce a mist aerosol. The exposure was performed under the following conditions: air pressure sprayed was 2.0 kg/cm^2, an air flow rate of 50 l/minute, injection rate of test material into the atomizer of 0.14 ml/minute, and an exposure period of 4 hours/day for 28 continuous days.
3.Statistics	Bw, food and water consumption, organ weights: F-test was used to analyse the homogeneity and data from exposure groups were compared to the control using Student's t-test or Fisher-Behrens test. Haematology and clinical chemistry: The difference of variances was analysed using the analysis of variance. If a significant difference was observed the 5% level, the exposure group data were further compared to the control by the LSD method. Urinalysis: The data of the exposure groups were compared to the control by Mann-Whitney U-test.

C. methods

1.Observations	Observations were done every day throughout the exposure period 0.5, 1, 2, 3 and 4 hours after the start of exposure. Additional observation was made about 20 hour after the end of exposure (before start of exposure on the following day).
2.Body weight	Body weight was measured immediately before the start of the first exposure and thereafter twice a week.
3.Food and water consumption	Food and water consumptions/cage during two consecutive days including a day for body weight measurement were measured once a week throughout the exposure period.
4.Urinalysis	Before the start of the 25th exposure, fresh urine was collected from rats of each group for urinalysis.
5.Ophthalmoscopy	Ophthalmoscopical examination was performed at the end of the 25th exposure in rats of group 1 (vehicle control), 2 (negative control) and 5 (highest pyriproxifen dose).

6.Haematology and clinical chemistry	All surviving rats were fasted after the end of the final exposure and blood was collected haematological and clinical chemistry examination.
7.Pathology	Surviving animals were sacrificed and organs and tissues were removed for gross observation. Selected organs were weighed and examined for histopathology.

Results

Aerial concentration and particle size: The mean aerial concentration was 269 mg/m^3 for group 3, 482 mg/m^3 for group 3 and 1000 mg/m^3 for group 5. The median particle size during the exposure period was 0.88 µm for group 1, 0.84 µm for group 3, 0.84 µm for group 4 and 0.71 µm for group 5. There was no significant difference in particle size between exposure groups.

Observations: 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.

Body weight: 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.

Food and water consumption: No treatment-related effect on food or water consumption was observed.

Haematology: There were no significant treatment-related changes in haematology parameters.

Clinical chemistry: 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.

Table B.6.3.3-1 Clinical chemistry findings

	Males				Females			
Parameter	**0**	**269**	**482**	**1000**	**0**	**269**	**482**	**1000**
Albumin	3.2	3.3	3.2	3.3*	3.5	3.5	3.6	3.6
LDH	72	59	88	104*	71	66	83	79
Calcium	9.8	10.0	10.0	10.1*	9.9	10.0	10.0	10.0
Glucose	132	126	127	145	121	120	101*	128

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

Urinalysis: No treatment-related findings.

Ophthalmoscopy: There were no abnormal findings in any group.

Organ weights: 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).The lower spleen weights are likely related to the reduced body weight in the high dose males.

Table B.6.3.3-2 Organ weight findings

	Males	Females

Dose (mg/m³)	0	269	482	1000	0	269	482	1000
Absolute organ weights (g)								
Liver	10.62	10.80	11.12	10.85	6.65	6.95	6.69	7.11
Spleen	0.86	0.78	0.80	0.75*	0.52	0.58	0.51	0.51
Lung	1.53	1.46	1.45	1.36**	1.10	1.16	1.09	1.08
Thyroid	0.61	0.59	0.62	0.55	0.44	0.45	0.44	0.43
Testes	3.68	3.53	3.65	3.62	-	-	-	-
Prostate	0.52	0.54	0.56	0.50	-	-	-	-
Ovaries	-	-	-	-	89	86	80	90
Relative organ weights (organ weights/body weight, in g)								
Liver	2.90	2.98	3.04	3.15**	3.06	3.11	3.09	3.23
Spleen	0.23	0.22	0.22	0.22	0.24	0.26	0.24	0.23
Lung	0.42	0.40	0.40	0.40	0.51	0.52	0.50	0.49
Thyroid	0.17	0.16	0.17	0.16	0.20	0.20	0.21	0.20
Testes	1.00	0.98	1.00	1.05	-	-	-	-
Prostate	0.14	0.15	0.15	0.15	-	-	-	-
Ovaries	-	-	-	-	41.1	38.8	36.8	41.1

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

Pathology: Macroscopic and microscopic examinations revealed no treatment-related findings.

Acceptability

The study is considered acceptable for evaluation.

Conclusions

The NOAEL was established at 482 mg/m³, based on salivation, reduced body weight gain, increased LDH and changes in organ weights.

RMS conclusion: In the original conclusion, changes in organ weight were considered in the setting of the NOAEL. However, the decrease in spleen weight could be a result of the lower body weight in the high dose males. The increase in liver weight (only 9%) is not considered adverse. Furthermore, the organ weight changes were not accompanied by (histo)pathological findings. Therefore, RMS does not consider the changes in organ weights for the setting of the NOAEL.

RMS does still agree with the NOAEL value of 482 mg/m³ (equivalent to 86.8 mg/kg bw/day), based on salivation, reduced body weight gain and increased LDH.

3.12.2 Human data

No human data are available

3.12.3 Other data

No other data are available.

3.13 Aspiration hazard

3.13.1 Animal data

No animal data are available

3.13.2 Human data

No human data are available

3.13.3 Other data

No other data are available.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

4.1.1.1 K. Itoh, A Tanoue, T. Matsuda (1988) Biotic degradation of 1-(4-phenoxyphenoxy)-2-(2-pylidyloxy)propane (code name: S-31183) by activated sludge

Study reference:

K. Itoh, A Tanoue, T. Matsuda (1988) Biotic degradation of 1-(4-phenoxyphenoxy)-2-(2-pylidyloxy)propane (code name: S-31183) by activated sludge, Sumitomo Chemical Co., Ltd., report No.: NNM-0064

Detailed study summary and results:

The ready biodegradability of pyriproxyfen was studied in a closed bottle test according to EHWD No. 5, PAB No. 615, BIB No. 392 (which is comparable to OECD 301C). Test solutions containing pyriproxyfen (100 mg/L) and activated sludge inoculum (30 mg/L) were kept in bottles in the dark for 28 days at 24.0 to 25.5°C. Single flasks for the inoculum blank control (inoculum, no test substance), "sterile" control (test substance, no inoculum) and the reference substance (aniline, 100 mg/L) were included. The test solution was stirred continuously. Oxygen consumption was continuously monitored. The residual (28 days) pyriproxyfen concentration was determined.

The pass level for the reference substance (60% ThOD) was reached within 7 days. Pyriproxyfen was not readily biodegradable in this test (<1% biodegradation after 7 and 28 days).

Test type:

Ready bio-degradability of chemical substance by microbe (EHWD No. 5, PAB No. 615, BIB No. 392[‘74] which is comparable to OECD 301C). The study was conducted to GLP.

Test substance:

Pyriproxyfen, purity 99.4%, Batch No. PTG-86013

Study design

The ready biodegradability of pyriproxyfen was studied in a closed bottle test according to EHWD No. 5, PAB No. 615, BIB No. 392 (which is comparable to OECD 301C).
Test solutions (300 mL, triplicate) containing pyriproxyfen (99.4% pure, 100 mg/L) and activated sludge inoculum (30 mg/L) were kept in bottles in the dark for 28 days at 24.0 to 25.5°C. Single flasks for the inoculum blank control (inoculum, no test substance), "sterile" control (test substance, no inoculum) and the reference substance (aniline, 100 mg/L) were included. The test solution was stirred continuously. Oxygen consumption was continuously monitored using a coulometer. The residual (28 days) pyriproxyfen concentration was determined by HPLV-UV (analysis of concentrated dichloromethane extract).

Results:

The pass level for the reference substance (60% ThOD) was reached within 7 days. Pyriproxyfen was not readily biodegradable in this test (<1% biodegradation after 7 and 28 days respectively). The residual pyriproxyfen concentration was 96% and 98% of applied (inoculum + test substance and "sterile" control, respectively). The oxygen consumption of the inoculum control was 4.7 mg and 8 mg in the system after 7 and 28 days respectively.

Details on the experimental design lacked (e.g., method of preparation of test solutions, actual measured dissolved oxygen concentrations, handling and composition of the inoculum). Therefore the study is regarded supplementary.

Pyriproxyfen was not readily biodegradable in a modified MITI test. In the RAR, the study was regarded supplementary and the substance was considered not readily biodegradable based on the results of this study and by default.

4.1.2 BOD_5/COD

No information provided.

4.1.3 Aquatic simulation tests

4.1.3.1 T. Katagi, N. Takahashi (1989). Hydrolysis of S-31183 in buffered aqueous solutions at 50°C

Study reference:

T. Katagi, N. Takahashi (1989). Hydrolysis of S-31183 in buffered aqueous solutions at 50°C. Sumitomo Chemical Co., Ltd., report No.: NNM-90-0013

Detailed study summary and results:

A hydrolysis study was conducted in duplicate with [phenyl-^{14}C] pyriproxyfen and [pyridyl-^{14}C] pyriproxyfen. The test substance was dissolved at 0.1 mg/L in the acetate buffer at pH 4.0 and borate buffers at pHs 7.0 and 9.0 by using 1% acetonitrile as a co-solvent, and aseptically incubated at 50±0.1°C for 7 days in darkness. The recovery of ^{14}C was 96-109%. Pyriproxyfen was degraded with half-lives of greater than 367 days in all buffer solutions and is, therefore, hydrolytically stable under the conditions tested.

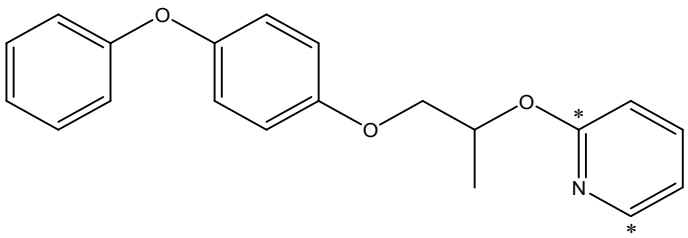
Materials and methods:

Test type:

OECD 111 (1981). The study was conducted to GLP.

Test substance:

1a. Test Materials (radiolabelled):	[Pyridyl-^{14}C] pyriproxyfen
Description:	Not stated
Lot/Batch No.:	C-86-063
Specific activity:	257 mCi/g
Radiopurity:	98%



* indicates position of radiolabel

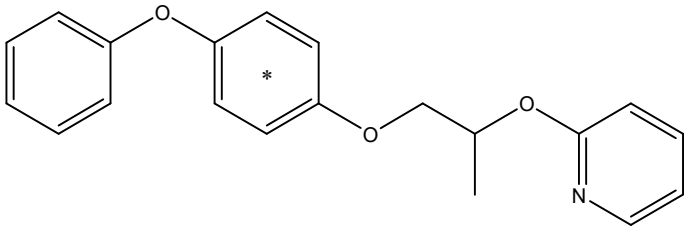
b. (radiolabelled): [Phenyl-^{14}C] pyriproxyfen

Description: Not stated

Lot/Batch No.: C-86-092

Specific activity: 181 mCi/g

Radiopurity: 97%



* indicates position of radiolabel

Study design: The hydrolysis of pyriproxyfen at 50°C in buffered aqueous solutions at pH 4, 7 and 9 was studied in compliance with GLP.

Aliquots (7 mL) of [phenyl-^{14}C] pyriproxyfen and [pyridyl-^{14}C] pyriproxyfen in acetonitrile were added to duplicate samples (700 mL, 50°C) of buffered aqueous solutions at pH 4 (0.05 M sodium acetate/acetic acid), 7 (0.05 M borate/KCl) and 9 (0.05 M borate/NaOH). The buffer solutions were sterilised before use and dissolved oxygen was removed by N_2 gas. The concentration of pyriproxyfen was 0.1 mg/L. Samples were taken after 0, 1, 2, 3, 4 and 7 days of incubation (50°C, dark) for total radioactivity measurements (LSC) and extraction. Sampled aliquots were extracted three times with ethyl acetate. The combined extract was concentrated prior to analysis by TLC. Radioactivity remaining in the aqueous phase was determined by LSC. Location of radioactivity on TLC (normal phase) plates was by autoradiography and quantification by scraping off spots and LSC.

Identity of pyriproxyfen was confirmed by HPLC-RAM (reversed phase) and comparison with a reference standard. Efficiency of the extraction procedure and analysis was checked at 0.01 and 0.1 mg/L and found to be acceptable (recovery (all pHs) 91.4-98.8% AR). Sterility of the buffer solutions was confirmed (plate count) and pH was confirmed to be within 0.1 unit of the target.

Results:

The mass balance (all pHs) was 96-109% AR. No radioactivity (<0.1% AR) remained in the aqueous phase after extraction. Extractable radioactivity consisted of mainly pyriproxyfen and ≤2.7% AR (unidentified) "others". The results indicated that pyriproxyfen was hydrolytically stable at pH 4, 7 and 9 (50°C, 7 days). No meaningful half-lives could be calculated.

4.1.3.2 M. Ponte (2015) Determination of the quantum yield of [14C]pyriproxyfen in pH 7 buffer solution under artificial sunlight

Study reference:

M. Ponte (2015) Determination of the quantum yield of [14C]pyriproxyfen in pH 7 buffer solution under artificial sunlight. Sumitomo Chemical Co., Ltd., Unpublished report No.: NNM-0087

Detailed study summary and results:

An aqueous photolysis study of [pyridyl-^{14}C]pyriproxyfen was conducted at 0.020 µg/mL (with <1% acetonitrile co-solvent) in sterilized phosphate buffer at pH 7 to determine the photolysis quantum yield. Samples were prepared in quartz tubes (15 mm i.d.) for irradiation with a Suntest CPS+ apparatus equipped with a Xenon lamp with filters blocking infrared light and irradiation below 290 nm. The samples were subjected to continuous irradiation for up to 7 days. The average integrated intensities of the light source for the 290-400 nm and 290-800 nm ranges were 47.0 and 379 W/m^2, respectively. Light exposed samples were placed in a temperature controlled deionized water bath maintained at 25±1°C throughout the study period. Dark control samples in Pyrex tubes wrapped with aluminium foil were placed in an incubator maintained at 25±1°C. Volatile gasses were trapped continuously during the exposure period using ethylene glycol to trap organic volatiles, and two 10% aqueous NaOH solutions to trap CO_2. Chemical actinometer samples (PNAP-PYR) were prepared and exposed concurrently with the test solutions for quantum yield determination.

Duplicate light exposed samples were collected at time 0 and 7 additional time points. In order to evaluate the effect of hydrolysis on the degradation of pyriproxyfen, duplicate dark control samples were also collected at each interval. In light exposed samples, recoveries in the NaOH traps represented an average of 7.3% AR by Day 7, while radiocarbon recovered in the ethylene glycol traps represented 0.4% AR at the end of the study. Radiocarbon recovered in the traps for volatiles in dark control samples represented ≤0.5% AR throughout the study period.

Pyriproxyfen degraded rapidly in light exposed samples and represented 7.1% AR following 40 hours of irradiation, declining below detection after 4 days of continuous irradiation. Pyriproxyfen was stable to hydrolysis and represented 90.4% AR in dark control samples following 7 days of incubation in the dark.

The half-life of pyriproxyfen in light exposed samples was determined as 10.5 hours of continuous irradiation based on the percent pyriproxyfen in solution using pseudo-first order kinetics. The half-life of pyriproxyfen in solar day equivalents is presented below. The quantum yield of pyriproxyfen was determined to be 1.42 x 10^{-2}.

Table 4.1.3.2-01 Summary of results

Sample set	DT_{50} in Suntest hours	R^2	DT_{50} in sunlight days		
			US (40 °N summer)	OECD (30-50 °N, summer)	JMAFF (35 °N spring)
Light exposed	10.5	0.976	0.9	0.8	2.6

Test type:

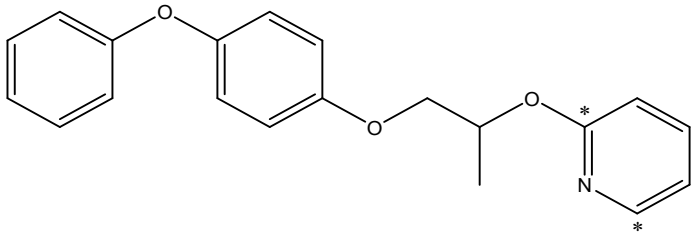
OECD 316 (2008). The study was conducted to GLP.

Materials and methods:

Test substance:

Test Materials (radiolabelled): [Pyridyl-^{14}C]-pyriproxyfen

Description:	Not stated
Lot/Batch No.:	CFQ13438
Specific activity:	14.8 MBq/mg
Radiopurity:	≥96.9% (determined in application solution)



* indicates position of radiolabel

Test System: 0.01 M Phosphate pH 7 buffer sterilized by passing through a 0.2 µm filter

Study design:

The apparatus utilized for exposure of [^{14}C] pyriproxyfen in aqueous solutions to artificial light was a Heraeus Suntest CPS+ unit. The Suntest CPS+ was equipped with a Xenon arc lamp fitted with a quartz glass filter with IR-reflective coating and a special UV glass filter blocking the radiation below approximately 290 nm, to simulate outdoor sunlight exposure. Light intensity and spectral distribution of the Xenon light source were determined prior to the experimental start and after its use in the study with a StellarNet Black Comet CXR-100 spectrometer. The light intensity was set at 600 W/m^2. The spectral distribution was measured at the level of the samples and recorded in the 290-400 nm and 290-800 nm ranges.

Quartz sample tubes (15 mm i.d., shown in pre-test to give insignificant adsorption of the test material, 99-103% in aqueous phase) equipped with septum screw caps with a Teflon-lined silicon septum were used for the irradiated samples. For the dark control samples, Pyrex sample tubes (15 mm i.d.) were used. All glassware was autoclaved at 121°C and 0.10 MPa for 30 minutes prior to use.

The dose solution was prepared in acetonitrile. The nominal concentration of pyriproxyfen in the definitive experiment samples was 0.02 µg/mL, with 1% acetonitrile co-solvent. Aliquots of representative samples were plated on trypticase soy agar for sterility assay at the time of application and at the final sampling time point.

To determine the quantum yield for pyriproxyfen in aqueous solutions, autoclaved tubes containing PNAP-PYR actinometer (1 x 10^{-5} M for PNAP and 2.11 x 10^{-2} M for PYR) in sterile water were run concurrently. At each sampling event, an aliquot of each actinometer sample was taken along with the samples and stored. Aliquots of the samples were transferred to autosampler vials and analysed by HPLC at the end of the exposure. For time zero, the actinometer stock solution was analysed.

Light exposed and dark samples were connected to an air supply. Light exposed samples were placed in the Suntest CPS+ for continuous irradiation. Light exposed sample tubes were placed in a deionized water bath and maintained at a temperature of 25±1°C. Dark control samples were placed in an incubator maintained at 25±1°C for the incubation period. Volatiles were trapped in one ethylene glycol for organic volatiles and two 10% aqueous NaOH solutions for CO_2. At each sampling time (0, 2, 4, 7, 24 and 40 hours and 4 and 7 days), duplicate light exposed samples and duplicate dark control samples were collected and processed. The pH of the samples were measured at sampling. The entire aqueous sample was transferred to a 100 mL graduated cylinder and the sample tubes were rinsed with 5 mL of acetonitrile. The rinse was combined with the aqueous sample, the volume was measured and aliquots (3 × 0.5 mL) were radioassayed by LSC. Samples were analyzed directly by reverse HPLC. Compound identification was based on co-chromatography with non-radiolabelled pyriproxyfen reference standard. Volumes were measured for all traps solutions and aliquots taken (3 × 0.5 mL) for radioassay by LSC. Additionally, aliquots (2 mL) of the light and dark actinometer samples were also taken at each sampling event and analysed by HPLC.

The UV/Visible absorption spectrum of a pyriproxyfen solution (1.56 x 10^{-5} M) in acetonitrile and of the pH 7 phosphate buffer used as test system were obtained and used to determine the theoretical maximum half-life of photolysis and quantum yield in aqueous solutions.

Results:

The spectral distribution of the Xenon lamp was similar to that of natural sunlight. The average intensity of the light measured at the start and the end of the test at the level of the samples was the same at the start and the end of the test and was 47.0 W/m^2 in the 290-400 nm range and 379 W/m^2 in the 290-800 nm range.

The pH of the samples averaged 7.10 (range 7.04-7.18) in light exposed samples and 7.11 (range 7.06-7.15) in dark controls. Plating aliquots of representative samples in trypticase soy agar and incubation for 2 days at 35°C showed that sample sterility was maintained throughout the study period.

The recovery of radioactivity is summarized in Table 4.1.3.2-02 and 4.1.3.2-03, and the concentration of pyriproxyfen in irradiated and dark solutions in Table 4.1.3.2-04.

Mass balances of individual replicates were in the range 93.2-101.6% AR. Individual replicate traps for organic volatiles contained ≤0.5% AR in irradiated samples and 0.0% AR in dark controls. Mean radioactivity in NaOH traps increased to 7.3% AR at study end in irradiated samples, whilst none was found in NaOH traps of dark controls.

[^{14}C] Pyriproxyfen degraded rapidly in light exposed samples, representing an average of 7.1% AR following 40 hours of exposure, and declining to below detection by Day 4. In dark control samples, no measurable degradation of pyriproxyfen was observed by the end of the 7-day study period.

HPLC column recovery was determined for the day 4 replicate A sample and found to be 113.9%.

Table 4.1.3.2-02 Mass balance following the aqueous photolysis of [^{14}C] pyriproxyfen in pH 7 buffer solutions – light exposed samples

Sample	% of applied radiocarbon			
	Aqueous sample	Volatile traps		Total recovery %
		Ethylene glycol	NaOH	
T0dA	98.1	NA	NA	98.1
T0dB	100.0	NA	NA	100.0
Average	99.1	NA	NA	99.1
LT2hrA	94.0	0.0	0.0	94.0
LT2hrB	94.7	0.0	0.0	94.7
Average	94.4	0.0	0.0	94.4
LT4hrA	96.0	0.0	0.0	96.0
LT4hrB	95.3	0.0	0.0	95.3
Average	95.7	0.0	0.0	95.7
LT7hrA	97.3	0.0	0.0	97.3
LT7hrB	99.6	0.0	0.0	99.6
Average	98.5	0.0	0.0	98.5
LT1dA	99.2	0.0	0.8	100.0
LT1dB	99.7	0.0	0.5	100.2
Average	99.5	0.0	0.7	100.1

Sample	% of applied radiocarbon			
	Aqueous sample	Volatile traps		Total recovery %
		Ethylene glycol	NaOH	
LT40hrA	95.6	0.0	1.1	96.7
LT40hrB	100.1	0.0	1.5	101.6
Average	97.9	0.0	1.3	99.2
LT4dA	92.0	0.3	4.9	97.2
LT4dB	93.6	0.5	5.6	99.7
Average	92.8	0.4	5.3	98.5
LT7dA	89.9	0.2	6.2	96.3
LT7dB	89.4	0.5	8.4	98.3
Average	89.7	0.4	7.3	97.3

NA = Not Applicable

Table 4.1.3.2-03 Mass balance following the aqueous photolysis of [^{14}C] pyriproxyfen in pH 7 buffer solutions – dark controls

Sample	% of applied radiocarbon			
	Aqueous sample	Volatile traps		Total recovery %
		Ethylene glycol	NaOH	
DT2hrA	96.7	0.0	0.0	96.7
DT2hrB	99.4	0.0	0.0	99.4
Average	98.1	0.0	0.0	98.1
DT4hrA	94.1	0.0	0.0	94.1
DT4hrB	98.3	0.0	0.0	98.3
Average	96.2	0.0	0.0	96.2
DT7hrA	98.5	0.0	0.0	98.5
DT7hrB	98.2	0.0	0.0	98.2
Average	98.4	0.0	0.0	98.4
DT1dA	95.4	0.0	0.4	95.8
DT1dB	97.9	0.0	0.3	98.2
Average	96.7	0.0	0.4	97.0
DT40hrA	96.1	0.0	0.6	96.7
DT40hrB	93.8	0.0	0.3	94.1
Average	95.0	0.0	0.5	95.4
DT4dA	99.0	0.0	0.3	99.3
DT4dB	100.1	0.0	0.3	100.4

Sample	% of applied radiocarbon			
	Aqueous sample	Volatile traps		Total recovery %
		Ethylene glycol	NaOH	
Average	99.6	0.0	0.3	99.9
DT7dA	93.2	0.0	0.0	93.2
DT7dB	93.8	0.0	0.0	93.8
Average	93.5	0.0	0.0	93.5

Table 4.1.3.2-04 Concentration of [^{14}C] pyriproxyfen in pH 7 buffer solutions

Light Exposed			Dark Controls		
Sample	Pyriproxyfen		Sample	Pyriproxyfen	
	% AR	ppm		% AR	ppm
T0dA	96.2	0.0192	-	-	-
T0dB	97.8	0.0196	-	-	-
Average	97.0	0.0194	-	-	-
LT2hrA	88.1	0.0176	DT2hrA	96.7	0.0193
LT2hrB	77.1	0.0154	DT2hrB	96.3	0.0193
Average	82.6	0.0165	Average	96.5	0.0193
LT4hrA	73.4	0.0147	DT4hrA	94.1	0.0188
LT4hrB	72.0	0.0144	DT4hrB	96.0	0.0192
Average	72.7	0.0146	Average	95.1	0.0190
LT7hrA	57.4	0.0115	DT7hrA	96.9	0.0194
LT7hrB	54.9	0.0110	DT7hrB	95.0	0.0190
Average	56.2	0.0113	Average	96.0	0.0192
LT1dA	19.3	0.0039	DT1dA	92.0	0.0184
LT1dB	28.4	0.0057	DT1dB	97.9	0.0196
Average	23.9	0.0048	Average	95.0	0.0190
LT40hrA	9.0	0.0018	DT40hrA	93.3	0.0187
LT40hrB	5.2	0.0010	DT40hrB	91.1	0.0182
Average	7.1	0.0014	Average	92.2	0.0185
LT4dA	0.0	0.0000	DT4dA	96.6	0.0193
LT4dB	0.0	0.0000	DT4dB	97.6	0.0195
Average	0.0	0.0000	Average	97.1	0.0194
LT7dA	0.0	0.0000	DT7dA	89.9	0.0180
LT7dB	0.0	0.0000	DT7dB	90.9	0.0182

Light Exposed			Dark Controls		
Sample	Pyriproxyfen		Sample	Pyriproxyfen	
	% AR	ppm		% AR	ppm
Average	0.0	0.0000	Average	90.4	0.0181

The half-life of pyriproxyfen in pH 7 buffer was determined based on the percent pyriproxyfen in solution using first-order kinetics and Microsoft Excel®. The degradation rate constants (*k*) and half-lives for light-exposed and dark control samples are summarized in Table 4.1.3.2-05. Note that the DT_{50} of 96 days determined in the dark control is not acceptable due to the poor SFO fit (r^2 0.325). The first order fit for degradation of pyriproxyfen in light exposed samples is shown in Figure 4.1.3.2-01.

Table 4.1.3.2-05 Rate constants and half-life of pyriproxyfen in light and dark units

Sample Set	Rate constant ($hours^{-1}$)	$T_{1/2}$	r^2
Light exposed	0.0661	10.5 hours	0.976
Dark control	0.0003	96 days	0.325

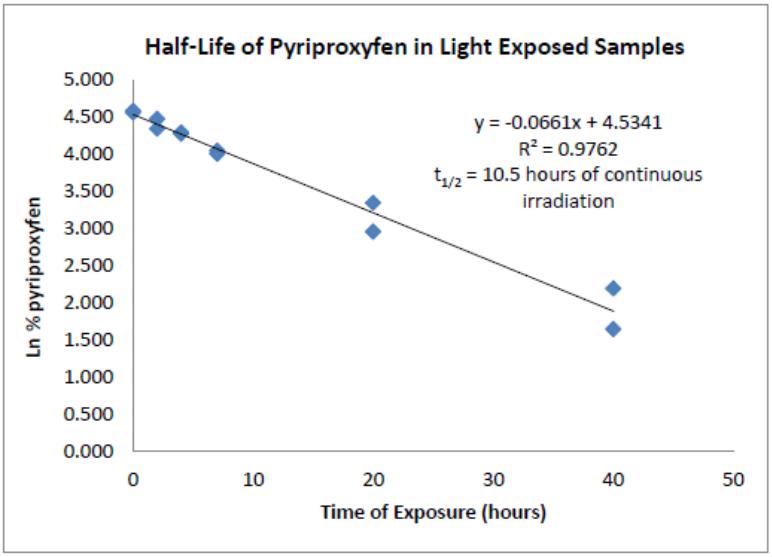


Figure 4.1.3.2-01 First order linear fit for degradation of pyriproxyfen in light exposed samples (ln transformed data)

The half-life under artificial light was converted to natural solar days using global irradiation, see Table 4.1.3.2-06.

Table 4.1.3.2-06 Conversion to natural solar days

Sample Set	DT_{50} in suntest hours[1]	r^2	DT_{50} in Sunlight days		
			US (40°N summer)[2]	OECD (30-50°N summer)[3]	JMAFF (35°N spring)[4]
Light exposed	10.5 hours	0.976	0.9	0.8	2.6

[1] continuous suntest irradiation; [2] average summer irradiation in the 300-800 nm range at 40 °N latitude; 1 US solar day = 4502 W*h/m^2/day [3] average summer irradiation in the 300-400 nm range at 30-50 °N latitude; 1

OECD solar day = 603 W*h/m^2/day based on 67 W/m2 maximum intensity in the 300-400 nm region x 0.75 (factor for varying light intensities throughout the day) x 12 hours of average sunlight per day [4] average spring irradiation in the 300-400 nm range at 35 °N latitude in Tokyo; 1 JMAFF solar day = 187.6 W*h/m^2/day.

The quantum yield of pyriproxyfen was calculated using the formula:

$$\phi_{ts} = (k_{ts} / k_{ac}) \frac{\sum_{\lambda} \varepsilon_{\lambda ac} L_{\lambda}}{\sum_{\lambda} \varepsilon_{\lambda ts} L_{\lambda}} \phi_{ac}$$

where ϕ= quantum yield (for actinometer calculated as 0.0169 x conc[PYR]), ac = actinometer, ts = test substance, k_{ts}/k_{ac} = ratio of rate constants for degradation of test substance and actinometer, respectively (determined experimentally), ε = molar absorptivity (determined from UV spectrum of test substance and actinometer), L_{λ} = light irradiance (measured light intensity in relevant range of Xenon lamp in W/m^2).

The quantum yield of pyriproxyfen was determined to be 1.42×10^{-2}.

4.1.4 Other degradability studies

4.1.4.1 D. Adam. (2015). [^{14}C] Pyriproxyfen – aerobic mineralisation in surface water – simulation biodegradation test

Study reference:

D. Adam. (2015). [^{14}C] Pyriproxyfen – aerobic mineralisation in surface water – simulation biodegradation test. Sumitomo Chemical Co., Ltd., report No.: NNM-0086

Detailed study summary and results:

Aerobic mineralisation of [^{14}C] pyriproxyfen in surface water was investigated under defined laboratory conditions in the dark. The radiolabelled test item, separately labelled in two positions (pyridyl and phenyl positions), was applied to 100 mL of natural pond water at nominal test item concentrations of 0.05 and 0.005 mg/L. Additionally, the high concentration experiment conducted with the pyridyl-label was performed under sterile conditions in order to gain information about abiotic degradability of the test item. The test flasks were incubated in the dark for a period of 63 days at 20.9±0.2°C under aerobic conditions by gently stirring the water. Radiolabelled benzoic acid (with and without solvent control) was used as reference substance to check for sufficient microbial activity of the test water.

Pyriproxyfen degraded rapidly in the tested non-sterile water system. The half-life (DT_{50}) in biologically active surface water system was 14.5 days for the high dose (0.05 mg/L) and 5.0 days for the low dose (0.005 mg/L) test systems, while under sterile conditions pyriproxyfen was stable.

Metabolites found at >10% AR were 4'-OH-Pyr (both labels and doses, max 23.9% AR), DPH-Pyr (both labels and doses, max 42.8 %AR), PYPAC (pyridyl label, both doses, max 13.1% AR), PYPA (pyridyl label, both doses, max 51.0% AR), POP (phenyl label, both doses, max 13.0% AR) and 4'-OH-POP (phenyl label, both doses, max 16.5% AR). POPA was identified in phenyl-labeled systems (both doses) but never exceeded 4.9% AR. The identity of the above compounds was confirmed in representative extracts using 2D-TLC.

Unidentified compounds found at >10% AR were M12 (pyridyl-label, low dose only, max 15.5% AR in any replicate, replicate mean 11.1% AR), M14 (pyridyl-label, low dose only, max 11.7% AR in any replicate, replicate mean 6.3% AR), M20 (phenyl-label, low and high dose, max 15.9% AR in any replicate, replicate mean 12.1 and 12.6% AR for low and high dose, respectively) and M24 (phenyl-label, high dose only, max

16.1% AR, replicate mean 8.0% AR). Several other unidentified were detected but never exceeded 10% AR in any replicate.

Fraction M20 consists of very low-molecular compounds as it was not possible to retard M20 by HPLC or resolve from the application spot on TLC plates with several solvent systems optimized for polar components.

Unidentified compound M12 was found at >10% AR in pyridyl-label systems only treated with the low dose, at a maximum of 15.5% AR in any replicate, with a maximum replicate mean of 11.1% AR. M12 was found in the organic extracts and eluted just after DPH-Pyr.

Chiral HPLC analysis of 0 and 14 DAT high dose samples demonstrated that the 1:1 ratio of R and S enantiomers remained approximately constant (R:S ratio 0.99 at start, 1.06-1.12 after 14 days), indicating that no significant enantio-selective degradation occurred.

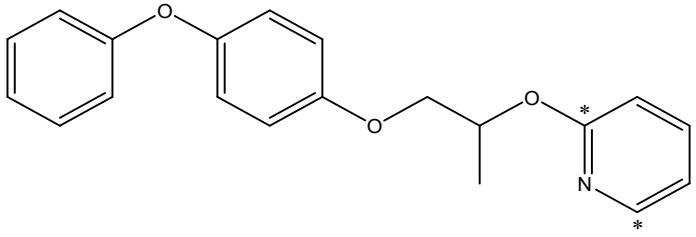
Test type:

OECD 309 (2004). The study was conducted to GLP.

Materials and methods:

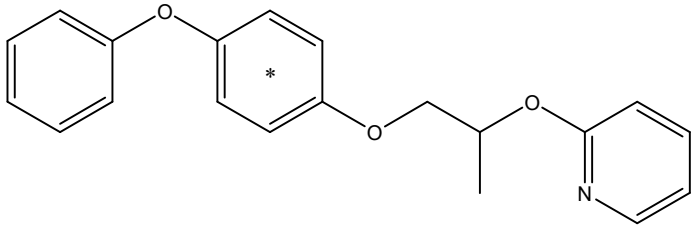
Test substance:

1a.	**Test Materials (radiolabelled):**	[Pyridyl-2,6-^{14}C]-pyriproxyfen
	Description:	Not stated
	Lot/Batch No.:	CFQ13438
	Specific activity:	14.8 MBq/mg
	Radiopurity:	≥95.5% (determined in application solutions)



* indicates position of radiolabel

b.	**(radiolabelled):**	[Phenyl-^{14}C]-pyriproxyfen
	Description:	Not stated
	Lot/Batch No.:	CFQ13879
	Specific activity:	13.3 MBq/mg
	Radiopurity:	≥96.7% (determined in application solutions)



* indicates position of radiolabel

Test System: One freshly sampled Swiss pond water ("Fröschenweiher" in Rheinfelden) was used and its characteristics are presented in Table 4.1.4.1-01. The sampling location was in an area not subject to effluent discharges and located far from human activity. The sample was transported to the test facility in sealed

containers and filtered through a 0.1 mm sieve. The water was then stored at about 4°C in the dark, until use (maximum of 14 days)

Table 4.1.4.1-01 Water parameter for the test system

Parameter	Value
Origin/Source	Fröschenweiher Rheinfelden, Switzerland
Batch no.	Fröschenweiher 06/15
Sampling depth	On the surface (5-20 cm)
Colour	Grey/green
Turbidity	100 cm
Temperature	21°C
pH	8.2
Redox potential	182.4 mV
Oxygen content	7.4 mg/L
Total organic carbon (TOC)	3.63 mg/L
Dissolved organic carbon (DOC)	2.09 mg/L
NO_3	<0.10 mg/L
NO_2	<0.82 mg/L
N total	<1.00 mg/L
P total	0.19 mg/L
Dissolved orthophosphate	0.004 mg/L
Ammonium (NH^{4+})	<0.13 mg/L

Study design:

Each test system consisted of an open gas-flow-system with 350 mL Erlenmeyer flasks, containing 100 ml of natural water. The set of experimental conditions are illustrated below.

Table 4.1.4.1-02 Study lay-out

Treatment	Number of sampling intervals	Number of samples per sampling interval	Reserves (no. of flasks)[1]	Total number of flasks
High dose	7 per label	Duplicate	6 per label	40 (covering both labels)
Low dose	7 per label	Duplicate	6 per label	40 (covering both labels)
High dose, sterilised[2]	7 (one label only)	Duplicate	-	14
Control of microbial activity	3 (one label only)	Duplicate	-	2
Solvent control[3]	3 (one label only)	Duplicate	-	2

Treatment	Number of sampling intervals	Number of samples per sampling interval	Reserves (no. of flasks)[1]	Total number of flasks
Non-treated[4]	3	Duplicate	-	2

[1] Reserve samples used for additional intervals, if necessary, [2] Sterilization performed by autoclaving, [3] Serves as a check to see if co-solvent affects microbial activity, [4] Non-treated samples used to determine physico-chemical parameters

The application rate for the low dose (LD) samples was 0.005 mg/L for both labels and for the high dose (HD) samples 0.050 mg/L and 0.052 mg/L for [pyridyl-^{14}C] and [phenyl-^{14}C] pyriproxyfen respectively. The test systems were treated with a solution of the test item in ethanol/water (1:1 v/v) for high dose or acetonitrile for low dose. After treatment, samples were connected to a trapping system equipped with a total of two absorption traps, one containing ethylene glycol and the other 2N NaOH (in this sequence) to trap organic volatiles and $^{14}CO_2$, respectively. Samples were incubated at a controlled temperature of 20.9±0.2°C in the dark, under aerobic conditions. Each flask was aerated with moistened air. The samples were continuously and gently stirred to maintain particles and micro-organisms in suspension.

Degradation of [^{14}C] benzoic acid (treated at rate 0.12 mg/L) was monitored using the same experimental set-up in order to test the microbial activity of the test water (with and without co-solvent ethanol).

Duplicate samples treated with the test item(s) were taken from each test system (HD, HD sterile and LD) immediately after treatment (time 0) and after 3, 7, 14, 28, 42 and 63 days of incubation. Aliquots of samples treated with benzoic acid were removed from the flasks and analysed immediately after treatment (time 0) and after 7 and 14 days.

At each sampling interval, entire samples (100mL) were taken and the radioactivity present determined by measuring dulplicate aliquots of up to 1 mL by LSC (0 to 14 DAT). For 28 to 63 DAT samples, a 2 mL aliquot of the water phase was initially acidified with 30 µL trifluoroacetic acid (TFA) following LSC measurement of respective duplicate 500 µL aliquots. Subsequently, the oxygen content and pH in the sample was determined and the water adjusted to pH 3 to 4 with 300 µL TFA. Thereupon, for all intervals, the water sample was partitioned three times with 50 mL of ethyl acetate. The volumes of the water and organic phases were recorded and the radioactivity present determined by LSC. Aliquots of the organic (for all intervals) and water (only when containing >3% AR) phases were then separately concentrated to dryness under reduced pressure by rotary evaporation and re-constituted with 1 to 2 mL of acetonitrile/water (1/1; v/v) and acetonitrile/water (9/1; v/v), respectively.

Additionally, a vessel wash with 10 mL of acetonitrile was performed for all test vessels.

Radioactivity in the trapping solutions, i.e. ethylene glycol and sodium hydroxide, was quantified by LSC at each sampling interval.

After LSC measurement, all individual concentrated organic extracts and concentrated water phases were subjected to HPLC analysis (reversed phase and (day 0 and 14 only) chiral analysis to determine the enantiomer ratio). Selected samples were additionally analysed by TLC to confirm the results obtained by HPLC. Compound identification was based on co-chromatography with non-radiolabelled reference standards.

Samples treated with benzoic acid: At the respective sampling intervals, aliquots were removed from the water and analysed by LSC to determine dissolved radioactivity and volatile radioactivity in trapping solutions. Amounts of benzoic acid in the water phase were determined by HPLC analysis.

Results:

pH and oxygen content: Oxygen levels high, fairly constant, and comparable in all treatments and controls (means over entire incubation period in range 8.42-8.67 mg/L). Mean pH was in the range 8.50-8.61, with limited variation (overall range in individual replicates 8.01-8.70 except for high dose sterile controls: 7.97-9.92).

Microbial activity: Within 14 days of incubation, the mean amount of radioactivity applied as benzoic acid to the control samples (no co-solvent) decreased rapidly from the water phase from initially 102.3% to 47.1% under formation of 42.2% of radioactive carbon dioxide. Benzoic acid, with an initial mean amount of 101.6% AR at time zero, degraded in the respective samples to 16.0% AR within 14 days of incubation.

In the solvent control samples (benzoic acid, co-solvent ethanol), faster degradation of benzoic acid was observed when compared to the control samples missing co-solvent. Within 14 days of incubation, the mean amount of benzoic acid decreased rapidly in the water phase from initially 100.9% to 9.1% under formation of 58.2% of radioactive carbon dioxide. Benzoic acid, with an initial mean amount of 100.2% AR at time zero, completely dissipated from the respective samples within 7 days of incubation.

As more than 90% of benzoic acid degraded within 7 days in the solvent control samples, and evolved carbon dioxide was simultaneously observed during the 14-day incubation period in each of the test systems, the test water was considered to be microbially active.

The recovery of radioactivity is summarized in Table 4.1.4.1-03 to Table 4.1.4.1-07. Mass balances of individual replicates were in the range 89.9-109.5% AR, with the exception of the day 42 low dose phenyl-label sample A: 82.7% AR. The report did not comment on the low mass balance for this sample. It may be associated with incomplete CO_2 trapping, the radioactivity levels in the day 14-42 CO_2 traps did not show the monotonous increase, which was observed in all other incubations.

At the end of incubation (63 days) CO_2 was evolved to levels of 12.4-15.6% AR in pyridyl-labelled systems, and 18.2 and 32.4% AR in phenyl-labelled systems (high and low dose, respectively), whilst formation of CO_2 was insignificant (0.2% AR) in sterilized systems. Radioactivity in ethylene glycol samples was insignificant (≤0.5% AR) except for phenyl-labelled systems (2.5% AR).

Table 4.1.4.1-03 Balance of radioactivity after the application of [pyridyl-^{14}C] pyriproxyfen (high dose)

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
0	A	1.6	97.1	<0.1	NP	NP	98.7
	B	1.8	95.0	<0.1	NP	NP	96.8
	Mean	1.7	96.0	<0.1	NP	NP	97.8
3	A	1.9	88.6	0.2	0.1	<0.1	90.8
	B	1.8	98.9	0.2	0.1	<0.1	101.1
	Mean	1.8	93.8	0.2	0.1	<0.1	95.9
7	A	3.7	96.5	0.3	0.2	<0.1	100.6
	B	3.7	92.2	0.3	0.2	<0.1	96.4
	Mean	3.7	94.3	0.3	0.2	<0.1	98.5
14	A	4.0	94.2	<0.1	1.3	0.2	99.7
	B	5.5	100.9	0.1	0.6	<0.1	107.1
	Mean	4.8	97.6	0.1	0.9	0.1	103.4
28	A	7.8	87.3	0.4	4.2	0.2	99.9
	B	6.8	90.7	0.5	3.7	0.1	101.8
	Mean	7.3	89.0	0.4	3.9	0.2	100.8
42	A	11.8	77.3	0.2	5.2	0.1	94.7
	B	9.3	88.3	0.6	5.4	0.1	103.7
	Mean	10.6	82.8	0.4	5.3	0.1	99.2
63	A	12.5	65.6	0.6	14.5	0.4	93.5

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
	B	10.5	70.4	0.2	10.4	0.3	91.8
	Mean	11.5	68.0	0.4	12.4	0.3	92.7

NP= not performed

Table 4.1.4.1-04 Balance of radioactivity after the application of [pyridyl-^{14}C] pyriproxyfen (high dose sterile)

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
0	A	1.3	97.4	<0.1	NP	NP	98.7
	B	1.3	98.0	<0.1	NP	NP	99.4
	Mean	1.3	97.7	<0.1	NP	NP	99.0
3	A	1.2	96.1	<0.1	<0.1	<0.1	97.4
	B	1.4	105.2	0.1	<0.1	<0.1	106.7
	Mean	1.3	100.6	0.1	<0.1	<0.1	102.
7	A	1.4	98.0	<0.1	0.1	0.1	99.6
	B	1.4	99.5	0.1	0.1	<0.1	101.0
	Mean	1.4	98.8	0.1	0.1	<0.1	100.3
14	A	1.4	92.1	0.2	0.1	<0.1	93.7
	B	1.6	96.2	0.7	0.1	0.1	98.6
	Mean	1.5	94.1	0.4	0.1	<0.1	96.2
28	A	1.7	100.5	0.1	0.2	0.2	102.7
	B	1.7	99.8	0.1	0.2	0.3	102.
	Mean	1.7	100.2	0.1	0.2	0.3	102.4
42	A	1.7	92.6	0.2	0.2	0.2	94.9
	B	1.8	93.7	0.4	0.2	0.2	96.3
	Mean	1.8	93.2	0.3	0.2	0.2	95.6
63	A	1.8	99.4	<0.1	0.2	0.2	101.6
	B	1.8	94.1	0.1	0.2	0.8	97.1
	Mean	1.8	96.8	0.1	0.2	0.5	99.3

NP= not performed

Table 4.1.4.1-05 Balance of radioactivity after the application of [pyridyl-^{14}C] pyriproxyfen (low dose)

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
0	A	1.3	105.2	<0.1	NP	NP	106.5
	B	1.4	105.0	0.1	NP	NP	106.5
	Mean	1.3	105.1	0.1	NP	NP	106.5

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
3	A	3.6	97.3	0.1	0.2	<0.1	101.3
	B	3.7	106.7	0.2	0.1	<0.1	110.7
	Mean	3.7	102.0	0.2	0.2	<0.1	106.0
7	A	8.0	93.3	0.3	0.8	<0.1	102.4
	B	7.7	85.1	2.6	1.2	<0.1	96.5
	Mean	7.8	89.2	1.4	1.0	<0.1	99.5
14	A	7.9	83.5	5.5	3.9	<0.1	100.8
	B	7.8	89.4	5.1	4.7	<0.1	107.0
	Mean	7.8	86.4	5.3	4.3	<0.1	103.9
28	A	10.2	85.6	0.5	6.3	<0.1	102.6
	B	11.4	77.5	0.6	9.2	0.1	98.8
	Mean	10.8	81.5	0.6	7.8	<0.1	100.7
42	A	13.8	66.4	0.1	9.6	<0.1	89.9
	B	11.1	82.4	0.1	8.8	<0.1	102.3
	Mean	12.4	74.4	0.1	9.2	<0.1	96.1
63	A	20.6	67.3	0.1	15.1	0.1	103.3
	B	19.0	66.1	0.2	16.2	0.2	101.6
	Mean	19.8	66.7	0.2	15.6	0.2	102.5

NP= not performed

For the phenyl-labelled test item, total mean recoveries were 97.6 ± 3.9% AR for the high dose and 96.4 ± 6.8% AR for the low dose experiment

Table 4.1.4.1-06 Balance of radioactivity after the application of [phenyl-^{14}C] pyriproxyfen (high dose)

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
0	A	0.8	94.4	<0.1	NP	NP	95.1
	B	0.7	93.0	<0.1	NP	NP	93.7
	Mean	0.7	93.7	<0.1	NP	NP	94.4
3	A	2.1	94.5	0.2	0.3	0.1	97.5
	B	1.9	94.8	0.1	0.2	<0.1	97.1
	Mean	2.0	94.6	0.2	0.2	0.1	97.2
7	A	4.6	90.2	0.2	0.5	<0.1	95.5
	B	2.8	99.1	0.4	0.7	<0.1	103.0
	Mean	3.7	94.7	0.3	0.6	<0.1	99.3
14	A	4.2	87.5	0.6	0.3	<0.1	92.6
	B	3.9	98.4	0.6	0.5	<0.1	103.5
	Mean	4.0	93.0	0.6	0.4	<0.1	98.1
28	A	7.9	89.6	0.3	2.7	<0.1	100.5

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
	B	12.3	85.1	0.2	4.9	0.2	102.7
	Mean	10.1	87.4	0.2	3.8	0.1	101.6
42	A	16.0	68.3	0.1	16.1	0.5	101.0
	B	20.4	62.9	1.1	10.8	1.4	96.6
	Mean	18.2	65.6	0.6	13.4	1.0	98.8
63	A	22.9	51.7	2.4	18.0	1.3	96.3
	B	25.2	42.6	1.6	18.3	3.6	91.4
	Mean	24.0	47.2	2.0	18.2	2.5	93.9

NP= not performed

Table 4.1.4.1-07 Balance of radioactivity after the application of [phenyl-^{14}C] pyriproxyfen (low dose)

Incubation Time	Replicate	Aqueous phase	Organic phase	Vessel Wash	$^{14}CO_2$	Organic volatiles	Total
0	A	0.8	94.9	<0.1	NP	NP	95.7
	B	1.1	93.0	0.1	NP	NP	94.1
	Mean	1.0	93.9	<0.1	NP	NP	94.9
3	A	4.8	86.4	0.2	0.7	<0.1	92.1
	B	5.8	89.0	0.2	0.2	<0.1	95.1
	Mean	5.3	87.7	0.2	0.4	<0.1	93.6
7	A	7.9	94.6	0.4	2.6	<0.1	105.5
	B	7.3	99.7	0.4	2.1	<0.1	109.5
	Mean	7.6	97.1	0.4	2.4	<0.1	107.5
14	A	10.0	72.8	0.5	12.7	<0.1	96.0
	B	10.6	71.0	0.6	15.7	<0.1	98.0
	Mean	10.3	71.9	0.5	14.2	<0.1	97.0
28	A	18.3	78.7	0.2	6.4	<0.1	103.5
	B	18.4	67.6	0.2	10.4	<0.1	96.6
	Mean	18.3	73.1	0.2	8.4	<0.1	100.1
42	A	20.7	45.5	1.8	14.8	<0.1	82.7
	B	24.3	57.3	0.2	17.5	<0.1	99.4
	Mean	22.5	51.4	1.0	16.2	<0.1	91.1
63	A	27.6	30.0	0.9	32.9	0.2	91.5
	B	26.8	31.2	0.1	31.8	<0.1	90.0
	Mean	27.2	30.6	0.5	32.4	0.1	90.8

NP= not performed

The identification of radioactivity is summarized in Table 4.1.4.1-08 to Table 4.1.4.1-12. Pyriproxyfen degraded in non-sterile samples while remaining stable in the sterile water system. Degradation observed in non-sterile samples resulted in formation of higher amounts of radioactive carbon dioxide and higher number

of metabolites when compared with sterile samples. In the summary below, % AR value pertain to individual replicates unless indicated differently.

Pyriproxyfen levels decreased from 93.0-105.2% AR at the start to ≤6.8% AR at study end (day 63).

Metabolites found at >10% AR were 4'-OH-Pyr (both labels and doses, max 23.9% AR), DPH-Pyr (both labels and doses, max 42.8% AR), PYPAC (pyridyl label, both doses, max 13.1% AR), PYPA (pyridyl label, both doses, max 51.0% AR), POP (phenyl label, both doses, max 13.0% AR) and 4'-OH-POP (phenyl label, both doses, max 16.5% AR). POPA was identified in phenyl-labeled systems (both doses) but never exceeded 4.9% AR. The identity of the above compounds was confirmed in representative extracts using 2D-TLC.

Unidentified compounds found at >10% AR were M12 (pyridyl-label, low dose only, max 15.5% AR in any replicate, replicate mean 11.1% AR), M14 (pyridyl-label, low dose only, max 11.7% AR in any replicate, replicate mean 6.3% AR), M20 (phenyl-label, low and high dose, max 15.9% AR in any replicate, replicate mean 12.1 and 12.6% AR for low and high dose, respectively) and M24 (phenyl-label, high dose only, max 16.1% AR, replicate mean 8.0% AR). Several other unidentified compounds were detected but none exceeded 10% AR in any replicate.

It was concluded that fraction M20 consists of very low-molecular compounds as it was not possible to retard M20 by HPLC or resolving from the application spot on TLC plates with several solvent systems optimized for polar components.

Chiral HPLC analysis of 0 and 14 DAT high dose samples demonstrated that the 1:1 ratio of R and S enantiomers remained approximately constant (R:S ratio 0.99 at start, 1.06-1.12 after 14 days), indicating that no significant enantio-selective degradation occurred.

Table 4.1.4.1-08 Pattern of degradation and formation of metabolite after the application of [pyridyl-^{14}C] pyriproxyfen (high dose)

Compound	Replicate	0	3	7	14	28	42	63
Pyriproxyfen	A	97.1	77.9	83.7	69.7	18.1	6.9	1.2
	B	95.0	89.4	79.4	68.0	12.6	2.2	ND
	Mean	96.0	83.7	81.6	68.8	15.3	4.5	0.6
4'-OH-Pyr (M1)	A	ND	5.2	7.8	13.1	8.2	0.9	ND
	B	ND	4.3	5.7	14.5	9.9	ND	ND
	Mean	ND	4.8	6.7	13.8	9.1	0.4	ND
DPH-Pyr (M2)	A	ND	ND	1.4	3.1	21.3	24.2	9.4
	B	ND	ND	ND	4.5	28.4	40.3	18.0
	Mean	ND	ND	0.7	3.8	24.7	32.3	13.7
PYPAC (M3)	A	ND	ND	ND	2.9	11.3	8.0	12.3
	B	ND	ND	1.9	5.5	9.5	7.1	10.0
	Mean	ND	ND	0.9	4.2	10.4	7.5	11.2
PYPA (M4)	A	ND	3.3	3.0	2.2	17.6	24.8	40.5
	B	ND	4.0	5.4	2.5	18.5	27.2	33.0
	Mean	ND	3.7	4.2	2.3	18.1	26.0	36.8
M8	A	ND	2.1	1.9	0.5	1.7	1.6	1.6
	B	ND	1.2	1.4	ND	0.6	0.9	1.3
	Mean	ND	1.7	1.6	0.2	1.2	1.3	1.5

Compound	Replicate	0	3	7	14	28	42	63
M9	A	ND	ND	ND	3.4	ND	1.8	1.9
	B	ND	ND	ND	3.1	0.6	1.1	2.0
	Mean	ND	ND	ND	3.3	0.3	1.4	2.0
M10	A	ND	ND	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	0.5	ND	ND
	Mean	ND	ND	ND	ND	0.2	ND	ND
M11	A	ND	ND	ND	ND	ND	0.9	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	0.4	ND
M12	A	ND	ND	ND	ND	4.1	3.5	1.3
	B	ND	ND	ND	ND	ND	3.0	2.4
	Mean	ND	ND	ND	ND	2.0	3.3	1.8
M13	A	ND	ND	1.6	1.6	4.8	1.5	ND
	B	ND	ND	1.4	3.8	4.6	ND	ND
	Mean	ND	ND	1.5	2.7	4.7	0.7	ND
M14	A	ND	ND	0.7	0.9	0.7	0.7	0.9
	B	ND	ND	0.5	3.9	0.6	0.5	0.8
	Mean	ND	ND	0.6	2.4	0.6	0.6	0.8
M15	A	ND	ND	ND	ND	ND	0.9	1.7
	B	ND	ND	ND	ND	0.7	0.6	1.2
	Mean	ND	ND	ND	ND	0.3	0.7	1.5
M19	A	ND	ND	ND	ND	1.9	1.9	1.6
	B	ND	ND	ND	ND	2.5	3.3	3.1
	Mean	ND	ND	ND	ND	2.2	2.6	2.3
M20	A	ND	ND	ND	ND	ND	1.1	1.2
	B	ND	ND	ND	ND	ND	0.7	0.7
	Mean	ND	ND	ND	ND	ND	0.9	0.9
M22	A	ND	ND	ND	ND	ND	ND	0.7
	B	ND	ND	ND	ND	ND	ND	0.6
	Mean	ND	ND	ND	ND	ND	ND	0.7
M23	A	ND	ND	ND	0.4	3.0	2.9	1.8
	B	ND	ND	ND	ND	4.8	3.2	2.5
	Mean	ND	ND	ND	0.2	3.9	3.0	2.2
M24	A	ND	ND	ND	0.2	2.5	7.6	2.0
	B	ND	ND	ND	ND	2.9	6.2	4.5
	Mean	ND	ND	ND	0.1	2.7	6.9	3.2

ND = not detected

Table 4.1.4.1-09 Pattern of degradation and formation of metabolite after the application of [pyridyl-^{14}C] pyriproxyfen (high dose sterile)

Compound	Replicate	0	3	7	14	28	42	63
Pyriproxyfen	A	97.4	95.1	96.6	85.9	97.0	90.0	95.6
	B	98.0	105.2	97.0	93.9	94.6	91.1	90.7
	Mean	97.7	100.1	96.8	89.9	95.8	90.6	93.1
4'-OH-Pyr (M1)	A	ND	ND	ND	1.5	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	0.7	ND	ND	ND
DPH-Pyr (M2)	A	ND	ND	ND	2.0	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	1.0	ND	ND	ND
PYPA (M4)	A	ND	1.0	1.5	2.7	3.5	2.6	3.8
	B	ND	ND	2.6	2.3	5.2	2.6	3.4
	Mean	ND	0.5	2.0	2.5	4.3	2.6	3.6

ND = not detected

Table 4.1.4.1-10 Pattern of degradation and formation of metabolite after the application of [pyridyl-^{14}C] pyriproxyfen (low dose)

Compound	Replicate	0	3	7	14	28	42	63
Pyriproxyfen	A	105.2	62.7	32.1	9.3	ND	3.2	ND
	B	105.0	73.5	23.4	11.6	ND	4.4	ND
	Mean	105.1	68.1	27.7	10.4	ND	3.8	ND
4'-OH-Pyr (M1)	A	ND	23.9	16.2	13.3	ND	ND	ND
	B	ND	20.7	7.3	4.6	2.5	4.9	ND
	Mean	ND	22.3	11.8	8.9	1.2	2.4	ND
DPH-Pyr (M2)	A	ND	4.3	6.5	26.4	34.5	4.8	ND
	B	ND	4.0	8.6	38.2	20.6	5.0	4.7
	Mean	ND	4.2	7.5	32.3	27.6	4.9	2.4
PYPAC (M3)	A	ND	ND	6.2	6.7	10.8	8.1	13.1
	B	ND	ND	10.9	7.2	7.5	10.6	11.9
	Mean	ND	ND	8.5	6.9	9.2	9.3	12.5
PYPA (M4)	A	ND	3.3	10.6	17.5	34.5	42.4	50.8
	B	ND	5.0	21.0	16.6	33.7	51.0	39.3
	Mean	ND	4.1	15.8	17.0	34.1	46.7	45.1
M8	A	ND	ND	ND	7.9	4.4	6.5	6.7
	B	ND	ND	4.0	7.8	7.8	7.0	5.9
	Mean	ND	ND	2.0	7.8	6.1	6.8	6.3
M9	A	ND	ND	6.5	ND	ND	ND	3.5
	B	ND	ND	ND	ND	ND	ND	2.2

Compound	Replicate	0	3	7	14	28	42	63
	Mean	ND	ND	3.2	ND	ND	ND	2.8
M12	A	ND	ND	6.7	3.2	ND	ND	ND
	B	ND	ND	15.5	3.9	2.6	ND	ND
	Mean	ND	ND	11.1	3.5	1.3	ND	ND
M13	A	ND	3.0	3.0	ND	ND	ND	ND
	B	ND	3.5	ND	ND	ND	ND	ND
	Mean	ND	3.3	1.5	ND	ND	ND	ND
M14	A	ND	ND	11.7	ND	ND	ND	2.7
	B	ND	ND	0.9	ND	ND	ND	6.4
	Mean	ND	ND	6.3	ND	ND	ND	4.5
M16	A	ND	ND	1.8	ND	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	0.9	ND	ND	ND	ND
M18	A	ND	ND	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	2.2	ND	ND
	Mean	ND	ND	ND	ND	1.1	ND	ND
M20	A	ND	ND	ND	ND	ND	7.2	3.7
	B	ND	ND	ND	ND	ND	ND	4.9
	Mean	ND	ND	ND	ND	ND	3.6	4.3
M21	A	ND	ND	ND	ND	ND	ND	2.5
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	1.3
M23	A	ND	ND	ND	7.1	3.4	ND	ND
	B	ND	ND	ND	7.3	5.2	ND	2.3
	Mean	ND	ND	ND	7.2	4.3	ND	1.1
M24	A	ND	ND	ND	ND	7.9	8.0	5.1
	B	ND	ND	ND	ND	3.2	3.5	7.4
	Mean	ND	ND	ND	ND	5.6	5.8	6.2

ND = not detected

Table 4.1.4.1-11 Pattern of degradation and formation of metabolite after the application of [phenyl-^{14}C] pyriproxyfen (high dose)

Compound	Replicate	0	3	7	14	28	42	63
Pyriproxyfen	A	94.4	86.2	75.8	59.0	33.4	6.8	6.8
	B	93.0	84.1	80.4	66.3	3.0	2.3	1.3
	Mean	93.7	85.2	78.1	62.7	18.2	4.6	4.0
4'-OH-Pyr (M1)	A	ND	4.5	9.8	13.1	18.5	5.3	1.3
	B	ND	5.6	12.6	13.2	2.2	1.6	2.2
	Mean	ND	5.1	11.2	13.2	10.4	3.5	1.8

Compound	Replicate	0	3	7	14	28	42	63
DPH-Pyr (M2)	A	ND	ND	2.1	5.5	13.7	15.2	17.4
	B	ND	1.6	1.6	7.7	30.5	27.9	14.8
	Mean	ND	0.8	1.9	6.6	22.1	21.6	16.1
POPA (M5)	A	ND	1.6	1.3	2.3	2.7	2.4	3.6
	B	ND	1.7	1.6	2.3	2.0	ND	3.2
	Mean	ND	1.7	1.4	2.3	2.4	1.2	3.4
POP (M6)	A	ND	2.1	3.7	6.9	11.0	7.2	5.8
	B	ND	1.7	3.2	4.4	9.9	8.1	4.6
	Mean	ND	1.9	3.5	5.7	10.5	7.7	5.2
4’-OH-POP (M7)	A	ND	ND	ND	ND	4.7	16.0	16.5
	B	ND	ND	ND	2.0	9.9	14.4	7.3
	Mean	ND	ND	ND	1.0	7.3	15.2	11.9
M8	A	ND	ND	ND	ND	0.3	ND	3.7
	B	ND	ND	ND	ND	ND	ND	9.4
	Mean	ND	ND	ND	ND	0.1	ND	6.5
M10	A	ND	ND	ND	ND	ND	1.4	ND
	B	ND	ND	ND	1.9	ND	ND	ND
	Mean	ND	ND	ND	1.0	ND	0.7	ND
M13	A	ND	ND	2.1	2.2	3.5	ND	ND
	B	ND	ND	2.4	2.4	3.3	ND	ND
	Mean	ND	ND	2.3	2.3	3.4	ND	ND
M16	A	ND	ND	ND	ND	ND	2.1	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	1.1	ND
M17	A	ND	ND	ND	ND	ND	2.9	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	1.5	ND
M19	A	ND	ND	ND	ND	ND	2.4	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	1.2	ND
M20*	A	ND	ND	ND	2.6	1.1	4.8	8.3
	B	ND	ND	ND	2.0	8.8	5.2	15.8
	Mean	ND	ND	ND	2.3	5.0	5.0	12.1
M21	A	ND	ND	ND	ND	2.3	ND	ND
	B	ND	ND	ND	ND	5.4	ND	ND
	Mean	ND	ND	ND	ND	3.9	ND	ND
M23	A	ND	ND	ND	ND	1.8	ND	3.7
	B	ND	ND	ND	ND	2.9	2.4	2.2
	Mean	ND	ND	ND	ND	2.3	1.2	3.0

Compound	Replicate	0	3	7	14	28	42	63
M24	A	ND	ND	ND	ND	ND	6.9	7.6
	B	ND	ND	ND	ND	16.1	2.2	5.3
	Mean	ND	ND	ND	ND	8.0	4.5	6.4
M25	A	ND	ND	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	ND	4.0	1.7
	Mean	ND	ND	ND	ND	ND	2.0	0.8

ND = not detected
* M20 was highly polar fraction eluted at 3.5 min in HPLC, consisting of very low-molecular compounds.

Table 4.1.4.1-12 Pattern of degradation and formation of metabolite after the application of [phenyl-^{14}C] pyriproxyfen (low dose)

Compound	Replicate	0	3	7	14	28	42	63
Pyriproxyfen	A	94.9	52.4	29.4	35.6	4.6	ND	ND
	B	93.0	52.7	45.5	33.2	ND	ND	ND
	Mean	93.9	52.6	37.4	34.4	2.3	ND	ND
4'-OH-Pyr (M1)	A	ND	17.5	23.9	12.3	3.7	3.4	4.2
	B	ND	18.9	22.1	ND	ND	7.1	2.3
	Mean	ND	18.2	23.0	6.1	1.9	5.2	3.2
DPH-Pyr (M2)	A	ND	7.6	19.9	10.7	36.6	15.7	8.7
	B	ND	5.4	17.9	28.7	42.8	22.6	7.2
	Mean	ND	6.5	18.9	19.7	39.7	19.1	8.0
POPA (M5)	A	ND	2.7	4.9	2.8	ND	ND	2.2
	B	ND	2.5	3.2	ND	ND	ND	7.2
	Mean	ND	2.6	4.1	1.4	ND	ND	4.7
POP (M6)	A	ND	4.4	13.0	6.6	8.8	5.4	4.3
	B	ND	7.1	8.3	6.2	6.1	ND	5.8
	Mean	ND	5.7	10.7	6.4	7.4	2.7	5.0
4'-OH-POP (M7)	A	ND	ND	3.8	ND	13.1	9.9	9.3
	B	ND	ND	9.9	3.0	14.0	15.5	8.0
	Mean	ND	ND	6.8	1.5	13.5	12.7	8.6
M8	A	ND	ND	ND	ND	ND	ND	7.9
	B	ND	ND	ND	ND	ND	ND	6.3
	Mean	ND	ND	ND	ND	ND	ND	7.1
M10	A	ND	ND	ND	ND	5.1	ND	ND
	B	ND	ND	ND	ND	3.7	ND	ND
	Mean	ND	ND	ND	ND	4.4	ND	ND
M12	A	ND	1.9	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	0.9	ND	ND	ND	ND	ND

Compound	Replicate	0	3	7	14	28	42	63
M13	A	ND	ND	3.4	2.4	ND	ND	ND
	B	ND	2.4	ND	ND	ND	ND	ND
	Mean	ND	1.2	1.7	1.2	ND	ND	ND
M20*	A	ND	ND	ND	10.0	13.1	8.0	13.8
	B	ND	ND	ND	10.6	7.0	15.9	11.3
	Mean	ND	ND	ND	10.3	10.1	12.0	12.6
M21	A	ND	ND	ND	ND	ND	6.1	ND
	B	ND	ND	ND	ND	4.7	12.2	ND
	Mean	ND	ND	ND	ND	2.4	9.1	ND
M22	A	ND	ND	4.2	2.5	ND	ND	ND
	B	ND	ND	ND	ND	7.7	ND	ND
	Mean	ND	ND	2.1	1.3	3.9	ND	ND
M23	A	ND	ND	ND	ND	4.4	5.1	ND
	B	ND	ND	ND	ND	ND	ND	3.2
	Mean	ND	ND	ND	ND	2.2	2.5	1.6
M24	A	ND	ND	ND	ND	7.4	ND	4.3
	B	ND	ND	ND	ND	ND	ND	3.8
	Mean	ND	ND	ND	ND	3.7	ND	4.0
M25	A	ND	ND	ND	ND	ND	ND	3.0
	B	ND	ND	ND	ND	ND	ND	3.0
	Mean	ND	ND	ND	ND	ND	ND	3.0

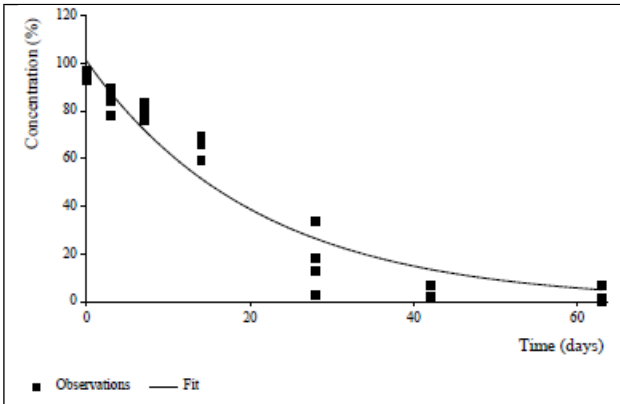
ND = not detected
* M20 was highly polar fraction eluted at 3.5 min in HPLC, consisting of very low-molecular compounds.

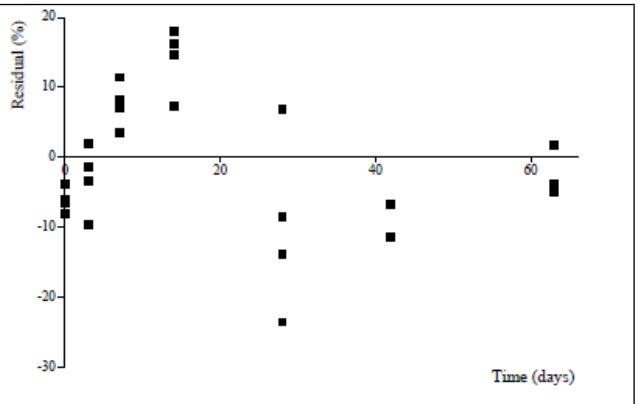
The degradation/dissipation rate for pyriproxyfen in pond water was calculated according to FOCUS Kinetics Guidance (2006) using a single first-order (SFO) kinetics model with CAKE software (version 3.1). Input data sets for modelling were derived from individual data for each time-point. All data points were unweighted. The calculated degradation half-lives (DT_{50}) and DT_{90} values obtained for the total system are shown in the table below.

Table 4.1.4.1-13 Rate of degradation for pyriproxyfen in natural water

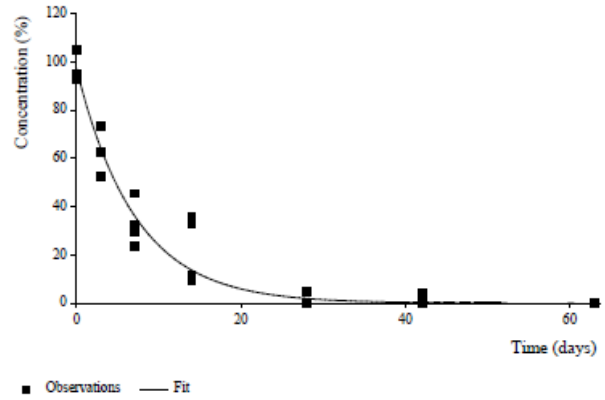
Pond: Test conc	Model	M_0	Parameter	Prob > t	DT_{50} (days)	DT_{90} (days)	Chi Square error %
High Dose	SFO Parent	100.9	k = 0.0478	3.87E-11	14.5	48.2	13.3
Low Dose		97.02	k = 0.1398	1.66E-11	5.0	16.5	10.2

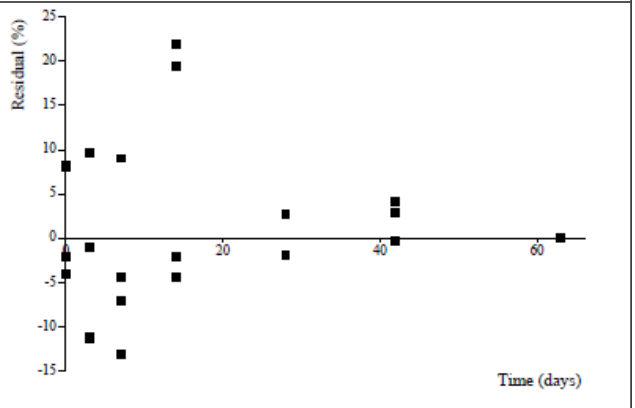
Figure 4.1.4.1-01 Modelled versus fitted data and residuals plots (SFO kinetics)





Observations and fitted model	Residuals
High dose	





Observations and fitted model	Residuals
Low dose	

4.1.4.2 C. J. Lewis. (2000a). [^{14}C] Pyriproxyfen: Degradation and retention in water-sediment systems

Study reference:

C. J. Lewis. (2000a). [^{14}C] Pyriproxyfen: Degradation and retention in water-sediment systems. Sumitomo Chemical Co., Ltd., report No.: NNM-0076

Detailed study summary and results:

The degradation of [phenyl-^{14}C] pyriproxyfen (PP) and [pyridyl-^{14}C] pyriproxyfen (PYR) was investigated in two water/sediment systems (Mill Stream Pond and Emperor Lake) which were sieved through a 2 mm (sediment) or 0.2 mm (water) sieve and stored for 2 days at ~4°C before use. Water/sediment systems (2.5 cm sediment layer – 6 cm water) were equilibrated for 54 days at 20±2°C. Test substance was applied in acetonitrile (~0.1% v/v) to the water layer at a concentration of 73 µg/L (equivalent to a surface application of 225 g a.s./ha distributed in a 30 cm deep water layer). The water/sediment systems were incubated at 20±2°C in the dark under continuous slight agitation (on an orbital shaker). Humidified CO_2-free air was passed through the headspace of the incubation flasks and volatiles and CO_2 were trapped. Sediment biomass was determined (fumigation extraction) at the beginning and end of the study.

Single flasks were analysed at 0, 1, 2, 3, 7, 14, 50 and 100 days after treatment. The water and sediment layer were separated. The water layer was acidified to pH 3-4 (acetic acid) and extracted three times with ethyl acetate, concentrated and analysed by HPLC-RAM (reversed phase) and TLC (normal phase). Radioactivity

was determined by LSC. The sediment was extracted three times with MeOH:water and three times with acetone:acetic acid. The combined extracts were concentrated to the aqueous phase and extracted three times with ethyl acetate after acidification to pH 3-4. Extracts were concentrated prior to analysis by HPLC-RAM (reversed phase) and TLC (normal phase). The sediment (only samples with maximum amount of unextractables) was further extracted by reflux and unextractable residues were fractionated into fulvic and humic acids and humin. Unextractables were determined by combustion/LSC. Radioactivity in trapping solutions was determined by LSC. CO_2 in the NaOH trap was confirmed by $BaCl_2$ precipitation. The recoveries of the concentration procedures were 97-99%. During extraction of the water layer, 2-3% AR was lost as a result of dissolved CO_2 losses (experimentally demonstrated). Identification of pyriproxyfen and metabolites was performed by comparison with reference standards.

Mass balances were 90-98% AR. The radioactivity level in the Mill stream pond water decreased from 57/54% AR (PP/PYR) on day 0 to 2.1/3.4% AR on day 100. The radioactivity level in the Emperor Lake water decreased from 73/75% AR (PP/PYR) on day 0 to 2.7/27% AR on day 100. The extractable amount of radioactivity partitioning into the Mill stream pond sediment increased to 63/59% AR (PP/PYR) on day 7/3 and was 5/20% AR on day 100. The amount of extractable radioactivity in the Emperor Lake sediment increased to 47/52% AR (PP/PYR) on day 7/7 and was 11/23% AR on day 100. The unextractable fraction in the Mill stream pond sediment increased to 39/37% AR (PP/PYR) on day 50/100 and was 37% AR on day 100. The unextractable fraction in the Emperor Lake sediment increased to 51/31% AR (PP/PYR) on day 100. CO_2 was a major degradation product (53/36% AR in Mill stream pond after 100 days and 25/11% AR in Emperor Lake after 100 days). Volatile organics were insignificant (≤0.2% AR). Of the unextractable sediment radioactivity, 3.2-5.3% AR was released by reflux extraction, 9.9-16.2% AR, 4.4-8.3% AR and 13-23% AR was associated with fulvic acids, humic acids and humin, respectively.

Pyriproxyfen in Mill stream pond water fell from 56/53% AR (PP/PYR) on day 0 to <1% AR on day 14. The level of parent pyriproxyfen in Emperor Lake water fell from 72/74% AR (PP/PYR) on day 0 to 3.1/1.7% AR on day 14 and <0.1% AR on day 100. The main metabolites in the Mill stream pond system were 4'-OH-Pyr (max. 4.8/15% AR on day 1/7 in water/sediment and PYPAC (max. 6.8/4.3% AR on day 50 in water/sediment). The main metabolites in the Emperor Lake system were 4'-OH-Pyr (max. 3.0/9.8% AR on day 2/14 in water/sediment), DPH-Pyr (max. 12/3.2% AR on day 2/50 in water/sediment) and PYPAC (max. 24/7.6% AR on day100/100 in water/sediment). Minor (≤1.6% AR in any system/compartment) identified metabolites were POP, POPA, 4'-OH-POP, PYPAC-Me and PYPA. The sum of unidentified components was ≤3.6/3.0% in water/sediment.

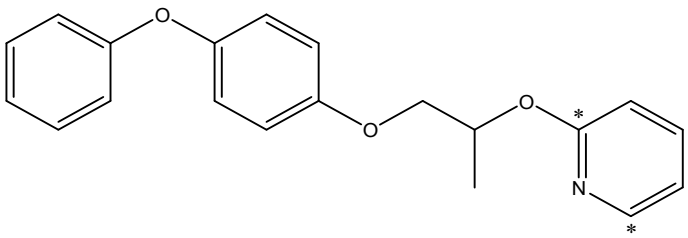
Test type:

SETAC Procedures for assessing the environmental fate and ecotoxicity of pesticides, Section 8.2 (1995).The study was conducted to GLP.

Materials and methods:

Test substance:

1a. Test Materials (radiolabelled):		[Pyridyl-^{14}C] pyriproxyfen
	Description:	Not stated
	Lot/Batch No.:	RIS99031
	Specific activity:	13.0 MBq/mg
	Radiopurity:	99.6%



* indicates position of radiolabel

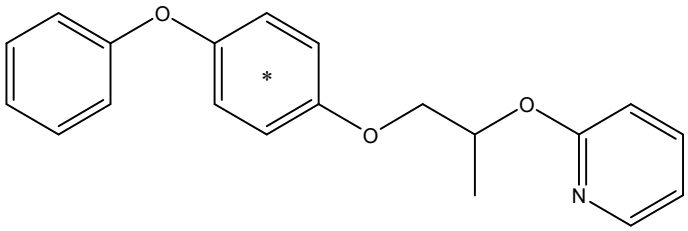
b. **(radiolabelled):** [Phenyl-^{14}C] pyriproxyfen

Description: Not stated

Lot/Batch No.: RIS99030

Specific activity: 12.4 MBq/mg

Radiopurity: 99.6%



* indicates position of radiolabel

Test System: Two UK water /sediment systems (Wareham, Dorset and Chatsworth, Derbyshire) were used and their characteristics are presented in Table B.4.1.4.2-01

Table 4.1.4.2-01 Water and sediment characteristics

Parameter	Mill stream pond		Emperor lake	
	Water	Sediment	Water	Sediment
Textural class[1]	NA	clay loam	NA	sandy loam
% sand/silt/clay[1]	NA	31/43/26	NA	72/11/17
CEC [meq/100 g]	NA	42.5	NA	7.8
Organic carbon	NA	9.2	NA	3.6
pH[2]	~8.5	~7.5	~8.0	~6.5
Microbial biomass [µg C/g] (start/end)	NA	727/1155	NA	197/163
Microbial biomass [% OC] (start/end)	NA	0.8/1.3	NA	0.6/0.5
Redox potential [mV][2]	~350	~100	~400	~200
Dissolved oxygen (% saturation)[2]	80	-	90	-

NA = not applicable, [1] according to DIN 19683, [2] determined at the end of acclimatisation

Study design:

The behaviour of [phenyl-^{14}C] and [pyridyl-^{14}C] pyriproxyfen was studied in two water/sediment systems, and in compliance with GLP.

Sediment and associated water were sampled from two locations, sieved through a 2 mm (sediment) and 0.2 mm (water) sieve and stored for 2 days at ~4°C before use. The water/sediment properties are listed in Table 4.1.4.2-01. Water/sediment systems (2.5 cm sediment layer - 6 cm water) were equilibrated for 54 days at 20±2°C prior to spiking (continuous slight agitation and aeration). Aliquots (90 µL) of [phenyl-^{14}C] and [pyridyl-^{14}C]pyriproxyfen in acetonitrile (~0.1% v/v) were added to the water layer at a concentration of 73 µg/L (equivalent to a surface application of 225 g as/ha distributed in a 30 cm deep water layer). The water/sediment systems were incubated at 20±2°C in the dark under continuous slight agitation (on an orbital shaker). Humidified CO_2-free air was passed through the headspace of the incubation flasks. Volatiles and CO_2 were trapped in ethanediol, 2% paraffin in xylene and 2M NaOH traps. Sediment biomass was determined (fumigation extraction) at the beginning and end of the study. Redox potential (sediment and water), pH (water) and dissolved oxygen (water) were measured at various time points in the equilibration and incubation phase of the study. The sediment redox potential (incubation phase) was -50 to +50 mV (Mill stream pond) and -20 to +100 mV (Emperor Lake). Oxygen saturation in the water (incubation phase) was 60-90% (Mill stream pond) and 70-90% (Emperor Lake). Water redox potentials (incubation phase) were +250 to +400 mV (Mill stream pond) and +225 to +475 mV (Emperor Lake).

Single flasks were analysed at 0, 1, 2, 3, 7, 14, 50 and 100 days after treatment. The water and sediment layer were separated (by aspiration). The water layer was acidified to pH 3-4 (acetic acid) and extracted three times with ethyl acetate. Radioactivity in the water layer, ethyl acetate extract and remaining aqueous phase was determined by LSC. Combined extracts were concentrated prior to analysis by HPLC-RAM (reversed phase) and TLC (normal phase). The sediment was extracted three times with MeOH:water and three times with acetone:acetic acid. The combined extracts were concentrated to the aqueous phase and extracted three times with ethyl acetate after acidification to pH 3-4. Radioactivity in the MeOH/acetone extracts, ethyl acetate extract and remaining aqueous phase was determined by LSC. Combined ethyl acetate extracts were concentrated prior to analysis by HPLC-RAM (reversed phase) and TLC (normal phase). The sediment (only samples with maximum amount of unextractables) was further extracted by acetone:acetic acid under reflux conditions and unextractable residues were fractionated into fulvic and humic acids and humin. Unextractables were determined by combustion/LSC. Radioactivity in trapping solutions was determined by LSC. CO_2 in the NaOH trap was confirmed by $BaCl_2$ precipitation. The recoveries of the concentration procedures were 97-99%. During extraction of the water layer, 2-3% AR was lost as a result of dissolved CO_2 losses (experimentally demonstrated).

Identification/quantification of pyriproxyfen and metabolites was performed by HPLC-RAM and TLC analysis and comparison with reference standards.

Results:

The distribution of radioactivity is shown in Tables 4.1.4.2-02 to -05. Mass balances were 90-98% AR. The radioactivity level in the Mill stream pond water decreased from 57/54% AR (phenyl/pyridyl) on day 0 to 2.1/3.4% AR (phenyl/pyridyl) on day 100. The radioactivity level in the Emperor Lake water decreased from 73/75% AR (phenyl/pyridyl) on day 0 to 2.7/27% AR (phenyl/pyridyl) on day 100. The extractable amount of radioactivity partitioning into the Mill stream pond sediment increased to 63/59% AR (phenyl/pyridyl) on day 7/3 and was 5/20% AR on day 100. The amount of extractable radioactivity partitioning into the Emperor Lake sediment increased to 47/52% AR (phenyl/pyridyl) on day 7/7 and was 11/23% AR on day 100. The unextractable fraction in the Mill stream pond sediment increased to 39/37% AR (phenyl/pyridyl) on day 50/100 and was 37/37% AR on day 100. The unextractable fraction in the Emperor Lake sediment increased to 51/31% AR (phenyl/pyridyl) on day 100. CO_2 was a major degradation product (53/36% AR in Mill stream pond after 100 days and 25/11% AR in Emperor Lake after 100 days). Volatile organics were insignificant (≤0.2% AR). Of the unextractable sediment radioactivity, 3.2-5.3% AR was released by reflux extraction, 9.9-16.2% AR, 4.4-8.3% AR and 13-23% AR was associated with fulvic acids, humic acids and humin, respectively.

Table 4.1.4.2-02 Distribution of radioactivity (% of applied) in Mill stream pond water and sediment treated with [phenyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied								
	Water			Sediment			CO_2	Volatiles	Mass balance[1]
	Extractable	Unextractable	Total	Extractable	Unextractable	Total			
0	56.6	0.4	57.0	40.0	0.1	40.1	NA	NA	97.1
1	41.0	3.4	44.4	44.8	5.3	50.1	0.3	0.0	95.8
2	29.6	1.4	30.5	61.7	3.8	65.5	0.3	0.0	97.4
3	30.7	2.1	30.5	57.3	4.1	61.4	0.8	0.0	93.9
7	5.4	1.2	9.3	63.1	15.3	78.4	7.4	0.0	96.0
14	2.6	2.9	5.9	36.2	30.3	66.5	21.8	0.0	94.5
50	2.3	1.7	4.4	17.4	38.5	55.9	31.1	0.0	97.5
100	0.5	1.3	2.1	5.1	36.8	41.9	52.5	0.1	97.7

NA = not applicable, [1] includes glass rinsates (0.0-1.2% AR, except day 50: 6.1% AR)

Table 4.1.4.2-03 Distribution of radioactivity (% of applied) in Emperor Lake water and sediment treated with [phenyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied								
	Water			Sediment			CO_2	Volatiles	Mass balance[1]
	Extractable	Unextractable	Total	Extractable	Unextractable	Total			
0	72.8	0.3	73.1	24.6	0.1	24.7	NA	NA	97.8
1	40.3	0.5	40.8	46.5	7.8	54.3	0.1	0.0	96.0
2	27.3	3.7	33.5	28.0	27.2	55.2	1.3	0.0	91.7
3	10.7	1.1	13.3	45.3	29.4	74.7	1.9	0.0	90.2
7	17.8	2.1	20.8	47.1	20.4	67.5	2.7	0.0	91.8
14	11.6	2.3	14.9	43.2	29.4	72.6	4.4	0.0	94.3
50	3.2	3.5	7.1	22.9	47.3	70.2	14.1	0.1	92.0
100	1.1	1.3	2.7	10.8	51.4	62.2	24.6	0.1	90.6

NA = not applicable, [1] includes glass rinsates (0.0-2.4% AR)

Table 4.1.4.2-04 Distribution of radioactivity (% of applied) in Mill stream pond water and sediment treated with [pyridyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied								
	Water			Sediment			CO_2	Volatiles	Mass balance[1]
	Extractable	Unextractable	Total	Extractable	Unextractable	Total			
0	53.6	0.8	54.4	42.0	0.1	42.1	NA	NA	96.5
1	36.8	2.1	38.9	52.5	3.5	56.0	0.3	0.0	96.3
2	34.4	2.3	36.7	50.9	3.7	54.6	0.5	0.0	93.5
3	22.7	2.5	25.6	58.8	7.9	66.7	1.1	0.0	95.5
7	20.2	3.8	25.4	50.4	14.5	64.9	2.6	0.0	93.2
14	11.0	2.1	13.4	55.9	18.0	73.9	8.9	0.0	96.7
50	6.8	1.8	9.9	22.2	32.6	54.8	30.0	0.1	96.5
100	1.1	1.1	3.4	19.7	36.7	56.4	35.9	0.2	96.4

NA = not applicable, [1] includes glass rinsates (0.0-2.1% AR)

Table 4.1.4.2-05 Distribution of radioactivity (% of applied) in Emperor Lake water and sediment treated with [pyridyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied								
	Water			Sediment			CO_2	Volatiles	Mass balance[1]
	Extractable	Unextractable	Total	Extractable	Unextractable	Total			
0	74.4	0.4	74.8	23.7	0.1	23.8	na	na	98.6
1	59.2	0.6	59.8	31.6	3.3	34.9	0.0	0.0	95.6
2	37.6	1.0	38.6	50.1	7.0	57.1	0.1	0.0	96.3
3	33.4	2.5	36.4	43.9	13.3	57.2	0.8	0.0	94.9
7	25.4	1.9	27.7	52.1	13.8	65.9	1.3	0.0	97.1
14	18.1	2.5	22.1	50.0	19.4	69.4	2.2	0.0	96.0
50	21.5	3.2	25.8	33.5	27.7	61.2	6.2	0.0	93.3
100	23.8	2.9	26.9	23.0	30.9	53.9	11.0	0.0	92.6

NA = not applicable, [1] includes glass rinsates (0.0-2.3% AR)

Identified compounds in water or sediment are shown in Tables 4.1.4.2-06 to -09. The level of parent pyriproxyfen in Mill stream pond water fell from 56/53% AR (phenyl/pyridyl) on day 0 to <1% AR on day 14. The level of parent pyriproxyfen in Emperor Lake water fell from 72/74% AR (phenyl/pyridyl) on day 0 to 3.1/1.7% AR on day 14 and <0.1% AR on day 100. The main metabolites (>10% AR and/or 2x>5% AR and/or increasing trend) in the Mill stream pond system were 4'-OH-Pyr (max. 4.8/15% AR on day 1/7 in water/sediment and PYPAC (max. 6.8/4.3% AR on day 50/50 in water/sediment). The main metabolites (>10% AR and/or 2x>5% AR and/or increasing trend) in the Emperor Lake system were 4'-OH-Pyr (max. 3.0/9.8%

AR on day 2/14 in water/sediment), DPH-Pyr (max. 12/3.2% AR on day 2/50 in water/sediment) and PYPAC (max. 24/7.6% AR on day 100/100 in water/sediment). Minor (≤1.6% AR in any system/compartment) identified metabolites were POP, POPA, 4'-OH-POP, PYPAC-Me and PYPA. The sum of unidentified components was ≤3.6/3.0% in water/sediment.

Table 4.1.4.2-06 Compounds (% of applied) in Mill stream pond water and sediment treated with [phenyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	POP	POPA	4'-OH-POP	Sum unidentified
Water							
0	56.4	ND	ND	ND	ND	ND	ND
1[1]	17.8	9.0	9.7	1.6	1.6	1.1	ND
2	22.2	3.8	2.4	0.4	ND	ND	0.5
3	20.8	4.5	3.2	ND	1.0	ND	1.0
7	2.9	0.9	0.7	ND	ND	ND	1.0
14	0.8	0.2	ND	ND	ND	ND	1.6
50	0.5	0.4	0.4	ND	ND	ND	1.0
100	SNA	SNA	SNA	SNA	SNA	SNA	SNA
Sediment							
0	38.2	ND	ND	ND	ND	ND	ND
1[1]	30.8	8.8	2.2	ND	ND	ND	ND
2	48.5	9.3	0.9	ND	ND	ND	0.9
3	47.7	6.6	1.1	ND	ND	ND	0.8
7	44.4	10.7	1.2	0.5	ND	ND	1.9
14	21.2	7.8	2.6	ND	ND	ND	1.7
50	10.1	3.1	2.0	ND	ND	0.5	ND
100	1.6	0.5	2.4	ND	ND	ND	ND
Total							
0	94.6	ND	ND	ND	ND	ND	ND
1[1]	48.6	17.8	11.9	1.6	1.6	1.1	ND
2	70.8	13.2	3.3	0.4	ND	ND	1.4
3	68.5	11.1	4.3	ND	1.0	ND	1.8
7	47.3	11.6	1.9	0.5	ND	ND	2.9
14	22.0	8.0	2.6	ND	ND	ND	3.4
50	10.6	3.5	2.3	ND	ND	0.5	1.0
100	1.6	0.5	2.4	ND	ND	ND	ND

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	POP	POPA	4'-OH-POP	Sum unidentified

ND = not detected, SNA = sample not analysed, [1] data point was considered an outlier (deviating results for pyriproxyfen)

Table 4.1.4.2-07 Compounds (% of applied) in Emperor Lake water and sediment treated with [phenyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	POP	POPA	4'-OH-POP	Sum unidentified
Water							
0	72.2	ND	ND	ND	ND	ND	ND
1	39.5	0.7	ND	ND	ND	ND	ND
2	12.4	3.0	11.8	ND	ND	ND	ND
3	9.5	1.0	ND	ND	ND	ND	ND
7	14.8	1.9	0.9	ND	ND	ND	ND
14	3.1	1.9	4.4	ND	ND	1.0	1.1
50	0.2	ND	0.8	ND	ND	ND	2.2
100	ND	ND	ND	ND	ND	ND	1.1
Sediment							
0	23.8	ND	ND	ND	ND	ND	ND
1	44.7	ND	ND	ND	ND	ND	ND
2	24.9	1.0	0.9	ND	ND	ND	ND
3	39.9	2.2	ND	ND	ND	ND	ND
7	RS	RS	RS	RS	RS	RS	RS
14	30.2	9.8	3.1	ND	ND	ND	ND
50	13.5	4.8	3.2	ND	ND	ND	ND
100	5.7	2.3	2.2	ND	ND	ND	ND
Total							
0	96.1	ND	ND	ND	ND	ND	ND
1	84.3	0.7	ND	ND	ND	ND	ND
2	37.3	3.9	**12.7**	ND	ND	ND	ND
3	49.5	3.2	ND	ND	ND	ND	ND
7	RS	RS	RS	RS	RS	RS	RS
14	33.3	11.7	7.5	ND	ND	1.0	1.1
50	13.8	4.8	4.0	ND	ND	ND	2.2

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	POP	POPA	4'-OH-POP	Sum unidentified
100	5.7	2.3	2.2	ND	ND	ND	1.1

ND = not detected, RS = rejected sample

Table 4.1.4.2-08 Compounds (% of applied) in Mill stream pond water and sediment treated with [pyridyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	PYPAC	PYPAC-Me	PYPA	Sum unidentified
Water							
0	53.3	ND	ND	ND	ND	ND	ND
1	27.4	4.8	2.3	2.2	ND	ND	ND
2	22.7	4.7	2.5	3.0	ND	ND	1.2
3	13.9	1.5	2.3	4.8	ND	ND	ND
7	3.6	1.1	4.2	6.7	0.7	0.2	3.6
14[1]	0.7	0.2	ND	9.6	ND	ND	0.5
50	ND	ND	ND	6.8	ND	ND	ND
100	ND	ND	ND	ND	ND	ND	1.1
Sediment							
0	40.2	ND	ND	ND	ND	ND	ND
1	45.3	5.3	ND	ND	ND	ND	ND
2	42.9	5.8	0.9	ND	ND	ND	1.0
3	39.5	11.5	1.8	1.0	ND	ND	2.0
7	22.3	14.8	4.3	2.3	ND	ND	3.0
14[1]	40.4	8.2	1.0	4.1	ND	ND	ND
50	8.7	4.6	1.4	4.3	ND	ND	0.3
100	8.8	5.5	2.6	0.7	ND	ND	ND
Total							
0	93.5	ND	ND	ND	ND	ND	ND
1	72.7	10.2	2.3	2.2	ND	ND	ND
2	65.6	10.5	3.4	3.0	ND	ND	2.2
3	53.4	13.0	4.1	5.8	ND	ND	2.0
7	26.0	**15.9**	8.5	9.0	0.7	0.2	6.7
14[1]	41.1	8.4	1.0	13.6	ND	ND	0.5

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	PYPAC	PYPAC-Me	PYPA	Sum unidentified
50	8.7	4.6	1.4	11.1	ND	ND	0.3
100	8.8	5.5	2.6	0.7	ND	ND	1.1

ND = not detected, [1] data point was considered an outlier (deviating results for pyriproxyfen)

Table 4.1.4.2-09 Compounds (% of applied) in Emperor Lake water and sediment treated with [pyridyl-^{14}C] pyriproxyfen at 73 µg/L and incubated at 20°C in the dark

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	PYPAC	PYPAC-Me	PYPA	Sum unidentified
Water							
0	74.3	ND	ND	ND	ND	ND	ND
1	55.4	0.9	ND	2.5	ND	ND	ND
2	29.9	0.5	ND	6.7	ND	0.4	ND
3	20.7	1.1	0.9	10.1	ND	0.6	ND
7	11.9	ND	ND	12.4	ND	0.8	ND
14	1.7	1.4	1.4	13.5	ND	ND	ND
50	ND	ND	ND	19.4	1.6	0.3	ND
100	ND	ND	ND	23.6	ND	ND	ND
Sediment							
0	22.5	ND	ND	ND	ND	ND	ND
1	30.6	ND	ND	ND	ND	ND	ND
2	46.2	1.3	ND	ND	ND	ND	ND
3	38.4	3.1	ND	1.2	ND	ND	ND
7	43.6	4.7	0.8	2.5	ND	ND	ND
14	35.8	7.0	2.0	4.1	ND	ND	ND
50	13.5	9.2	2.9	6.2	ND	ND	ND
100	9.2	3.3	1.8	7.6	ND	ND	ND
Total							
0	96.9	ND	ND	ND	ND	ND	ND
1	86.0	0.9	ND	2.5	ND	ND	ND
2	76.1	1.8	ND	6.7	ND	0.4	ND
3	59.2	4.2	0.9	11.3	ND	0.6	ND
7	55.6	4.7	0.8	14.9	ND	0.8	ND

Days of incubation	% of applied						
	Pyriproxyfen	4'-OH-Pyr	DPH-Pyr	PYPAC	PYPAC-Me	PYPA	Sum unidentified
14	37.5	8.4	3.4	17.6	ND	ND	ND
50	13.5	9.2	2.9	25.6	1.6	0.3	ND
100	9.2	3.3	1.8	31.2	ND	ND	ND

ND = not detected, [1] data point was considered an outlier (deviating results for pyriproxyfen)

4.1.4.3 J. Cooke (2016b) Pyriproxyfen: Kinetic assessment of water/sediment studies

Study reference:

J. Cooke (2016b) Pyriproxyfen: Kinetic assessment of water/sediment studies. Sumitomo Chemical Co., Ltd., Unpublished report No.: NNM-0096

Detailed study summary and results:

The degradation/dissipation behaviour of pyriproxyfen in water/sediment systems has been investigated in a laboratory study (Lewis, 2000a, 4.1.4.2 – Study 2).

The data from this study have been used to determine the degradation/dissipation half-lives of pyriproxyfen in water/sediment. The data were considered appropriate for calculation of both persistence and modelling endpoints.

The data from this study were analysed using the CAKE v3.2 (2016) software package according to guidance provided by FOCUS (2006, 2011) based on level P-1 kinetics (single compartment kinetics). DT_{50} and DT_{90} values were calculated for comparison with relevant study triggers and persistence criteria and separate DT_{50} values were calculated for use as modelling endpoints.

The FOCUS (2006, 2011) flowcharts for calculating persistence and modelling endpoints have been followed. Each compartment of the water/sediment systems has been considered following the steps in the flowchart.

The pyriproxyfen persistence DT_{50} values in the total system ranged from 4.8 to 5.7 days and DT_{90} values ranged from 53.5 to 61.0 days. Modelling total system DT_{50} values ranged from 16.1 to 18.4 days, with a geometric mean of 17.2 days.

The pyriproxyfen persistence DT_{50} values in the surface water ranged from 0.4 to 0.9 days and DT_{90} values ranged from 5.0 to 7.5 days. Modelling surface water DT_{50} values ranged from 1.5 to 2.3 days, with a geometric mean of 1.9 days.

The pyriproxyfen persistence DT_{50} values in the sediment ranged from 22.1 to 36.8 days and DT_{90} values ranged from 73.5 to 122 days. Modelling sediment DT_{50} values also ranged from 22.1 to 36.8 days, with a geometric mean of 28.5 days.

Test type:

FOCUS (2011). Version 1.0, 436 pp amending FOCUS (2006) version 2.0, 434 pp. GLP is not a requirement for this assessment.

Test substance:

For full details see 4.1.4.2 – Study 2

Materials and methods:

Input data were generated according to the data handling recommendations made in the FOCUS guidance for degradation kinetics (FOCUS, 2006, 2011).

Two separate radiolabelling positions were used in the Lewis (2000) study. These were treated as true replicates for kinetic fitting.

The limit of detection (LOD) for HPLC was recorded as *ca* 0.2% of applied radioactivity in the Lewis (2000a) study. The report stated that, at this level, the handling of non-detects according to the procedure recommended by FOCUS (2006, 2011) has no influence on estimated degradation/dissipation endpoints and was not necessary (i.e., the non-detects were omitted from modelling).

The initial percent recovery of pyriproxyfen, M_0, in the total system and the water phase was set equivalent to the initial mass balance value for the total system.

The sediment values used in the calculations were those from the maximum onwards and the time at which the maximum occurred was set to 0 days.

The model input data are shown in Table 4.1.4.3-01 and Table 4.1.4.3-02.

The kinetic modelling was conducted using CAKE version 3.2 (2016). In the first instance, the data were directly fitted, un-weighted, with the complete usable data set and unconstrained initial concentration (M_0). The acceptability of kinetic fits was judged both visually and according to the χ^2 error and the t-test functions as recommended by FOCUS (2006, 2011). It is recommended that a χ^2 error of 15% or less and a t-test probability of greater than 90% ($p < 0.1$) for estimated degradation rate constants indicate acceptable fits. When fitting the first-order multi-compartment model (FOMC), the t-test is not appropriate as a measure of confidence (FOCUS, 2006, 2011). In this case, the confidence intervals of the α and β parameter estimates were assessed and a fit was considered acceptable if the 95th percentile intervals did not include zero.

The FOCUS (2006, 2011) Level P-I flowcharts for calculating persistence and modelling endpoints have been followed.

The Lewis (2000) study was performed at 20 ± 2°C, so normalisation was not required.

Table 4.1.4.3-01 Model input data from Lewis (2000) pyriproxyfen in Mill stream pond water/sediment

Radiolabel	Time (DAT)	Pyriproxyfen (%)		
		Total system	Surface water	Sediment
Phenoxy	0	97.1	97.1	NA
	1	48.6	17.8	NA
	2 (0)	70.8	22.2	48.5
	3 (1)	68.5	20.8	47.7
	7 (5)	47.3	2.9	44.4
	14 (12)	22.0	0.8	21.2
	50 (48)	10.6	0.5	10.1
	100 (98)	1.6	SNA	1.6
Pyridyl	0	96.5	96.5	NA
	1	72.7	27.4	NA
	2 (0)	65.6	22.7	42.9
	3 (1)	53.4	13.9	39.5
	7 (5)	26.0	3.6	22.3
	14 (12)	41.1	0.7	40.4
	50 (48)	8.7	ND	8.7
	100 (98)	8.8	ND	8.8

NA = not applicable (sediment values before maximum not used in the kinetics assessment), SNA = sample not analysed, ND = not detected, ( ) = values are days after the maximum in sediment.

Table 4.1.4.3-02 Model input data from Lewis (2000) pyriproxyfen in Emperor lake water/sediment

Radiolabel	Time (DAT)	Pyriproxyfen (%)		
		Total system	Surface water	Sediment
Phenoxy	0	97.8	97.8	NA
	1	84.3	39.5	NA
	2 (0)	37.3	12.4	24.9
	3 (1)	49.5	9.5	39.9
	7 (5)	RS	14.8	RS
	14 (12)	33.3	3.1	30.2
	50 (48)	13.8	0.2	13.5
	100 (98)	5.7	ND	5.7

Radiolabel	Time (DAT)	Pyriproxyfen (%)		
		Total system	Surface water	Sediment
Pyridyl	0	98.6	98.6	NA
	1	86	55.4	NA
	2 (0)	76.1	29.9	46.2
	3 (1)	59.2	20.7	38.4
	7 (5)	55.6	11.9	43.6
	14 (12)	37.5	1.7	35.8
	50 (48)	13.5	ND	13.5
	100 (98)	9.2	ND	9.2

NA = not applicable (sediment values before maximum not used in the kinetics assessment), RS = rejected sample,
ND = not detected, ( ) = values are days after the maximum in sediment.

Results:

The persistence endpoints for pyriproxyfen are summarised in Table 4.1.4.3-03.

Table 4.1.4.3-03 Summary of persistence endpoints for pyriproxyfen from water/sediment study

Water/ sediment system	Temp (°C)	Total system (degradation)		Water (dissipation)		Sediment[1] (dissipation)		Kinetics
		DT_{50}/DT_{90} (days)	χ^2 error (%)	DT_{50}/DT_{90} (days)	χ^2 error (%)	DT_{50}/DT_{90} (days)	χ^2 error (%)	
Mill stream pond	20	4.8 / 53.5	12.2	0.4 / 5.0	7.6	22.1 / 73.5	6.6	DFOP/ HS/ SFO
Emperor lake	20	5.7 / 61.0	8.0	0.9 / 7.5	5.3	36.8 /122	10.3	HS/ HS/ SFO
Worst case		5.7 / 61.0		0.9 / 7.5		36.8 / 122		
Geometric mean		5.2 / 57.1		0.6 / 6.1		28.5 / 94.7		

[1] calculated as decline from maximum observed

The modelling endpoints for pyriproxyfen are summarised in Table 4.1.4.3-04.

Table 4.1.4.3-04 Summary of modelling endpoints for pyriproxyfen from water/sediment study

Water/ sediment system	Temp (°C)	Total system (degradation)		Water (dissipation)		Sediment[1] (dissipation)		Kinetics
		DT_{50} (days)	χ^2 error (%)	DT_{50} (days)	χ^2 error (%)	DT_{50} (days)	χ^2 error (%)	
Mill stream pond	20	16.1	12.2	1.5	7.6	22.1	6.6	DFOP/ HS/ SFO
Emperor lake	20	18.4	8.0	2.3	5.3	36.8	10.3	HS/ HS/ SFO
Geometric mean		17.2		1.9		28.5		

[1] calculated as decline from maximum observed

4.1.4.4 M. Yoshida, R. Kodaka, T. Fujisawa (2013) Stability of pyriproxyfen in air (calculation by Atkinson's method)

Study reference:

M. Yoshida, R. Kodaka, T. Fujisawa (2013) Stability of pyriproxyfen in air (calculation by Atkinson's method). Sumitomo Chemical Co., Ltd., Unpublished report No.: NNP-0120

Detailed study summary and results:

The decomposition rate constant of pyriproxyfen in the atmosphere is estimated according to the Atkinson's method, using the Atmospheric Oxidation Program (AOPWIN version 1.92, US EPA). The AOPWIN is a computer program calculating the rate constants of the gas-phase reactions between photochemically produced hydroxyl radical and organic chemicals, and between ozone and olefinic/acetylenic compounds.

The reaction rate constant of pyriproxyfen with hydroxyl radical was calculated to be 52.2359×10^{-12} cm^3 $molecule^{-1}$ sec^{-1}. Assuming that a 12-hour daytime hydroxyl radical concentration is 1.5×10^6 molecules cm^{-3}, the corresponding half-life was calculated to be 2.457 hours (0.205 days).

Test type:

Calculation of stability of Pyriproxyfen in air, there is no guideline for this test. GLP is not a requirement for this assessment.

Test substance:

Pyriproxyfen, however, this is a calculation only, therefore no actual test substance.

Materials and methods:

The decomposition rate constant of pyriproxyfen in the atmosphere is estimated according to the Atkinson's method, using the Atmospheric Oxidation Program (AOPWIN version 1.92, US EPA). The AOPWIN is a computer program calculating the rate constants of the gas-phase reactions between photochemically produced hydroxyl radical and organic chemicals, and between ozone and olefinic/acetylenic compounds

Results:

The reaction rate constant of pyriproxyfen with hydroxyl radical was calculated to be 52.2359 × 10-12 cm^3 $molecule^{-1}$ sec^{-1}. Assuming that a 12-hour daytime hydroxyl radical concentration is 1.5×10^6 molecules cm^{-3}, the corresponding half-life was calculated to be 2.457 hours (0.205 days).

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

4.2.1.1 CA 8.2.2.3/01a, CA 8.2.2.3/01b, CA 8.2.2.3/01c

Report: CA 8.2.2.3/01a (1993) Uptake, depuration and bioconcentration of ^{14}C-pyriproxyfen by bluegill sunfish. Sumitomo Chemical Co., Ltd., Unpublished Report No. NNM-31-0027 CA 8.2.2.3/01b (1994) Characterization of ^{14}C-residues in bluegill sunfish treated with ^{14}C-pyriproxyfen. Sumitomo Chemical Co., Ltd., Unpublished Report No. NNM-41-0031 CA 8.2.2.3/01c (1999) Calculation of ^{14}C-pyriproxyfen clearance time (CT_{90}, CT_{95}) in bluegill sunfish. Sumitomo Chemical Co., Ltd., Unpublished Report No. NNM-0061	
Previous evaluation	Sumbited in the DAR (November 2005). A new robust summary is provided below because this study is used to derive endpoints for the fish risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion. Re-evaluated by the RMS for the re-registration and considered acceptably conservative for use in risk assessment, despite short-comings (see comments below).
Conclusion	Bioconcentration factor = 1379 - 1495 L/kg wwt

Guidelines

U.S. Environmental Protection Agency, 1982. Pesticide Assessment Guidelines, Subdivision N - Chemistry: Environmental Fate. Series 165-4, Laboratory Studies of Pesticide Accumulation in Fish

Deviations: None reported.

GLP

Yes (certified laboratory), except for MULTI calculation

Materials and Methods

Materials

1. Test Material:	[phenyl-^{14}C]pyriproxyfen, [pyridyl-^{14}C]pyriproxyfen
Description:	Not stated
Lot/Batch #:	[phenyl-^{14}C]pyriproxyfen: C-90-019; [pyridyl-^{14}C]pyriproxyfen: C-90-018
Radiochemical purity:	[phenyl-^{14}C]pyriproxyfen: 100.0 ± 0.1%, specific radioactivity: 181 µCi/mg; [pyridyl-^{14}C]pyriproxyfen: 99.1 ± 0.6%, Specific radioactivity: 257 µCi/mg
Expiry date:	Not stated, but the structure was confirmed by NMR and mass spectra analyses in the study
2a. Test animals:	Bluegill sunfish (*Lepomie macrochirus*), used in the test were obtained from Kurtz's Fish Hatchery, Pennsylvania, USA.

2b. Maintenance:	The fish were maintained under controlled laboratory conditions in dilution water until they were placed into test chambers on June 6, 1990. On Day -3, the mean weight of these fish was 0.84 ± 0.22 g (mean ± SD) with a range of 0.58 - 1.53 g. These fish had a mean standard length of 32.1 ± 2.6 mm (mean ± SD) with a range of 28 - 39 mm. Fish were fed frozen brine shrimp during acclimation, holding, and throughout the study at a daily rate of approximately 2 percent of their body weight. During the acclimation period, the diet was periodically supplemented with Purina Trout Chow (#2) and/or Tetramin Flakes.

Study Design

Bluegill sunfish (*Lepomis macrochirus*) at an initial loading of 250 fish per 60 L exposure chambers were exposed to [phenyl-^{14}C] or [pyridyl-^{14}C] pyriproxyfen for 28 days at a nominal concentration of 0.02 mg a.s./L in a flow-through system. An additional group of 250 fish were exposed to dilution water/carrier solvent (0.09 mL dimethyl formamide/2.5 L water) as a control: the dilution water was blended well water with a hardness of 56-80 mg/L as $CaCO_3$. The flow rate of test solution (480 L/day/test chamber) supplied to the chambers was sufficient to replace the approximately 44 L test volume about 11x per day.

At the end of the 28-day exposure period, the delivery of ^{14}C-pyriproxyfen was stopped and then dilution water only was delivered into the control and test chambers. The remaining fish were then exposed to flowing untreated water for a 14-day depuration phase. The nominal test conditions were: 16 hours light, 8 hours dark; a temperature of 20-22°C, pH 6.8-6.9, dissolved oxygen concentration 7.0mg/L.

The bluegill sunfish used for this experiment had an initial mean weight of 0.84 (±0.22) g and initial mean standard length of 32.1 (±2.6) mm. Fish samples were taken at 3, 7, 14, 21 and 28 days at the exposure phase (days 0, 21 and 28 in the case of the control) and 1, 3, 7, 10 and 14 days of the depuration phase. Water samples were taken on days 0, 3, 7, 14, 21 and 28 days and days 1 and 3 of the depuration phase.

On each sampling date, six fish were collected from each test chamber. Three fish were dissected into fillet/edible (body, muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). The other three fish from each aquarium were used for whole fish analysis. The weights of dissected parts and whole fish were measured. An additional 85 fish were taken for metabolite identification at 21 and 28 days.

For determination of ^{14}C concentrations (ppm) in the fish and the subsequent calculation of BCFs (bioconcentration factors), individual samples were homogenised with liquid nitrogen, aliquots were combusted and radioactivity measured using Liquid Scintillation Counting (LSC). The depuration rate constant (K_2), CT_{90} and CT_{95} were determined by the MULTI computer program.

For metabolite identification, each treated sample was separately homogenised in liquid nitrogen. The homogenate was extracted with methanol. The remaining solid fractions were further extracted for the analysis of unextractable residues. Remaining post extraction solids (PES) were combusted for LSC analysis. The methanol extracts were analysed by TLC and HPLC co-chromatography with reference standards.

Results and Discussion

Physical and Chemical Measurements of Water

Temperature in the control and treatment chambers ranged from 20.4 to 22.1°C during the study. The dissolved oxygen concentration ranged from 4.7 to 8.9 mg/L and was more than 68 percent of saturation at 22°C. The pH values in the test chambers were in good accordance with those in the control throughout the study, ranging from 6.4 to 7.1.

Measurement of Test Concentrations

The radioactivity of water calculated as mg/l of ^{14}C-pyriproxyfen in phenyl and pyridyl labelled test chambers during the 28 day exposure period were 0.0191 (±0.0023) mg/l and 0.0200 (±0.0027) mg/l, respectively. During the depuration period, concentrations of radioactivity were negligible.

Biological Findings

No abnormal behaviour was observed for the control and treated fish. No fish mortality was observed during the depuration phase, 17 fish (7 percent) in the control, 10 fish (4 percent) in the [phenyl-^{14}C] chamber and 9 fish (4 percent) in the [pyridyl-^{14}C] chamber died during the uptake phase. Fish showed no visible signs of stress during the uptake or depuration phase of the study and accepted food readily each day. The ^{14}C concentration in fish during the uptake phase reached equilibrium between days 3-7 of exposure. When the fish were exposed to clean water, the radiocarbon was eliminated rapidly from the fish body and the percentages of the [phenyl-^{14}C] label eliminated by day 14 in the whole fish, edible tissues and non-edible tissues were 98.1, 93.1 and 97.8%, respectively. The corresponding figures for the [pyridyl-^{14}C] label were 89.6, 69.5 and 89.7%, respectively.

The concentrations of radioactivity in fish during the uptake and depuration phases and the resultant bioconcentration factors (BCFs) are presented in Tables B.9.2.8.1-1and -2.

Table B.9.2.8.1-01 Bioconcentration factors of radioactivity in bluegill sunfish treated with ^{14}C-pyriproxyfen during uptake period

Radio-label	Period (days)	Whole fish		Fillet		Viscera	
		mg/kg *)	BCF	mg/kg *)	BCF	mg/kg *)	BCF
phenyl-^{14}C	3	20.878		9.447		31.898	
	7	27.438		11.080		39.845	
	14	26.928		9.448		35.415	
	21	22.464		8.950		72.464	
	28	34.025		5.464		57.364	
	Average	26.346	1379	8.878	465	47.397	2482
pyridyl-^{14}C	3	28.778		6.881		46.043	
	7	28.873		10.889		36.060	
	14	26.439		10.424		48.567	
	21	28.858		8.507		48.616	
	28	36.536		11.108		59.713	
	Average	29.897	1495	9.562	478	47.800	2390

* Concentration of radioactivity (mg/kg ; equivalent to pyriproxyfen)

Table B.9.8.2.1-02 Elimination of radioactivity from bluegill sunfish treated with ^{14}C-pyriproxyfen during depuration period

Period (days)	Concentration of radioactivity in fish (mg/kg ; equivalent to pyriproxyfen)					
	phenyl-^{14}C			pyridyl-^{14}C		
	Whole fish	Fillet	Viscera	Whole fish	Fillet	Viscera
0	34.025	5.464	57.364	36.536	11.108	59.713
1	22.629	5.423	25.675	28.552	6.600	35.449
3	3.314	1.007	6.337	10.606	3.925	12.120
7	1.060	0.466	2.572	4.581	3.452	6.964
10	0.580	0.215	1.158	4.438	2.598	4.436
14	0.657	0.377	1.265	3.793	3.386	6.176

The clearance times (CT_{50}, CT_{90} and CT_{95}) were calculated using the computer program MULTI. The results of these calculations are shown in the Table below.

Table B.9.8.2.1-03 The clearance times (CT_{50}, CT_{90} and CT_{95}) of radioactivity in whole fish during depuration phase

Radiolabel	Clearance time (days)		
	CT_{50}*	CT_{90}	CT_{95}
phenyl-^{14}C	0.86	3.4	4.4
pyridyl-^{14}C	1.63	8.4	11.0

* Calculated using least squares method

The metabolite distribution in fish is shown in Tables B.9.8.2.1-4A and -4B. Pyriproxyfen in fish is primarily metabolised by hydroxylation to form 4'-OH-Pyr, which occurs as sulfate and glucronide conjugates. In fish tissue, pyriproxyfen plus 4'-OH-Pyr and its conjugates together accounted for 37-70% of the radioactive residue. Direct hydroxylation also led to 5'',4'-OH-Pyr, found as the sulfate conjugate.

Two metabolites, 4'-OH-POP and 5-OH-PYPAC, were found also as conjugates. Other metabolites (POPA, DPH-Pyr, PYPAC, 2-OH-PYPA and PYPA) were found in relatively small amounts as simple non-conjugated fragments of pyriproxyfen. Post extracted solids (PES) were also analysed (Table CA 8.2.7/01-5). The majority of the radioactivity in the PES was associated with proteins (extracted with pronase). Both the hydroxylated and conjugated products were eliminated by the fish and were found in the exposure water. None of the products, or pyriproxyfen, accumulated irreversibly in fish as shown by the high rate of depuration.

The chemical name and abbreviation of the metabolites are shown in Table B.9.2.8.1-06. The proposed metabolic pathways of pyriproxyfen are shown in Figure B.9.2.8.1-01.

Table B.9.2.8.1-4A Quantitation of ^{14}C-components in methanol extracts of fish ([phenyl-^{14}C] pyriproxyfen)

Components	Fillet				Viscera			
	Day 21		Day 28		Day 21		Day 28	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Pyriproxyfen	48.2	3.93	47.1	4.95	24.0	12.3	23.3	13.2
POPA	2.0	0.17	0.8	0.09	1.7	0.89	0.7	0.42
4'-OH-Pyr	1.3	0.11	1.3	0.14	3.3	1.67	2.8	1.57
DPH-Pyr	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.03	0.02
Non-polar Unknown 1	1.0	0.08	0.6	0.06	0.7	0.33	0.4	0.24
Non-polar Unknown 2	0.3	0.02	0.1	0.01	0.2	0.10	0.4	0.24
4'-OH-Pyr Sulfate	15.5	1.26	10.7	1.13	12.2	6.25	7.2	4.06
4'-OH-Pyr Glucronide	5.4	0.44	6.8	0.72	12.7	6.48	11.5	6.50
5'',4'-OH-Pyr Sulfate	7.7	0.63	13.3	1.40	30.0	15.3	34.0	19.2
4'-OH-POP Sulfate	3.7	0.30	9.0	0.95	11.0	5.61	12.2	6.87

Table B.9.2.8.1-4B Quantitation of ^{14}C-components in methanol extracts of fish [pyridyl-^{14}C] pyriproxyfen)

Components	Fillet				Viscera			
	Day 21		Day 28		Day 21		Day 28	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Pyriproxyfen	31.4	3.96	38.9	4.05	18.3	9.84	21.8	12.0
4'-OH-Pyr	1.6	0.20	0.4	0.04	1.3	0.71	0.5	0.30
PYPAC	0.5	0.06	0.4	0.04	0.4	0.19	0.4	0.22
2-OH-PY	4.5	0.56	5.3	0.56	0.8	0.44	0.9	0.47
PYPA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Non-polar Unknown 1	0.8	0.10	0.4	0.04	0.6	0.32	0.4	0.22
Non-polar Unknown 2	0.2	0.03	N.D.	N.D.	0.1	0.06	0.1	0.08
4'-OH-Pyr Sulfate	18.0	2.27	15.0	1.56	16.8	9.03	15.7	8.63
4'-OH-Pyr Glucronide	1.6	0.20	2.0	0.20	1.0	0.52	1.2	0.67
5'',4'-OH-Pyr Sulfate + 5-OH-PYPAC Sulfate	11.5	1.45	5.4	0.56	22.7	12.2	34.6	19.0
Polar Unknown 1	6.5	0.82	7.2	0.75	3.2	1.73	6.2	3.4
Polar Unknown 2	1.6	0.20	1.0	0.10	3.3	1.77	4.0	2.2

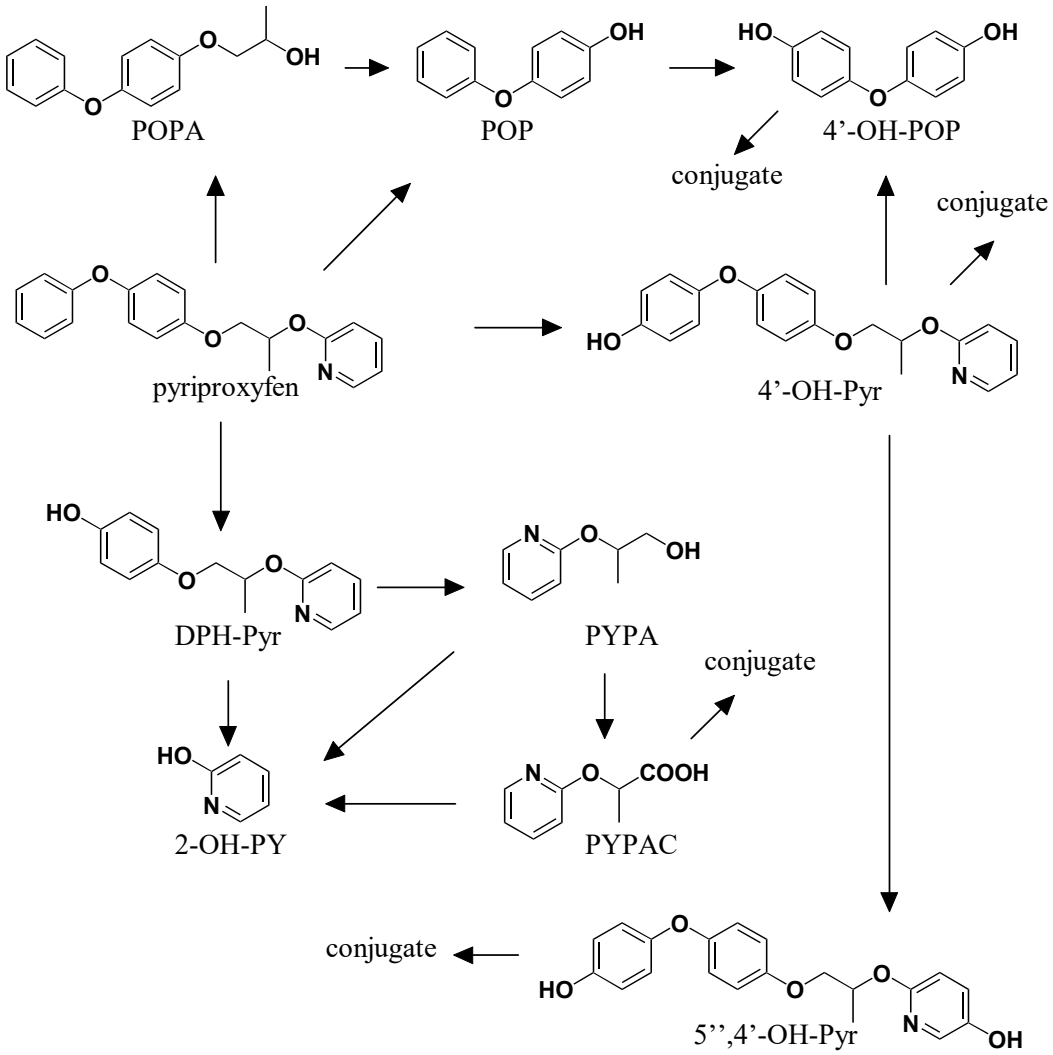
Table B.9.2.8.1-05 Characterization of unextracted ^{14}C-residue in day 28 edible post extraction solids (PES)

Fraction	Class of material extracted	phenyl		pyridyl	
		%TRR	mg/kg	%TRR	mg/kg
Bligh-Dyer, Aqueous	Peptides, Sugars, Soluble proteins	0.1	0.02	0.9	0.09
Bligh-Dyer, Organic	Lipids	0.2	0.02	0.3	0.03
Hexane	Lipids	0.1	0.01	<0.05	<0.005
Methanol	Rinse to remove hexane and facilitate drying tissue	0.2	0.02	0.3	0.03
Pronase	Proteins	2.5	0.27	14.3	1.49
Acid hydrolysis	Covalently bonded material	Not performed	--	0.5	0.05
Bound residue	--	0.4	0.04	0.1	0.01
Total		3.5	0.38	16.4	1.70
Radioactive residue in PES		4.1	0.43	23.0	2.40

Table B.9.2.8.1-06 Metabolism of ^{14}C pyriproxyfen in fish: Identity of metabolites identified

Designation	Chemical name
Pyriproxyfen (Parent)	4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether
4'-OH-Pyr	4-(4-hydroxyphenoxy)phenyl (*RS*)-2-(2-pyridyloxy) propyl ether
5",4'-OH-Pyr	4-(4-hydroxyphenoxy)phenyl (*RS*)-2-(5-hydroxypyridyl-2-oxy) propyl ether
DPH-Pyr	4-hydroxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether
POPA	(*RS*)-2-hydroxypropyl 4-phenoxyphenyl ether
PYPA	(*RS*)-2-(2-pyridyloxy) propanol
PYPAC	(*RS*)-2-(2-pyridyloxy) propionic acid
POP	4-phenoxyphenol
4'-OH-POP	4-hydroxyphenoxy phenol
2-OH-PY	2-hydroxypyridine

Figure B.9.2.8.1-01 Metabolic pathways of pyriproxyfen in fish



Conclusions

Pyriproxyfen shows a relatively low bioaccumulation potential in fish. Under flow-through exposure conditions at nominal concentration of 0.020 mg/l, a steady state BCF was reached within approximately 7 days of exposure. The average BCFs of ^{14}C material at the 28-day exposure period for whole fish was 1379-1495, respectively. Pyriproxyfen was also readily cleared away from fish tissues (1.9-10% of ^{14}C residues remaining in whole fish after the 14 day depuration phase) with the CT_{95} calculated to be in the range 4.4-11.0 days. It is concluded that pyriproxyfen will not be persistent in fish.

In fish tissue, pyriproxyfen plus 4'-OH-Pyr and its conjugates together accounted for 37 - 70% of the radioactive residue. The major metabolic process in the fish is the hydroxylation and the major portions of the hydroxylated products undergo conjugation. Both the hydroxylated and conjugated products were eliminated by the fish and were found in the exposure water. None of the products, or pyriproxyfen, accumulated irreversibly in fish as shown by the high rate of depuration.

Comments RMS (Renewal)

This is an old study, which does not comply with the OECD 305 guideline. The BCF was not normalized to 5% lipid content, which is considered a major deficiency. However, the lipid content in bluegill sunfish is typically between 7-9% and the BCF here is expressed assuming 5% lipid content (it was not normalized for it). Therefore, the BCF expressed for 5% lipid content is considered more conservative. Furthermore, the levels of pyriproxyfen in bluegill at the accumulation steady-state were determined to be 50% or less. By applying 50% degradation, the definitive BCF of pyriproxyfen is reduced to 690-748, and this value should be considered as a starting point for the bioaccumulation evaluation, according to the applicant. In addition, although not considered valid, the study with the carp (see below), which contains 3-6.3% lipids, results in BCF of 512-669 (normalized for 5% lipid content). To address uncertainty in the study with the bluegill sunfish, a conservative BCF of 1379-1495 for pyriproxyfen is considered acceptable by the RMS.

It is noted that only one concentration was used and it was not argued by the applicant why is this considered sufficient. However, another study presented in B.9.2.8.2 (not suitable for risk assessment) provides an indication of no strong dependency between the BCF and exposure concentration (BCF of 669 and 512 for exposure to 5 and 0.5 ug as/L, respectively). Therefore, testing only one concentration is considered acceptable. Furthermore, the body mass appears not to change substantially over the course of the test. Therefore, not accounting for the residue dilution by growth is acceptable. Finally, the uptake was rather fast – steady state was achieved within 3-7 day, but the uptake and elimination rates constants were not calculated. This is considered not to have an impact on the validity of the BCF. Nevertheless, the OECD guideline 305 advises to calculate them if possible. In response, the applicant provided the rates as calculated in RStudio. They were 1135.8 and 0.8002 (uptake and elimination rates, respectively) for the BCF of 1419.3.

4'-OH-pyr (and its conjugates) was identified as a major metabolite in the study and so its bioaccumulation potential can be taken into account in the risk assessment by assuming the BCF value based on total ^{14}C.

4.2.2 Bioaccumulation test with other organisms

No data are available.

4.3 Acute toxicity

In case a study is considered acceptable for the risk assessment it implies that the endpoint can also be used for the classification and labelling purposes. Hence, in the conclusions below, the text 'Study results can be used for risk assessment' should also be read as 'Study results can be used for classification purposes'.

4.3.1 Short-term toxicity to fish

4.3.1.1 CA 8.2.1/01 (1989a) Acute flow-through toxicity of Sumilarv to rainbow trout (Salmo gairdneri)

Report: CA 8.2.1/01 (1989a) Acute flow-through toxicity of Sumilarv to rainbow trout (*Salmo gairdneri*). Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-91-0035	
Previous evaluation	In DAR (November 2005) for original approval

Remark by RMS	Considered acceptable at the time of original inclusion and is acceptable for the renewal. For the renewal, a very short original study summary has been replaced by an extended summary.
Conclusion	LC_{50} > 0.325 mg a.s.s/L

Guidelines & deviations: US EPA 72-1. No deviations reported

GLP: Yes

Acceptability: Acceptable

Materials and Methods

Test Material

Test material: Sumilarv T.G. (technical pyriproxyfen)

Description: Pale yellow viscous liquid

Lot/batch no.: PYG87074

Purity: 95.3%

Expiry date: Not stated

Test animals: The rainbow trout (*Salmo gairdneri*) used in the test were obtained from Mt. Lassen Farms, Red Bluff, California.

Maintenance: The fish were reared and maintained at the test facility in well water and were fed newly hatched brine shrimp or a commercial fish food, daily. They were maintained on a 16-hour daylight photoperiod. Forty eight hours before test initiation the rainbow trouts were removed from the culture tank and placed in a temperature acclimation unit. During this time the fish were held without food at 13° C.

Study Design

Analytical measurement of pyriproxyfen were made at 0 and 96 hours (gas-liquid chromatography).

A flow-through test system was used to determine the 96 hour LC_{50} value for the rainbow trout (*Salmo gairdneri*) exposed to technical pyriproxyfen in freshwater. Twenty fish per test concentration plus water and solvent controls were used in a nominal dosing regime of 22.5, 45, 90, 180 and 360 µg a.s./L. Mean weight and length of fish (control group) were 2.3 (±1.5) g and 54 (±3.5) mm, respectively, at the end of the study. Observations for mortality and sub-lethal effects were made every 24 hours during the 96-hour exposure period.

The flow rate in the test aquaria was equivalent to 7.2 volume exchanges per day (aquaria were 30 L).

Results and Discussion

The measured test concentrations at the 0-hour sampling were on average 74 ± 11% of the nominal values and at 96 hours they were on average 74 ± 26% of the nominal values. The mean of the 0 and 96 hour measurements were 20.3, 32, 54, 102 and 325 µg a.s./L. These results yielded an average of 74 ±16% of the nominal test concentrations of 22.5, 45, 90, 180 and 360 µg a.s./L.

Water temperature was 13° C and dissolved oxygen ranged between 8.2 to 8.9 mg/L (81 and 88% saturation at 13° C).

The results of survival are presented in the table below. No mortality was observed in any treatment, except that one fish out of 20 died at 96 h in the 325 µg a.s./L treatment. LC_{50} is > 325 µg a.s./L based on the mean measured concentration at 0 and 96 h.

Behavioural/sublethal effects were observed at the highest tested concertation and included erratic swimming, loss of equilibrium and surfacing. Therefore, the NOEC for survival was established at 102 µg a.s./L

Mortality and Behavioral Observations During the Acute Flow-Through Toxicity Test of Sumilarv with Rainbow Trout (Salmo gairdneri)

Mean Measured Test Concentration µg/l (ppb)	No. Placed in test	24-Hour		48-Hour		72-Hour		96-Hour	
		Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations
Control	20	0	20 N	0	20 N	0	20 N	0	20 N
Solvent Control	20	0	20 N	0	20 N	0	20 N	0	20 N
20.3	20	0	20 N	0	20 N	0	20 N	0	20 N
32	20	0	20 N	0	20 N	0	20 N	0	20 N
54	20	0	20 N	0	20 N	0	20 N	0	20 N
102	20	0	20 N	0	20 N	0	20 N	0	20 N
325	20	0	20 N	0	20 N	0	2 SUR; 1 ES; 17 N	1	1 SUR; 16 N; 2 LOE/SUR

Key to Observations: Mort. = Mortality; Cum. Mort. = Cummulative Mortality; N = Normal; SUR = Surfacing; LOE = Loss of Equilibrium; ES = Erratic Swimming.

Conclusions

Pyriproxyfen 96 h LC_{50} for rainbow trout was >0.325 mg a.s./L and 96 h NOEC was 0.102 mg a.s./L, based on the absence of any effects seen at all test concentrations. The endpoints were based on mean measured concentrations of pyriproxifen.

Comments RMS (RAR)

The study is valid and acceptable for risk assessment.

4.3.1.2 CA 8.2.1/02 (1989b) Acute flow-through toxicity of Sumilarv to bluegill (Lepomis macrochirus)

Report: CA 8.2.1/02 (1989b) Acute flow-through toxicity of Sumilarv to bluegill (*Lepomis macrochirus*). Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-91-0034	
Previous evaluation	In DAR (November 2005) for original approval. A new robust summary is provided below for the acute toxicity with bluegill sunfish because this study is used to derive endpoints for the fish risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion.
Conclusion	96-hr LC_{50} > 0.270 mg a.s./L

Guidelines & deviations: U.S. EPA Pesticide Assessment Guidelines No. 72-1 (June 1985). Deviations: None reported.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Sumilarv T.G. (technical pyriproxyfen)
Description:	Beige solid
Lot/Batch #:	PYG-87074
Purity:	95.3%
Expiry date:	Not stated
2a. Test animals:	The bluegill sunfish (*Lepomis macrochirus*) used in the test were obtained from Osage Catfisheries, Missouri, USA.
2b. Maintenance:	The fish were reared and maintained at the test facility in well water and were fed newly hatched brine shrimp or a commercial fish food, daily. They were maintained on a 16-hour daylight photoperiod. Forty eight hours before test initiation the bluegill were removed from the culture tank and placed in a temperature acclimation unit. During this time the fish were held without food at 22 ± 1°C.

Study Design

A flow-through test system was used to determine the 24, 48, 72 and 96 hour LC_{50} values for bluegill sunfish (*Lepomis macrochirus*) exposed to technical pyriproxyfen (purity: 95.3%) in freshwater.

The test material was dissolved in acetone to prepare a diluter stock solution which was dispensed via a proportional diluter system, together with the test medium (test facility well water prepared to a total hardness of between 40 to 48 mg/L (as $CaCO_3$)) to prepare nominal test concentrations of 0, 0.0225, 0.045, 0.090, 0.180 and 0.360 mg a.s./L (based on a preliminary range-finding tests). The diluter delivered 1 litre of test solution at an average rate of 8.85x per hour (equivalent to 7.0 volume changes per day). Twenty fish (mean weight - 1.2 ± 0.77 g; mean length - 34 ± 5.9 mm) at each test concentration were placed in a single 40-litre glass aquaria containing 30 litres of test solution.

Observations for mortality and sub-lethal effects were made every 24 hours during the 96-hour exposure period. Test concentrations were analysed at 0 and 96 hours and temperature, dissolved oxygen and pH were measured in the control, solvent control, low, middle and high test concentrations at 0, 48 and 96 hours.

Results and Discussion

Physical and Chemical Measurements of Water

Water chemistry parameters were within the specified limits: temperature remained at 23°C, pH remained constant at 7.1 and dissolved oxygen ranged from 7.8 to 8.4 mg/L (95 and 102% saturation, respectively).

Measurement of Test Concentrations

The measured test concentrations at the 0-hour sampling were on average 81.4 ± 8.2% of the nominal values and at 96 hours they were on average 71.6 ± 4.5% of the nominal values, indicating that the test material was relatively stable under the test conditions. The mean measured concentrations were 0.0191, 0.0315, 0.070, 0.135 and 0.270 mg a.s./L (77 ± 5.5% of the nominal values) and the results are presented as these values.

Biological Findings

No mortality or behavioural/sublethal effects were observed in any of the test concentrations, up to and including 0.270 mg a.s./L, during the 96-hour exposure period.

Conclusions

Pyriproxyfen 96-hr LC_{50} to bluegill sunfish was >0.270 mg a.s./L and 96-hr NOEC was 0.270 mg a.s./L, based on the absence of any effects seen at all test concentrations.

Comments RMS

No comments.

4.3.1.3 CA 8.2.1/05 (1991) Acute flow-through toxicity of Sumilarv T.G. to the Sheepshead minnow (Cyprinidon variegatus)

Report: CA 8.2.1/05 (1991) Acute flow through toxicity of Sumilarv T.G. to the sheepshead minnow (*Cyprinodon variegatus*). Sumitomo Chemical Co., Ltd. Unpublished report No.: 38644 (company code NNW-11-0070).	
Previous evaluation	New study
Remark by RMS	The study is acceptable.
Conclusion	96-hr LC_{50} > 1.02 mg a.s./L

Guidelines & deviations: US EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-3. No deviations reported.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Sumilarv T.G. (technical pyriproxyfen)
Description:	Yellow solid
Lot/Batch #:	PYG-87074
Purity:	95.3%
Expiry date:	Not stated
2a. Test animals:	Sheepshead minnow (*Cyprinodon variegatus*), used in the test were obtained from Aquatic Research Organisms, New Hampshire, USA.
2b. Maintenance:	The test fish were acclimated to test conditions at the test facility for 30 days. Prior to testing, fish were maintained in 100% dilution water under flow-through conditions. They were not treated for disease and they were free of apparent sickness, injuries and abnormalities at the beginning of the test. During the acclimation period the temperature ranged from 21.0 to 22.1°C and the dissolved oxygen concentration was maintained above 7.3 mg/L. Fish were fed dry commercial fish food once or twice daily before the test.

Study Design

A dose-response test was conducted under flow-through conditions (daily renewal of the test media) with five test concentrations of 0.7, 1.2, 2.0, 3.0 and 5.0 mg test item/L nominal, a solvent control and a dilution water (filtered natural seawater) control. The test substance was supplied to the test vessels by an intermittent flow proportional diluter (average 6.0 media exchanges per 24 hours in each test vessel) which was observed at least twice daily for normal operation. The study was conducted over a duration period of 96 hours. Twenty

fish were equally distributed among two replicates of each treatment. The test was performed in 19.6 glass aquaria (approximately 20 cm x 40 cm x 25 cm) that contained 15 litres of test solution (water depth of approximately 18 cm). Test vessels were randomly arranged in a water bath during the 96 hours exposure period. A 16-hour light and 8-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 12 $\mu Es^{-1}m^{-2}$. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels. Fish were not fed during the 48 hours before the test or during the 96-hour exposure.

The number of surviving fish and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discolouration or change in behaviour) were determined visually and recorded initially and at 24-hour intervals. Dead fish were removed when first observed. After 96 hours of exposure the total length and average wet weight of surviving control fish was obtained after blotting dry. Dissolved oxygen, salinity and temperature were measured and recorded daily in each test chamber. The temperature in one test vessel was recorded continuously during the test. Analytical determination of the test item concentration was performed on samples from each test vessel collected at the initiation and termination of the test.

Results and Discussion

Physical and Chemical Measurements of Water

Insoluble material in the form of a surface slick was initially observed in test vessels containing the two highest concentrations of test substance and the highest concentration was cloudy. After 24 hours, insoluble material in the form of white solids was always present in all non-control vessels and the highest concentration remained cloudy. Control and solvent control test media were clear throughout the test. Insoluble material (white solids) was also noted throughout the test in the secondary stock solution, which was prepared in dilution water by the proportional diluter.

Salinity, dissolved oxygen, temperature and pH ranged from 15 -16 ppt, 6.0 – 7.7 mg/L, 22.2 – 22.9 ^{o}C and 7.8 – 8.2, respectively. These water quality parameters were within acceptable limits and appeared to be unaffected by the test compound.

Measurement of Test Concentrations

Mean measured concentrations of the test substance ranged from 20 to 35% of nominal values, indicating that the test was conducted above the solubility limit in seawater. The initial stock solution, prepared in acetone, had a nominal concentration of 50,000 mg/L and a mean measured concentration of 50,500 mg/L. The secondary stock solution, prepared by the proportional diluter in dilution water, had a nominal concentration of 5 mg/L and a mean measured concentration of 1.75 mg/L.

Biological Findings

One hundred percent survival and no abnormal behaviour was observed in the control and solvent control exposure. Control fish had an average standard length of 26.0 mm and an average wet weight (blotted dry) of 0.36 g at the end of the test, resulting in a loading rate of approximately 0.24 g/L at any time and 0.04 g/L/24 hours. At the highest test concentration (1.02 mg a.s./L, mean measured concentration) there were 3 dead fish (out of 20) after 96 hours and a single dead fish at 0.617 mg a.s./L. No abnormal behaviour was observed in any surviving fish.

Conclusions

The LC_{50} value (96 h) value for sheepshead minnow (*Cyprinodon variegatus*) exposed to technical pyriproxyfen was greater than 1.02 mg a.s./L (mean measured concentration), the highest concentration tested. On the basis of the observations made during the test, the NOEC was determined to be 0.617 mg a.s./L (mean measured concentration).

Comments RMS:

The validity criteria of OECD 203 were met (<10% mortality in the control, dissolved oxygen ≥60% air saturation throughout the test and results were based on measured concentrations).

The concentrations of the test item in the test solution in each vessel at the study initiation and after 96 hours of exposure were determined by a sufficiently validated gas liquid chromatography (GLC) method (recoveries of 95%, 96% and 95% were observed at fortification levels of 0.599, 2.99 and 5.99 ppm, respectively). Endpoints were based on arithmetic mean measured concentrations, which is acceptable. The measured concentrations of the test substance during the toxicity test are presented below:

Table B.9.2.1.5-01 Nominal and measured concentrations of Sumilarv T.G.

Nominal concentration (mg/L)	Measured concentration of Sumilarv T.G. (mg/L)			Mean measured concentration as % of nominal
	0 hours	96 hours	Mean	
0.00 (control)	ND*	ND*	ND*	-
0.00 (solvent control)	ND*	ND*	ND*	-
0.7	0.164	0.328	0.246	35
1.2	0.295	0.303	0.299	25
2.0	0.547	0.308	0.428	21
3.0	0.906	0.327	0.617	21
5.0	1.47	0.575	1.02	20

*ND = not detected

Mortality did not exceed 15% in any tested concentration. The LC50 was >1.02 mg/L, based on mean measured concentrations. The study is acceptable, and the LC50 may be used for risk assessment.

4.3.1.4 CP 10.2.1/01 (1992) Static Acute Toxicity of S-71639 10EC to Rainbow Trout (Oncorhynchus mykiss)

Report	CP 10.2.1/01 (1992)
Title	Static Acute Toxicity of S-71639 10EC to Rainbow Trout (*Oncorhynchus mykiss*).
Report number	NNW-21-0076
Guidelines	U.S. EPA-FIFRA 40 CFR Section 158.145 Guideline 72-1, OECD Guideline 203
GLP	Yes
Previous evaluation	DAR (2005)
RMS comment	The applicant submitted a new, more extensive summary. Study considered acceptable for use in risk assessment

Conclusion	LC50 = 0.22 mg a.s./L

Materials and Methods

Materials

1. Test Material: S-71639 (Pyriproxyfen) 10EC

Description: Liquid

Lot/Batch #: C611L13

Purity: 10.4%

Expiry date: Not stated

2a. Test animals: The rainbow trout (*Oncorhynchus mykiss*) used in the test were obtained from Mt. Lassen Trout farm in Red Bluff, California, USA

2b. Maintenance: Prior to testing, the fish were held on a 16 hours daylight photoperiod with a 30 minute transition period. The fish were cultured in hard blended water. The fish were fed daily newly hatched brine shrimp (*Artemia* sp.), aquatic invertebrates, or a commercially available fish food. Fish were not fed during the 48-hour period prior to test initiation and during the exposure period.

Study Design

A static test system was used to determine the 24, 48, 72 and 96 hour LC_{50} values for rainbow trout (*Oncorhynchus mykiss*) exposed to the test material. The dilution water used was prepared by blending naturally hard well water with demineralised well water, and had a pH of 8.3, total hardness and alkalinity as CaCO3 of 146 mg/L and 164 mg/L, respectively, and a conductivity of 380 µMhos/cm.

Rainbow trout were exposed to S-71639 10EC under static conditions at nominal test concentrations of 0.7, 1.3, 2.3, 4.7 and 7.5 mg S-71639 10EC/L, together with a dilution water control and formulation blank. One test vessel per treatment level, control and formulation blank were prepared, each containing 10 fish. The test vessels were 5 gallon glass vessels, each containing 15 L of test solution and kept in a water bath at 15°C (±2°C). The mean organism loading was 0.08 g of biomass per litre of test solution. Assessments of mortality and abnormal (sublethal) effects were carried out after 0, 24, 48, 72 and 96 hours.

The pH, dissolved oxygen concentration and temperature were measured at 0, 48, and 96 hours in all test chambers. Continuous temperature monitoring was performed in the control solution throughout the exposure

period. At test initiation (0 hour) and termination (96 hours), samples were removed from the test vessels for analysis of S-17639 10EC concentrations using gas-liquid chromatography (GLC).

Results and Discussion

Physical and Chemical Measurements of Water

Water chemistry parameters were considered to be within acceptable limits: temperature ranged from 14.5 to 15.5^{o}C; pH ranged from 8.1 to 8.3 (80% to 93% saturation); and dissolved oxygen ranged from 7.6 to 8.8 mg/L.

Measurement of Test Concentrations

Fortification samples were prepared at a concentration range of 0.612 to 8.16 mg/L S-17639 and analysed at 0 and 96 hours. Recoveries of these samples averaged 102% (± 4.6%).The mean concentrations of S-17639 10 EC at 0 and 96 hours were 0.65,1.2, 2.3, 4.6 and 7.2mg/L respectively, representing 96% (±3.3%) of the nominal concentrations of 0.7, 1.3, 2.3, 4.7 and 7.5 mg/L.

Biological Findings

The results are summarised in Table B.9.3.1.1-01. All results were based on the mean measured concentrations of 0.65, 1.2, 2.3, 4.6 and 7.2 mg/L. Following 96 hours of exposure, no mortality was found in the control, formulation blank, 0.65 and 1.2 mg/L treatment levels. At 2.3, 4.6 and 7.2 mg/L a cumulative mortality of 60, 100 and 100%, respectively, was found during the 96 hours exposure period.

Sublethal effects of surfacing, loss of equilibrium, dark discoloration, fish on the bottom of the test vessel, labored respiration, erratic swimming, and/or vertical orientation were observed in the formulation blank, 1.2, 2.3, 4.6 and 7.2 mg/L treatment levels. No abnormal effects were found in the control and lowest concertation of 0.65 mg/L.

Table B.9.3.1.1-01 Mortality and sublethal effects

Mean measured test concentration (mg/L)	24-Hour		48-Hour		72-Hour		96-Hour	
	Mor.	Obs.	Mor.	Obs.	Mor.	Obs.	Mor.	Obs.
Control	0	10 N	0	10 N	0	10 N	0	10 N
Formulation blank	0	2 DK/OB 1 DK/SUR 5 SUR 2 N	0	1 DK/SUR 5 SUR 3 OB/LR 1 N	0	1 DK/SUR 1 OB/LR 4 SUR 4 N	0	5 SUR 5 N
0.65	0	10 N	0	10 N	0	10 N	0	10 N
1.2	0	1 DK/SUR 2 SUR 2 LOE 5 N	0	4 SUR/LR 1 DK/SUR/LR 2 V/LR 3 N	0	1 DK/SUR/LR 3 SUR/LR 3 V/LR 3 N	0	1 OB/LOE/LR 1 SUR/LR 2 DK/OB/LR 2 V/LR 4 N
2.3	0	2 ES/LR 3 LOE/LR 5 OB/LR/LOE	1	9 OB/LR/LOE	5	5 OB/LR/LOE	6	4 OB/LR/LOE
4.6	5	5 OB/LOE/LR	10	-	10	-	10	-
7.2	10	-	10	-	10	-	10	-

Mor. = Mortality; Obs. = Observations; N = Normal; SUR = Surfacing; LOE = Loss of Equilibrium; ES = Erratic Swimming; DK = Dark Discolouration; OB = Fish on the Bottom; V = Vertical Orientation; LR = Laboured Respiration

Conclusion

The LC_{50} value (96 h) value for rainbow trout (*Onchorhynchus mykiss*) exposed to S-17639 10EC was 2.1 mg/L (95% confidence limits of 1.2 to 4.6 mg/L), equivalent to 0.22 mg a.s./L, based on the average measured concentrations. The NOEC was determined to be 0.65 mg/L based on the lack of mortality and sublethal effects at this concentration.

Comments RMS

No comments

4.3.2 Short-term toxicity to aquatic invertebrates

4.3.2.1 CA 8.2.4.1/01 (1989) Acute flow-through toxicity of Sumilarv to Daphnia magna

Report: CA 8.2.4.1/01 (1989) Acute flow-through toxicity of Sumilarv to *Daphnia magna*. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-91-0036	
Previous evaluation	Sumbited in the DAR (November 2005). A new robust summary is provided below for the acute toxicity of technical pyriproxyfen with *Daphnia magna* because this study is used to derive endpoints for the aquatic invertebrate risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion.
Conclusion	EC_{50} = 0.40 mg a.s./L

Guidelines:

U.S. EPA Pesticide Assessment Guidelines No. 72-2

Deviations: None reported.

GLP

Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Sumilarv T.G. (technical pyriproxyfen)
Description:	Beige solid
Lot/Batch #:	PYG-87074
Purity:	95.3%
Expiry date:	Not stated
2a. Test animals:	The *Daphnia magna* used in this study were obtained from an in-house daphnid culture maintained at the test facility.
2b. Maintenance:	All daphnids were held in a temperature controlled area at 20 (±2) °C. The lighting was 50-70 footcandles on a l6-hour daylight photoperiod, with 30 minute dawn and dusk transition periods. During the holding period, the daphnids were fed a suspension of algae (*Selenastrum capricornutum*) supplemented with a Tetramin®/cereal leaves/yeast suspension. Only first instar daphnids <24 hours old) were selected for testing.

Study Design

The 48-hour LC_{50} of pyriproxyfen to *Daphnia magna* was determined in four replicate exposure chambers (1 litre glass beakers), each containing ten first instar *D. magna* (less than 24 hours old). The test material was dissolved in acetone to prepare a diluter stock solution which was dispensed via a proportional diluter system, together with the test medium (surface impoundment and soft blended water with a total hardness of between 160 to 180 mg/L (as $CaCO_3$)) to prepare nominal test concentrations of 0, 0.06, 0.12, 0.25, 0.50 and 1.0 mg a.s./L (based on an initial range-finding test). The diluter delivered 4.0 mL/chamber/minute, which was sufficient to replace the 1 L test volume about 5.8x per day.

Assessments of mortality (immobilisation) and abnormal effects were made at 24 and 48 hours after initiating exposure. Test concentrations were analysed at 0 and 48 hours and temperature, dissolved oxygen and pH were measured in the control, low, middle and high test concentrations at 0 and 48 hours.

Results and Discussion

Physical and Chemical Measurements of Water

Water chemistry parameters were within the specified limits: temperature ranged from 20 to 21°C, pH ranged from 7.9 to 8.0 and dissolved oxygen from 7.6 to 8.0 mg/L (87 and 94% saturation, respectively).

Measurement of Test Concentrations

The measured test concentrations at the 0-hour sampling were on average 80 ± 8.9% of the nominal values and at 48 hours they were on average 66 ± 16% of the nominal values indicating that the test material degraded slightly under the test conditions. The mean measured concentrations were 0.043, 0.089, 0.19, 0.43 and 0.60 mg a.s./L (72 ± 8.5% of the nominal values) and the results are presented as these values.

Biological Findings

There was little or no mortality at test concentrations up to and including 0.19 mg a.s./L after 48 hours, but sublethal effects e.g. *Daphnia* swimming on the bottom or quiescent, were seen at concentrations of 0.089 mg a.s./L and above. The results are shown in the Table below.

Table B.9.2.4.1-01 Mortality (immobilisation) and behavioural observations during the acute toxicity test of technical pyriproxyfen to *Daphnia magna*

Mean measured test concentration (mg a.s./L)	24 Hours		48 Hours	
	Cumulative mortality (%)	Observations* (nos. daphnids affected)	Cumulative mortality (%)	Observations* (nos. daphnids affected)
Control	0	---	0	---
Solvent control	0	---	0	---
0.043	0	---	0	---
0.089	0	---	0	31 OB
0.19	0	---	3	30 OB/Q, 9 OB
0.43	0	36 OB	35	26 OB/Q
0.60	82.5	5 OB/Q, 2 OB	93	3 OB/Q

* Unless otherwise indicated, the test water was clear and free of precipitate and all daphnids were normal in appearance and behaviour. OB: on the bottom; Q: quiescent.

Conclusions

Pyriproxyfen 48-hr EC_{50} to *Daphnia magna* - 0.40 mg a.s./L (95% C.I. of 0.35 to 0.46 mg a.s./L); 48-hr NOEC - 0.043 mg a.s./L based on immobility and sublethal effects seen at test concentrations of 0.089 mg a.s./L and above.

Comments RMS (DAR)

No comments.

Comments RMS (renewal)

Based upon the request of EFSA, the RMS confirms that the data fit well the dose response curve. The RMS did analysis using ToxRat (not available at the time of the study conduction) and a slightly higher endpoint was estimated, *i.e.* 48 h LC_{50} = 0.462 mg a.s./L (95% C.I. of 0.418 to 0.494 mg a.s./L). The difference in the endpoint values is likely a result of a different fitting model / statistical method used. The endpoint proposed by the applicant, LC_{50} = 0.40 mg a.s./L, can be used in risk assessment.

4.3.2.2 CA 8.2.4.2/01 (1998) – Acute Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions

Report: CA 8.2.4.2/01 (1998)– Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions. Sumitomo Chemical Co., Ltd. Unpublished report 98-8-7445 (Company Code No.: NNW-0138)	
Previous evaluation	New study.
Remark by RMS	The study is acceptable.
Conclusion	EC_{50} = 0.092 mg a.s./L

Guidelines

FIFRA Guideline 72-3; OPPTS Guideline (draft) 850.1025

Deviations: There were no significant deviations.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Sumilarv T.G. (technical pyriproxyfen)
Description:	White solid
Lot/Batch #:	70104
Purity:	97.1%
Expiry date:	March 02, 1999
2a. Test animals:	The Eastern oysters (*Crassostrea virginica*) used in the test were obtained from a commercial supplier. They were checked for damage and parasites before use. The oysters were determined to be reproductively immature by pressing the tissue in the area where the gametes are stored to confirm that none were present. They were of similar age and had a mean valve height of 34 ± 2.8 mm.
2b. Maintenance:	The oysters were held in natural unfiltered seawater (as used in the test) at the test facility for 10 days prior to testing. During this acclimation period, the seawater temperature was gradually decreased from 24 to 20°C, and salinity was gradually increased from 10 to 32‰. The pH ranged from 6.9 to 7.7 and the dissolved oxygen concentration ranged from 81 to 100% of saturation. During acclimation and throughout the testing period, oysters were fed a supplementary algal diet of *Isochrysis galbana*. Prior to testing, 3 mm of the new peripheral shell growth of each oyster was removed. The test organisms were then held for ca. 24 hours and examined for any sign of stress.

Study Design

The objective of this study was to estimate the acute toxicity of technical pyriproxyfen to the Eastern oyster (*Crassostrea virginica*) under flow-through conditions. Reduction of shell deposition was used as the indicator of toxicity. The test was conducted using an exposure system consisting of a constant-flow serial diluter with a dilution factor of 60% between concentrations, a temperature controlled water bath and a set of 14 exposure aquaria. There were five test concentrations (nominally 18, 30, 50, 84 and 140 µg a.s./L) together with dilution water and solvent (acetone) controls. Test vessels consisted of glass aquaria (49.5 x 25.5 x 29 cm) with a test volume maintained at approximately 18 L. The flow of exposure solution to each aquarium (75 mL/minute) provided approximately 6 volume replacements every 24 hours.

The exposure of oysters was initiated by impartially selecting and placing 20 oysters in each aquarium (40 per treatment level and the controls). Biological observations (e.g. visible abnormalities such as excessive mucous production or a failure to siphon and feed, as evidenced by a lack of faeces) were made at test initiation and at each subsequent 24-hour interval until termination of the test (after 96 hours exposure). Sublethal effects were determined by a comparison of the performance and appearance of the exposed oysters to that of the control oysters. After 96 hours of exposure, the oysters were removed from the test aquaria and the new shell growth was measured to the nearest 0.1 mm using a calibrated micrometer.

The pH, dissolved oxygen concentration, salinity and temperature were measured daily in each replicate aquarium. Continuous temperature monitoring was performed in one replicate of the solvent control. At test

initiation (0 hour) and termination (96 hours), samples were removed from one replicate for analysis of pyriproxyfen.

Results and Discussion

The validity criteria for the study were met: shell growth in the control should be 2.0 mm (actual – 3.3 mm average); dissolved oxygen should exceed 60% of air saturation value (actual 58 to 85%). While values of 58 to 59% were recorded in several aquaria between 48 and 96 hours these were only slightly below the guideline requirement of 60% these dissolved oxygen concentrations did not adversely affect the oysters.

Physical and Chemical Measurements of Water

Dissolved oxygen concentrations ranged from 58 to 85% of saturation over the 96-hour exposure period. The remaining water quality parameters measured were unaffected by the test substance concentrations and remained within acceptable levels for the survival and growth of Eastern oysters. Daily monitoring of the temperature of the test solutions established a range of 20 to 21°C during the exposure period and continuous monitoring of one replicate of the solvent control established a range of 21 to 22°C. Salinity ranged from 31 to 32‰ and pH ranged from 7.3 to 7.7.

Measurement of Test Concentrations

The measured concentrations at 0-hour were 16, 18, 40, 53 and 110 µg/L while the 96-hour values were 11, 15, 36, 54 and 110 µg/L (for the nominal concentrations of 18, 30, 50, 84 and 140 µg/L, respectively). The mean measured concentrations ranged from 56 to 77% of the nominal values and defined the exposure concentrations as 13, 17, 38, 53 and 110 µg a.s./L.

Biological Findings

Growth among dilution water control oysters at test termination averaged 3.3 mm, which is greater than the stated guideline minimum of 2.0 mm and within the historical range of 0.9 to 5.5 mm established at the test facility. There was no statistically significant difference between the control and solvent control oyster growth data and so all calculations to establish treatment effect levels were performed using the pooled control data (3.2 mm). At test termination, no mortality was observed in any treatment level tested. Oysters exposed at 110 µg/L showed reduced feeding and faecal matter production. Mean shell growth for oysters exposed at 13, 17 and 38 µg/L was 3.1, 2.9 and 3.1 mm, respectively, which was not significantly reduced compared to the pooled control. Mean shell growth for oysters exposed at 53 and 110 µg/L was 2.4 and 1.2 mm, respectively, which was significantly reduced compared to the pooled control (Williams' Test) by 26 and 62%, respectively.

Conclusions

Based on the results of this study, the 96-hour EC_{50} value was calculated by linear regression to be 92 µg a.s./L (95% confidence interval of 49 to 170 µg a.s./L). The 96-hour LC_{50} value was >110 µg a.s./L. The No-Observed-Effect-Concentration (NOEC) for this study was 38 µg a.s./L.

Comments by RMS:

The concentrations of the test item in the test solution from one replicate per concentration at the study initiation and after 96 hours of exposure were determined by a sufficiently validated gas chromatography (GC) method (recoveries of 102-113% at nominal fortification levels of 20.0-140 µg/L in three quality control samples). Endpoints were based on arithmetic mean measured concentrations, which is acceptable. The measured values were rounded to two significant figures. The measured concentrations of the test substance during the toxicity test are presented below:

Table B.9.2.4.6-01 Nominal and measured concentrations of Sumilarv T.G.

Nominal concentration (µg/L)	Measured concentration of Sumilarv T.G. (µg/L)		
	0 hours	96 hours	Mean
0.00 (control)	< 3.1	< 2.8	NA*
0.00 (solvent control)	<3.1	<2.8	NA*
18	16	11	13
30	18	15	17
50	40	36	38
84	53	54	53
140	110	110	110

* NA – not applicable

The mean shell deposition and percent reduction values (average of 40 oysters per each tested concentration) are presented below:

Table B.9.2.4.6-02 Mean shell deposition and mean percent reduction in oysters at different test concentrations

Mean measured concentration (µg/L)	Mean shell deposition (mm)	Mean percent reduction
Control	3.3	NA*
Solvent control	3.1	NA*
Pooled control	3.2	NA*
13	3.1	4
17	2.9	9.0
38	3.1	5.0
53	2.4	26**
103	1.2	62**

* NA – not applicable

** Significantly reduced compared to the pooled control (Williams' test)

The EC50 was calculated by linear regression analysis based on the mean measured concentrations. The validity criteria of the study were met. The study is considered to be acceptable and the EC50 value (92 µg/L; 95% CI 49-170 µg/L) can be used for the risk assessment.

4.3.2.3 CA 8.2.4.2/02 (1999) – Acute Toxicity to Mysids (Mysidopsis bahia) Under

Report: CA 8.2.4.2/02, J.V. Sousa (1999) Sumilarv T.G. – Acute Toxicity to Mysids (*Mysidopsis bahia*) Under Flow-Through Conditions.

Sumitomo Chemical Co., Ltd. Unpublished report 98-6-7349 (Company Code No.: NNW-0139)

Previous evaluation	New study.
Remark by RMS	The study is acceptable.
Conclusion	EC_{50} = 0.065 mg a.s./L

Guidelines

OPPTS Guideline (draft) 850.1035

Deviations: There were no deviations.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Sumilarv T.G. (technical pyriproxyfen)
Description:	White solid
Lot/Batch #:	70104
Purity:	97.1%
Expiry date:	March 02, 1999
2a. Test animals:	The mysid shrimps (*Mysidopsis bahia*) used in the test were obtained from laboratory cultures maintained at the test facility.
2b. Maintenance:	The mysids were cultured in glass aquaria with a closed-loop recirculating filtration system providing artificial seawater to the aquaria. The seawater was characterised as having a salinity range of 23 to 30‰ during the 14-day period prior to test initiation. Over the same period the pH ranged from 7.8 to 7.9 and the temperature was maintained at 26°C. Mysids were fed live brine shrimp (*Artemia salina*) nauplii (≤48 hours old) twice daily. Juvenile mysids, ≤24 hours old, were collected for use in the study.

Study Design

The objective of this study was to estimate the acute toxicity of technical pyriproxyfen to the mysid shrimp (*Mysidopsis bahia*) under flow-through conditions. Mortality was used as the indicator of toxicity. The test was conducted using an exposure system consisting of a constant-flow serial diluter with a dilution factor of 60% between concentrations, a temperature controlled water bath and a set of 14 exposure vessels. There were five test concentrations (nominally 26, 43, 72, 120 and 200 µg a.s./L) together with dilution water and solvent (acetone) controls. Natural filtered seawater was used as dilution and control water during this study. Test vessels consisted of glass aquaria (39 x 20 x 25 cm) with a test volume maintained between approximately 8 and 11 L. The flow of exposure solution to each aquarium (50 mL/minute) provided approximately 6.5 volume replacements every 24 hours.

The exposure of the mysids was initiated by impartially selecting and introducing 10 mysids into each test aquarium (20 per treatment level and the controls). Biological observations of the exposed mysids and observations of the physical characteristics of the test solutions (e.g. precipitate, film on test solution surface) were recorded at test initiation and at each subsequent 24-hour interval. Death was determined by the absence

of mobility and failure to respond to gentle prodding. Live brine shrimp nauplii (*Artemia salina*) were added to each test vessel containing live test organisms twice daily during the exposure period.

The pH, dissolved oxygen concentration, salinity and temperature were measured daily in each replicate aquarium. Continuous temperature monitoring was performed in one replicate of the dilution water control. At test initiation (0 hour) and termination (96 hours), samples were removed from one replicate for analysis of pyriproxyfen.

Results and Discussion

Physical and Chemical Measurements of Water

Dissolved oxygen concentrations ranged from 77 to 100% of saturation over the 96-hour exposure period. The remaining water quality parameters measured were also unaffected by the test substance concentrations and remained within acceptable levels for the survival of mysids. Daily monitoring of the temperature of the test solutions established a range of 24 to 25°C during the exposure period and continuous monitoring of one replicate of the solvent control established a range of 24 to 26°C. Salinity ranged from 19 to 20‰ and pH ranged from 7.7 to 7.9.

Measurement of Test Concentrations

The measured concentrations were similar between sampling intervals: at 0-hour they were 16, 30, 50, 77 and 150 µg/L while the 96-hour values were 22, 45, 71, 84 and 150 µg/L (for the nominal concentrations of 26, 43, 72, 120 and 200 µg/L, respectively). The mean measured concentrations ranged from 67 to 88% of the nominal values and defined the exposure concentrations as 19, 38, 61, 80 and 150 µg a.s./L.

Biological Findings

Following 48 hours exposure, 100% mortality was observed among mysids exposed to the highest treatment level, 150 µg/L. At test termination (96 hours) 100% mortality was observed among mysids exposed to the second highest concentration, 80 µg/L. During the same period, 30% mortality was observed among mysids exposed to the 61 µg/L treatment level. Mortality of 5% was observed among the dilution water control mysids, which was considered to be within the expected range of naturally occurring variability, and there was no mortality in the solvent control. Sublethal effects (e.g. lethargy) were observed among surviving mysids exposed at 61 µg/L. No mortality or sublethal effects were observed among mysids exposed to the 19 and 38 µg/L treatment levels.

Conclusions

Based on the results of this study, the 48 and 96-hour EC_{50} values were calculated by nonlinear interpolation to be 69 and 65 µg a.s./L, respectively (95% confidence intervals of 61 to 80 and 61 to 80 µg a.s./L, respectively). The No-Observed-Effect-Concentration (NOEC) for this study was 38 µg a.s./L.

Comments by RMS

The concentrations of the test item in the test solution in one vessel per tested concentration at the study initiation and after 96 hours of exposure were determined by a sufficiently validated gas chromatography (GC) method (recoveries of 84.5-129% at fortification levels of 25-200 µg/L). Endpoints were based on arithmetic mean measured concentrations, which is acceptable. The measured concentrations of the test substance during the toxicity test are presented below:

Table B.9.2.4.7-01 Nominal and measured concentrations of Sumilarv T.G.

Nominal concentration (µg/L)	Measured concentration of Sumilarv T.G. (µg/L)		
	0 hours	96 hours	Mean
0.00 (control)	ND*	ND*	ND*

0.00 (solvent control)	ND*	ND*	ND*
26	16	22	19
43	30	45	38
72	50	71	61
120	77	84	80
200	150	150	150

*ND = not detected

The measured concentrations were rounded to two significant figures.

The cumulative mean mortality results (per two replicates per tested concentration) are summarized below:

Table B.9.2.4.7-02 Mean cumulative mortality of mysids at 24, 48, 72 and 96 hours at different test substance concentrations

	Mean cumulative mortality (%)			
Mean measured concentration (µg/L)	24 hour	48 hour	72 hour	96 hour
Control	0	5	5	5
Solvent control	0	0	0	0
19	0	0	0	0
38	0	0	0	0
61	0	20	25	30
80	0	85	95	100
150	50	100	100	100

The 96-hour LC50 value of 65 µg/L (95% CI 61-80 µg/L) was estimated by nonlinear interpolation, with 95% CI calculated by binomial probability. The study is considered to be acceptable and the calculated LC50 (65 µg/L) can be used in the risk assessment.

4.3.2.3 CP 10.2.1/02 (1992) Acute Toxicity of S-71639 10EC to Daphnia magna

Report	CP 10.2.1/02, G.C. Blakmore, J. Stratton (1992)
Title	Acute Toxicity of S-71639 10EC to *Daphnia magna*
Report number	NNW-21-0096
Guidelines	U.S. EPA-FIFRA 40 CFR Section 158.145 Guideline 72-2, OECD Guideline 202
GLP	Yes
Previous evaluation	DAR (2005)
RMS comment	The applicant submitted a new, more extensive summary. Considered acceptable for use in risk assessment
Conclusion	EC_{50} = 1.8 (CI: 1.1 - 2.5) mg S-71639 10EC/L (= 0.19 mg a.s./L)

Materials and Methods

Materials

1. **Test Material:** S-71639 (Pyriproxyfen) 10EC

   Description: Liquid

   Lot/Batch #: C611L13

   Purity: 10.4%

   Expiry date: Not stated

2. **Test animals:** The *Daphnia magna* used in the test were obtained from laboratory cultures maintained at the test facility. Test organisms were < 24 hours old at the initiation of the test.

2. **Maintenance:** Prior to testing, the daphnids were held in glass containers under a photoperiod of 16 hours light with 30 dawn and dusk transition periods and lighting maintained at 50-70 foot-candles. The culture water consisted of hard blended water, which combines well water and reverse osmosis water blended to a hardness od 130-160 mg/L as CaCO3. All daphnids were cultured and tested in a temperature controlled area at 20 (±1°C). Daphnids were fed a suspension of at least one algae species: *Selenastrum capricornutum*, *Ankistrodesmus falcatus* and/or *Chlamydomonas*

reinhardtii., along with a supplement consisting of trout chow and active dry yeast.

Study Design

A static test system was used to determine the 24 and 48 hour EC_{50} values for daphnids (*Daphnia magna*) exposed to the test material in freshwater. The hard blended water used during the definitive test had a pH of 8.3, total hardness and alkalinity as CaCO3 of 152 and 166 mg/L, respectively, and a specific conductivity range of µMhos/cm.

Daphnia magna were exposed to S-71639 10EC under static conditions at nominal test concentrations of 0.13, 0.25, 0.5, 1.0 and 2.0 mg S-71639 10EC /L, together with a control and formulation blank. Two replicate test vessels per treatment level, control and formulation blank were prepared, each containing 10 *D.magna* neonates (< 24 hours old). The test vessels were 250 mL glass beakers, each containing 200 mL of test solution. The number of immobilized daphnids observed in each replicate test vessel was recorded at test initiation and after 24 and 48 hours of exposure. Observation on abnormal effects such as surfacing, clumping of the daphnids together, and daphnids tending to the bottom of the test chambers were also made and recorded after 0, 24 and 48 hours of exposure.

The pH, dissolved oxygen concentration and temperature were measured at 0 and 48 hours in each treatment level, control and formulation blank. Continuous temperature monitoring was performed throughout the exposure period. At test initiation (0 hour) and termination (48 hours), samples were removed from the test vessels for analysis of S-17639 10EC concentrations using gas-liquid chromatography (GLC).

Results and Discussion

Physical and Chemical Measurements of Water

Water chemistry parameters were considered to be within acceptable limits: temperature was recorded 20.2°C throughout the test; pH ranged from 8.0 to 8.3 and dissolved oxygen from 7.9 to 8.4 mg/L.

Measurement of Test Concentrations

Fortification samples were prepared at concentrations of 0.107, 1.07 and 2.14 mg/L, and analysed at 0 and 48 hours. Recoveries of these samples averaged 108% (± 5.9%).The mean measured concentrations of S-17639 10 EC at 0 and 48 hours were 0.13,0.27, 0.56, 1.1 and 2.5 mg/L respectively, representing 100, 108, 112, 110 and 125% of the nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L, respectively.

Biological Findings

The results are summarised in Table B.9.3.1.2-01. All results were based on the mean measured concentrations of 0.13, 0.27, 0.56, 1.1 and 2.5 mg/L. Following 48 hours of exposure, no immobilization was observed among daphnids exposed to the control, formulation blank, 0.13, 0.27, 0.56, 1.1 mg/L treatment levels. In the 2.2

mg/L treatment level, nine immobilized daphnids were observed. The no-effect concentration based on the absence of immobility and other abnormal effects was the mean measured concentration of 0.27 mg/L after 48 hours.

Table B.9.3.1.2-01 Immobilisation and sublethal effects

Mean measured test concentration (mg/L)	24 Hours		48 Hours	
	Cumulative immobility (%)	Observations* (nos. daphnids affected)	Cumulative immobility (%)	Observations* (nos. daphnids affected)
Control	0	-	0	-
Formulation blank	0	-	0	-
0.13	0	-	0	-
0.27	0	-	0	-
0.56	0	-	0	8 N; 2 OB
1.1	0	8 N; 12 OB	0	3 N; 17 OB
2.5	25	15 OB	90	2 ERR

* Unless otherwise indicated, the test water was clear and free of precipitate and all daphnids were normal in appearance and behaviour. OB: On the Bottom; ERR = Erratic Swimming

Conclusions

The EC_{50} (48 h) value for daphnids (*Daphnia magna*) exposed to S-71639 10EC was 1.8 mg/L (95% confidence limits of 1.1 to 2.5 mg/L), equivalent to 0.19 mg a.s./L, based on the average measured concentrations. The 48 hour NOEC was estimated at 0.27 mg/L.

Comments RMS

No comments

4.3.3 Algal growth inhibition tests

4.3.3.1 CA 8.2.6.1/01 (1991) Acute toxicity of pyriproxyfen to Selenastrum capricornutum Prinz

Report: CA 8.2.6.1/01, (1991) Acute toxicity of pyriproxyfen to *Selenastrum capricornutum* Prinz. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-11-0068	
Previous evaluation	In DAR (November 2005) for original approval. A new robust summary is provided below for the study to assess the effects of technical pyriproxyfen on the growth of *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata*) because this study is used to derive endpoints for the aquatic invertebrate risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion.
Conclusion	72-hr E_rC_{50} = 0.111 (CI: 0.090 - 0.136) mg a.s./L 72-hr E_bC_{50} = 0.064 (CI: 0.058 - 0.069) mg a.s./L

Guidelines

OECD 201 (adopted 7 June 1984) - equivalent to EEC C.3.

Deviations: There were no deviations reported.

GLP

Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Technical pyriproxyfen
Description:	Not stated
Lot/Batch #:	007204
Purity:	97.2%
Expiry date:	Not stated
2. Test organism:	The freshwater green algae (*Selenastrum capricornutum*) was selected as a test organism. The algae used during this study were obtained from the Department of Botany, University of Texas, USA.
2. Culturing:	The algal culture used for this study was three days old at test initiation. It was conducted in a synthetic algal culture medium prepared in sterile reverse-osmosis water. After preparation the medium was pH-adjusted to 7.7 ± 0.3.

Study Design

The test vessels were 250 ml conical flasks containing 100 mL test solution, stoppered with a foam plug to reduce evaporation. Five test concentrations were prepared in triplicate by the addition of 1.0 mL of algal

inoculum (*Selenastrum capricornutum*) to aliquots of sterile nutrient medium containing the test substance (prepared using acetone as the vehicle) so that they contained approximately 1.0 x 10^4 cells/mL. The nominal test concentrations were 0.025, 0.05, 0.10, 0.20 and 0.40 mg a.s./L (based on an initial range-finding test). An untreated control and a vehicle blank (acetone at 0.1 mL/L, the level in the highest test concentration) were also included. A continuous photoperiod was maintained for the 72-hour incubation period, with a test temperature of 24 ± 2°C. Gaseous exchange and suspension of algal cells in the test vessels were maintained by an orbital shaker.

Temperature and pH were measured at 0 and 72 hours in the controls and all test solutions. Concentrations of pyriproxyfen in the test system were measured at 0 and 72 hours by means of GLC. Samples of the test solutions were taken daily and algal cell counts were conducted using a haemacytometer.

Results and Discussion

Measurement of Test Concentrations

The mean measured concentrations were 0.020, 0.038, 0.069, 0.15 and 0.33 mg a.s./L. These represented 69 to 83% of the nominal concentrations and the overall mean was 77 ± 5.3% of the nominal values. The results are presented as these values.

Physical and Chemical Measurements of Water

Temperature ranged from 23 to 24°C, pH ranged from 7.2 to 8.4 (this range reflected an increase from 0 to 72 hours, which was attributed to algal growth because an increase was also seen in the control, which did not contain pyriproxyfen).

Biological Findings

Growth (Cell counts): Logarithmic phase growth was confirmed at 72 hours in both controls. The control and solvent control cell count data were not significantly different (student t-test; $p \leq 0.05$) after 72 hours and so pooled values were used for comparison with the test concentrations. The growth data were subjected to a one-way ANOVA and multiple means test (Dunnett's Test). This indicated a significant inhibitory effect ($p \leq 0.05$) on growth for the 0.038, 0.069, 0.15 and 0.33 mg a.s./L concentrations of pyriproxyfen to *Selenastrum capricornutum* Prinz, compared to the pooled controls after 72 hours (see Table).

Table B.9.2.6.1-01Measured cell counts of *Selenastrum capricornutum* Prinz

Mean measured concentration (mg a.s./L)	Mean Cell Counts (cells/mL/10^4)			
	0-hours	24-hours	48-hours	72-hours
Control	0.93	3.5	22	100
Solvent control	0.67	3.1	20	110
0.020	-	2.3	21	100
0.038	-	2.8	19	84*
0.069	-	2.9*	11*	47*
0.15	-	1.7*	3.0*	3.8*
0.33	-	0.85*	0.81*	1.1*

* Denotes a significant ($p \leq 0.05$) inhibition effect from the control and solvent control as calculated using transformed (square root) cell counts by Dunnett's Test

Conclusions

Pyriproxyfen 72-hr EC_{50} to *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata*) was calculated to be 0.064 mg a.s./L (95% C.I. of 0.058 to 0.069 mg a.s./L); 72-hr NOEC was determined to be 0.020 mg a.s./L, based on a significant inhibition of absolute cell numbers at concentrations of 0.038 mg a.s./L and above.

E_bC_{50}, E_rC_{50}, NOE_bC and NOE_rC values were estimated by the RMS (November 2005) from the reported raw data on cell counts using the procedures in OECD 201 (based on nominal concentrations, as initial measured concentrations were ≥80% of nominal), as the report presented only EC_{50} and NOEC values based on the percentage reduction of absolute cell numbers relative to the pooled control. On this basis, the 72-hr E_bC_{50} is 0.094 mg a.s./L (95% C.I. of 0.051 to 0.127 mg a.s./L) and the 72-hr E_rC_{50} is 0.15 mg a.s./L (95% C.I. of 0.063 to 0.34 mg a.s./L).

Comments RMS (DAR)

No comments.

Comments RMS (renewal)

Validity criteria were met. Since the concentration of the active substance was not maintained during the test, the endpoint should be based on the mean measured concentration. The E_yC_{50} is 0.064 mg a.s./L (mean measured, 95% C.I. of 0.058 to 0.069 mg a.s./L) and E_rC_{50} is 0.111 (mean measured, 95% C.I. of 0.090 to 0.136 mg a.s./L).

4.3.3.2 CP 10.2.1/03 (1992) Acute Toxicity of S-71639 10EC to Selenastrum capricornutum Printz

Report	CP 10.2.1/03, J.W. Blasberg, S.L. Hicks, J. Stratton (1992)
Title	Acute Toxicity of S-71639 10EC to *Selenastrum capricornutum*
Report number	NNW-21-0077
Guidelines	OECD Guideline 201
GLP	Yes
Previous evaluation	DAR (2005)
RMS comment	The applicant submitted a new, more extensive summary. Considered acceptable for use in risk assessment
Conclusion	ErC50 = 1.100 mg S-71639 10EC/L (= 0.110 mg a.s./L) EbC50 = 0.710 mg S-71639 10EC/L (= 0.074 mg a.s./L)

Materials and Methods

Materials

1. **Test Material:** S-71639 10EC

   Description: Liquid

   Lot/Batch #: C611L13

Purity:	10.4%
Expiry date:	Not stated
2a. Test animals:	The freshwater green algae (*Selenastrum capricornutum*) was selected as a test organism. The algae used during this study were obtained from the Department of Botany, University of Texas, USA.
2b. Maintenance:	The algal culture used for this study was four days old at test initiation. It was conducted in a synthetic algal culture medium prepared in sterile Milli-Q water. After preparation the medium was pH-adjusted to 7.7 ± 0.3 (using 1 N NaOH).

Study Design

The test vessels were 250 ml Erlenmeyer flasks containing 100 mL of appropriate solution for each test vessel and stoppered with a foam plug to reduce evaporation. Five test concentrations were prepared in triplicate by the addition of 1.0 mL of algal inoculum (*Selenastrum capricornutum*) containing approximately 1.0×10^6 cells/mL resulting in approximately 1.0×10^4 cells/mL in each flask. The nominal test concentrations were 0.25, 0.5, 1.0, 2.0 and 4.0 mg/L (based on an initial range-finding test). A control and a formulation blank were also included. A continuous cool-white fluorescent lighting and constant rotary agitation was maintained for the 72-hour incubation period, with a test temperature of 24±2°C. Light intensity was maintained at 800 ± 10% ft-c (approximately 8600 lux) and the agitation rate at approximately 100 rpm.

Temperature and pH were measured at 0 and 72 hours in the control, formulation blank and all test solutions. Concentrations of S-71639 10EC in the test system were measured at 0 and 72 hours using a gas chromatograph with a nitrogen-phosphorus detector (NPD). Samples of the test solutions were taken daily and algal cell counts were conducted using a haemacytometer.

Results and Discussion

Physical and Chemical Measurements of Water

0 and 72 hours measurements of temperature ranged from 23 to 24°C and pH ranged from 7.2 to 7.7.

Measurement of Test Concentrations

Fortification samples were prepared at concentrations of 0.214, 2.14 and 4.28 mg/L, and analysed at 0 and 72 hours. Recoveries of these samples averaged 110% (± 2.1%).The mean measured concentrations were 0.24, 0.47, 0.97 and 1.9 mg/L, representing a mean of 96% ± 1.3% of the nominal values. Test level 5 was disregarded due to the unusually low result obtained at 72 hours.

Biological Findings

All results were based on the mean measured concentrations of 0.24, 0.47, 0.97 and 1.9 mg/L

Growth (Cell counts): Logarithmic phase growth was confirmed at 72 hours in control and formulation blank. The control and formulation blank cell count data were not significantly different (Student t-test; $p \leq 0.05$) after 72 hours and so pooled values were used for comparison with the test concentrations. The growth data were subjected to an ANOVA and multiple means test (Dunnett's Test). This indicated a significant inhibitory effect ($p \leq 0.05$) on growth for the 0.47, 0.97 and 1.9 mg/L concentrations of S-71639 10EC to *Selenastrum capricornutum* Prinz, compared to the pooled control and formulation blank after 72 hours (see Table B.9.3.1.3-01).

Table B.9.3.1.3-01 Measured cell counts of *Selenastrum capricornutum* Prinz

Mean measured concentration (mg S-71639 10EC/L)	Mean Cell Counts (cells/mL/10^4)			
	0-hours	24-hours	48-hours	72-hours
Control	0.82	3.3	14	73
Formulation blank	0.85	3.4	15	84
0.24	-	2.6	13	71
0.47	-	2.1	12*	55*
0.97	-	2.3*	4.2*	18*
1.9	-	1.6*	1.3*	1.7*
4.0[a]	-	0.93[b]	0.82[b]	0.59[b]

[a] Nominal test concentration

[b] Values were obtained from the cell count data forms. Statistical analysis was not conducted on this data

* Denotes a significant ($p \leq 0.05$) inhibition effect from the control and formulation blank as calculated using transformed (square root) cell counts by Dunnett's Test

Conclusions

S-17639 10EC 72-hr EC_{50} to *Selenastrum capricornutum* - 0.63 mg /L (95% C.I. = 0.60 and 0.67 mg /L) equivalent to about 0.066 mg a.s./L; 72-hr NOEC - 0.24 mg /L, equivalent to about 0.025 mg a.s./L, based on a significant inhibition of growth at concentrations of 0.47 mg/L and above.

Comments RMS

Validity criteria were met. In the Annex I EFSA DAR (November 2005), the RMS calculated the endpoints according to OECD 201, based on nominal concentrations, since initial measured concentrations were ≥80% of nominals. It was stated: "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." Result presented in DAR, in mg a.s./L with 95% CL: 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. These values were also included in the final LoEP on which the Inclusion was based. In line with this, the RAR concluded that the following values can be used for risk assessment: 72 hour E_bC_{50} 0.074 mg a.s./L (0.710 mg formulation/L); E_rC_{50} 0.11 mg a.s./L (1.1 mg formulation/L). These values can also be used for classification purposes.

4.3.4 Lemna sp. growth inhibition test

4.3.4.1 CA 8.2.7/01 (1996) Pyriproxyfen - Toxicity to duckweed, Lemna gibba

Report: CA 8.2.7/01 (1996) Pyriproxyfen - Toxicity to duckweed, *Lemna gibba*. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-0126	
Previous evaluation	In DAR (November 2005) for original approval. A new robust summary is provided below for the study to assess the effects of technical pyriproxyfen on the growth of *Lemna gibba* because this study is used to derive endpoints for the aquatic risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion.
Conclusion	14-day EC_{50} >0.18 mg a.s./L (frond density) 14-day EC_{50} >0.18 mg a.s./L (biomass)

Guidelines

FIFRA guidelines 122-2 and 123-2: US EPA (1982); Urban & Cook (1986) Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget plants. EPA 540/9-82-020. US EPA, Washington, DC.

Deviations: A minor protocol deviation occurred. Light intensity ranged from 3200-4300 lux on test day 2 (instead of 4250-5750 lux). It was not considered that this deviation had any impact on the results of the study (guideline requirement is >3200 lux).

GLP

Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Technical pyriproxyfen
Description:	Yellow-white crystalline solid
Lot/Batch #:	50401G
Purity:	98.4%
Expiry date:	Not stated
2. Test organism:	The freshwater vascular plant used in this toxicity study was the duckweed *Lemna gibba,* strain 03. The duckweed was originally obtained from the University of California, USA and was maintained in stock culture at the test facility.
2. Culturing:	The stock cultures were maintained in an environmental chamber with the following conditions for a minimum of two days before testing: a temperature of 25 ± 1 °C and continuous illumination of approximately 300 to 500 footcandles at the surface of the medium, Temperature was controlled using an environmental chamber. The inoculum used to initiate the toxicity test with pyriproxyfen was taken from a stock culture that had been transferred to fresh medium two days prior to testing,

Study Design

Stock solutions were prepared by dissolving pyriproxyfen in acetone and the diluting these in Hoagland's medium, as appropriate, to produce the test solutions (nominally, 0.023, 0.045, 0.090, 0.18 and 0.36 mg a.s./L. A solvent control was prepared by diluting acetone in Hoagland's medium to give the same concentration as in each test solution (100 μL/L). Additional untreated Hoagland's medium was prepared and designated the control.

One hour after the test solutions were prepared and added to the test vessels, an inoculum of five plants with three fronds each was aseptically introduced into each vessel. At 3-day intervals fronds were counted and observations made of any abnormal fronds. Following the observations on days 3, 6, 9 and 12, the fronds were transferred to the appropriate freshly prepared test or control solutions. At test termination (day 14), the fronds for each replicate were counted and then removed from each vessel, blotted dry and dried at 105°C for three days prior to weight determination.

Temperature was measured continuously with a minimum/maximum thermometer located in a flask of water adjacent to the test vessels within the environmental chamber. The minimum and maximum temperature was recorded daily. Light intensity was measured at 0 hour and each subsequent 24-hour interval during the exposure period. The pH of the test and control solutions was measured at test initiation (new solutions), in the aged and new solutions at the 3-, 6-, 9- and 12-day renewals, and at test termination (aged solutions). Pyriproxyfen concentrations were measured on day 6 in newly prepared test solutions (prepared from the refrigerated stock solutions formulated at test initiation). Pyriproxyfen concentrations were also measured in the 3-day old (aged) solutions at the end of the renewal period (day 9).

Results and Discussion

Measurement of Test Concentrations

Measured concentrations decreased by approximately 50% between sampling intervals (0.023, 0.039, 0.072, 0.11 and 0.25 mg a.s./L on day 6 and 0.0095, 0.014, 0.025, 0.044 and 0.11 mg a.s./L on day 9). The mean measured concentrations ranged from 44 to 70% of the nominal values and defined the exposure levels as 0.016, 0.026, 0.049, 0.078 and 0.18 mg a.s./L.

Physical and Chemical Measurements of Water

The pH of the freshly prepared test and control solutions ranged from 4.5 to 5.2, while the pH of the aged test and control solutions ranged from 4.7 to 6.2. Continuous temperature monitoring showed that the test solution

temperature ranged from 24 to 25°C. Light intensity over the test period ranged from 400 to 520 footcandles (4300 to 5600 lux), with a single exception on test day 2 when it ranged from 300 to 400 footcandles (3200 to 4300 lux).

Biological Findings

Frond production (density) and observations of the fronds recorded during the 14-day exposure to pyriproxyfen are presented in Table B.9.2.7.1-01. At test termination, the control and solvent control averaged 392 and 357 fronds per replicate, respectively. Statistical analysis determined no significant difference in frond density between the control and solvent control and so the statistical analysis of the treatment frond density data used the pooled control values. Frond production in the treatment groups at the end of the 14-day exposure period ranged between 358 and 500 fronds. Statistical analysis determined no significant reduction in frond production at any treatment concentration when compared to the pooled control data ($p < 0.05$). Since no concentration tested resulted in >50% reduction in frond density, the 14-day EC_{50} value was empirically estimated to be >0.18 mg a.s./L, the highest concentration tested.

Frond biomass (dry weight) measured at test termination is presented in Table B.9.2.7.1-02. The 14-day biomass for the control and solvent control averaged 0.1185 and 0.1190 g per replicate, respectively. Statistical analysis determined no significant difference in frond biomass between the control and solvent control and so the statistical analysis of the treatment frond biomass data used the pooled control values. Frond biomass in the treatment groups at the end of the 14-day exposure period ranged between 0.1140 and 0.1302 g. Statistical analysis determined no significant reduction in frond biomass at any treatment concentration when compared to the pooled control data ($p < 0.05$). Since no concentration tested resulted in >50% reduction in frond biomass, the 14-day EC_{50} value was empirically estimated to be >0.18 mg a.s./L, the highest concentration tested.

Table B.9.2.7.1-01 Frond production (density) and observations recorded for *Lemna gibba* after exposure to pyriproxyfen

Mean measured concentration (mg a.s./L)	Fronds/replicates (mean ± S.D, n = 3)					% reduction (Day 14)
	Day 3	Day 6	Day 9	Day 12	Day 14	
Control	37 ± 4	80 ± 17	186 ± 5	274 ± 26	392 ± 88	Not applicable
Solvent control	29 ± 6[2]	68 ± 14[2]	138 ± 41[2]	250 ± 49[2]	357 ± 33[2]	Not applicable
Pooled control	33 ± 6	74 ± 15	162 ± 37	262 ± 37	374 ± 62	Not applicable
0.016	28 ± 9[2]	64 ± 16[12]	137 ± 25[12]	216 ± 49[1]	358 ± 69[1]	4.4
0.026	33 ± 4[2]	76 ± 6[12]	153 ± 7[12]	279 ± 16[2]	500 ± 68[1]	-33
0.049	34 ± 3[1]	69 ± 3[1]	149 ± 5[1]	220 ± 21[1]	424 ± 46[1]	-13
0.078	35 ± 8[1]	83 ± 5[1]	152 ± 12[1]	224 ± 23[1]	398 ± 35[1]	-6
0.18	35 ± 4[1]	85 ± 2[3]	154 ± 14[3]	234 ± 5[2]	380 ± 36[2]	-1.6

[1] Fronds observed to have reduced root formation in comparison to control

[2] Fronds observed to be slightly chlorotic in comparison to control

[3] Fronds observed to be larger in comparison to control

Table B.9.2.7.1-02 Frond biomass (dry weight) for *Lemna gibba* after exposure to pyriproxyfen

Mean measured concentration (mg a.s./L)	Frond biomass/g (mean ± S.D, n = 3)	% reduction (Day 14)
Control	0.1185 ± 0.0337	Not applicable
Solvent control	0.1190 ± 0.0287	Not applicable
Pooled control	0.1188 ± 0.0280	Not applicable
0.016	0.1176 ± 0.0572	1.0
0.026	0.1302 ± 0.0194	-9.6
0.049	0.1252 ± 0.0122	-5.4
0.078	0.1140 ± 0.0242	4.0
0.18	0.1147 ± 0.0130	3.5

Conclusions

Based on the results of this study:

The 14-day EC_{50}, based on frond density and biomass, was estimated to be >0.18 mg a.s./L. The corresponding NOEC was determined to be 0.18 mg a.s./L, the highest concentration tested.

Comments RMS

Validity criteria were met.

4.4 Chronic toxicity

Please note that only the studies evaluated as acceptable for the risk assessment in Vol. 3 B9 are included in this document. The studies summaries are as per Vol. 3 B9. In the case a study is considered acceptable for the risk assessment it implies that the endpoint can also be used for the classification and labelling purposes.

4.4.1 Fish early-life stage (FELS) toxicity test

4.4.1.1 CA 8.2.2.1/01 (1991) Early life-stage toxicity of Sumilarv technical to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions

Report: CA 8.2.2.1/01 (1991) Early life-stage toxicity of Sumilarv technical to the rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. Sumitomo Chemical Co., Ltd. Unpublished report No.: 39377 (Company Code No. NNW-11-0062)	
Previous evaluation	In DAR (November 2005) for original approval. A new robust summary is provided below for the fish early life stage study with rainbow trout because this study is used to derive endpoints for the fish risk assessment**.**
Remark by RMS	Considered acceptable at the time of original inclusion. The endpoint derived at original inclusion does not change.

Conclusion	95-d NOEC = 0.0043 mg a.s./L

Guidelines & deviations: U.S. EPA Pesticide Assessment Guidelines No. 72-4 (June 1985). Deviations: None reported.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Sumilarv T.G. (technical pyriproxyfen)
Description:	Light tan liquid
Lot/Batch #:	#007024
Purity:	97.2%
Expiry date:	Not stated
2a. Test animals:	Unfertilised rainbow trout eggs (*Oncorhynchus mykiss*) and milt used in the test were obtained from Mt. Lassen Trout farm, California, USA.
2b. Maintenance:	The unfertilized eggs and milt were acclimated from 1.5°C to 8.5°C over two hours. Upon acclimation, the eggs were transferred to a dry plastic container and the milt was mixed with the eggs. The egg-milt mixture was covered with isothermal dilution water, and gently stirred to facilitate fertilization. Approximately one minute after mixing, the mixture was rinsed several times with isothermal control dilution water to remove excess milt and ovarian fluid. After a final rinse, the newly fertilized eggs were covered with isothermal control dilution water. The eggs were shielded from excess light and allowed to water-harden for approximately one hour before they were distributed to incubation cups in the test system.

Study Design

Eggs from female rainbow trout (*Oncorhynchus mykiss*) and milt from adult males were used to produce newly fertilised eggs (< 3 hours post-fertilisation) for the study. The developing embryos were incubated in glass cups placed in replicate chambers (12 L glass aquaria). A diluter stock solution was prepared which was mixed with soft blended well water (total hardness of 162-190 mg/L (as $CaCO_3$)) via a proportional diluter system to prepare nominal test concentrations of 0, 0.0019, 0.0038, 0.0075, 0.015 and 0.030 mg a.s./L. Dimethylformamide (DMF) was used as a carrier and there were blank and solvent controls. The diluter delivered an average rate of 98.25 L/replicate/day which was increased to 134.04 L/replicate/day during the last two weeks (to ensure adequate oxygen supply).

Thirty five newly fertilised eggs were placed in incubator cups in each of 4 replicate exposure aquaria per concentration (total of 140/concentration). An additional 50 eggs were placed into each of 4 control exposure chambers in separate incubation cups to assess fertilisation success on day 12. Total exposure time under the flow-through conditions was 95 days. When hatching commenced, the number of embryos hatched in each incubation cup was recorded daily until day 36 (when hatch was judged to be complete). Feeding was commenced on test day 48 (14 days post-hatch) when the sac-fry began to exhibit normal swim-up behaviour. Abnormal behavioural, normal swim-up behaviour, abnormal physical change and mortality were monitored daily. Growth was determined on days 35 and 61 post-hatch: growth was determined on day 35 by the photographic method and on day 95 (61 days post-hatch) the fish were sacrificed and measured for length and weight. Test concentrations were analysed and temperature, dissolved oxygen, conductivity and pH were measured on days 0, 1 and 7 and weekly thereafter. There was a 16-hour light: 8-hour dark cycle.

Results and Discussion

Physical and Chemical Measurements of Water

Water chemistry parameters were generally within the specified limits: temperature ranged from 9.8 to 11.3°C, pH ranged from 7.98 to 8.57 and dissolved oxygen ranged from 6.8 to 10.6 mg/L (64 and 98% saturation, respectively). Dissolved oxygen concentrations fell below 75% intermittently in the solvent control and each test concentration from day 84 onwards. This was attributed to the presence of DMF as the vehicle used to dissolve the test compound (the highest biomass loading was observed in the control where oxygen concentrations did not fall below 75% saturation). However, the declining oxygen concentrations were not considered to be entirely attributable to biomass loading, but overall it was considered that it did not have any impact on the validity of the data.

Measurement of Test Concentrations

The mean measured concentrations were 0.0018 ± 0.00017, 0.0043 ± 0.00040, 0.0067 ± 0.00056, 0.014 ± 0.0011 and 0.026 ± 0.0015 mg a.s./L, and the results are presented as these values. These figures ranged from 87-113% of the nominal test concentrations.

Biological Findings

Egg hatchability: Viability for the egg lot used to initiate the study averaged 98%, indicating a high degree of fertilisation success (the effect of the test material on this was not assessed). Control and solvent control responses were pooled after a two-tailed Fisher's exact test indicated no significant difference between the groups. Frequency analysis, coupled with a one-tailed Fisher's exact test, indicated no significant reduction in hatch at any test concentration when compared to the pooled control group (see Table B.9.2.2.2-01).

Fry survival: Fry survival was determined for the interval between sac-fry reduction and days 35 and 61 post-hatch.

35-Day post-hatch: Control and solvent control responses were pooled after a two-tailed Fisher's exact test indicated no significant difference between the groups. Single replicates in the 0.0067 and 0.014 mg a.s./L test concentrations exhibited 67 and 47% survival, respectively. This was attributed to natural variability and was not considered to be a compound-related effect. Frequency analysis, coupled with a one-tailed Fisher's exact test, identified a statistically significant reduction in survival in the 0.014 mg a.s./L test concentration when compared to the pooled control group, but this was largely due to the single replicate (see Table B.9.2.2.2-01).

61-Day post-hatch: Control and solvent control responses were pooled after a two-tailed Fisher's exact test indicated no significant difference between the groups. As during the first 35 days, single replicates in the 0.0067 and 0.014 mg a.s./L mean measured test concentrations exhibited 64 and 47% survival, respectively. Again this was attributed to natural variability and was not considered to be a compound-related effect. Frequency analysis, coupled with a one-tailed Fisher's exact test, identified a statistically significant reduction in survival in the 0.014 mg a.s./L test concentration when compared to the pooled control group, but again this was largely due to the single replicate (see Table B.9.2.2.2-01).

Growth - standard length measured on days 35 and 61 and wet weight on day 61:

35-Day and 61-Day post hatch - standard length: A t-test comparison between the control and solvent control indicated no significant difference between the groups, and so pooled values were used. ANOVA, coupled with Dunnett's one-tailed mean comparison test, indicated a significant reduction in length in the 0.0067, 0.014 and 0.026 mg a.s./L mean measured test concentrations when compared to the pooled control group (see Table B.9.2.2.2-02). The values on day 61 were used for the final conclusions, and on this occasion a single replicate from each of the 0.0067 and 0.014 mg a.s./L concentrations were excluded from the analysis due to low survival.

61-Day post hatch - wet weight: A t-test comparison between the control and solvent control indicated no significant difference between the groups and so pooled values were used. ANOVA, coupled with Dunnet's one-tailed mean comparison test, indicated a significant reduction in 61-days post-hatch blotted wet weight in the 0.0067, 0.014 and 0.026 mg a.s./L mean measured test concentrations when compared to the pooled control

group (see Table B.9.2.2.2-02). Again, because of significantly unequal sample size in the two replicates from the 0.0067 and 0.014 mg a.s./L concentrations due to low survival, they were excluded from the analysis.

Morphological and behavioural observation: Swim-up began at approximately the same time throughout all test concentrations and appeared to proceed at the same rate in all test concentrations. Compound-related physical and behavioural effects that were noted during this study included the following: 1) fish resting on the bottom of the test chamber, 2) quiescence, 3) discoloration, 4) spinal curvature, and 5) irregular respiration. These effects were noted primarily in the 0.0067, 0.014, and 0.026 mg a.s./L test concentrations, and they had effectively disappeared by day 77 (43-Day post-hatch).

Table B.9.2.2.2-01 Egg hatchability and fry survival

Mean measured test concentration (mg a.s./L)	Replicate	Egg Hatch (%)	35-Day Post-Hatch Survival (%)	61-Day Post-Hatch Survival (%)
Control	A	100	93	93
	B	97	100	100
	C	100	100	100
	D	100	93	93
	Mean	99	97	97
Solvent Control	A	91	100	100
	B	100	100	100
	C	97	93	93
	D	94	93	93
	Mean	96	97	97
Pooled Controls	Mean	97	97	97
0.0018	A	100	100	100
	B	100	100	100
	C	100	80	80
	D	100	87	87
	Mean	100	92	92
0.0043	A	100	100	100
	B	100	93	93
	C	100	100	100
	D	97	100	100
	Mean	99	98	98
0.0067	A	97	93	93
	B	100	100	100
	C	100	67	64[a]
	D	94	100	100
	Mean	98	90	90
0.014	A	100	47	47
	B	97	100	100

Mean measured test concentration (mg a.s./L)	Replicate	Egg Hatch (%)	35-Day Post-Hatch Survival (%)	61-Day Post-Hatch Survival (%)
	C	100	93	93
	D	97	93	93
	Mean	99	83*	83*
0.026	A	100	100	100
	B	100	93	93
	C	97	100	100
	D	94	100	100
	Mean	98	98	98

* Statistically significant reduction (P≤0.05) when compared to pooled control group

[a] Initial n=14 due to escape from chamber

Table B.9.2.2.2-02: Mean standard length and blotted wet weight

Mean measured test concentration (mg a.s./L)	35-Day Post -Hatch	61-Day Post-Hatch	
	Mean Standard Length (mm)	Mean Standard Length (mm)	Mean Wet weight (g)
Control	33.106 ± 1.28	48.563 ± 2.73	1.711 ± 0.33
Solvent Control	33.481 ± 1.73	48.403 ± 3.46	1.706 ± 0.38
Pooled Controls	33.294 ± 1.52	48.482 ± 3.11	1.709 ± 0.36
0.0018	32.847 ± 1.29	48.041 ± 2.96	1.632 ± 0.32
0.0043	32.947 ± 1.19	48.197 ± 2.26	1.666 ± 0.27
0.0067	32.462* ± 1.75	47.148* ± 3.45	1.603* ± 0.35
0.014	32.075* ± 1.58	45.708* ± 4.24	1.432* ± 0.39
0.026	31.763* ± 1.46	45.055* ± 3.37	1.350* ± 0.33

* Statistically significant reduction (P≤0.05) when compared to pooled control group

Conclusions

Based on the most sensitive endpoint evaluated during this rainbow trout (*Oncorhynchus mykiss*) early life stage toxicity study, growth (length and wet weight), the NOEC for technical pyriproxyfen is 0.0043 mg a.s./L and the LOEC is 0.0067 mg a.s./L.

Comments by RMS

The study summary was included in the original DAR. The study was assessed by the RMS against the current version of OECD Guideline 210 (2013). Overall the study was conducted in agreement with the guideline requirements, and the validity criteria were met. The test substance concentrations were determined by a sufficiently validated gas-liquid chromatography (mean recoveries in quality control samples of 105%, 99% and 100% at fortification levels of 1.52, 15.2 and 35.4 µg/L (n = 16)). As no statistical differences were observed between control and solvent control responses for all endpoints, the results from the control and solvent control groups were pooled, which is considered to be acceptable.

Based on the statistically significantly reduced mean standard length and mean wet weight the NOEC was set at 4.3 µg/L.

The percentages of the decrease in standard length and weight caused by pyriproxyfen after 60 days of exposure are presented in Table B.9.2.2.2-03:

Table B.9.2.2.2-03 Percentage of decrease in mean standard length and mean weight of rainbow trout after 60 days of exposure to pyriproxyfen#

Treatment (µg/L)	n	Mean standard length (mm)	% Reduction	Mean weight (g)	% Reduction
Pooled control	8	48.482	-	1.709	-
1.8	4	48.041	0.9	1.632	4.5
4.3	4	48.197	0.6	1.666	2.5
6.7	4	47.148*	2.8	1.603*	6.2
14.0	4	45.708*	5.7	1.432*	16.2
26.0	4	45.055*	7.1	1.35*	21.0

* Significantly different from control at 5% level

The applicant submitted a calculation of reduction of mean length and weight, based on reported replicate values for length and weight. Resulting mean values (and hence reduction %) differed from those included in the study report, which may be the result of rounding. As mean values calculated from reported replicate values yield lower reductions compared to reported mean values, the latter were used to calculate reductions by RMS.

The NOEC of 4.3 µg/L can be used for risk assessment.

4.4.2 Full life cycle toxicity test

CA 8.2.2.2/01 (2007) Pyriproxyfen: Full Life Cycle Toxicity Test with Medaka (Oryzias latipes) under Flow-Through Conditions

Report: CA 8.2.2.2/01, (2007) Pyriproxyfen: Full Life Cycle Toxicity Test with Medaka *(Oryzias latipes)* under Flow-Through Conditions. Sumitomo Chemical Co., Ltd. Unpublished report 1043.035.123 (Company Code No.: NNW-0181)	
Previous evaluation	New study
Remark by RMS	The study is acceptable.
Conclusion	189-d NOEC = 0.0027mg a.s./L

Guidelines

The Medaka *(Oryzias latipes)* Full Life Cycle Test Guideline (Ministry of the Environment, Japan, Annex 6-2, November 2002)

Deviations: On days 89 and 90, the temperature was 27.1 and 27.2°C, respectively (instead of 25 ± 2 °C); on several days the air saturation value was above 100% and on day 119, the air saturation in replicate A of mean measured 0.84 µg test item/L and in replicate B of mean measured 8.6 µg test item/L was below 60% (instead of 60 to 100%). During the in-life phase, total hardness generally ranged between 10 and 80 mg/L (expressed

as $CaCO_3$) and the conductivity ranged from 420 to 850 µS/cm (instead of 20 to 40 mg/L and 650 and 910 µS/cm, respectively). These deviations were not considered to have adverse effects on the results and conclusions derived from this study.

GLP

Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Pyriproxyfen
Description:	Solid
Lot/Batch #:	040803G
Purity:	98.7%
Expiry date:	August 31, 2007
2a. Test animals:	Medaka *(Oryzias latipes)* S-rR strain used during this study were from a laboratory culture, originally obtained from Sumika Technoservice Corporation. The genotypic sex of this strain can be identified using a colour marker so that genetic females are white and genetic males are orange/red.
2b. Maintenance:	Macroscopically healthy and normal fish were selected as brood culture for obtaining the eggs for the P-generation. The fish were cultured under flow through conditions similar to the test conditions. The fish were about 4 months old when the eggs for starting the P-generation were collected. They were cultured for more than 14 days under test conditions. The culture was fed at least once daily with brine shrimp nauplii, the same type of food which was used for the test. In order to obtain eggs with an age of < 24 hours, all eggs including the eggs attached to the females were removed from the culture tanks on the evening prior to collecting the eggs used for the test. On the day of the start of the exposure, eggs were collected about 3 hours after light on. Thereafter, eggs were separated from each other and fertilised eggs were selected for the start of the exposure.

Study Design

The objective of the study was to determine lethal and sublethal effects, including reproduction, of technical pyriproxyfen on Medaka *(Oryzias latipes)* in a full life cycle test (over two generations) under flow-through conditions.

Nominal exposure concentrations were 1.0, 3.2 and 10 µg test item/L along with water and solvent controls. A continuous flow-through system under which actual flow rates were maintained at +/- 10 % of the nominal flow rates was used. The delivery rate of the test solution in each of the test vessels was approximately 13 volume replacements per day. The diluter system was checked at least once daily for proper function.

The duration of exposure was 189 days in total. In the P-generation, the fertilised egg to adult stage (60 days after hatching) was exposed to the chemical and the effect on following parameters was determined for 20 impartially selected fish from each treatment group:

• Total length (tl)

• Wet weight (blotted dry)

• Condition factor (calculated as [body weight/total length^3) x 1000)

• Deformities

• Genetic sex (colouration of the body and the outer rays of the caudal fin)

• Secondary sex characteristics (fin morphology)

• Gonad weight

• Gonadosomatic index (GSI) (calculated as (gonad weight/body weight) x 100)

• Liver weight

• Hepatosomatic index (HSI)

• Hepatic Vitellogenin (VTG) concentration

• Functional sex (histology of gonads, ovo-testis)

The fin morphology was characterised with following ratios:

• *dm/tl,*

• *dc/dm,*

• *am/tl,*

• *a2/tl.*

With: tl: total length of the individual fish,

dm: maximum length of dorsal fin,

dc: cleft depth between the last ray and preceding one of dorsal fin ,

am: maximum length of anal fin,

a2: length of the second ray from the last one of the anal fin.

Furthermore, in order to determine the fecundity and fertility, an additional 30 day exposure (reproduction phase) was conducted with 8 breeding pairs per treatment level. On day 114 post hatch, i.e., after the reproduction phase, the parameters determined on day 60 post hatch were determined from all surviving fish from the P-generation.

In the F1-generation, eggs which were collected prior to termination of the P-Generation (days 99 to 101 after hatch of the P-generation) were exposed to the chemical for 60 days after hatching, and the effects on the parameters listed for day 60 of the P-generation were investigated.

Numbers of fish tested in the P and F1-generation were 4 replicates of 15 organisms per test concentration. For the reproduction phase 8 pairs (1 male and 1 female) per test concentration were impartially selected.

Actual test concentrations in all test concentrations were measured once a week. Water quality measurements, i.e., water temperature, dissolved oxygen concentration, and pH were measured weekly. Water hardness and alkalinity at the highest concentrations as well as the water control were measured at test initiation and termination. Conductivity was determined at test termination.

Results and Discussion

Physical and Chemical Measurements of Water

Water temperature, dissolved oxygen concentration and pH ranged from 23.9 to 26.1°C, 5.16 to 9.12 mg/L (corresponding to 66 to 117% air saturation), and 6.98 to 8.95, respectively. In the control and at the highest test concentration, total hardness at start of the exposure was 30 and 31 mg/L, respectively, and at end of the exposure 80 and 78 mg/L, respectively. Alkalinity ranged between 391 and 392 mg/L at start and 383 and 385 mg/L at the end of exposure. Conductivity ranged between 845 and 850 µS/cm at the end of exposure.

Measurement of Test Concentrations

In control and solvent control samples no test item was detected at the retention time of pyriproxyfen which confirmed the proper operation of the test system during this study. Treatment concentrations showed that the concentration in each test solution was kept appropriately during the test period, with the mean recoveries of 83.9% (n = 52, C.V. = 17.5%), 82.8% (n = 51, C.V. = 18.0%) and 86.3% (n = 52, C.V. = 16.9%) at nominal treatment levels of 1.0, 3.2 and 10.0 µg test item/L, respectively. Analyses of these samples indicated that all procedures during the test were properly conducted. Based on the analytical results, mean measured concentrations of test solutions were 0.84, 2.7 and 8.6 µg test item/L for nominal treatment levels of 1.0, 3.2 and 10.0 µg test item/L.

Results from the P-Generation

Embryonic phase

Control hatchability was 70%. Average hatchability ranged from 70 to 86% and the time of hatching ranged from 10 to 12 days. For both parameters no statistically significant differences were found when compared to the solvent control and pooled control, respectively.

Larval - Mature Phase

There were no behavioural changes or deformities when compared to the controls.

Control and solvent control survival rates on day 60 post hatch were equal or greater 91.8%. The average survival rates in the control, solvent control and 0.84, 2.7 and 8.6 µg test item/L treatment levels were 91.8, 100, 96.7, 93.5 and 93.8%, respectively. There were no statistically significant differences of the survival when compared to the solvent control.

For males the average total length for control, solvent control, 0.84, 2.7 and 8.6 µg test item/L treatment levels were 30.9, 31.3, 32.7, 31.0 and 30.8 mm, respectively. The difference of the pooled control to 0.84 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-response). Average total length of the females ranged from 31.3 to 32.5 mm and there were no significant differences when compared to the pooled control. Average body weight was 350 to 403 mg in males and 433 to 499 mg in females and average condition factors were 11.6 to 12.3 for males and 13.7 to 14.8 for females (no significant differences compared to the pooled control).

Average gonad weight for males was 3.39, 3.04, 4.05, 3.90 and 3.42 mg corresponding to control, solvent control, 0.84, 2.7 and 8.6 µg test item/L, respectively. The difference of the pooled control to 0.84 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-response). Average gonad weight for females ranged from 62.0 to 76.4 mg (no significant differences compared to the pooled control). Average gonado-somatic indices (GSI) for males were 0.93, 0.84, 1.02, 1.13 and 1.00 corresponding to control, solvent control, 0.84, 2.7 and 8.6 µg test item/L, respectively. The difference of the pooled control to 2.7 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-response). Average GSIs for females ranged from 13.4 to 15.9 (no significant differences). No test item related sex-reversal occurred.

The average maximum length of the dorsal fin / total length (dm/tl) ranged from 0.145 to 0.151 for males and from 0.109 to 0.113 for females (no significant differences within sexes compared to pooled control). The average cleft depth between the last ray and the preceding one of the dorsal fin / maximum length of the dorsal fin (dc/dm) for males was 0.326, 0.311, 0.314, 0.357 and 0.312 corresponding to control, solvent control, 0.84, 2.7 and 8.6 µg test item/L, respectively. The difference of the pooled control to 2.7 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-response). For females, dc/dm ranged from 0.079 to 0.096 (no significant differences). The average maximum length of the anal fin / total length (am/tl) ranged from 0.147 to 0.152 for males and from 0.106 to 0.112 for females, respectively (no significant differences). The average length of the second ray from the last one of the anal fin / total length (a2/tl) ranged from 0.105 to 0.110 for males and from 0.065 to 0.069 for females (no significant differences).

The average liver weight ranged from 10.2 to 12.5 mg in males (no significant differences compared to the pooled control). For females, the corresponding values for the control, solvent control, 0.84, 2.7 and 8.6 µg test item/L were 29.2, 25.1, 19.2, 25.7 and 21.4 mg, respectively. The difference of the pooled control to 0.84 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-

response). The average hepatic somatic index (HSI) ranged from 2.7 to 3.3 in males (no significant differences). For females the average HSI for control, solvent control, 0.84, 2.7 and 8.6 µg test item/L treatment levels were 5.9, 5.4, 4.2, 5.4 and 4.9, respectively. The difference of the pooled control to 0.84 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-response). For males, the average hepatic vitellogenin concentrations for control, solvent control, 0.84, 2.7 and 8.6 µg test item/L treatment levels were 0.17, 0.27, 0.54, 0.57 and 0.65 ng/mg, respectively. Differences of the pooled control to 0.84 and 8.6 µg test item/L were statistically significant but considered to be caused by unusual low control and solvent control hepatic vitellogenin concentrations and hence as not test item related. In females, the average hepatic vitellogenin concentration ranged from 556 to 1202 ng/mg (no significant differences).

Reproduction phase

Average number of eggs ranged from 34.2 to 39.2 eggs per female per day within the 30 days reproduction phase (no significant differences). Average fertility was 89.4, 94.3, 96.0, 89.8 and 91.3% for control, solvent control, 0.84, 2.7 and 8.6 µg test item/L, respectively. The difference of the pooled control to 2.7 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-response).

Control survival rates from day 61 post hatch to day 114 post hatch were 100%. The average survival rates from start of the reproduction assay (i.e., day 72 post-hatch) to day 114 post-hatch in the control, solvent control and 0.84, 2.7 and 8.6 µg *test item/L* treatment levels were 100, 100, 100, 87.5 and 93.8%, respectively (no significant differences). The average total length ranged from 35.9 to 37.3 mm for males and 36.5 to 37.6 mm for females, respectively, while average body weight ranged from 508 to 554 mg in males and 648 to 775 mg in females, respectively (no significant differences). Average condition factors were 10.6 to 11.3 for males and 13.3 and 14.4 for females (no significant differences).

Average gonad weight ranged from 4.55 to 5.59 mg for males and 91.1 to 107.4 mg for females (no significant differences within the sexes). Average gonadosomatic indices (GSI) ranged from 0.84 to 1.03 for males and 13.4 to 14.4 for females (no significant differences within the sexes). The average maximum length of the dorsal fin / total length (dm/tl) ranged from 0.138 to 0.145 for males and from 0.113 to 0.119 for females (no significant differences within the sexes). The average cleft depth between the last ray and the preceding one of the dorsal fin / maximum length of the dorsal fin (dc/dm) ranged from 0.339 to 0.363 for the males and from 0.048 to 0.058 for females (no significant differences within the sexes). The average maximum length of the anal fin / total length (am/tl) ranged from 0.141 to 0.147 in males and from 0.104 to 0.108 in females, respectively (no significant differences within the sexes). The average length of the second ray from the last one of the anal fin / total length (a2/tl) ranged from 0.102 to 0.114 for males and from 0.066 to 0.069 for females (no significant differences within the sexes). All males showed papillary processes on the anal fin while none of the females showed these processes.

The average liver weight ranged from 9.1 to 9.9 mg in males and from 34.8 to 55.6 mg in females, respectively (no significant differences within the sexes). The average hepatosomatic index (HSI) ranged from 1.7 to 1.8 in males and from 5.3 to 7.8 in females (no significant differences within the sexes). The average hepatic vitellogenin concentrations for ranged from 0.11 to 0.41 ng/mg for males and from 723 to 1310 ng/mg for females (no significant differences within the sexes).

Results from the F1-Generation

Embryonic phase

Hatchability of the eggs spawned on days 99, 100, and 101 after hatching of the F1-generation ranged from 71 to 94% and the time to hatch ranged from 9 to 12 days. Hatchability on day 99 at 8.6 µg test item/L was statistically significantly different compared to the solvent control but this was not considered to be treatment-related (hatchability for the treated test groups was within the control range and no significant differences were found for the hatchability from days 100 and 101 post hatch). For the time of hatching no significant effects were found for the eggs spawned on days 99, 100 and 101 post hatch.

Larval - Mature Phase

Control and solvent control survival rates on day 60 post hatch were greater than 90%. Average post-hatch survival for the control, solvent control and mean measured 0.84, 2.7 and 8.6 µg test item/L was 93.3, 100, 98.3, 98.3 and 96.7%, respectively (no significant differences).

There were no behavioural changes or deformities when compared to the controls.

For males the average total length for control, solvent control, 0.84, 2.7 and 8.61 µg test item/L treatment levels were 28.2, 28.8, 28.2, 27.9 and 28.0 mm, respectively. The difference of the pooled control to 2.7 µg test item/L was statistically significant but this was not considered to be treatment-related (no dose-response). The average total length of female fish ranged from 28.2 to 29.1 mm (no significant differences). Average body weight ranged from 257 to 284 mg for males and 297 to 316 mg for females (no significant differences within the sexes). Average condition factors were 11.4 to 11.9 for males and 12.2 to 13.5 for females (no significant differences within the sexes).

Average gonad weight ranged from 2.11 to 2.84 mg for males and 31.3 to 34.9 mg for females (no significant differences within the sexes). Average gonadosomatic indices (GSI) ranged from 0.80 to 1.04 for males and 9.9 to 10.8 for females (no significant differences within the sexes). For all fish, histopathological analysis of the gonads verified the sex determined based on the colouration, i.e. no sex-reversal occurred. Only one male and one female with non-functional gonads (solvent control and 0.84 µg test item/L, respectively) were observed, i.e. there was no effect of the test item on gonad histopathology.

The average maximum length of the dorsal fin / total length (dm/tl) ranged from 0.144 to 0.153 for males and from 0.109 to 0.115 for females (no significant differences within the sexes). The average cleft between the last ray and the preceding one of the dorsal fin / maximum length of the dorsal fin (dc/dm) ranged from 0.251 to 0.306 for the males and from 0.063 to 0.106 for females, respectively (no significant differences within the sexes). The average maximum length of the anal fin / total length (am/tl) ranged from 0.142 to 0.153 in males and from 0.107 to 0.113 in females, respectively (no significant differences within the sexes). The average length of the second ray from the last one of the anal fin / total length (a2/tl) ranged from 0.106 to 0.112 for males and from 0.068 to 0.069 for females, respectively (no significant differences within the sexes). Except for two male fish (one at 0.84 and one at 8.6 µg test item/L), all males showed papillary processes on the anal fin while none of the females showed these processes.

The average liver weight ranged from 7.2 to 8.3 mg in males and from 12.3 to 18.5 mg in females, respectively (no significant differences within the sexes). The average hepatic somatic index (HSI) ranged from 2.7 to 2.9 in males (no significant differences).For females the average hepatic somatic index (HSI) for control, solvent control, 0.84, 2.7 and 8.6 µg test item/L treatment levels were 5.8, 3.8, 5.6, 4.7 and 4.7, respectively. Differences of the pooled control to 0.84 µg test item/L were statistically significant but considered to be due to an unusually low HSI for the solvent control and hence not test item related (no dose-response). The average hepatic vitellogenin concentrations ranged from 0.26 to 0.88 ng/mg for males and from 482 to 765 ng/mg for females, respectively (no significant differences within the sexes).

Conclusions

Performance of the control and solvent control fish assured that the test conditions were appropriate for Medaka growth and reproduction. None of the treatment levels tested, i.e., concentrations ranging from 0.84 to 8.6 µg test item/L caused statistically significant and scientifically consistent differences of survival, growth, secondary sexual characteristics, hepatic vitellogenin concentration, gonad histology, and reproduction. Therefore, the overall NOEC for this 2-generation long-term study is the highest concentration tested, 8.6 µg test item/L based on mean measured concentrations. Since no test item related effects were found for this study, the LOEC is higher than the highest test concentration and ECx values could not be calculated for any of the parameters.

The findings from this long-term study indicate that the test item has no toxic and no endocrine disrupting effects on Medaka exposed for two generations at concentrations up to 8.6 µg test item/L.

Comments RMS

The study was assessed by the RMS according to the requirements of the OECD Review Paper No. 95 on Fish Life-Cycle Tests (2008).

The concentrations of the test substance in water were determined by a sufficiently validated GC method (recoveries 77.3% (n = 5), 111% (n = 5) and 98.7% (n = 5) for 0.3, 1.0 and 20 µg/L). Actual concentrations of the test substance in the test solution during the study were determined once weekly from two replicates of each test concentration. Three quality control samples of different concentrations (1.0, 3.0 and 10.0 µg/L) were prepared on each sampling day and analysed with the set of samples (mean recoveries 95.1% (n = 23), 82.8% (n = 26) and 85.8% (n = 27)). The concentrations of the test substance during the study were reported as arithmetic mean measured concentrations, which is acceptable.

The OECD Review Paper No. 95 does not present a protocol for a full life cycle study with Medaka; however, the protocol used in the current study resembles to a large extent the protocol suggested for a multigeneration study with Medaka (OECD guideline 240), but without an assessment of the F2 generation. The study used lower number of fish than recommended by the OECD Review Paper No. 95, i.e. 15 embryos/fish per replicate with a total of 4 replicates per concentration in the P and F1-generations (60 fish in total per concentration), instead of 100 per concentration, as recommended by the OECD, and the effects were assessed on 20 fish per concentration only. For the reproduction phase 8 pairs (1 male and 1 female) per test concentration were used, instead of 12 males/20 females per replicate (recommended ratio of males to females 4:6), as recommended by the OECD for a multigeneration study with Medaka. The time frames used in the study also deviated somewhat from the recommendations of the OECD; e.g. 30 days reproduction phase instead of two weeks, the P1 generation was assessed until 114 days post-hatching. These deviations are not expected to have affected the validity of the study. The adequate parameters have been assessed.

The summary tables of the results reported in the study are presented below. For all parameters, the data for the untreated control and solvent control were statistically analysed. When the data were not statistically significantly different from each other, the data for the controls were pooled for statistical analysis of the treatments. When the control and solvent control were statistically significantly different from each other, treated groups were compared to the solvent control.

Table B.9.2.2.4-01 Hatchability and time of hatching of P-generation medaka exposed to pyriproxyfen

Mean measured concentration (µg/L)	Hatchability (%)	Time of hatching (day)
Control	70	10.0
Solvent control	86	10.3
0.84	86	10.5
2.7	74	10.0
8.6	76	12.0

Table B.9.2.2.4-02 Summary of the parameters of P-generation determined at 60 days post-hatching

Mean measured conc. (µg/L)	Mean survival (%)	Sex ratio		Total length (mm)		Body weight (mg)		Condition factor		Gonad weight (mg)		GSI (%)	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	91.8	9	11	32.5	30.9	499	366	14.6	12.3	76.4	3.39	15.3	0.93
Solvent control	100	10	10	31.9	31.3	464	361	14.3	11.7	62.0	3.04	13.4	0.84
0.84	95.7	8	11	31.3	32.7*	454	403	14.8	11.6	67.2	4.05*	14.6	1.02
2.7	93.5	8	11	32.0	31.0	468	352	14.2	11.8	66.2	3.90	14.2	1.13*
8.6	93.8	10	10	31.6	30.8	433	350	13.7	11.9	71.9	3.42	15.9	1.00

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-03 Summary of the fin morphology characteristics of P-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	dm/tl		dc/dm		am/tl		a2/tl		% appearance of small papillary processes on the posterior region of the anal fin	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	0.109	0.147	0.096	0.326	0.110	0.152	0.065	0.109	0	91
Solvent control	0.110	0.148	0.093	0.311	0.108	0.149	0.066	0.105	0	100
0.84	0.113	0.145	0.079	0.314	0.112	0.147	0.067	0.109	0	100
2.7	0.111	0.147	0.088	0.357*	0.111	0.149	0.069	0.109	0	100
8.6	0.111	0.151	0.082	0.312	0.106	0.150	0.067	0.110	0	100

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-04 Mean liver weights, hepatosomatic indices and hepatic vitellogenin concentrations of P-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	Liver weight (mg)		HSI (%)		Hepatic VTG (ng/mg)	
	♀	♂	♀	♂	♀	♂
Control	29.2	12.5	5.9	3.3	1202	0.17
Solvent control	25.1	12.0	5.4	3.3	907	0.27
0.84	19.2*	10.8	4.2*	2.7	778	0.54*
2.7	25.7	10.2	5.4	2.9	1012	0.57
8.6	21.4	10.6	4.9	3.1	556	0.65*

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-05 Histopathological evaluation of P-generation Medaka determined at 60 days post-hatching

Mean measured concentration (µg/L)	Number of fish		Ovotestis		Other male deformities		Non-functional gonad	
	♀	♂	♀	♂	♀	♂	♀	♂
Control	9	11	N	N	N	N	N	N
Solvent control	10	10	N	N	N	N	N	N
0.84	9	11	N	N	N	N	Immature ovary: 1	N
2.7	8	11	N	N	N	N	N	N
8.6	10	10	N	1Y[a]	N	N	Immature ovary: 1	N

[a] One male had ovotestis

N: none

Y: yes

Table B.9.2.2.4-06 Mean fecundity and fertility of P-generation during the reproduction phase

Mean measured concentration (µg/L)	Mean fecundity (eggs/female/day)	Mean fertility
Control	35.0 ± 7.8	89.4 ± 8.9
Solvent control	35.9 ± 10.8	94.3 ± 8.8
0.84	38.2 ± 8.4	96.0 ± 1.4
2.7	39.2 ± 15.1	89.8 ± 11.1**
8.6	34.2 ± 7.2	91.3 ± 9.9

** Statistically significant when compared to the solvent control ($p < 0.05$)

Table B.9.2.2.4-07 Summary of the parameters of P-generation determined at 61-114 days post-hatching

Mean measured concentration (µg/L)	Mean survival on day 114 (%)	Sex ratio		Total length (mm)		Body weight (mg)		Condition factor		Gonad weight (mg)		GSI (%)	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	100	8	8	37.6	36.1	775	508	14.4	10.8	105.3	4.99	13.4	0.98
Solvent control	100	8	8	37.5	36.6	743	549	14.0	11.3	107.4	5.59	14.4	1.03
0.84	100	8	8	37.5	37.3	735	554	13.9	10.7	98.9	5.38	13.4	0.98
2.7	87.5	6	8	36.8	36.9	706	538	14.2	10.6	97.3	4.68	13.6	0.86
8.6	93.8	7	8	36.5	35.9	648	531	13.3	11.3	91.1	4.55	13.8	0.84

Table B.9.2.2.4-08 Summary of the fin morphology characteristics of P-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	dm/tl		dc/dm		am/tl		a2/tl		% appearance of small papillary processes on the posterior region of the anal fin	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	0.115	0.139	0.056	0.363	0.104	0.141	0.069	0.102	0	100
Solvent control	0.117	0.145	0.055	0.349	0.108	0.146	0.068	0.114	0	100
0.84	0.119	0.141	0.048	0.339	0.108	0.144	0.068	0.109	0	100
2.7	0.116	0.138	0.058	0.362	0.106	0.144	0.066	0.113	0	100
8.6	0.113	0.141	0.056	0.362	0.107	0.147	0.068	0.112	0	100

Table B.9.2.2.4-09 Mean liver weights, hepatosomatic indices and hepatic vitellogenin concentrations of P-generation determined at 61-114 days post-hatching

Mean measured concentration (µg/L)	Liver weight (mg)		HSI (%)		Hepatic VTG (ng/mg)	
	♀	♂	♀	♂	♀	♂
Control	41.1	9.2	5.4	1.8	1310	0.41
Solvent control	39.1	9.9	5.4	1.8	767	0.21
0.84	42.5	9.7	5.8	1.8	1043	0.13
2.7	55.6	9.4	7.8	1.7	772	0.19
8.6	34.8	9.1	5.3	1.7	723	0.11

Table B.9.2.2.4-10 Histopathological evaluation of P-generation Medaka determined at 114 days post-hatching

Mean measured concentration (µg/L)	Number of fish		Ovotestis		Other male deformities		Non-functional gonad	
	♀	♂	♀	♂	♀	♂	♀	♂
Control	8	8	N	N	N	N	N	N
Solvent control	8	8	N	1Y[a]	N	N	N	N
0.84	8	8	N	N	N	N	N	N
2.7	6	8	N	N	N	N	N	N
8.6	7	8	N	N	N	N	N	N

[a] One male had ovotestis

N: none

Y: yes

Table B.9.2.2.4-11 Summary of the hatchability and time of hatching of F1 generation started on days 99, 100 and 101 and the pooled results for hatchability calculated by the RMS

Mean measured concentration (µg/L)	Hatchability (%)				Time of hatching (day)		
	99 dph	100 dph	101 dph	99-101 dph	99 dph	100 dph	101 dph
Control	76	88	83	82.08	9.5	9.5	10
Solvent control	94	86	88	89.17	9.8	9.3	9.8
0.84	90	91	88	89.58	9.8	9.8	10
2.7	94	94	93	93.33	9.8	9.5	9.8
8.6	71**	78	76	75.00*, **	9.5	10	10

* Statistically significant when compared to the control ($p < 0.05$)

** Statistically significant when compared to the solvent control ($p < 0.05$)

Table B.9.2.2.4-12 Summary of the parameters of F1-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	Mean survival (%)	Sex ratio		Total length (mm)		Body weight (mg)		Condition factor		Gonad weight (mg)		GSI (%)	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	93.3	21	35	28.9	28.2	297	257	12.2	11.4	31.3	2.11	9.9	0.80
Solvent control	100	25	35	28.8	28.8	315	284	13.2	11.9	32.2	2.63	10.1	0.97
0.84	98.3	33	26	29.1	28.2	316	263	12.8	11.7	32.8	2.54	10.3	0.86
2.7	98.3	33	26	28.3	27.9*	305	259	13.4	11.8	34.9	2.78	10.8	1.04
8.6	96.7	32	26	28.2	28.0	302	261	13.5	11.7	31.5	2.84	10.3	1.01

* Statistically significant when compared to pooled controls ($p < 0.05$)

Table B.9.2.2.4-13 Summary of the fin morphology characteristics of F1-generation determined at 60 days post-hatching

Mean measured concentration (µg/L)	dm/tl		dc/dm		am/tl		a2/tl		% appearance of small papillary processes on the posterior region of the anal fin	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	0.115	0.152	0.106	0.306	0.113	0.147	0.068	0.109	0	100
Solvent control	0.115	0.151	0.065	0.270	0.112	0.153	0.068	0.112	0	100
0.84	0.109	0.146	0.069	0.259	0.109	0.151	0.068	0.106	0	90
2.7	0.115	0.144	0.063	0.251	0.111	0.142	0.069	0.112	0	100
8.6	0.109	0.153	0.067	0.258	0.107	0.151	0.069	0.107	0	89

Table B.9.2.2.4-14 Mean liver weights, hepatosomatic indices and hepatic vitellogenin concentrations of F1-generation determined at 61-114 days post-hatching

Mean measured concentration (µg/L)	Liver weight (mg)		HSI (%)		Hepatic VTG (ng/mg)	
	♀	♂	♀	♂	♀	♂
Control	18.5	7.2	5.8	2.8	482	0.57
Solvent control	12.3	7.6	3.8	2.7	596	0.26
0.84	17.4	8.3	5.6**	2.8	765	0.44
2.7	15.2	7.8	4.7	2.9	520	0.88
8.6	14.3	8.2	4.7	2.9	603	0.65

Table B.9.2.2.4-15 Histopathological evaluation of F1-generation Medaka determined at 60 days post-hatching

Mean measured concentration (µg/L)	Number of fish		Ovotestis		Other male deformities		Non-functional gonad	
	♀	♂	♀	♂	♀	♂	♀	♂
Control	10	10	N	N	N	N	N	N
Solvent control	11	9	N	N	N	N	N	Immature testis: 1
0.84	10	10	N	N	N	N	Immature ovary: 1	N
2.7	10	10	N	N	N	N	N	N
8.6	11	9	N	N	N	N	N	N

[a] One male had ovotestis

N: none

Y: yes

Hepatic vitellogenin concentration increased slightly in P-generation males at 60 days post-hatching with a dose-response trend, with values at 0.84 and 8.6 µg test item/L being statistically significantly different from the pooled controls. However, this trend was not observed at 61-114 days post-hatching in P-generation and in F1 generation at 60 days post-hatch.

A slight reduction of gonad weights following a dose-response trend (without achieving statistical significance) was observed in P-generation at 60 days post-hatch; however, this trend was not obvious at 114 days post-hatch and in F1-generation at 60 days post-hatch. Histopathological evaluation of gonads of P-generation performed on day 60 and day 114 post-hatch and of F1 generation on day 60 post-hatch gave no indication of test item-related sex-reversal.

Hatchability success of the F1 generation was reported to be statistically significantly reduced for eggs collected on day 99 post-hatch of the P-generation. A reduction was also observed for eggs collected on days 100 and 101 post-hatch, but these were not reported to be statistically significant. A statistical analysis for the pooled batches was conducted by RMS. The data for the different batches were pooled per treatment group and analyzed using Shapiro-Wilk's test for normal distribution, Levene's test for homogeneity of variances, trend analysis and Williams Multiple sequential t-test (α 0.05). A statistically significant difference was found between the control and the solvent-control, therefore the analysis for the pyriproxyfen treatments was based on comparison with both controls separately. The overall hatchability at the highest tested concentration was statistically significantly reduced in both cases (by 8.6% from the untreated control and by 15.9% from the solvent-control). As hatchability is a population relevant endpoint, the NOEC from this study is concluded to be 2.7 µg a.s./L. As hatchability at the highest tested concentration was reduced by more than 10%, attempts were made to calculate an EC10. None of the models returned statistically significant fits, and a reliable EC10 could thus not be derived.

The current study does not allow for the calculation of ECx values. According to Regulation (EU) No 283/2013, the statistical power of the NOEC shall be assessed in case no ECx values can be derived. However, this is not possible as long as default type I and II errors have not been fixed, default variance for any response variable have not been established and typical detectable effect sizes of each parameter have not been calculated (EFSA Supporting publication 2015:EN-924). Considering the level of effect on which the NOEC was based (i.e. an 8.6%-15.9% reduction in hatchability at the highest tested concentration, compared to the untreated or solvent control) and the complete absence of effects at the next lower level (i.e. the NOEC), the NOEC is considered sufficiently protective. The NOEC of pyriproxyfen in a fish full life cycle test in medaka was 2.7 µg a.s./L and may be used for risk assessment.

Additional note RMS: EFSA proposed an expert meeting to discuss whether the effect on hatchability percentage is treatment related.

When the clean water and solvent controls were statistically different, the test results should be compared with the solvent control (unless there are strong reasons to consider otherwise). In the solvent treatment, the overall hatchability (results for overall 3 days) was 15.9% higher than in the 8.6 µg a.s./L treatment and the difference was statistically significant.

The biological relevance of the results and the endpoint can be discussed at the expert meeting. The RMS provides below raw data to supplement the discussion.

Table 13. Hatchability and Time of Hatching of F1-Generation Medaka Exposed to Pyriproxyfen Started with Eggs from Day 118 after Start of the Exposure (Day 99 Post Hatch).

Mean Measured Concentration [µg test item/L]		Hatchability [%]	Time of Hatching[a] [Day]
Control	1	80	10
	2	80	10
	3	65	9
	4	80	9
	Mean	**76**	**9.5**
Solvent Control	1	100	10
	2	90	10
	3	95	9
	4	90	10
	Mean	**94**	**9.8**
0.84	1	95	10
	2	90	10
	3	85	9
	4	90	10
	Mean	**90**	**9.8**
2.7	1	90	10
	2	95	10
	3	95	9
	4	95	10
	Mean	**94**	**9.8**
8.6	1	65	10
	2	60	9
	3	85	9
	4	75	10
	Mean	**71****	**9.5**

[a] Time of hatching defines the day when hatchability of living embryos was above 70%
** Statistically significant difference when compared with the solvent control ($p < 0.05$)

Table 14. Hatchability and Time of Hatching of F1-Generation Medaka Exposed to Pyriproxyfen Started with Eggs from Day 119 after Start of the Exposure (Day 100 Post Hatch).

Mean Measured Concentration [µg test item/L]		Hatchability [%]	Time of Hatching[a] [Day]
Control	1	95	10
	2	80	9
	3	90	9
	4	85	10
	Mean	**88**	**9.5**
Solvent Control	1	70	10
	2	100	9
	3	90	9
	4	85	9
	Mean	**86**	**9.3**
0.84	1	95	9
	2	95	10
	3	75	10
	4	100	10
	Mean	**91**	**9.8**
2.7	1	95	10
	2	95	9
	3	95	9
	4	90	10
	Mean	**94**	**9.5**
8.6	1	75	12
	2	80	9
	3	90	9
	4	65	10
	Mean	**78**	**10**

[a] Time of hatching defines the day when hatchability of living embryos was above 70%

Table 15. Hatchability and Time of Hatching of F1-Generation Medaka Exposed to Pyriproxyfen Started with Eggs from Day 120 after Start of the Exposure (Day 101 Post Hatch).

Mean Measured Concentration [µg test item/L]		Hatchability [%]	Time of Hatching[a] [Day]
Control	1	80	11
	2	80	9
	3	80	10
	4	90	10
	Mean	**83**	**10**
Solvent Control	1	90	10
	2	90	10
	3	85	10
	4	85	9
	Mean	**88**	**9.8**
0.84	1	90	9
	2	90	10
	3	85	12
	4	85	9
	Mean	**88**	**10**
2.7	1	100	10
	2	95	9
	3	85	10
	4	90	10
	Mean	**93**	**9.8**
8.6	1	85	11
	2	85	10
	3	70	9
	4	65	10
	Mean	**76**	**10**

[a] Time of hatching defines the day when hatchability of living embryos was above 70%

4.4.3 Fish short-term toxicity test on embryo and sac-fry stages

No data are available

4.4.4 Aquatic Toxicity-Fish, juvenile growth test

No data are available

4.4.5 Chronic toxicity to aquatic invertebrates

4.4.5.1 CA 8.2.5.1/01a (1992) Chronic toxicity of 14C-Sumilarv to Daphnia magna under flow-through test conditions

Report: CA 8.2.5.1/01a, (1992) Chronic toxicity of ^{14}C-Sumilarv to *Daphnia magna* under flow-through test conditions. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-21-0075 CA 8.2.5.1/01b, (2016) Derivation of endpoints (EC_{10} and EC_{20} values) for *Daphnia magna* chronic toxicity study with pyriproxyfen. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-0247	
Previous evaluation	Submitted in the DAR (November 2005). A new robust summary is provided below for the acute toxicity of technical pyriproxyfen with *Daphnia magna* because this study is used to derive endpoints for the aquatic invertebrate risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion. Derivation of endpoints (EC_{10} and EC_{20} values) was done for RAR and results are shown in the summary, but ECx values were not checked by the RMS.
Conclusion	NOEC = 0.000015 mg a.s./L EC10 = 0.0000088 mg a.s./L

Guidelines

U.S. EPA Pesticide Assessment Guidelines, 72-4(b)

Deviations: None reported.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	[Pyridyl-2,6-^{14}C]-pyriproxyfen
Description:	Not stated
Lot/Batch #:	C-90-061
Radiochemical purity:	100% (working specific radioactivity: 817,000 dpm/µg)
Expiry date:	Not stated
2a. Test animals:	The *Daphnia magna* used in this study were obtained from an in-house daphnid culture maintained at the test facility.
2b. Maintenance:	All daphnids were cultured and tested in a temperature-controlled area at 20 (±2)°C. The lighting was provided by cool white fluorescent bulbs at an intensity of 40-80 footcandles on a 16-hour daylight and 8-hour darkness photoperiod, with 30-minute dawn and dusk transition periods. During the holding period, the daphnids were fed a suspension of algae *(Selenastrum capricornutum* and *Ankistrodesmus falcatus),* which was supplemented with a trout chow (Rangens@ Salmon Starter #2) and yeast

(Saccharomyces sp.) suspension. Only first-instar daphnids « 24 hours old) were selected for testing.

Study Design

Four replicates of ten first instar *Daphnia magna* (<24 hours old) per concentration were exposed to [pyridyl-2,6-^{14}C]-pyriproxyfen for 21 days at nominal concentrations of 0 (dilution water control), 0 (solvent control), 0.0000024, 0.0000048, 0.000010, 0.000020 and 0.000040 mg a.s./L in a flow-through system (study #1). These dose levels were based on a preliminary 17-day study. However, the results of the first definitive study showed that another study was needed to identify the wide toxicity slope for this compound with *D. magna* and so a second flow-through test was conducted with nominal concentrations of 0 (dilution water control), 0 (solvent control), 0.000018, 0.000036. 0.000075, 0.000150 and 0.000300 mg a.s./L (study #2).

The system contained seven sets of four replicate 1-litre test chambers, designated as control, vehicle blank, and level #1 through level #5. The ten first-instar daphnids were randomly placed in each test chamber at the test start. The test material was dissolved in dimethyl formamide (DMF) to prepare a stock solution which was dispensed via a proportional diluter system, together with the test medium (blended well water and reverse osmosis water with a total hardness of between 160 to 180 mg/L (as $CaCO_3$)) to prepare the nominal test concentrations. The diluter delivered 3.5 (study #1) or 3.4 (study #2) mL/chamber/minute, which was sufficient to replace the 1 L test volume about 5.0x per day. The test daphnids were uniformly fed twice daily (an algal suspension of, *Ankistrodesmus falcatus/Selenastrum capricornutum* supplemented by a suspension consisting of yeast and fish food).

Survival, abnormal effects and observance of first brood were recorded every day throughout the study. Reproductive success was measured by counting and discarding the offspring produced in each test vessel three times a week. At the end of the tests on day 21, the surviving adults were measured from the apex of the helmet to the base of the posterior spine. Test concentrations were analysed on study days -N (pre-treatment samples to check that the system had equilibrated) 0, 4, 7, 14 and 21 (studies #1 and #2): composite samples were taken from the 4 replicate beakers of each treatment level and analysed by LSC. Temperature, dissolved oxygen and pH were measured in the control, low, middle and high test concentrations in alternating duplicate chambers on days 0, 4, 7, 14 and 21. Water quality parameters of temperature, hardness, dissolved oxygen, pH, alkalinity and conductivity were measured on the dilution water daily. During definitive study #1, concentrations of ^{14}C-Sumilarv in test system water were determined on study days -N, 0, 4, 7, 14, and 21. 400 mL water samples were collected by compositing 100 mL from each of the four replicate beakers for the control, the vehicle blank, and the five test levels. The concentrations of ^{14}C-activity calculated as ^{14}C-Sumilarv in water during definitive study #2 were calculated by LSC analysis of samples pipetted directly from the control and treated tanks.

Results and Discussion

Physical and Chemical Measurements of Water

Study #1: Water chemistry parameters were within the specified limits: temperature ranged from 19 to 20°C, pH ranged from 8.3 to 8.4 and dissolved oxygen from 7.8 to 8.4 mg/L (90 and 97% saturation, respectively). Two minor deviations occurred: on day 11 the water hardness rose to 188 mg/L and on day 13 the turnover rate fell to 3.7x. These deviations were not considered to have adversely affected the study.

Study #2: Water chemistry parameters were within the specified limits: temperature ranged from 19 to 20°C, pH ranged from 8.2 to 8.4 and dissolved oxygen from 7.9 to 8.5 mg/L (91 and 98% saturation, respectively). A minor deviation occurred: on day 20 the turnover rate fell to 1.0x. This deviation was not considered to have adversely affected the study.

Measurement of Test Concentrations

Study #1: Mean measured concentrations (days 0-21) of ^{14}C-pyriproxyfen were 0.0000018, 0.0000044, 0.0000071, 0.000015 and 0.000031 mg a.s./L. These values range from 71 - 92% of the nominal test concentrations.

Study #2: Mean measured concentrations of ^{14}C-pyriproxyfen were 0.000020, 0.000027, 0.000056, 0.000120 and 0.000240 mg a.s./L. These values range from 75-111% of the nominal test concentrations.

Biological Findings

Adult mortality:

Studies #1 and #2: The control and solvent control survival rates were not significantly different and were therefore pooled. Survival of *Daphnia magna* after the 21-day exposure to ^{14}C-pyriproxyfen in all test concentrations was not significantly affected as compared to the pooled controls (see Table B.9.2.5.1-01). Day 21 EC_{50} values were therefore >0.000031 and >0.000240 mg a.s./L in the two tests, respectively.

Adult behavioural/sublethal effects:

Study #1: Most daphnids were normal in appearance and behaviour throughout the study and any observations noted were incidental to the treatments (see Table B.9.2.5.1-01).

Study #2: Most daphnids were normal in appearance and behaviour throughout the study and any observations noted were incidental to the treatments, with the exception of the treatment effect on size (see Table B.9.2.5.1-01).

Adult Daphnid length:

The control and solvent control groups were not significantly different, so they were pooled.

Study #1: When compared to the pooled controls, there was no significant difference in adult lengths for any of the concentrations tested. However, while not significant, daphnid length was slightly reduced at the mean measured concentration of 0.000031 mg a.s./L (see Table B.9.2.5.1-01). This is in agreement with the results for Study #2, where significant effects on length of adult daphnids were seen at concentrations of 0.000027 mg a.s./L and above.

Study #2: When compared to the pooled controls, there was a significant difference in adult lengths for all of the concentrations tested. The statistical analysis of adult daphnid length was repeated, using the solvent control for comparison, in order to see if the value for 0.000020 mg a.s./L was still significantly different (on the basis that this comparison was more relevant). In this case, there was no significant difference ($p > 0.05$) and so 0.000020 mg a.s./L was considered to be the NOEC for adult daphnid length (see Table B.9.2.5.1-01).

Young/Adult reproduction days (Total number of young produced/Total number of adult reproduction days):

The control and solvent control were not significantly different, so they were pooled.

Study #1: Young/Adult reproduction days was shown not to be affected when compared to the pooled controls. However, while not significant, young / adult reproduction days was slightly reduced at the mean measured concentration of 0.000031 mg a.s./L (see Table B.9.2.5.1-01). This is in agreement with the results for Study #2, where significant effects on young/adult reproduction days were seen at concentrations of 0.000027 mg a.s./L and above.

Study #2: When compared to the pooled controls, there was a significant difference in young/adult reproduction days for all concentrations tested. The statistical analysis of young/adult reproduction days was repeated, using the solvent control as the level for comparison, in order to see if the value for 0.000020 mg a.s./L was still significantly different (on the basis that this comparison was more relevant). In this case, there was no significant difference ($p > 0.05$) and so 0.000020 mg a.s./L was considered to be the NOEC for young/adult reproduction days (see Table B.9.2.5.1-01).

Time to first brood:

Control and solvent control were again pooled since there was no significant difference between them.

Study #1: Exposure to ^{14}C-pyriproxyfen for 21 days had no significant effect on time to first brood at any of the test concentrations, when compared to the pooled controls (see Table).

Study #2: Exposure to ^{14}C-pyriproxyfen for 21 days had a significant effect on time to first brood at 0.000056, 0.000120 and 0.000240 mg a.s./L, when compared to the pooled controls.

Table B.9.2.5.1-01 Percent survival, behavioural observations, adult Daphnid length, young/adult reproduction days and time to first brood of *Daphnia magna* continuously exposure to ^{14}C-pyriproxyfen for 21 Days

Mean measured test concentration (mg a.s./L)	Day-21 Adult Survival[a] (%)	Day-21 Observation [d]	Day-21 Adult Daphnid Length[b] (mm)	Young/Adult Reprod. Days[b]	Time to First Brood[b]
Study #1					
Control	38 (97.5 ± 5.00)	---	3.91 ± 0.19	8.35 ± 0.35	8.00 ± 0.0
Solvent control	38 (95.0 ± 10.0)	2LD	3.91 ± 0.14	7.87 ± 0.74	7.00 ± 0.0
Pooled controls [c]	(96.3 ± 7.44)	---	3.91 ± 0.16	8.11 ± 0.60	7.50 ± 0.53
0.0000018	37 (92.5 ± 5.00)	---	3.99 ± 0.09	8.68 ± 0.30	7.00 ± 0.0
0.0000044	37 (92.5 ± 9.57)	---	3.89 ± 0.13	8.27 ± 0.34	7.50 ± 0.58
0.0000071	38 (95.0 ± 5.77)	1LD	3.96 ± 0.13	9.07 ± 0.68	8.00 ± 0.0
0.000015	38 (95.0 ± 5.77)	---	3.93 ± 0.10	9.12 ± 0.70	8.00 ± 0.0
0.000031	38 (95.0 ± 5.77)	1LD	3.81 ± 0.14	7.84 ± 0.74	8.00 ± 0.0
Study #2					
Control	40 (100.0 ± 0.00)	---	4.10 ± 0.13	9.02 ± 0.99	8.00 ± 0.0
Solvent control	40 (100.0 ± 0.00)	---	4.03 ± 0.16	8.09 ± 0.87	8.00 ± 0.0
Pooled controls [c]	(100.0 ± 0.00)	---	4.06 ± 0.15	8.55 ± 0.99	8.00 ± 0.0
0.000020	40 (100.0 ± 0.00)	---	3.93 ± 0.14**	7.43 ± 0.61**	8.00 ± 0.0
0.000027	40 (100.0 ± 0.00)	---	3.88 ± 0.12**/*	5.99 ± 0.32**/*	8.00 ± 0.0
0.000056	40 (100.0 ± 0.00)	---	3.67 ± 0.19**/*	3.72 ± 0.58**/*	8.8 ± 0.5**
0.000120	40 (100.0 ± 0.00)	1SM	3.54 ± 0.15**/*	2.93 ± 0.51**/*	10.8 ± 0.5**
0.000240	40 (100.0 ± 0.00)	40SM	3.33 ± 0.18**/*	1.58 ± 0.38**/*	11.0 ± 0.0**

a: Data were subjected to frequency analysis coupled with a one-tailed Fisher's exact test.

b: Data were subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple means comparison test.

c: Control and solvent control were compared by one-tailed Fisher's exact test or t-test. If significantly different, comparison was made with solvent control; otherwise, controls were pooled.

d: Unless otherwise indicated, the test water was clear and free of precipitate and all daphnids were normal in appearance and behaviour. The following abbreviations were used for observations: LD = Light Discoloration, SM = Smaller than Control

*: Denotes values significant different (P≤0.05) from the solvent control.

**: Denotes values significant different (P≤0.05) from the pooled controls.

Conclusions

Pyriproxyfen 21-day EC_{50} to *Daphnia magna* - >0.000240 mg a.s./L (based on immobilization); 21-day NOEC - 0.000015 mg a.s./L (based on mortality, sublethal effects reduced reproductive success seen at test concentrations of 0.000027 mg a.s./L and above) and 0.000020 mg a.s./L (based on no significant compound-related effects); 21-day LOEC - 0.000027 mg a.s./L (based on significant effects on growth and reproduction seen at this concentration).

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. This was however not addressed in the study report. Therefore, the RMS requested the applicant to perform the necessary calculations to obtain these values. The results of these calculations, which were performed with ToxRatPro Version 3.2.1@, are shown below. Reproduction and growth (as measured by length) were the most sensitive biological parameters.

Table B.9.2.5.1-02 Summary of results for reproduction and growth endpoints at the end of exposure period (ng/L)

Endpoint	Mean	95% confidence limit
Adult length		
EC_{10}	76	10-180
EC_{20}	273	56-520
Reproduction (number of young)		
EC_{10}	8.8	2.6-16
EC_{20}	18	7.2-28

Comment by RMS (DAR 2005)

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.

Comment by RMS (Renewal)

EC10 of 8.8 ng/L was concluded based upon effects on reproduction, but the ECx derivation was not repeated by RMS.

4.4.5.2 CA 8.2.5.2/06 (1995) Sumilarv (pyriproxyfen) - Chronic toxicity to mysids (Mysidopsis bahia) under flow-through test conditions

Report: CA 8.2.5.2/06, (1995) Sumilarv (pyriproxyfen) - Chronic toxicity to mysids (*Mysidopsis bahia*) under flow-through test conditions. Sumitomo Chemical Co., Ltd. Unpublished report No.: 93-5-5262 (Company code NNW-51-0119) CA 8.2.5.2/06b, (2016) Derivation of endpoints (EC_{10} and EC_{20} values) for *Mysidopsis bahia* chronic toxicity study with pyriproxyfen. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-0249	
Previous evaluation	In DAR (November 2005) for original approval. A new robust summary is provided below for the chronic toxicity of pyriproxyfen with *Mysidopsis bahia* because this study is used to derive endpoints for the fish risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion.
Conclusion	NOEC = 0.00081 mg a.s./L

Guidelines

FIFRA guideline 72-4: ASTM (1987) Standard Practice for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids. ASTM Committee E-47 on Biological Effects and Environmental Fate. Designation: E 1191-87.

Deviations: None reported.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material: Sumilarv (pyriproxyfen, S-31183)

Description: White solid crystal

Lot/Batch #: PYG-87074

Purity: 95.3%

Expiry date: Not stated

2a. Test animals: The mysids (*Mysidopsis bahia*) used in this study (≤24 hours old at test initiation) were obtained from laboratory cultures maintained at the test facility.

2b. Maintenance: The culture water was laboratory well water and was characterized as soft water with general ranges as total hardness of 30 to 60 mg/L as CaCO3, total alkalinity of 25 to 45 mg/L as CaCO3, a pH of 6.9 to 7.7 and specific conductance of 110 to 160 micromhos/cm.

Mysids were cultured in one of several 76-L glass aquaria with a closed-loop recirculating filtration system providing artificial seawater to the aquaria. The artificial seawater in the aquaria was characterized as having a salinity of 26 to 28 ‰ and a pH of 8.1 to 8.2 during the 14-day period prior to test initiation. The area in which the mysids were cultured received a regulated photoperiod of 16 hours of light and 8 hours of darkness. Commercial aquarium heaters were used to maintain the culture solutions at 24 to 25°C. The artificial seawater was formulated in the same manner as the water used in the preliminary and definitive tests. Juvenile

mysids (≤24 hours old) were collected. Mysids were fed live brine shrimp *(Artemia salina)* nauplii, ≤ 48 hours old (post-hydration), twice daily.

Study Design

Artificial seawater was used as dilution water during the study (commercially prepared salt formula mixed with soft freshwater, hardness 20-40 mg/L as $CaCO_3$). Thirty mysids were added to two replicate vessels for each treatment: a solvent control (acetone at the same concentration as in each test solution, 100 µL/L), a dilution water control and nominal exposure concentrations of 0.63, 1.3, 2.5, 5.0 and 10 µg a.s./L (based on the results of a range-finding test). The exposure system consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 14 exposure aquaria (39 x 20 x 25 cm). The diluter provided the test solutions to each vessel at a rate of approximately 11 aquarium volume additions per day (90% test solution replacement rate of approximately 5 hours).

For test initiation, 15 mysids (≤24 hours) were each transferred to one of 28 retention chambers. The test was initiated when the retention chambers were placed in their respective test aquaria. Each test aquarium (two per treatment level) contained two retention chambers, resulting in 30 mysids per replicate vessel and 60 mysids for each treatment level. Throughout the test, the mysids were fed live brine shrimp (*Artemia salina*). A photoperiod of 16 hours light and 8 hours darkness was applied throughout the test. In order to count the mysids during the exposure period, each retention chamber was gently lifted from the aquaria and placed in a dish containing water. Counts were made of the number of dead and living organisms together with observations of any abnormal appearance or behaviour, although during the first 16 days of the test, exact numbers of living organisms could not be ascertained.

When the mysids reached sexual maturity and had paired on day 17, ten pairs (one male and one female) were each placed in a glass jar with the remaining mysids in each aquarium being placed in one of the initial retention chambers. Males from this pool were used to replace any males that died in the jars but any females dying were not replaced. After pairing, the number of offspring produced by each female, in addition to the other observations, was recorded. Observations were made daily throughout the study and dead parental mysids and offspring were removed and discarded. At test termination, all mysids were sacrificed by immersion in cold, deionized water and then separated into male and female groups before body lengths and dry weights (60°C for approximately 72 hours) were taken. Individual body length to the nearest 0.1 mm was determined using a dissecting microscope calibrated with a stage micrometer. Individual total dry body weight to the nearest 0.01 mg was determined using an analytical balance. Reproductive success was calculated for each replicate aquarium as the ratio of the total number of offspring produced to the total number of females contained within each chamber per reproductive day.

Temperature, pH, salinity and the dissolved oxygen concentration were measured daily in each replicate of each treatment level throughout the exposure period. In addition, exposure solution temperature was continuously monitored in one replicate of the dilution water control. The dilution water control and the high, middle and low test concentrations were sampled and analysed for pyriproxyfen concentration twice prior to the initiation of the definitive test. During the in-life phase of the test, water samples were removed and analysed on test days 0, 7, 14, 15, 21 and 28.

Results and Discussion

Physical and Chemical Measurements of Water

Water quality parameters measured during the 28-day exposure period remained within acceptable limits for the survival, reproduction and growth (total length and dry weight) of mysids. Environmental conditions during the test were as follows: salinity 24 - 26%; temperature 26 - 27°C, dissolved oxygen 77 - 114% of saturation and pH 8.0 - 8.5.

Measurement of Test Concentrations

Results of the analyses of the pyriproxyfen exposure solutions during the in-life phase of the definitive test established that the measured concentrations were generally consistent between replicate vessels and that the expected concentration gradient (50% dilutions) was maintained. Day 0 results averaged 100% of the nominal

concentrations (ranging between 80 and 131%, on average) but are not considered representative of exposure conditions maintained over the 28-day exposure period. At the day 0 sampling, the exposure vessels did not contain the retention chambers and the test organisms and food were not present. With the introduction of these items, the test conditions changed rapidly (within less than 24 hours), so altering the equilibrium present on day 0.

Measured concentrations on days 7, 15, 21 and 28 were generally consistent between intervals, averaging approximately 65% of the nominal concentrations (ranging between 49 and 87%). Mean measured concentrations were calculated both with and without the day 0 measurements (excluding them lowered the mean measured concentrations by about 11%). The reported mean measured concentrations were based on the analyses for days 7, 15, 21 and 28 as this is considered to be more conservative and more representative of the mysid exposure period. Based on thisthe mean measured concentrations were 0.48, 0.81, 1.6, 3.1 and 6.4 µg a.s./L, ranging from 62 to 77% of the nominal values. Coefficients of variation averaged 12% for all mean measured concentrations.

Biological Findings

A summary of the survival and reproductive success data, together with the measurements for growth (body length and dry weight) at test termination is presented in Table B.9.2.5.11-01. After 28 days of exposure, survival of 77 and 90% was observed among mysid shrimps exposed to the control and solvent control solutions, respectively (pooled control data = 83%). During the same period, survival of 75 to 92% was observed among mysids exposed to mean measured concentrations of pyriproxyfen ranging from 0.48 to 6.4 µg a.s./L. No statistically significant differences in survival were found comparison to the pooled control.

Reproductive success at the end of the test in the control and solvent control averaged 0.31 and 0.32 offspring/female/reproductive day, respectively (pooled control data = 0.32 offspring/female/reproductive day). Reproductive success among mysids exposed to the 0.48 and 0.81 µg a.s./L treatment levels averaged 0.30 and 0.33 offspring/female/reproductive day, respectively, and was not significantly different from the pooled control data. Reproductive success in the 1.6, 3.1 and 6.4 µg a.s./L treatment levels averaged 0.19, 0.16 and 0.08 offspring/female/reproductive day, respectively, and was significantly different from the reproductive success in the pooled controls ($p \leq 0.05$, Williams' Test).

The average body lengths of mysid shrimps exposed to the control and solvent control were 7.1 and 7.0 mm (males) and 7.1 and 6.9 mm (females), respectively (pooled control: 7.1 mm males and 6.9 mm females). The average body length of male and female mysids exposed to pyriproxyfen concentrations of 0.48 to 6.4 µg a.s./L ranged from 6.8 to 7.4 mm. No statistically significant differences in survival were found in comparison to the pooled control. The average dry body weights of mysid shrimps exposed to the control and solvent control were 0.79 and 0.87 mg (males) and 0.84 and 0.85 mg (females), respectively (pooled control: 0.81 mg for males and 0.86 mg for females). The average dry body weight of male and female mysids exposed to pyriproxyfen concentrations of 0.48 to 6.4 µg a.s./L ranged from 0.76 to 0.94 mg. No statistically significant differences in survival were found in comparison to the pooled control.

Table B.9.2.5.11-01 Summary of the survival, reproductive success and growth during the 28-day life-cycle exposure of mysids (*Mysidopsis bahia*) to pyriproxyfen

Mean measured concentration (µg a.s./L)	% survival[1]	Reproductive success[1,2]	Total body length (mm)[1,3]		Dry body weight (mg)[1,3]	
			Males	Females	Males	Females
Control	77	0.31	7.1±0.29	7.0±0.39	0.79±0.12	0.87±0.14
Solvent control	90	0.32	7.1±0.58	6.9±0.40	0.84±0.15	0.85±0.20
Pooled control	83	0.32	7.1±0.49	6.9±0.39	0.81±0.14	0.86±0.17
0.48	83	0.30	7.4±0.43	7.3±0.45	0.80±0.14	0.94±0.18
0.81	92	0.33	7.2±0.40	7.1±0.72	0.76±0.10	0.87±0.23
1.6	75	0.19*	7.2±0.40	6.9±0.33	0.83±0.09	0.88±0.20
3.1	82	0.16*	7.2±0.34	7.0±0.34	0.83±0.13	0.92±0.17
6.4	82	0.08*	7.1±0.42	6.8±0.41	0.78±0.13	0.79±0.15

[1] Mean of 2 replicates

[2] Total number of offspring produced by total number of females per reproductive day

[3] Replicate values = mean ± S.D. (n=15 pairs)

* Significantly different from the pooled control ($p \leq 0.05$)

Conclusions

Based on the results of this study (using mean measured concentrations): The 28 days no-observed effect concentration (NOEC) was determined to be 0.81 µg a.s./L. (based on reproductive success).

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. This was however not addressed in the study report. Therefore, the RMS requested the applicant to perform the necessary calculations to obtain these values. The results of these calculations, which were performed with ToxRatPro® (Version 3.2.1), are shown in Table B.9.2.5.11-02. Results were based on mean measured concentrations of pyriproxyfen, provided in the earlier conducted study. Reproduction was the most sensitive biological parameter.

Table B.9.2.5.11-02 Summary of results for reproduction endpoints at the end of exposure period (µg/L)

Endpoint	Mean	95% confidence limit
Reproduction (number of offspring)		
EC_{10}	0.87	0.003 - n.d.
EC_{20}	1.2	0.002 - n.d.

n.d.: not determined due to mathematical reasons or inappropriate data

Comments by RMS (renewal)

The study was conducted in general agreement with the FIFRA Guideline 72-4 (1987). The reproductive success was reported as the number of offspring/female/reproductive day, whereas the guideline indicates that reproduction should be expressed as total number of offspring/female. This is acceptable as the number of reproductive days was comparable for all treatments (i.e. 100-110, see Table CA 9.2.5 /11-3 below). The

validity criteria were met (i.e. less than 25% of the females in the control group failed to produce 3 or more young). Results were based on the mean measured concentrations, which is acceptable. The analytical method used was sufficiently validated (recoveries at fortification levels 0.750, 2.00 and 10 µg a.s./L were 92-121%, 71-123% and 85-120%, respectively, all n=5).Therefore, this study is considered valid.

Table B.9.2.5.11-03 Reproductive success and number of reproductive days during the 28-day life-cycle exposure of mysids (*Mysidopsis bahia*) to pyriproxyfen

Mean measured concentration (µg a.s./L)	Reproductive success[1,2]	Reproductive days[1]
Control	0.31	109
Solvent control	0.32	107
Pooled control	0.32	108
0.48	0.30	106
0.81	0.33	105
1.6	0.19*	106
3.1	0.16*	110
6.4	0.08*	107

[1] Mean of 2 replicates

[2] Total number of offspring produced by total number of females per reproductive day

Based on the results for reproductive success presented in Table 9.2.5/11-1, the NOEC for pyriproxyfen towards mysids (*Mysidopsis bahia*) was determined to be 0.81 µg a.s./L. EC_{10} and EC_{20} values were calculated by the applicant and were 0.87 and 1.2 µg a.s./L respectively.

Reliability of endpoints

To assess the reliability of the estimated ECx values, two approaches are described in EFSA Supporting publication 2015:EN-924:

- Normalised width of the confidence interval (NW = (upper limit – lower limit) / median estimate); rating of the NW ranges from excellent (<0.2) to bad (>2)
- Relationship between EC10 and EC20/EC50 confidence intervals: the best case (high certainty of protection level) is achieved when EC10 is lower than the lower limit of the EC20; the worst case (low certainty of protection level) occurs when the median EC10 is greater than the lower confidence limit for the EC50.

For this study, the upper limits of the confidence intervals for all EC_x values could not be determined. Therefore, above mentioned rules cannot be applied. The EC_{10} and EC_{20} are considered unreliable due to very large confidence intervals.

According to Regulation (EU) No 283/2013, the statistical power of the NOEC shall be assessed in case no

reliable ECx values can be derived. However, this is not possible as long as default type I and II errors have not been fixed, default variance for any response variable have not been established and typical detectable effect sizes of each parameter have not been calculated (EFSA Supporting publication 2015:EN-924). Considering the level of effect on which the NOEC was based (i.e. an increase of the mean number of offspring/surviving female rather than a decrease), the NOEC is considered sufficiently protective.

The NOEC for Pyriproxyfen was 0.81 µg /L and may be used for risk assessment.

4.4.5.3 CA 8.2.5.3/01 (2003) Pyriproxyfen - The full life-cycle toxicity to midge (Chironomus riparius) under static conditions

Report: CA 8.2.5.3/01, (2003) Pyriproxyfen - The full life-cycle toxicity to midge (*Chironomus riparius*) under static conditions. Sumitomo Chemical Co., Ltd. Unpublished report No.: 13048.6330 (Company code NNW-0157)	
Previous evaluation	In DAR (November 2005) for original approval. A new robust summary is provided below for the chronic toxicity of technical pyriproxyfen with *Chironomusa riparius* because this study is used to derive endpoints for the aquatic invertebrate risk assessment.
Remark by RMS	Considered acceptable at the time of original inclusion. No confidence intervals for midge emergence or development rate could be calculated by RMS with ToxRAT using either Probit or Weibull analysis. Therefore, the EC10 values are not considered reliable.
Conclusion	NOEC = 0.010 mg a.s./L (nominal), NOEC = 0.031 mg a.s./L (mean measured)

Guidelines

OECD (2001) Proposal for a New Guideline No. 219. Sediment Water Chironomid Toxicity Test Using Spiked Water. February 2001. Paris, France.

Deviations: A number of minor deviations occurred: (1) Midge larvae reared at 19-23°C (instead of 18-22°C), (2) Light intensity ranged from 970-1100 lux (instead of 500-1000 lux), (3) pH measured on days -1, 0, 1 and 28 (instead of -1, 0 and 28), (4) Water quality samples taken from solvent control and highest treatment level rather than control and highest treatment level, (5) Samples taken from replicates A-D taken on day 8 to check that samples taken at initiation had been spiked appropriately. It is not considered that these deviations had any impact on the results of the study.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. **Test Material (radiolabeled):** [pyridyl-2,6-^{14}C]pyriproxyfen

   Description: Not stated

Lot/Batch #:	RIS 99031
Radiochemical purity:	99.6% (13.0MBq/mg)
Expiry date:	Not stated
Test Material (non-radiolabeled):	Pyriproxyfen
Description:	Not stated
Lot/Batch #:	80301G
Purity:	97.9%
Expiry date:	Jan 18, 2004
2a. Test animals:	The midge (*Chironomus riparius*) used in this study (approximately 2 days old at test initiationwere obtained from laboratory cultures maintained at the test facility.
2b. Maintenance:	The culture water was laboratory well water and was characterized as soft water with general ranges as total hardness of 30 to 60 mg/L as CaCO3, total alkalinity of 25 to 45 mg/L as CaCO3, a pH of 6.9 to 7.7 and specific conductance of 110 to 160 micromhos/cm. Five days prior to test initiation, egg masses were removed from culture aquaria and each individual egg mass was placed in a 30-mL plastic cup with 25 mL of culture water. The egg masses were observed daily until hatching was complete (approximately 24 to 48 hours after release of egg masses by the female midge). Hatched midge larvae were transferred to a shallow glass bowl containing 1 L of culture water (laboratory well water) and 10 mL of *Ankistrodesmus falcatus* (4 x 10^7 cell/mL) to serve as a substrate. During the rearing of the midge larvae, the temperature was 19 to 23 ºC and the dissolved oxygen ranged from 9.8 to 12.8 mg/L. The culture area was illuminated at an intensity range of 60 to 80 footcandles (650 to 860 lux) with a photoperiod of 16 hours light, 8 hours dark. The larvae were reared in the culture bowls for 2 days after hatching to provide first-instar larvae for use during the exposure to pyriproxyfen. No mortality of midge larvae was observed 48 hours prior to test initiation. During rearing, the midge larvae were fed a finely-ground suspension of flaked fish food (i.e., 10 mg/mL). Midge larvae were fed daily during the culturing and rearing period.

Study Design

A water-sediment system was set up using natural sediment (consisting of 94% sand, 6% silt, 0% clay, 2.4% organic carbon content and a pH of 6.2) and laboratory well water (total hardness of 49 to 50 m/L as CaCO3, total alkalinity of 33 to 36 mg/L as CaCO3, pH of 7.4 to 7.7 and specific conductivity of 140 µmhos/cm). Sediment and overlaying water were added to 600 mL glass beakers (1.5 cm layer of sediment equivalent to approximately 149 g sediment and 300 mL of overlying water) 7 days prior to test initiation. Twenty midge larvae were added to eight replicate vessels for each treatment (four biological and four analytical replicates): a solvent control (acetone at the same concentration as in each test solution, 100 µL/L), a water-only control and nominal exposure concentrations of 2.5, 5.0, 10, 20 and 40 µg a.s./L (based on the results of a range-finding test). Individual dosing stocks for the three highest treatment levels were prepared by adding a volume of the non-radiolabeled stock solutions, and a volume of the 50.4 µg/mL primary radiolabelled stock solution together for a final volume of 1000 µL for each stock. The dosing stocks for the two lowest treatment levels were prepared by diluting a volume of the 50.4 µg/mL primary radiolabelled stock solutions with solvent for a final volume of 1000 µL.

The test vessels were gently aerated for the duration of the study (except for the 24 hours after organism addition). The test animals were fed daily with a suspension of ground flaked fish food. Test vessels were observed daily and emergent adults counted and removed until test termination (day 28). In addition, daily monitoring was continued past day 28 for each treatment level (to distinguish between mortality and delayed emergence), with individual levels being terminated once there had been 7 consecutive days with no emergence. The sex of the emerging adults was recorded.

Water quality measurements made during the study were performed in the four replicate exposure vessels established for monitoring the biological performance of the exposed midge. Measurements of dissolved oxygen concentration, temperature and pH were made on the day the test organisms were added (day -1), the day of test substance application (day 0), test day 1, test day 28, and termination of each treatment level during the extension period (up to 37 days). In addition, dissolved oxygen concentration and temperature were measured daily in each replicate vessel of each treatment level and the controls during the 37-day exposure. The temperature was continuously monitored in one replicate at10 µg a.s./L. treatment vessel throughout the study. Total hardness, alkalinity, specific conductivity, and total ammonia of the test solutions were determined at test initiation and at test termination in a composite sample from the highest treatment level and solvent control solution. Samples of overlying water and sediment were taken for liquid scintillation counting (LSC) on days 0 (approximately 1 hour after application), 7, 14 and 28 (one analytical replicate for all treatments sampled on each occasion). Samples of pore water were taken from the 2.5, 10 and 40 µg a.s./L treatments on days 0 (approximately 1 hour after application), 7, 14 and 28 for LSC counting. One replicate of the 2.5, 10 and 40 µg a.s./L treatment levels was analysed at each interval for the concentration of pyriproxyfen in water and sediment by high performance liquid chromatography using radiochemical detection (HPLC/RAM).

Results and Discussion

Physical and Chemical Measurements of Water

Environmental conditions during the test were as follows: photoperiod - 16 hours light: 8 hours dark; light intensity 970 - 1100 lux; temperature 19 - 22°C; dissolved oxygen 7.2 - 9.3 mg/L and pH 7.6 - 8.2.

Measurement of Test Concentrations

Analysis of the stock solutions used to apply the test substance to the overlying water resulted in measured concentrations ranging from 107 to 113% of the nominal concentrations. The concentrations of pyriproxyfen in overlying water approximately 1 hour after application ranged between 88 and 96% of the nominal values (2.2, 4.5, 9.6, 19 and 35 µg a.s./L). Total residue concentrations decreased by day 7 and ranged from 17 to 27% of the nominal concentrations throughout the remainder of the study. The concentrations of pyriproxyfen showed corresponding increases in the pore water and in the sediment. Measured concentrations in the pore water at 1-hour were 0.057, 0.38 and 0.77 µg a.s./L at the 2.5, 10 and 40 µg a.s./L treatment levels, respectively, and by day 28 these had increased to 0.66, 2.7 and 9.4 µg a.s./L, respectively. Similarly, measured concentrations in the sediment at 1-hour were <LOQ, 1.6, 2.7, 5.0 and 10 µg a.s./kg at the 2.5, 5.0, 10, 20 and 40 µg a.s./L treatment levels, respectively, and by day 7 these had increased to 1.2, 3.0, 9.0, 18 and 29 µg a.s./kg, respectively (19 to 36% of the total applied radioactivity). Following day 7, sediment concentrations decreased slightly throughout the remainder of the study and ranged from 9 to 13% of the total applied radioactivity by the end of the test (day 28).

HPLC/RAM analysis of all overlying water samples on test day 0 showed that ≥98% of the extracted radioactivity was associated with parent material (pyriproxyfen). Similarly, analysis of the sediment samples on day 0 showed that 83 to 100% of the extracted radioactivity was associated with parent material. Analysis of the overlying water samples on test days 7, 14 and 28 showed that most of the extracted radioactivity (68-100%) was associated with PYPAC. Analysis of the sediment samples on test days 7, 14 and 28 showed that ≤41% of the extractable radioactivity was associated with parent material, with the amount declining over time. The remaining extracted radioactivity was associated with PYPAC, 4'-OH-Pyr and other unidentified degradates.

Biological Findings

A summary of the mean percent emergence and mean development rate at test termination is presented in Table B.9.2.5.13-1. Following 28 days of exposure, midge percent emergence and mean development rate for male, female and combined sexes in the control was 86% and 0.0567, 0.0505 and 0.0536, respectively, while in the solvent controls the values were 84% and 0.0587, 0.0554 and 0.0571, respectively. Pooled control data were used to assess treatment level effects. At test termination, the mean percent emergence in the treatment groups ranged from 74 to 88% and there were no significant differences compared to the pooled control. The mean development rate for male midges ranged from 0.0455 to 0.0636 and for females from 0.0439 to 0.0553, while for the combined sexes they ranged from 0.0439 to 0.0594. Mean development rates of male and female midges (combined) in the two highest treatment levels, 20 and 40 µg a.s./L, were statistically significantly different from the pooled controls ($p<0.05$).

Each treatment level and control group was extended until 7 days of no midge emergence was observed and then that group was terminated. This was done in order to see whether midge mortality or significant delays (past day 28) in development had occurred. Only 2 midges (female) emerged post day 28 (from the 20 and 40 µg a.s./L levels) and these did not change the results of the study.

Table B.9.2.5.13-1 Summary of results (mean percent emergence and mean development rates) calculated at test termination (day 28) of the midge (*Chironomus riparius*) full life-cycle exposure with pyriproxyfen

Nominal concentration (µg a.s./L)	Mean percent emerged (%)	Mean development rates		
		Male midges	Female midges	Male/female midges
Control	86	0.0567	0.0505	0.0536
Solvent control	84	0.0587	0.0554	0.0571
Pooled control	85	0.0577	0.0530	0.0553
2.5	80	0.0636	0.0553	0.0594
5.0	78	0.0613	0.0540	0.0572
10	88	0.0606	0.0519	0.0551
20	74 (75)[1]	0.0522 (0.0522)[1]	0.0486 (0.0484)[1]	0.0496* (0.0487)[1]
40	76 (78)[1]	0.0455 (0.0455)[1]	0.0439 (0.0436)[1]	0.0446* (0.0438)[1]

[1] Figures in brackets are results of extended exposure (day 37)

* Statistically significantly different compared to the pooled control data ($p<0.05$).

Conclusions

Based on the results of this study (using nominal concentrations):

The 28-day EC_{50} of pyriproxyfen to *Chironomus riparius* was estimated to be >40 µg a.s./L (based on midge emergence).

The 28-day no-observed effect concentration (NOEC) was established as 10 µg a.s./L (based on the midge development rates).

Comments by RMS

The study was conducted in general agreement with OECD 219 (2004). Since there was no statistically significant difference between the control and the solvent control, it was acceptable to use pooled data for statistical analysis.

The analytical method used was sufficiently validated (recoveries in sediment were 83.0-94.7% (LSC) and 83.9-104% (HPLC) at fortification levels of 0.606, 26.5 and 128 µg a.s./kg (n=5 at each level); recoveries in water were 102-105% (LSC) and 96.3-120% (HPLC) at fortification levels of 0.253, 2.51 and 40 µg a.s./L (n=5 at each level)).

In the above report effect concentrations were based on nominal concentrations. However, OECD 219 states that effect concentrations should preferably be based on the measured concentrations in overlaying water at the beginning of the test. Measured pyriproxyfen concentrations were in agreement with nominal concentrations at test initiation (86-95%) at 2.5, 10 and 40 µg a.s./L (as verified by HPLC). Therefore, using nominal concentrations for the calculations of endpoints is acceptable. However if this is used, processes as degradation/dissipation thus the real exposure to pyriproxyfen is not taken into consideration. The endpoint should then be expressed as mean measured of the water layer if the value is used to compare with PECsw in the RA. Total residue concentrations of pyriproxyfen decreased by day 7 and ranged from 17 to 27% of the nominal concentrations throughout the remainder of the study. The NOEC based on geomean measured concentration is 0.031 mg a.s./L.

From day 7, the parent component was not detectable as radiolabeled component. However, PYPAC was found at increasing levels of 68%, 81% and 100% of applied radioactivity on day 7, 14 and 28 respectively.

The EC_{50} for emergence was empirically estimated to be greater than the highest concentration tested. This is acceptable since none of the treatment levels tested reduced emergence by ≥50% compared to the pooled control data. RMS attempted to calculate EC_{10} and EC_{20} values using ToxRAT. No significant EC_{10} value for midge emergence could be calculated. ECx values for development rate of males + females were significant ($p<0.05$) using Probit and Weibull analysis. Please note that the guideline indicates that endpoints for development rate should be based on the pooled sexes, therefore ECx and NOEC values were not calculated for males and females separately. Results are shown in Table B.9.2.5.13-02.

Table B.9.2.5.13-02 Summary of results and endpoints

Nominal concentration (µg a.s./L)	Reduction of emergence (%) [1)]	Reduction of development rate (combined sexes) (%)
2.5	5.9	-7
5.0	8.8	-3
10	-3	0.4
20	13	10
40	10	19
NOEC	40	10
EC10	n.d.	23
EC20	>40	40
EC50	>40	>40

1) Compared to pooled control

Reliability of endpoints

To assess the reliability of the estimated ECx values, two approaches are described in EFSA Supporting publication 2015:EN-924:

- Normalised width of the confidence interval (NW = (upper limit – lower limit) / median estimate); rating of the NW ranges from excellent (<0.2) to bad (>2)
- Relationship between EC_{10} and EC_{20}/EC_{50} confidence intervals: the best case (high certainty of protection level) is achieved when EC_{10} is lower than the lower limit of the EC_{20}; the worst case (low certainty of protection level) occurs when the median EC_{10} is greater than the lower confidence limit for the EC_{50}.

No confidence intervals for midge emergence or development rate could be calculated by RMS with ToxRAT using either Probit or Weibull analysis. Therefore, the EC10 values are not considered reliable.

According to Regulation (EU) No 283/2013, the statistical power of the NOEC shall be assessed in case no reliable ECx can be derived. However, this is not possible as long as default type I and II errors have not been fixed, default variance for any response variable have not been established and typical detectable effect sizes of each parameter have not been calculated (EFSA Supporting publication 2015:EN-924). Considering the level of effect on which the NOEC was based in the current test, the NOEC is considered sufficiently protective.

The NOEC (in natural sediment) was 10 µg a.s./L and may be used for risk assessment.

4.4.5.4 CA 8.2.5.4/01 (2015) 42-Day Toxicity Test Exposing Freshwater Amphipods (Hyalella azteca) to Pyriproxyfen Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods

Report: CA 8.2.5.4/01, (2015) 42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Pyriproxyfen Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods. Valent U.S.A. Corporation. Unpublished report No.: 12709.6354 (Sumitomo Chemical Co., Ltd. Company code NNW-0243)	
Previous evaluation	New study
Remark by RMS	The study is acceptable.
Conclusion	NOEC = 15 mg a.s./kg (0.041 mg a.s./L based on mean measured pore water concentrations)

Guidelines

Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, 2nd Edition, test method 100.4 (U.S. EPA, 2000); draft OCSPP 850.1770 guideline (U.S. EPA, 2009).

Deviations: (1) Based on the lack of observed feeding activity early on in the exposure and comparison of day 28 survival results with the remaining replicates in this treatment group, it was concluded that amphipods were inadvertently not added to four replicates of the 17 mg/kg nominal treatment group at test initiation. Consequently, these replicates were omitted from all statistical analysis of both lethal and sublethal endpoints. (2) The sediment was mixed on the rolling mill for an additional two hours on two occasions as opposed to one during one week of the equilibration period. It is not considered that these deviations had any impact on the results of the study.

GLP: Yes (certified laboratory)

Materials and Methods

Materials

1. Test Material:	Pyriproxyfen T.G.
Description:	Not stated
Lot/Batch #:	080506G
Purity:	99.2% (analysed)
Expiry date:	August 24, 2017
2a. Test animals:	The freshwater amphipod, *Hyalella azteca,* used during this study were obtained from laboratory cultures maintained at the test facility,
2b. Maintenance:	Prior to exposure initiation, amphipods were maintained in 20-L glass aquaria containing approximately 15 L of culture water under flow-through conditions. The culture water was from the same source as the overlying water used during the test. Amphipods (8 days old at exposure initiation) used in the exposure were collected from reproducing adult amphipods removed from the main culture tanks 9 days prior to exposure initiation. The reproducing adult amphipods were placed in 9.5-L aquaria containing approximately 8 L of laboratory well water. Juvenile amphipods (< 24 hours old) produced by these isolated adults were then removed from the isolation tanks on the following day and pipetted into 1-L beakers containing approximately 0.80 L of laboratory well water. The juvenile amphipods were reared under static conditions for 8 days with gentle oil-free aeration. During the holding period, dissolved oxygen ranged from 4.3 to 7.9 mg/L and temperature ranged from 23 to 24 °C. The test organisms appeared healthy and no mortality was observed in the juvenile test population 48 hours prior to exposure initiation.

Study Design

The purpose of this study was to determine the effects of pyriproxyfen, applied to sediment, on the freshwater amphipod, *Hyalella azteca*. The study was performed under static-renewal conditions for a period of 42 days.

The test vessels used in the sediment test were 300-mL glass vessels containing 100 mL (approximately 4.0-cm layer) of sediment (equivalent to 162 g wet weight per vessel or 104 g dry weight per vessel). A turbulence reducer, consisting of a plastic disk, was used to minimize the disruption of the sediment layer during the introduction of 175 mL of overlying water. The total overlying water plus sediment volume was maintained at approximately 275 mL. During the 42-day study, the overlying water was renewed by adding two volume additions of water per test vessel per day using an intermittent delivery system in combination with a calibrated water-distribution system. The intermittent delivery system was calibrated to provide 1 L of water per cycle to the water-distribution system, which subsequently provided 50 mL of water per cycle to each replicate test vessel. The water delivery system cycled approximately 7 times per day, providing approximately 350 mL per vessel every 24 hours (i.e., approximately two overlying volume replacements per vessel per day).

Nominal treatment concentrations of 0.59, 1.8, 5.4, 17 and 50 mg/kg were used. One day prior to exposure initiation (day -1), the treated and control sediments were allocated to the replicate vessels. Twelve replicates were used to evaluate the biological response of the test organisms. Three additional replicates were established for the treated and solvent control sediments while six additional replicates were established for the negative control. These additional replicates were designated for chemical analysis. In the case of the negative control, additional replicates were also established for water quality measurements of the pore water. Overlying water was gently added to each vessel and each vessel was placed under the renewal system on day -1. Each replicate vessel established for monitoring biological response contained 10 amphipods (8 days old) with a total of 120

amphipods per concentration or control. The additional replicates established for chemical analysis and pore water quality measurements were maintained under the same conditions and contained test organisms, but were not used to evaluate the biological response of the test organisms.

Dissolved oxygen concentration, temperature and pH were measured in the overlying water of each available replicate vessel of each treatment level and control used for biological monitoring at days 0, 28, 29 and 42. On the remaining test days, dissolved oxygen and temperature were measured daily in one alternating available biological replicate of each treatment level and control. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature controlled water bath used to house the test vessels throughout the study. Total hardness, alkalinity, conductivity and total ammonia concentration of the overlying water were monitored at days 0 (exposure initiation), 28, 29 and 42 in each treatment level and the control groups from a composite sample of all available biological replicates. At exposure initiation, day 14 and day 28, pH and ammonia (as nitrogen) concentration were measured in one pore water sample from each of three replicates of the negative control. Exposure concentrations were measured on day 0 (exposure initiation), day 14 and day 28 (termination of sediment phase of the exposure) in the overlying water (all treatments and control), pore water (negative control) and sediment (all treatments and control).

Amphipod survival and growth (length) were determined in four of the twelve replicate vessels on day 28 by sieving the sediment to remove all surviving amphipods. The adults were preserved for up to two weeks prior to taking images for length determination. The amphipods in the remaining eight replicates following determination of survival and growth were also removed by sieving and survival of these organisms was recorded. The surviving amphipods from these replicates were then placed in 300-mL water-only exposure vessels containing a thin layer of silica sand. Reproduction and survival of the amphipods was measured on days 35 and 42 by removing and counting the adults and offspring in each replicate beaker. In addition, any offspring observed at the end of the sediment exposure phase (day 28) were counted and recorded. On day 35, adults were counted to assess day 35 survival and returned to their respective test vessels after reproduction had been assessed. At test termination (day 42), the adult amphipods were enumerated to assess day 42 survival and preserved for up to two weeks prior to taking images for length determination. The number of adult males and females were determined following preservation. Reproduction for both day 35 and 42 is expressed as the number of young per adult female amphipod in each replicate based on the number of females present at day 42. The day 42 growth (measured as length) of surviving, preserved amphipods was determined after test termination and measured using the same process to measure length on individuals from day 28.

Results and Discussion

Physical and Chemical Measurements of Water

Throughout the exposure period, pH (6.9-7.5), dissolved oxygen (3.1 – 7.3 mg/L) and ammonia concentration (≤0.32 mg/L) remained within acceptable limits. Dissolved oxygen was maintained above 2.5 mg/L throughout the exposure. Daily measurements of the temperature in the overlying water of each test vessel ranged from 22 to 24 ºC. Continuous monitoring in an auxiliary vessel established a temperature range of 22 to 24 ºC throughout the definitive study.

Measurement of Test Concentrations

Measured sediment concentrations on day 0 in the 0.59, 1.8, 5.4, 17 and 50 mg/kg nominal treatment levels were 0.54, 1.7, 6.3, 16 and 47 mg/kg, respectively, 0.54, 1.5, 5.4, 15 and 43 mg/kg, respectively, on day 14, and 0.48, 1.4, 4.4, 14 and 41 mg/kg, respectively, on day 28. Mean measured sediment concentrations ranged from 85 to 99% of nominal concentrations and defined the treatment levels tested as 0.52, 1.5, 5.4, 15 and 44 mg/kg.

Measured pore water concentrations on day 0 in the 0.59, 1.8, 5.4, 17 and 50 mg/kg nominal treatment levels were 0.0011, 0.0032, 0.017, 0.042 and 0.13 mg/L, respectively, 0.0012, 0.0043, 0.011, 0.029 and 0.15 mg/L, respectively, on day 14, and 0.0013, 0.0041, 0.016, 0.051 and 0.18 mg/L, respectively, on day 28. Mean measured pore water concentrations defined the treatment levels tested as 0.0012, 0.0039, 0.015, 0.041 and 0.15 mg/L.

Measured concentrations in the overlying water on day 0 in the 0.59, 1.8, 5.4, 17 and 50 mg/kg nominal treatment levels were 0.00064, 0.0029, 0.0077, 0.017 and 0.091 mg/L respectively. Overlying water concentrations were < 0.00030, < 0.00030, 0.00030, 0.0024 and 0.017 mg/L, respectively, on test day 14 and < 0.00030, < 0.00030, < 0.00030, 0.00079 and 0.0035 mg/L, respectively, on day 28.

Based on the results of the sediment and pore water analyses, the majority of pyriproxyfen applied remained associated with the sediment throughout the exposure and no significant degradation was observed.

Biological Findings

Amphipod survival and growth (day 28)

A summary of survival and growth data of the sediment exposure of *Hyalella azteca* to pyriproxyfen is presented in Table B.9.2.5.14-1. The exposure system provided test conditions that were appropriate for acceptable survival and growth of *Hyalella azteca*, as demonstrated by the control and solvent control organism performance,

On day 28, survival observed among amphipods for each treatment level tested (0.52, 1.5, 5.4, 15 and 44 mg/kg) resulted in a mean value of 98%. There was no significant difference in survival among amphipods exposed to any of the treatment levels tested compared to the control (99%, p≥0.05). Since no concentration tested resulted in ≥50% mortality when compared to the control data, the 28-day LC_{50} value for survival was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

On day 28, length among amphipods exposed to the 0.52, 1.5, 5.4, 15 and 44 mg/kg treatment levels averaged 5.62, 5.67, 5.40, 5.35 and 5.32 mm per amphipod, respectively. There was no significant difference in length in any of the treatment levels tested compared to the control organisms (5.62 mm per amphipod, p≥0.05). Since no concentration tested resulted in ≥50% reduction in length when compared to the control data, the 28-day EC_{50} value for growth was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

Amphipod survival and growth (day 35)

Survival and reproduction determined on day 35 are presented in Table B.9.2.5.14-2. Mean percent survival of 96, 96, 99, 96 and 96% was observed among amphipods exposed to the 0.52, 1.5, 5.4, 15 and 44 mg/kg treatment levels, respectively. There was no significant difference in survival among amphipods exposed to any of the treatment levels tested compared to the control (99%, p≥0.05). Since no concentration tested resulted in ≥50% mortality when compared to the control data, the 35-day LC_{50} value for survival was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

The mean number of offspring per female among amphipods exposed to the 0.52, 1.5, 5.4, 15 and 44 mg/kg treatment levels was 2.5, 6.4, 6.2, 4.1 and 5.1, respectively. There was no significant difference in reproduction among amphipods exposed to any of the treatment levels tested compared to the control (2.4 offspring per female, p≥0.05). Since no concentration tested resulted in ≥50% reduction in reproductive output compared to the control, the 35-day EC_{50} value for reproduction was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

Amphipod Survival, Growth, Reproduction and Sex Ratio (Day 42)

Survival, growth, reproduction and sex ratio determined on day 42 are presented in Table B.9.2.5.14-3. Mean percent survival of 95, 94, 99, 96 and 95% was observed among amphipods exposed to the 0.52, 1.5, 5.4, 15 and 44 mg/kg treatment levels, respectively. There was no significant difference in survival among amphipods exposed to any of the treatment levels tested compared to the control (96%, p≥0.05). Since no concentration tested resulted in ≥50% mortality compared to the control data, the 42-day LC50 value for survival was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

Mean growth among amphipods exposed to the 0.52, 1.5, 5.4, 15 and 44 mg/kg treatment levels averaged 6.24, 6.08, 6.15, 5.89 and 5.64 mm per amphipod, respectively. There was a significant difference (Bonferroni's Adjusted t-Test) in length among amphipods exposed to the 44 mg/kg treatment level compared to the control organisms (6.04 mm per amphipod, p<0.05). Since no concentration tested resulted in ≥50% reduction in

length when compared to the control data, the 42-day EC_{50} value for growth was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

The mean number of offspring per female among amphipods exposed to the 0.52, 1.5, 5.4, 15 and 44 mg/kg treatment levels was 9.8, 11, 16, 8.8 and 13, respectively. There was no significant difference in reproduction among amphipods exposed to any of the treatment levels tested compared to the control (5.1 offspring per female, p≥0.05). Since no concentration tested resulted in ≥50% reduction in reproductive output compared to the control, the 42-day EC_{50} value for reproduction was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

The mean male:female ratio among amphipods exposed to the 0.52, 1.5, 5.4, 15 and 44 mg/kg treatment levels was 1.1, 1.6, 1.4, 1.6 and 1.5, respectively. There was no significant difference in male:female ratio among amphipods exposed to any of the treatment levels tested compared to the control (1.1, p≥0.05). Since no concentration tested resulted in ≥50% reduction in male:female ratio when compared to the control data, the 42-day EC50 value for mean male:female ratio was empirically estimated to be >44 mg/kg, the highest mean measured sediment concentration tested.

Table B.9.2.5.14-1 Mean percent survival of adult amphipods and mean amphipod growth (length) during the chronic exposure of amphipods (*Hyalella azteca*) to pyriproxyfen on day 28

Mean Measured Sediment Concentration (mg/kg)	Day 28	
	Mean Percent Survival[a] (%)	Mean Length per Amphipod[a] (mm)
Control	99 (3)	5.62 (0.24)
Solvent control	99 (3)	5.54 (0.15)
0.52	98 (5)	5.62 (0.12)
1.5	98 (4)	5.67 (0.23)
5.4	98 (5)	5.40 (0.10)
15	98 (5)	5.35 (0.23)
44	98 (5)	5.32 (0.12)

[a] Standard deviation is presented in parentheses.

Table B.9.2.5.14-2 Mean percent survival of adult amphipods and mean number of offspring released per female amphipod during the chronic exposure of amphipods (*Hyalella azteca*) to pyriproxyfen on day 35

Mean Measured Sediment	Day 35

Concentration (mg/kg)	Mean Percent Survival[a] (%)	Mean Number of Offspring Released per Female[a]
Control	99 (4)	2.4 (1.9)
Solvent control	98 (7)	5.2 (3.4)
0.52	96 (5)	2.5 (2.0)
1.5	96 (5)	6.4 (6.3)
5.4	99 (4)	6.2 (4.1)
15	96 (5)	4.1 (2.2)
44	96 (5)	5.1 (2.6)

[a] Standard deviation is presented in parentheses.

Table B.9.2.5.14-3 Mean percent survival of adult amphipods and mean number of offspring released per female amphipod during the chronic exposure of amphipods (*Hyalella azteca*) to pyriproxyfen on day 42

Mean Measured Sediment Concentration (mg/kg)	Day 42			
	Mean Percent Survival[a] (%)	Mean Length per Amphipod[a] (mm)	Mean Number of Offspring Released per Female[a]	Mean Male:Female Ratio[a]
Control	96 54)	6.04 (0.38)	5.1 (5.2)	1.1 (0.62)
Solvent control	96 (7)	6.03 (0.25)	8.7 (5.0)	1.1 (0.37)
0.52	95 (5)	6.24 (0.24)	9.8 (6.2)	1.1 (0.73)
1.5	94 (7)	6.08 (0.18)	11 (6.9)	1.6 (1.1)
5.4	99 (4)	6.15 (0.28)	16 (7.0)	1.4 (1.6)
15	96 (5)	5.89 (0.23)	8.8 (2.2)	1.6 (0.53)
44	95 (5)	5.64[b] (0.29)	13 (5.7)	1.5 (0.90)

[a] Standard deviation is presented in parentheses
b Significantly reduced compared to the control, based on Bonferroni's Adjusted t-Test ($p<0.05$)

Conclusions

The most sensitive indicator of toxicity for pyriproxyfen and *Hyalella azteca* was 42-day growth. Based on this endpoint and the mean measured sediment concentrations of pyriproxyfen, the No-Observed-Effect Concentration (NOEC) was determined to be 15 mg a.s./kg (0.041 mg a.s./L based on mean measured pore water concentrations). The Lowest-Observed-Effect Concentration (LOEC) for amphipods was determined to be 44 mg a.s./kg (0.15 mg a.s./L based on mean measured pore water concentrations).

Since no concentration tested resulted in >50% mortality or reduction of amphipods survival, growth and reproduction when compared to the control data, both the LC_{50} and EC_{50} values were empirically estimated to be > 44 mg a.s./kg (> 0.15 mg a.s./L based on mean measured pore water concentrations), the highest mean measured sediment concentration tested.

Comments by RMS

The study was conducted in agreement with EPA Test Method 100.4, Section 14. Minimum survival in the control was 80% on day 28, at test initiation age was 7-8 days, on day 28 length was >3.2 mm for all individuals and between days 28 and 42 reproduction was >2 young/female for all individuals. Since the validity criteria were met, this study is considered valid.

The analytical method used was sufficiently validated (measured concentrations in aqueous QC samples and sediment QC samples ranged from 87.5 to 106% and 96.2 to 104% of the nominal fortified concentrations, respectively, both n=9). Measured concentrations are shown in the table below.

Table B.9.2.5.14-4 Measured concentrations during the chronic exposure of amphipods (*Hyalella azteca*) to pyriproxyfen

Nominal concentration (mg/kg)	**Measured concentration at**			
	Day 0	**Day 14**	**Day 28**	**Mean**
In sediment (mg/kg)				
0.59	0.54	0.54	0.48	0.52
1.8	1.7	1.5	1.4	1.5
5.4	6.3	5.4	4.4	5.4
17	16	15	14	15
50	47	43	41	44
In pore water (mg/L)				
0.59	0.0011	0.0012	0.0013	0.0012
1.8	0.0032	0.0043	0.0041	0.0039
5.4	0.017	0.011	0.016	0.015
17	0.042	0.029	0.051	0.041
50	0.13	0.15	0.18	0.15
In overlying water (mg/L)				
0.59	0.00064	<0.00030[a]	<0.00030	N/A[b]
1.8	0.0029	<0.00030	<0.00030	N/A
5.4	0.0077	0.00030	<0.00030	N/A
17	0.017	0.0024	0.00079	N/A
50	0.091	0.017	0.0035	N/A

[a] Concentrations expressed as less than values were below the minimum detectable limit (MDL). The MDL is dependent upon the lowest concentration calibration standard used and the dilution factor derived from the sample volume of the controls (i.e., 0.0000150 mg/L*20=0.00030 mg/L)

[b] Mean values for measured concentrations in overlying water were not reported

Since the majority of test substance (85 - 99% of nominal concentrations) remained associated with the sediment throughout the exposure and no significant degradation was observed, it was acceptable to base the endpoints on mean measured sediment concentrations.

Table B.9.2.5.14-5 Reduction in mean amphipod growth (length) on day 28 and on day 42

	Day 28 (N=4)		Day 42 (N=8)	
Mean Measured Sediment Concentration (mg/kg)	Mean Length per Amphipod[a] (mm)	% reduction compared to pooled controls	Mean Length per Amphipod[a] (mm)	% reduction compared to pooled controls
Control	5.62 (0.24)	N/A	6.04 (0.38)	N/A
Solvent control	5.54 (0.15)	N/A	6.03 (0.25)	N/A
0.52	5.62 (0.12)	-0.72	6.24 (0.24)	-3.40
1.5	5.67 (0.23)	-1.61	6.08 (0.18)	-0.75
5.4	5.40 (0.10)	3.23	6.15 (0.28)	-1.91
15	5.35 (0.23)[b]	4.13	5.89 (0.23)	2.40
44	5.32 (0.12)	4.66	5.64[c] (0.29)	6.55

[a] Standard deviation is presented in parentheses
[b] N=3, amphipods were not added to this replicate at test initiation and therefore the replicate was omitted from all statistical analysis
[c] Significantly reduced compared to the control, based on Bonferroni's Adjusted t-Test ($p<0.05$)

No statistically significant effects were seen in growth, survival or reproduction at and below 15 mg/kg throughout the exposure period (42 days).

Reliability of endpoints

According to Regulation (EU) No 283/2013, the statistical power of the NOEC shall be assessed in case no ECx values can be derived. However, this is not possible as long as default type I and II errors have not been fixed, default variance for any response variable have not been established and typical detectable effect sizes of each parameter have not been calculated (EFSA Supporting publication 2015:EN-924). Considering the level of effect on which the NOEC was based (i.e. a 6.5% reduction in length), the NOEC is considered sufficiently protective.

The 42-d NOEC for pyriproxyfen on the growth of Amphipods was 15 mg/kg (0.041 mg a.s./L based on mean measured pore water concentrations) and may be used for risk assessment.

4.4.6 Chronic toxicity to algae or aquatic plants

See short-term toxicity

4.5 Acute and/or chronic toxicity to other aquatic organisms

4.5.3. CP 10.2.3/01a (2004) Pyriproxyfen 10EC: plankton-dominated microcosm experiment

Study reference:

Report	CP 10.2.3/01a (2004)
Title	Pyriproxyfen 10EC: plankton-dominated microcosm experiment
Report number	NNW-0171
Guidelines	European Commission, Health & Consumer Protection Directorate-General, 2002. Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC, Working Document SANCO/3268/2001 rev.4 (final), 2002
GLP	Yes
Previous evaluation	DAR (2005)
RMS comment	Considered acceptable for use in risk assessment

Executive Summary

The impact of Pyriproxyfen 10EC was assessed in indoor laboratory plankton-dominated microcosms. Twenty-one microcosms were used for the effect evaluation located in a water bath for temperature regulation in a controlled-environment room. In addition, three microcosms were set up to provide analytical information on the rate of dissipation of pyriproxyfen from the water phase. Each microcosm consisted of an all-glass cylinder (total volume approximately 18 L). The microcosms contained a sediment layer of approximately 0.02 m and a water layer of 0.3 m. Sediment and water were collected from an uncontaminated eutrophic ditch. Dosage solutions were applied below the water surface by means of a stainless steel funnel, which was slowly moved during dosing and the water was gently stirred. Nominal concentrations of the active ingredient were 0 – 0.02 – 0.08 – 0.32 – 1.2 – 5 – 20 µg a.s./L (single application). The control microcosms received water only at application together with gentle stirring as for test item groups. There were three replicates for each treatment. Samples were taken at 8 and 1 days before application, and at 3, 7, 14, 21, 28, 35, 42, 49 and 56 days after application to assess effects on zooplankton (species composition and abundance).

The measured concentrations of pyriproxyfen in the water (mean values), deviated by 5% or less from the nominal concentrations. The dynamics of pyriproxyfen concentrations in the integrated water column of the fate microcosm showed a rapid decline over the first week after application, with levels falling below the LOD by the second week. A visual assessment of the data indicates that the half-life was about 1 day.

The results show that none of the treatments with the test substance Pyriproxyfen 10EC, indicated direct negative effects over the longer term (longer than 8 weeks). Effects were most explicit shortly after application of the test substance and showed a clear treatment - response relationship:

At the 0.02, 0.08 and 0.32 µg a.s./L-treatment levels: No treatment related effects observed.

At the 1.2 µg a.s./L-treatment level: No treatment related effects observed. $NOEC_{population}$ for the most sensitive taxon (*D.* gr. *galeata*) was at this treatment level.

At the 5 µg a.s./L-treatment level: A slight transient effect was observed with 1 cladoceran species, with a reduction in *D.* gr. *galeata* numbers on Day 7 but recovery occurred within 1 week. The $NOEC_{community}$ was at this treatment level, based on the lack of any statistically significant effects at the community level. It was also the NOEC for most of the other potentially sensitive endpoints.

At the 20 µg a.s./L-treatment level: Clear but transient direct effects on cladocerans in the form of *Daphnia* gr. *galeata* were observed. Recovery of the cladocerans occurred within 35 days. Clear indirect effects on some rotifers were observed. An increase in *Keratella quadrata* numbers occurred up to the end of the experiment. However, most rotifers as well as total rotifer abundance had recovered within 42 days. Effects on the community level were observed and had recovered within 28 days after treatment. No treatment related effects were observed on copepods, planktonic chlorophyll-a levels, nutrient levels or other community metabolism endpoints.

Materials and Methods

Materials

1. Test Material:	Pyriproxyfen 10EC
Description:	Not stated
Lot/Batch #:	B0200001
Purity:	102 g a.s./L (analysed)
Expiry date:	April 28, 2004
2a. Test animals:	The microcosm communities simulated a plankton-dominated nutrient-rich system. The planktonic seeding material originated from the plankton in the experimental ditch from which also the sediment and water was obtained and from other uncontaminated experimental ponds also located at the Sinderhoeve Experimental Station. The planktonic communities contained algae, cladocerans, copepods, ostracods and rotifers (any macroinvertebrates present were removed). Additional daphnids (*Daphnia* group *galeata*, containing the species *D. cucullata*, *D. galeata*, *D. hyalina*, *D. longispina* and *D. ambigua*) were added to each microcosm, as this is a group known to be particularly sensitive to pyriproxyfen.

2b. Preparation:	Plankton was collected by means of a 55-µm mesh plankton net by pouring about 125 L of ditch water through the net. Before pouring the water through the plankton net, it was filtered over a 0.5-mm mesh net to prevent collection of any macroinvertebrates. The plankton was transported in a plastic bucket to the laboratory. Before the water containing the plankton was added to the microcosms, it was checked for predators (e.g., phantom midge larvae, water bugs, water beetle larvae) which were then removed. Distribution of the plankton over the microcosms was done by dividing the water into aliquots of 0.5 L. These were poured into each of the systems. Introduction of the plankton was 29 days before the first sampling on day -8.

Study Design

The impact of Pyriproxyfen 10EC (emulsifiable concentrate) was assessed in indoor laboratory plankton-dominated microcosms. Twenty-one microcosms were used for the effect evaluation located in a water bath for temperature regulation in a controlled-environment room. In addition, three microcosms were set up to provide analytical information on the rate of dissipation of pyriproxyfen from the water phase. Each microcosm consisted of an all-glass cylinder (diameter 0.25 m, height 0.35 m, total volume approximately 18 L). The microcosms contained a sediment layer of approximately 0.02 m and a water layer of 0.3 m (approximately 14.7 L). Sediment and water were collected from an uncontaminated eutrophic ditch at the Sinderhoeve Experimental Station (Renkum, The Netherlands). After transport in plastic buckets to the laboratory, the sediment was thoroughly mixed. Before and during mixing, sticks, stones and large plant parts were removed by hand. The mixed sediment was distributed evenly over the bottom of the test vessels and 14,702 ± 4 g (mean ± SD) of water added to each one.

Daylight lamps provided artificial daylight for 14 hours per day (the remaining 10 hours were dark). Water temperature in the microcosms was regulated by placing them in a water bath with a temperature kept in a range of approximately 18 - 22 °C (increasing from minimum values to maximum values during the course of the day as a result of the irradiance and heat produced by the lamps). For each microcosm, water temperature (using a min-max thermometer, every working day) and light intensity (using a light meter twice a week) were recorded. Conditions for phytoplankton growth were maintained by adding nutrients to the microcosms (0.09 mg N/L and 0.015 mg P/L were added twice a week). To suppress periphyton growth, 5 snails per system (*Lymnaea stagnalis*, (sub)adults) were introduced into each microcosm. To prevent growth of a bacterial layer on the water surface of the microcosms and to stimulate a little water movement, compressed air was used to provide a light air flow over the water surface. Water losses due to evaporation were replenished with demineralised water and the water level in the surrounding water bath was maintained with tap water.

The application method chosen was to simulate a realistic worst-case environmental exposure. Accordingly, dosage solutions of Pyriproxyfen 10EC prepared in tap water were applied below the water surface by means of a stainless steel funnel, which was slowly moved during dosing. The water was then gently stirred by means of a glass rod to produce a uniform concentration throughout the water column. Care was taken to disturb the sediment as little as possible. Nominal concentrations of the active ingredient were 0 – 0.02 – 0.08 – 0.32 – 1.2 – 5 – 20 µg a.s./L (single application). The control microcosms received water only at application together with gentle stirring as for test item groups. There were three replicates for each treatment.

Observations

Samples were taken at 8 and 1 days before application, and at 3, 7, 14, 21, 28, 35, 42, 49 and 56 days after application to assess effects on zooplankton (species composition and abundance). On each occasion, water was sampled in each microcosm from several points by means of a perspex tube to obtain a total sample volume of approximately 1 litre. The water was filtered through a 55-µm mesh plankton net and the plankton preserved in formalin. The filtered water was poured back into microcosm from which it had originally been taken. Identification of the zooplankton was carried out under a microscope. Numbers of microzooplankton (i.e., Rotifera, copepod nauplii), were determined by counting a known volume. Numbers of macrozooplankton (i.e., Cladocera, (sub-)adult Copepoda, Ostracoda) were quantified by counting the total numbers of the sample under a binocular microscope. Species composition of the zooplankton was determined to the lowest practical taxonomic level.

Effects on phytoplankton (chlorophyll-a) was carried out at the same time as for the zooplankton, in order to avoid a dilution effect on the chlorophyll-a. An integral water sample of about 250 mL was collected from each microcosm, at three random locations. Water samples of *ca.* 100 mL were concentrated over a glass-fibre filter, using a vacuum pump and surplus water and filtrates were returned to the appropriate microcosms. The filters were stored in a deep freezer (up to 5 weeks) before measurement of chlorophyll-a content was carried out using a spectrophotometer.

Dissolved oxygen (DO), electrical conductivity, temperature and pH were measured at mid-water depth. The measurements were carried out in the morning (just before and/or at about the start of the photoperiod when the lowest DO levels occur) and later than 15.00 hrs when maximum DO levels were expected to occur. Measurements were performed in each microcosm, on 7 days and 1 to 0 days before application, and again 2, 8, 15, 22, 29, 36, 43, 50 and 57 days after application.

Prior to statistical analysis, zooplankton data were $\ln(2x+1)$ transformed, where x is the abundance value. $NOEC_{population}$ and $NOEC_{community}$ were determined by considering statistical information as well as taking biological significance into account. NOEC calculations at taxon or parameter level ($p \leq 0.05$) were carried out using the Williams test (ANOVA; Williams, 1972). In addition, the effects of the Pyriproxyfen 10EC treatment on the zooplankton community was analysed by the Principal Response Curves method (PRC), a

multivariate technique specially designed for the analysis of data from model ecosystem experiments (Van den Brink and Ter Braak, 1997; 1998; 1999).

Analytical data on concentrations in test media

Of the three microcosms used to provide analytical information, two were assigned to serve as blanks for fate quality control sampling purposes and the third was used to determine the concentration of the active ingredient in the water phase over time at a representative concentration (fate microcosm at 5 µg a.s./L). Samples were taken from the fate microcosm at -1d, < 1 h, 1 d, 3 d, 7 d, 14 d and every 2 weeks, until 2 consecutive time points gave an analysis below the limit of detection (approximately 0.01 µg a.s./L). Additional analysis of water samples taken from all microcosms shortly after application (about 1 hour) was conducted to verify the initial exposure concentrations. In all cases, depth-integrated water samples were taken by means of glass and stainless steel collecting material. The water samples were extracted with hexane, which was evaporated to dryness and the residue dissolved in toluene for analysis by GC-MSD.

The results are reported on the basis of the nominal test concentrations of the active ingredient of the test substance.

Results and Discussion

Deviations

Only minor deviations occurred, which had no significant impact on the study: a different Conductivity Meter was used on Day –8 for functional reasons; sampling was carried out from low to high concentrations to avoid contamination but from Day 22 onwards it was carried out from microcosms 1 to 24 (no contamination possible due to rapid dissipation and more pragmatic); chlorophyll-a samples from 4.11.03 stored in freezer for 5 weeks rather than 4 (estimated additional loss = 1%); for validation of the analytical method, 3 replicates were used for each fortified level and the 20 µg a.s./L level was validated during the study (in duplicate).

Environmental Conditions

Mean (± SD) light intensities were 316 ± 22 µE/m^2/s for the control and treated microcosms over the course of the study. For individual microcosms, mean light intensities ranged from 286 to 361 µE/m^2/s. Daily min. - max. water temperatures fluctuated between 18.6 ± 0.4 °C and 20.6 ± 0.5 °C (mean ± SD), respectively, in the water bath compartment. In water samples collected just before treatment and in the final phase of the experiment, concentrations of bicarbonate, N-total, ammonium, nitrate, P-total and Ortho-P were measured. At all treatment levels, bicarbonate values were lower at Day 49 than at Day -1 (mean concentrations in the range of 60.04 to 74.14 mg/L and 8.62 to 13.19 mg/L, respectively). Inorganic N increased over time in the microcosms (N-total: 0.90 to 1.06 mg/L and 30.17 to 47.06 mg/L, on Days –1 and 49, respectively; NH_4^+: <0.04 to 0.09 mg/L and 1.19 to 1.41 mg/L, on Days –1 and 49, respectively). Inorganic P concentrations (P-total, Ortho-P) were near the limits of detection and remained more or less constant over time. After statistical

analysis, no treatment-related effects on these parameters were observed and the NOEC for the water quality measurements was ≥ 20 µg a.s./L in all cases.

Measurement of Test Concentrations

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%), confirming the validity of the analytical measurements. Levels of pyriproxifen in dosing solutions ranged between 96 and 105% of nominal.

The mean concentrations of pyriproxyfen in the water, calculated on the basis of the measured concentrations in the dosage solutions, the doses applied and the water volumes of the microcosms were near the nominal concentrations, deviating by 5% or less from the nominal concentrations. Measured concentrations in the integrated samples from the water column 1 h after application indicated that mean initial concentrations generally were somewhat higher than the calculated concentrations, with values in the range of 100 to 137.5% of nominal (not including the lowest treatment level of 0.02 µg a.s./L). At 0.02 µg a.s./L, the concentrations showed a relatively high variation 1 h after application. Results of duplicate samples within the same test systems were found to differ by up to a factor of 13.4 and were considered less valid for the calculation of mean values. The relatively high variation shortly after application at the 0.02 µg/L-treatment level is considered to be an artefact of sampling at very low concentrations (at about the LOQ).

The dynamics of pyriproxyfen concentrations in the integrated water column of the fate microcosm (5 µg/L treatment level) showed a rapid decline over the first week after application, with levels falling below the LOD by the second week. As the number of measured values (>LOD) is limited (to 4), a calculated value for the half-life (t½) of pyriproxyfen in the water phase of the test system would not be very reliable. However, a visual assessment of the data indicates that the half-life was about 1 day.

Biological Findings

Zooplankton

In total, 24 zooplankton taxa were collected. Rotifers formed the majority of taxa, followed by crustaceans (Cladocera and Copepoda). Within the Copepoda, Cyclopoida, Calanoida and nauplii were found. In 204 cases, the Williams test ($p < 0.05$) was applied to data sets containing non-zeros. These tests yielded NOECs for 12 taxa. Of these, 6 taxa indicated more or less consistent treatment-related responses as they showed statistically significant deviations on several consecutive sampling dates. The NOECs obtained per sampling date for the zooplankton populations in microcosms are shown in Table B.9.3.3/1a-1 (NOECs based on poor data are not shown).

Of the six cladoceran species present, *Daphnia* gr. *galeata* was the most sensitive. Consistent, statistically significant reductions occurred at the 20 µg a.s./L-treatment level and lasted from the first sampling date post-

treatment (Day 3) up to and including Day 28. Reductions reached a maximum on Day 7. Thereafter, populations started to increase again and recovery was complete by Day 35 (i.e. within 35 days). There was also a statistically significant reduction at the 5 µg a.s./L-treatment level on Day 7. The duration of effects at this treatment level was less than 7 days, with no significant reduction in numbers on Day 14. The only other cladoceran showing significant deviations during the exposure period was *Simocephalus vetulus*. However, populations of this species occasionally (Days 14 and 35) showed significantly increased numbers at the 20 µg a.s./L-treatment level and these isolated deviations were considered inconsistent with no clear concentration-response relationship. Grouping all cladoceran species together into one group, consistent treatment-related responses were only observed at the 20 µg a.s./L-treatment level during the first week.

The NOEC for ostracods was at the 5 µg a.s./L-treatment level and this was a result of significant reductions in numbers occurring in the period of 28 – 35 days post-treatment. The NOEC, however, is based on low and scattered numbers, which were also seen before and after these sampling dates. Because of the low statistical quality of this information, this NOEC is considered not to be valid. No treatment-related effects were observed on copepods (i.e., nauplii and (sub)adult Cyclopoida and (sub)adult Calanoida) (NOEC ≥ 20 µg a.s./L).

Three of the 14 rotifer species encountered in the microcosms showed treatment-related effects (i.e. *Polyarhtra remata*, *Anuraeopsis fissa* and *Keratella quadrata*). Overall, consistent and statistically significant increases in rotifer populations occurred at the 20 µg a.s./L-treatment level. *P. remata* showed the most consistent increase in numbers. Overall, the NOEC for the rotifers was 5 µg a.s./L. On the first sampling date (Day 3) the NOEC for *A. fissa* was 0.02 µg a.s./L but there appeared to be no clear treatment-effect relationship as numbers were relatively high only at 0.08 and 0.32 µg a.s./L (i.e not at 1.2 µg a.s./L and above). This NOEC may therefore be unduly influenced by the relatively high numbers at these two treatment levels and is considered a statistical artefact. Except for *K. quadrata*, recovery at the 20 µg a.s./L-treatment level occurred within 42 days. *K. quadrata* abundance was significantly higher in the 20 µg a.s./L microcosms at the last two sampling dates, on Days 49 and 56 and although not statistically significant, numbers tended to be consistently higher from Day 14 onwards, indicating a treatment effect. However, the total rotifer abundance recovered within 28 days after treatment, which suggests that *K. quadrata* was not a dominant rotifer taxon.

The PRC analysis (see Figure B.9.3.3/1a-1) indicated that the community response was mainly dominated by the increase in the rotifers. This increase, which started on day 3, was clearest at the 20 µg a.s./L-treatment level. The rotifers mostly correlating with this response was the cluster made up of *P. remata*, *A. fissa*, *K. quadrata* and *Trichocerca* gr. *tigris/pusilla*. Responses of the cladoceran *Daphnia* gr. *galeata*, were negatively correlated with the treatment regime (indicating a decrease in numbers). Species in the cluster Calanoida – Cyclopoida had low species weights (b_k), indicating that they were less sensitive to the treatments, or that abundance numbers had too little statistical information density for an adequate analysis. The $NOEC_{community}$ was at the 5 µg a.s./L-treatment level with recovery occurring within 28 days at the 20 µg a.s/L level.

Chlorophyll

Phytoplankton chlorophyll-a concentrations were very low during the entire experimental period. Concentrations generally kept below, or near to the limit of detection (8.7 µg Chl-a/L) and remained below the limit of quantification (29 µg Chl-a/L). Chlorophyll-a did not show any treatment-related response. As the concentration values were below the LOQ, a proper NOEC calculation could not be performed but interpretation of the data indicated that a NOEC should be ≥ 20 µg a.s./L.

Community metabolism

The Pyriproxyfen 10EC treatment did not result in any significant deviations on the measured electrical conductivity values. Late in the experiment (Day 36), some isolated statistically significant deviations were found at the 20 µg a.s./L-treatment level for the DO and pH endpoints. Occasionally, oxygen production levels (max DO - min DO) were significantly reduced compared to control levels. This reduction was most severe on Day 2 (NOEC < 0.02 µg a.s./L), when negative production was between 0.4 and 1.5 mg/L compared to the mean control production. For all treatment levels, however, DO levels kept above 10 mg/L and are thus considered not to have been ecologically adverse effects. On one sampling date (Day 15), a NOEC of <0.02 µg a.s./L was determined for the afternoon pH values but although values were lower than those in the controls, they only varied by less than one pH-unit. For the majority of sampling dates, NOECs for the community metabolism endpoints were ≥ 20 µg a.s./L. Causality of the isolated statistical deviations with treatments was unclear and are considered to have no ecological significance.

Community interactions

Direct negative effects of the active ingredient and/or the adjuvants of Pyriproxyfen 10EC were seen on the most sensitive zooplankton species (particularly the dominant daphnid species *Daphnia* gr. *galeata*) with effects being most explicit at the 20 µg a.s./L-treatment level and, to a lesser extent, at the 5 µg a.s./L-treatment level. Consequently, it can be assumed that the overall grazing pressure on algae by cladocerans was temporarily reduced at these treatment levels. This, however, did not result in statistically significant increases of algal chlorophyll-a (levels remained below the LOQ at all treatment levels and the microcosms were generally maintained in their original clear-water state). This was most probably due to the associated increases seen in rotifer abundance. The community response was mainly dominated by the increase in the rotifers and this was clearest and of longest duration at the 20 µg a.s./L-treatment level. These responses of the rotifers are considered secondary effects, which can be explained as the result of a decrease of mechanistic filtering and/or a decrease of food source competition by sensitive cladocerans. At the highest treatment level, these indirect effects lasted longer than the direct effects, where recovery of sensitive species took place within 35 days. However, most rotifer populations, as well as total rotifer abundance, were back to normal levels within 42 days. In the case of one species only, abundance levels were higher until the end of the study.

Table B.9.3.3/1a-1 NOECs (Williams test, $p < 0.05$) per sampling date for zooplankton populations in microcosms (treatment levels, µg a.s./L). Concentrations > NOEC showed significant increases (↑) or

reductions (↓). Blank columns indicate no statistical significance at the highest treatment level, 20 µg a.s./L.

	-8	-1	3	7	14	21	28	35	42	49	56
Cladocera											
Daphnia gr. *galeata*			5(↓)	1.2(↓)	5(↓)	5(↓)	5(↓)				
Simocephalus vetulus					5(↑)*[1]			5(↑)*[1]			
Cladocera total			5(↓)	5(↓)		5(↓)	0.32(↓)*[2]				
Ostracoda							5(↓)*[3]	5(↓)*[3]			
Rotifera											
Anuraeopsis fissa			0.02(↑)*[4]	5(↑)	5(↑)	5(↑)	5(↑)				
Polyarthra remata			5(↑)	5(↑)	5(↑)	5(↑)	5(↑)	5(↑)			
Keratella quadrata	5(↓)*[5]				5(↑)					5(↑)	5(↑)
Keratella cochlearis								1.2(↑)*[6]			
Rotifera total			5(↑)	5(↑)	5(↑)	5(↑)					

*[1]: Isolated observations, lack of concentration-response relationship.

*[2]: No clear concentration-response relationship, isolated observation late in study.

*[3]: Low and scattered abundance numbers, no concentration-response relationship. NOEC not valid because data not suitable for adequate analysis.

*[4]: One isolated observation, no concentration-response relationship.

*[5]: Before treatment, not treatment related.

*[6]: Inconsistent observation late in study.

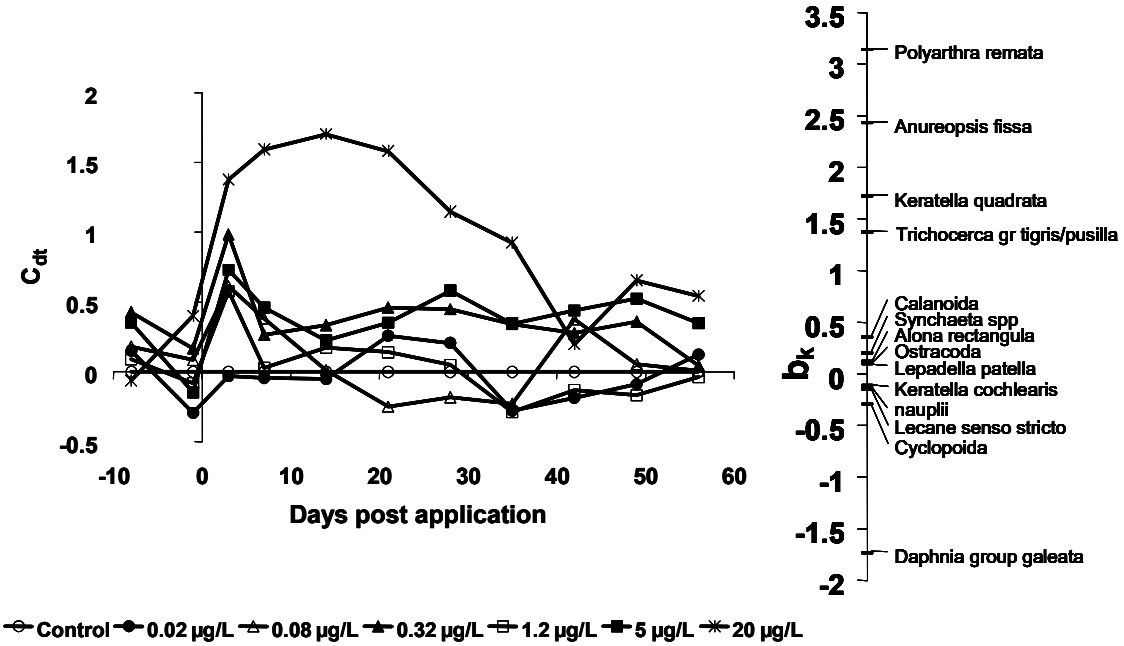


Figure B.9.3.3/1a-1 Principal Response Curves resulting from the analysis of the zooplankton data set, indicating the effects of the insecticide on the zooplankton community. Species with a species weight (b_k) between -0.1 and + 0.1 are not presented

Conclusions

The impact of Pyriproxyfen 10EC was tested on indoor plankton-dominated microcosms.

The microcosm communities consisted of cladocerans, copepods, ostracods, rotifers and algae. Nominal concentrations of the active ingredient were 0 – 0.02 – 0.08 – 0.32 – 1.2 – 5 – 20 µg a.s./L (single application). Effects on zooplankton (species composition and abundance), phytoplankton (chlorophyll-a), and community metabolism were measured. Effects reported were based on nominal test concentrations of the active ingredient of the test substance. The study is considered robust and valid according to the checklist for evaluating micro-/mesocosm studies provided in the EFSA aquatic guidance document (see Table B.9.3.3/1a-2).

Table B.9.3.3/1a-2 Checklist for evaluating micro-/mesocosm studies for regulatory purposes (adapted from De Jong *et al.*, 2008; Table 32 of the AGD)

Items	Notes	Reliability index 1–3 [a]
Methodology and test description		
1. Substance	**Properly characterised and reported?**	

Items	Notes	Reliability index 1–3 [a]
1.1 Concentration	Identity and amount of a.s. per litre test water?	1 (p.19)
1.2 Formulation and purity	Substances in the formulation influencing the working action of the a.s. should be reported	1 (although not reported formulation details are available.)
1.3 Vehicle	In case a vehicle—other than in the formulation—is used, identity and concentration?	1 (p.19 – no vehicle used)
1.4 Chemical analyses	Method, LOQ, LOD, recovery	1 (pp.21-22)
1.5 Properties	Relevant for potential fate and effects in test system	1 (p.19-20)
2. Test site, duration	**Properly characterised and reported?**	
2.1 Location	Necessary to make a link between the effects and local environmental conditions, representativeness	1 (indoor microcosm. Test organisms collected from natural source in The Netherlands)
2.2 Test date/duration	Application dates and experimental period?	1 (p.23)
2.4 General climatic conditions	Necessary to make a link between the effects and local climatic conditions	n.a. (indoor microcosm)
3. Application	**Properly characterised and reported?**	
3.1 Mode of application	Exposure route; spraying or homogenising the a.s. into the test medium?	1 (p.19)
3.2 Dosage and exposure	Actual concentrations during the test? Chemical analysis of dosing solution?	1 (pp.20-21 and 25-26)
3.3 Application scheme	Necessary to make a link between the test and the intended use of the PPP	1 (p.19. test substance added directly to water surface then mixed)

Items	Notes	Reliability index 1–3 [a]
3.4 Conditions during application	Weather conditions during application, wind speed and temperature?	n.a. (indoor microcosm)
4. Test design	**Properly designed and reported?**	
4.1 Type and size	e.g. outdoor microcosm, outdoor pond or mesocosm; dimensions	1 (p.17)
4.2 Pre-treatment	Proper equilibration?	1 (pp.17-18)
4.3 Treatment period	Number and spacing of treatments?	1 (p.19)
4.3 Post-treatment	Period long enough to allow expression of effects and recovery	1 (effects and recovery seen for sensitive groups (Cladocera and Rotifera) at highest concentration).
4.4 Untreated control	Sufficient number; solvent applied?	1 (in triplicate; no vehicle)
4.5 Replications	Sufficient replications for proper statistical analysis?	1 (in triplicate)
4.6 Statistics	Univariate and multivariate techniques applied	1 (p.24)
4.8 Dose–response	Number of test concentrations for finding a dose–response relation (controls excl.)	1 (6 concentrations were tested)
4.9 Quality assurance	Study conducted under GLP?	1
5. Biological system	**Representative and properly reported?**	
5.1 Populations	Enough sensitive/vulnerable species of the relevant taxonomic group?	2 (sensitive species in lab studies represented (Cladocera))
5.2 Community	The community/ecosystem representative and complete?	2 (presence of vulnerable species with long life history limited)
6. Sampling	**Is sampling adequate for risk assessment?**	
6.1 General features	Relevance selected measurement endpoints	1 (p.16)

Items	Notes	Reliability index 1–3 [a]
6.2 Actual concentration	Actual concentrations measured in medium and other compartments or biota?	1 (p.20)
6.3 Biological sampling	Appropriate methods and frequency?	1 (pp.22-23)
Results		
7. Endpoints	**Properly reported?**	
7.1 Type	Reported endpoints relevant for objective of study?	1 (effects of pyriproxyfen on chlorophyll, community metabolism and zooplankton)
7.2 Value	Are measured data consistently presented?	1
7.3 Verification of endpoint	Test results are verifiable and source data reported	1
8. Elaboration of results	**Are conclusions based on measured data? Methodology correct?**	
8.1 Statistical comparison	Data meet requirements for method used?	1
8.2 Dose–effect relationship	Minimal detectable difference; consistence of response	1 (in separate report)
8.3 Population-level responses	Sufficiently reported?	1
8.3 Community-level responses	Sufficiently reported?	1
9. Control		
9.1 Untreated control	Unexpected effects or disappearance of species?	1 (none)
9.2 Solvent control	Possible effects caused by solvent?	n.a. (no solvent control)
10. Classification of effects	**Properly derivable?**	1 (in separate report

Items	Notes	Reliability index 1–3 [a]
11. Biological meaning of statistically significant differences	**Sufficiently explained?**	1

[a] Relevant page and section nos. from the microcosm report provided in brackets for information purposes

1 Reliable - all data are reported, the methodology and the description are in accordance with internationally accepted test guidelines and/or the instructions, all other requirements fulfilled

2 Less reliable - not all data reported, the methodology and/or the description are slightly deviating from internationally accepted test guidelines or the instructions, without motivation, or not all other requirements fulfilled

3 Not reliable - essential data missing, the methodology and/or the description are not in accordance with internationally accepted test guidelines and/or the instructions without motivation, or not reported, or important other requirements are not fulfilled

Analysis of water samples taken shortly after application showed that the nominal concentrations were achieved (or slightly exceeded). The results show that none of the treatments with the test substance Pyriproxyfen 10EC, indicated direct negative effects over the longer term (longer than 8 weeks). Effects were most explicit shortly after application of the test substance and showed a clear treatment - response relationship:

At the 0.02 µg a.s./L-treatment level: No treatment related effects observed.

At the 0.08 µg a.s./L-treatment level: No treatment related effects observed.

At the 0.32 µg a.s./L-treatment level: No treatment related effects observed.

At the 1.2 µg a.s./L-treatment level: No treatment related effects observed. $NOEC_{population}$ for the most sensitive taxon (*D.* gr. *galeata*) was at this treatment level.

At the 5 µg a.s./L-treatment level: A slight transient effect was observed with 1 cladoceran species, with a reduction in *D.* gr. *galeata* numbers on Day 7 but recovery occurred within 1 week. The $NOEC_{community}$ was at this treatment level, based on the lack of any statistically significant effects at the community level. It was also the NOEC for most of the other potentially sensitive endpoints.

At the 20 µg a.s./L-treatment level: Clear but transient direct effects on cladocerans in the form of *Daphnia* gr. *galeata* were observed. Recovery of the cladocerans occurred within 35 days. Clear indirect effects on some rotifers were observed. An increase in *Keratella quadrata* numbers occurred up to the end of the experiment. However, most rotifers as well as total rotifer abundance had recovered within 42 days. Effects on the community level were observed and had recovered within 28 days after treatment. No treatment related effects

were observed on copepods, planktonic chlorophyll-a levels, nutrient levels or other community metabolism endpoints.

Comments by RMS

- The above study was also summarized in the DAR (November 2009), where it was noted that laboratory testing gave a very low chronic NOEC for *D. magna* of 0.015 µg a.s./L in a continuous flow system, and that this raised some concern, because *D. magna* was not present in the microcosm. However, on the basis of expert judgment and supplemental information from the notifier it was concluded that the microcosm study design was appropriate to negate the chronic effect on *D. magna.* Please refer to the DAR, page 436-437 for further detail. Furthermore, the Addendum to B9 on confirmatory data (2013) stated on page 8, that it was agreed by Member State experts in Praper 63 (January 2009) that the risk to aquatic invertebrates needs to be further addressed, as insects were not covered by the available study. EFSA noted after the peer review, that although the risk to sediment dwelling insects was addressed by the *C. riparius* study, this study could not be considered to cover effects on all other insects, given the mode of action (insect growth regulator). In response to these concerns, the applicant submitted a statement including data on four mesocosm studies from literature, and a chronic toxicity study with Mayfly nymphs. The Addendum to B9 on confirmatory data presents the evaluation of this information by the RMS, and the conclusion was reached that aquatic insects show a similar or lower level of susceptibility to pyriproxyfen when compared with zooplankton, particularly the crustaceans (cladocerans, copepods and ostracods), and that therefore the endpoints derived with pyriproxyfen for cladocerans (as a suitably sensitive group) at the population (microcosm) level are sufficient to represent the aquatic invertebrate group as a whole. Further details on the motivation for this conclusion are provided in the Addendum to B9 on confirmatory data (2013).
- 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.
- The concentrations of pyriproxyfen in the fate microcosm treated at 5 µg a.s./L are shown in Table B.9.3.3/1a-3. This data was implemented in CAKE version 3.1 in order to derive a SFO DT50 value. Two replicate samples from the fate microcosm were analysed, and the replicate mean data were directly fitted in CAKE un-weighted with the complete data set and unconstrained initial concentration. Non detectable

levels were set at 0.5xLOD, i.e. 0.0035 µg/L. The model fits are shown below (Figure B.9.3.3/1a-2). The rate constant was statistically significant (p=0.00109), the X^2 error % (7.71%) was <15%, and the visual fit was acceptable. The SFO DT50 was 0.931 days and the DT90 3.09 days.

Table B.9.3.3/1a-3 Concentrations of pyriproxyfen in depth-integrated water samples in a microcosm treated with a target concentration of 5 µg a.s./L (LOD 0.007 µg/L)

Day	Replicate sample (µg a.i./L)		Mean (µg a.i./L)
	1	2	
0.04	5.179	4.96	5.0695
1	2.636	2.783	2.7095
3	0.278	0.297	0.2875
7	0.019	0.005	0.012
14	n.d	n.d	--

n.d. = not detected

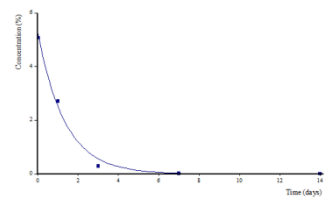
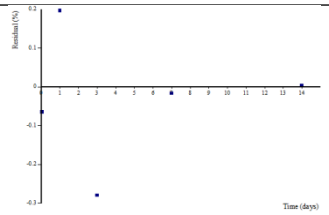
Model	Visual Fit	Residuals plot
SFO		

Figure B.9.3.3/1a-2 Visual SFO fit and SFO residuals plot

- The study summary and the study conclusions are acceptable. A re-analysis of the study data taking into account recent guidance from the Aquatic Guidance Document (EFSA, 2013) is provided in the two studies below (B.9.3.3/1b and B.9.3.3/1c).

B.9.1.1.1 B.9.3.3/1b Calculation of minimal detectable difference (MDD) values of a microcosm experiment performed with Pyriproxyfen 10EC

Report	CP 10.2.3/01b, J. Deneer, A. Rico, I. Roessink (2016)
Title	Calculation of minimal detectable difference (MDD) values of a microcosm experiment performed with Pyriproxyfen 10EC
Report number	NNW-0244
Guidelines	EFSA Aquatic Guidance Document (EFSA, 2013)
GLP	Not applicable
Previous evaluation	No, new study submitted for Renewal
RMS comment	Considered acceptable for use in risk assessment

Note: Experience and knowledge on the performance of microcosm and mesocosm studies continues to increase over time, and new developments and standardisations occur at fairly regular intervals. The latest knowledge has been included in the most recent EFSA Aquatic Guidance Document (EFSA, 2013). Proposals are given for the determination of a Regulatory Acceptable Concentration (RAC). One of the specific criteria is that at least eight taxa of the sensitive taxonomic group(s) should be present with an appropriate minimum detectable difference (MDD) to demonstrate potential treatment-related effects, i.e. for the insecticide pyriproxyfen at least 8 arthropod taxa of MDD Category 1 (see Brock et al. 2015) should be present. In addition, the community/ecosystem should be representative for communities in edge-of-field surface waters. These developments in regulatory requirements underpinning higher tier studies may have consequences for studies that have been performed before the adoption of these new requirements. Using methods in line with the aquatic effect assessment for plant protection products as described in the new Aquatic Guidance Document (EFSA, 2013), the following study re-analyses the data of a higher tier model ecosystem experiment with Pyriproxyfen 10EC performed by Van Wijngaarden (2004).

Executive Summary

A study was conducted to re-analyse the data of a higher tier model ecosystem experiment with Pyriproxyfen 10EC performed by Van Wijngaarden (2004). This used methods in line with the aquatic effect assessment for plant protection products as described in the new Aquatic Guidance Document (EFSA, 2013). In particular, it investigated the specific criterion that at least eight taxa of the sensitive taxonomic group(s) should be present with an appropriate minimum detectable difference (MDD) to demonstrate potential treatment-related effects.

In total, 24 zooplankton species were identified during the experiment. In addition, ‘total Cladocera’, ‘total Copepoda’ and ‘total Rotifera’ (whose abundances were calculated as the sum of abundances of individual

species within that group) were included in the analysis as additional zooplankton taxa. For 8 taxa the MDD criterion (Category 1) are met. However, 'aggregated groups' should not be counted as a separate potentially sensitive taxa in category 1 if one of the taxa contributing to that group has already been identified as one of the sensitive taxa. This means that only *Cylopoïda*, *Daphnia* gr. *galeata*, *Keratella cochlearis*, *Nauplii* and *Simocephalus vetulus* can be considered as Category 1 taxa. Of these Category 1 taxa, four were arthropods, the sensitive taxonomic group for the insecticide Pyriproxifen. For eight additional taxa, i.e: Ostracoda, *Anureopsis fissa*, *Filinia longiseta*, *Keratella aquadrata*, *Lecane 'senso stricto'*, *Polyarthra remata*, *Trichocerca* gr. *tigris*/gr. *pussila*, and *Trichotria pocillum*, a NOEC could be calculated on at least one sampling date (Category 2 taxa). For the remaining 11 taxa no NOEC could be established at any of the samplings (Category 3 taxa).

Methods

The data sets used in the analysis were taken from a microcosm study on pyriproxyfen: Van Wijngaarden, R.P.A. (April 26, 2004). Pyriproxyfen 10EC: plankton-dominated microcosm experiment (GLP-compliant study). Alterra confidential report ALT.RW.2003.2, Alterra Wageningen University and Research, Wageningen, The Netherlands.

Minimal Detectable Difference (MDD):

The power of a micro-/mesocosm test is the probability of finding treatment-related differences that do exist, as opposed to the likelihood of identifying treatment-related effects that do not exist (which is known as a Type I error or "false positive"). An indicator of the statistical power of a micro-/mesocosm test can be estimated *a posteriori*: viz. the minimum detectable difference (MDD). The MDD defines the mean amount of difference between a treatment and the control that must exist in order to conclude that there is a significant effect. This means that the lower the MDD, the less severe a difference needs to be to result in a significant effect. For the two-sample and multiple t-tests the MDD can be easily calculated by the rearranged formula of the t-test using Eq. 1 or Eq. 2 in cases where either the treatment/control variances, s^2_0 | s^2, or the pooled variance of all treatments, s^2 (≡ residual variance from a one-way ANOVA), are applied, respectively.

Equation 1: $$MDD = (\bar{x}_0 - \bar{x}\ )^* = t_{1-\alpha,df,a}\sqrt{\frac{s_0^2}{n_0} + \frac{s^2}{n}}$$

Equation 2: $$MDD = (\bar{x}_0 - \bar{x})^* = t_{1-\alpha,df,a}s\sqrt{\frac{1}{n_0} + \frac{1}{n}}$$

With: $t_{1-\alpha, df, a}$: quantile of the t-distribution;

df: degrees of freedom;

a: number of comparisons;

$(\bar{x_o} - \bar{x})^*$: corresponding difference between control and treatment mean;

n_0, n: sample sizes

The MDD usually is reported as a percentage of the control mean (MDD% = MDD / $\bar{x_o}$ * 100). Note that, although NOECs are calculated on the basis of Ln transformed data to normalize data, there is still a choice whether MDD% should be calculated on the basis of the Ln transformed abundance data or on the basis of the absolute abundance data. The MDD% based on the Ln transformed data (%MDD ln) are by definition lower than those based on the absolute data (%MDD_{abu}). Although the NOEC calculations are based on the Ln transformed abundance data, in this report the MDD values used in the evaluation of responses of zooplankton and phytoplankton populations are based on absolute abundance data, resulting in stricter requirements for taxa abundance data to be included in effect class derivation.

The resulting MDD values are ranked as follows (EFSA 2013):

Table B.9.3.3/1b-1 Classes of MDD values with associate effect detection for treatment-related declines in abundance/biomass (EFSA, 2013)

Class	MDD	Comment
0	> 100 %	No effects can be determined
I	90–100 %	Only large effects can be determined
II	70–90 %	Large to medium effects can be determined
III	50–70 %	Medium effects can be determined
IV	< 50 %	Small effects can be determined

If for a specific taxon on a specific sampling day the MDD is <100%, in theory a treatment-related decline in abundance can be demonstrated. If the MDD is ≥100%, however, the power of the test is too low to demonstrate treatment-related declines in abundance. However, in some cases of treatment-related increases a statistically significant effect may be demonstrated if the MDD is ≥100%, particularly when the abundance in control test systems is very low.

In the present analysis three categories of organisms were identified on the basis of their MDD, namely:

1. Category 1 taxa: characterized by sufficient statistical power to demonstrate treatment-related declines in abundance. For these taxa the MDD criterion (MDD_{abu} <100% at no less than five

samplings or MDD_{abu} <70% at no less than three samplings or MDD_{abu} <50% at no less than two samplings) is met. This category is used to evaluate the validity of the microcosm studies with respect to the requirement that at least eight taxa of the potentially sensitive taxonomic groups should be present with sufficiently low MDD's.

2. Category 2 taxa: these do not meet the MDD criterion mentioned above but for these taxa a NOEC could be calculated on at least one sampling. The NOECs calculated for both category 1 and category 2 taxa can be used to derive effect classes for RAC derivation.

3. Category 3 taxa: do not meet the MDD criterion mentioned above and no NOEC could be calculated on any of the samplings. These taxa cannot be used to derive effect classes for RAC derivation.

No Observed Effect Concentrations:

NOEC (No Observed Effect Concentration) estimations at taxon level ($p \leq 0.05$) were carried out using the Williams test (ANOVA; Williams, 1972). The analyses were performed with the Community Analysis (CA) computer program v4.3.14 (Hommen *et al.*, 1994), resulting in an overview of NOECs for each sampling day for the data analysed.

Results and Discussion

In total, 24 zooplankton species were identified during the experiment. In addition, 'total Cladocera', 'total Copepoda' and 'total Rotifera' (whose abundances were calculated as the sum of abundances of individual species within that group) were included in the analysis as additional zooplankton taxa. For each of 27 zooplankton taxa MDDs were calculated, and based on these an MDD-category was derived for each of the taxa (Table B.9.3.3/1b-2).

Table B.9.3.3/1b-2 Category taxa classification, NOECs and related % MDD_{abu} (in brackets) for zooplankton taxa. A '-' indicates a NOEC for a decrease, and a '+' for an increase in abundance. Cat. = Category based on MDDs.

		Sampling day relative to treatment										
Cat	Taxa	-8	-1	3	7	14	21	28	35	42	49	56
1	Cladocera (total)	≥20 (64)	≥20 (56)	5- (57)	5- (56)	≥20 (63)	5- (69)	0.32- (57)	≥20 (77)	≥20 (61)	≥20 (57)	≥20 (55)
1	Copepoda (total)	≥20 (70)	≥20 (61)	≥20 (75)	≥20 (71)	≥20 (64)	≤0.02+ (65)	≥20 (70)	≥20 (60)	≥20 (66)	≥20 (85)	≥20 (83)
1	Rotifera (total)	≥20 (82)	≥20 (88)	5+ (90)	5+ (86)	5+ (84)	5+ (101)	≥20 (90)	≥20 (98)	≥20 (87)	≥20 (89)	≥20 (96)
1	Cyclopoidea	≥20 (85)	≥20 (73)	≥20 (79)	≥20 (86)	≥20 (65)	≥20 (81)	≥20 (90)	≥20 (65)	≥20 (89)	≥20 (74)	≥20 (96)
1	Daphnia group galeata	≥20 (82)	≥20 (63)	5- (66)	1.2- (60)	5- (68)	5- (68)	5- (80)	≥20 (84)	≥20 (77)	≥20 (77)	≥20 (78)
1	Keratella cochlearis	≥20 (85)	≥20 (96)	≥20 (95)	≥20 (84)	≥20 (85)	≥20 (103)	≥20 (87)	1.2+ (n.c.)	≥20 (64)	≥20 (99)	≥20 (100)
1	Nauplii	≥20 (100)	≥20 (65)	≥20 (94)	≥20 (89)	≥20 (69)	≥20 (73)	≥20 (73)	≥20 (68)	≥20 (83)	≥20 (93)	≥20 (88)
1	Simocephalus vetulus	≥20 (102)	≥20 (66)	≥20 (91)	≥20 (71)	5+ (80)	≥20 (78)	≥20 (71)	5+ (87)	≥20 (79)	≥20 (84)	≥20 (90)
2	Ostracoda (total)	≥20 (119)	≥20 (97)	≥20 (121)	≥20 (106)	≥20 (158)	≥20 (110)	5- (97)	5- (97)	≥20 (109)	≥20 (118)	≥20 (149)
2	Anureopsis fissa	≥20 (n.c.)	≥20 (153)	0.02+ (n.c.)	5+ (n.c.)	5+ (n.c.)	5+ (n.c.)	5+ (n.c.)	≥20 (133)	≥20 (n.c.)	≥20 (148)	≥20 (n.c.)
2	Filinia longiseta	≥20 (102)			≤0.02- (97)			≥20 (n.c.)				

		Sampling day relative to treatment										
Cat	Taxa	-8	-1	3	7	14	21	28	35	42	49	56
2	Keratella quadrata	5- (98)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	5+ (142)	≥20 (n.c.)	≥20 (151)	≥20 (149)	≥20 (127)	5+ (n.c.)	5+ (135)
2	Lecane senso stricto				≥20 (n.c.)		≤0.02- (96)	≥20 (135)	≥20 (107)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)
2	Polyarthra remata	≥20 (n.c.)	≥20 (107)	5+ (154)	5+ (129)	5+ (125)	5+ (150)	5+ (n.c.)	5+ (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)
2	Trichocerca gr tigris/gr pusilla	≥20 (n.c.)		≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (128)	≥20 (101)	≥20 (100)	5- (99)	≥20 (101)	≥20 (101)
2	Trichotria pocillum		≥20 (n.c.)	≥20 (n.c.)		≥20 (n.c.)			≤0.02- (96)		≤0.02- (97)	≥20 (n.c.)
3	Alona affinis		≥20 (134)	≥20 (n.c.)	≥20 (221)	≥20 (184)	≥20 (143)	≥20 (n.c.)	≥20 (n.c.)	≥20 (112)	≥20 (143)	≥20 (n.c.)
3	Alona rectangula		≥20 (n.c.)		≥20 (232)	≥20 (213)	≥20 (134)			≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)
3	Bosmina longispina	≥20 (95)	≥20 (n.c.)	≥20 (n.c.)		≥20 (n.c.)			≥20 (n.c.)		≥20 (n.c.)	
3	Brachionus angularis							≥20 (n.c.)				
3	Calanoidea	≥20 (88)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (152)	≥20 (n.c.)	≥20 (166)
3	Cephalodella gibba										≥20 (n.c.)	≥20 (n.c.)
3	Ceriodaphnia sp.	≥20 (n.c.)										

		Sampling day relative to treatment										
Cat	Taxa	-8	-1	3	7	14	21	28	35	42	49	56
3	Lecane gr monostyla								≥20 (n.c.)		≥20 (n.c.)	≥20 (n.c.)
3	Lepadella patella				≥20 (n.c.)							
3	Synchaeta spp			≥20 (n.c.)	≥20 (n.c.)		≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)	≥20 (n.c.)		
3	Trichocerca longiseta	≥20 (124)	≥20 (n.c.)							≥20 (n.c.)		

For 8 taxa the MDD criterion (Category 1) are met. However, it should be noted that 'aggregated groups' (i.e. total cladocera, total copepoda, and total rotifera) should not be counted as a separate potentially sensitive taxa in category 1 if one of the taxa contributing to that group has already been identified as one of the sensitive taxa. This means that only *Cylopoïda*, *Daphnia* gr. *galeata*, *Keratella cochlearis*, *Nauplii* and *Simocephalus vetulus* can be considered as Category 1 taxa.

For eight additional taxa, i.e: Ostracoda, *Anureopsis fissa*, *Filinia longiseta*, *Keratella aquadrata*, *Lecane 'senso stricto'*, *Polyarthra remata*, *Trichocerca* gr. *tigris*/gr. *pussila*, and *Trichotria pocillum*, a NOEC could be calculated on at least one sampling date (Category 2 taxa).

For the remaining 11 taxa no NOEC could be established at any of the samplings (Category 3 taxa).

Conclusions

Of the 24 potentially sensitive zooplankton species present, five i.e. *Cylopoïda*, *Daphnia* gr. *galeata*, *Keratella cochlearis*, *Nauplii* and *Simocephalus vetulus* are classified as Category 1 taxa based on the MDD analysis. Of these Category 1 taxa, four were arthropods, the sensitive taxonomic group for the insecticide Pyriproxifen. For eight additional taxa, i.e: Ostracoda, *Anureopsis fissa*, *Filinia longiseta*, *Keratella quadrata*, *Lecane 'senso stricto'*, *Polyarthra remata*, *Trichocerca* gr. *tigris*/gr. *pussila*, and *Trichotria pocillum*, a NOEC could be calculated on at least one sampling date (Category 2 taxa). For the remaining 11 taxa no NOEC could be established at any of the samplings (Category 3 taxa).

Comments by RMS

The calculations are acceptable.

4.5.2. CA 8.1.4/01 Pyriproxyfen – Amphibian Metamorphosis Assay with African Clawed Frog (Xenopus laevis) Following OPPTS Test Guideline 890.1100 and OECD Test Guideline 231

Study reference:

CA 8.1.4/01 (2012) Pyriproxyfen – Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*) Following OPPTS Test Guideline 890.1100 and OECD Test Guideline 231. Sumitomo Chemical Co., Ltd. Unpublished report No.: NNW-0211

Guidelines

U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program Test Guidelines 890.1100, Amphibian Metamorphosis (Frog) (U.S. EPA, 2009) and OECD Guideline for Testing of Chemicals No. 231, Amphibian Metamorphosis Assay (OECD, 2009)

Deviations: Daily recording of temperature extremes determined that the maximum temperature reading (24 ºC) exceeded the range indicated in the protocol on exposure days 1 (23.5 ºC), 5 (23.6 ºC), 7 (23.6 ºC), 11 (23.6 ºC), 13 (23.9 ºC), 15 (23.5 ºC), 18 (23.5 ºC), 19 (23.5 ºC) and 20 (23.5 ºC). Daily test solution measurements were maintained within the specified range (22 ± 1ºC), indicating that these slight temperature deviations were not observed for an extended period of time. Hind limb length on day 7 was measured on the right side (instead of the left) for 5 tadpoles. These deviations were not considered to have had a negative impact on the results of the study.

GLP

Yes (certified laboratory)

Executive Summary

The Amphibian Metamorphosis Assay (AMA) is a screening assay intended to empirically identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. The AMA represents a generalized vertebrate model to the extent that is based on the conserved structures and function of the HPT axis. South African clawed frog (*Xenopus laevis*) tadpoles at stage 51 and 13 days old post fertilization at exposure initiation were exposed to technical pyriproxyfen in a 21-day AMA screening assay at nominal concentrations of 3.0, 30 and 300 µg a.s./L, together with dilution water and solvent controls. The endpoints evaluated were developmental stage, snout-vent length (SVL), hind limb length, hind limb length (normalized by SVL), whole body wet weight and thyroid gland histology.

Results of the test solution analyses showed that the measured concentrations decreased between sampling intervals; however the expected concentration gradient was maintained. The losses observed at each sampling interval appeared to be relatively similar across the dose range and were attributed to the increasing biomass and the increasing feed rate throughout the exposure period. Mean measured concentrations ranged from 57 to 75% of the nominal levels and defined the treatment levels tested as 1.7, 23 and 210 µg a.s./L. Pyriproxyfen was found to significantly decrease 7-day and 21-day hind limb length at 23 and 210 µg a.s./L; 7-day hind limb length (normalized by SVL) at 1.7, 23 and 210 µg a.s./L; and 7-day developmental stage, snout-vent length and wet weight at 210 µg a.s./L. Significant reductions were also observed in 21-day developmental stage, 21-day wet weight, 21-day snout-vent length, 21-day hind-limb length (normalized by SVL).

Effects on thyroid gland histopathology were not observed at any treatment level. Based on the reduction in developmental stage, and hind-limb length (normalized by SVL), pyriproxyfen did not result in advanced development of the African clawed frog. Additionally, asynchronous development was not observed during this exposure, based on developmental stage criteria. Effects on thyroid gland histopathology were not observed at any treatment level. Delayed development, based on developmental stage, hind-limb length, wet weight and snout-vent length was indicated and together with observations of reduced food consumption in the high treatment level, indicated overt morbity or general toxicity of pyriproxyfen to the African clawed frog. It was concluded that pyriproxyfen may not interact with the thyroid hormonal system.

Materials and Methods

Materials

1. Test Material:	Pyriproxyfen
Description:	Not stated
Lot/Batch #:	080506G
Purity:	99.5%
Expiry date:	December 17, 2012
2a. Test animals:	Stage 51 *Xenopus laevis* tadpoles were used to initiate the amphibian metamorphosis assay. The tadpoles originated from adult brood stock maintained at the test facility. The brood stock was originally obtained from ~~Nasco, Fort Atkinson, Wisconsin~~ and maintained in-house for > 14 days prior to use. It did not show any sign of sickness, disease, injuries or abnormalities from the day of receipt to the day of pre-exposure initiation.
2b. Maintenance:	The adult frogs were maintained in a closed-loop re-circulating culture unit. The culture water was FETAX solution and was characterized as having a dissolved oxygen range of 6.0 to 7.1 mg/L, a pH range of 6.9 to 7.2, a conductivity range of 1960 to 1990 µmhos/cm, a total hardness range of 180 to 200 mg/L as $CaCO_3$, and a total alkalinity range of 40 to 56 mg/L as $CaCO_3$. Brood stock and test organisms were cultured and

tested in FETAX solution from the same source. The culture unit was maintained in a temperature controlled room which maintained water temperature at 20 to 22 °C with a photoperiod of 12 hours of light and 12 hours of darkness.

Study Design

The Amphibian Metamorphosis Assay (AMA) is a screening assay intended to empirically identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. An AMA screening assay was conducted with pyriproxyfen and the South African clawed frog (*Xenopus laevis*). The endpoints were developmental stage, snout-vent length, hind limb length, hind limb length (normalized by SVL), whole body wet weight and thyroid gland histology.

The metamorphosis assay was conducted using an exposure system consisting of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 20 exposure aquaria. The exposure system was designed to provide three concentrations of the test substance (nominally 3.0, 30 and 300 µg a.s./L), a solvent control and a dilution water control to four replicate test aquaria each. FETAX solution was used as dilution and control water during this study. The exposure system was constructed of glass, silicone sealant, stainless steel, and Teflon®. Each 2.5-gallon exposure aquaria measured 30 x 14.5 x 20 cm with a 14.5-cm high side drain that maintained a constant exposure solution volume of approximately 6.5 L. During the study, the diluter delivered the control and test solutions to the exposure aquaria at a rate sufficient to provide approximately 6.1 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 9 hours.

On pre-exposure day 0, three pairs of adult male and female *X. laevis* were induced to breed. Once all tadpoles were at feeding stage (day 5 post fertilization), larvae from the highest quality spawn were transferred to nine, 10-L rearing tanks. On pre-exposure day 13 (day 13 post-fertilization) a sufficient number of tadpoles had reached stage 51 and so the exposure initiation process began. During the pre-exposure and in-life exposure periods, tadpoles were fed Xenopus Express Tadpole Food, a commercially available diet that is suitable for normal growth and development of *X. laevis* tadpoles. The daily feeding rates were increased as the study progressed to account for growth.

Dissolved oxygen concentration, pH and temperature measurements were taken in all vessels on day 0 and in alternating replicates daily thereafter. Total hardness, total alkalinity and specific conductance were measured in one replicate of the control, low and high test concentrations on day 0 and in alternating replicates weekly thereafter. Test solution temperature was continuously monitored in one of the solvent control. During the in-life phase of the definitive study, water samples were removed from each treatment level and control on test days 0, 8, 9, 10, 15 and 21. At exposure initiation (day 0), samples were removed from each replicate of each treatment and control solution and from alternating replicates weekly thereafter. Samples were removed on test day 9 due to inconsistent recoveries with the day 8 sampling. On test day 10, samples were removed from each replicate of the low concentration (3.0 µg a.s./L, nominal) for confirmation of lower than expected recoveries obtained in this treatment level on day 9.

All test vessels were examined daily for survival and behavioural assessment. On day 7, five tadpoles were removed randomly from each test vessel and euthanized. Developmental stage was then determined for each tadpole using a binocular dissection microscope. Each tadpole was then blotted dry prior to body weight determination to the nearest 0.1 mg. Digital photographs were taken of each tadpole for snout-vent length and hind limb length measurements. At test termination (day 21), the remaining tadpoles were removed from the test vessels and euthanized. Digital images were then taken of each tadpole for snout-vent length and hind limb length measurements. Developmental stage was then determined for each tadpole using a binocular dissection microscope. Each tadpole was then blotted dry and weighed to the nearest 0.1 mg. For histological analyses, a total of five tadpoles were selected from each replicate test concentration.

Results and Discussion

During this study, all validity criteria were met: For any given treatment (including controls), mortality should not exceed 10%, while for any given replicate, mortality should not exceed three tadpoles; mortality of no more than two tadpoles per replicate in the control group should occur; at least two treatment levels, with all

four uncompromised replicates, should be used for analysis; at least two treatment levels without overt toxicity should be available for analysis.

Physical and Chemical Measurements of Water

During the study, daily measurements of the test solutions established a dissolved oxygen concentration range of 3.6 to 8.8 mg/L (42 to 100% of saturation). Continuous temperature monitoring in one replicate of the solvent control demonstrated that the temperature ranged from 22 to 24 ºC throughout the exposure, while daily temperature monitoring established a range of 22 to 23 ºC. Measurements of pH ranged from 7.2 to 8.2. Weekly characterization of the high, low and control solutions established total hardness and alkalinity ranges as $CaCO_3$ of 140 to 160 mg/L and 74 to 80 mg/L, respectively, and a specific conductance range of 1600 to 1800 µmhos/cm. The measurements established that the water quality parameters maintained throughout the 21-day exposure remained within an acceptable range for the promotion of tadpole survival and growth.

Measurement of Test Concentrations

Results of the diluter stock solution analysis resulted in recoveries ranging from 96 to 120% of nominal concentration. Results of the test solution analyses showed that the measured concentrations decreased between sampling intervals; however the expected concentration gradient was maintained. The losses observed at each sampling interval appeared to be relatively similar across the dose range and were attributed to the increasing biomass and the increasing feed rate throughout the exposure period. The coefficient of variation (CV) for the low (30%) and mid dose (26%) slightly exceeded the guideline recommended of > 20%, which was attributed to the biomass in each vessel. On test day 8, sample recoveries were highly variable and inconsistent with previous analyses, which was attributed to a processing error so that the exposure system was re-sampled on day 9. The results from the day 9 sampling demonstrated that the 3.0 µg a.s./L nominal treatment level recoveries were lower than expected. On test day 10, samples were removed from each replicate of the low concentration (3.0 µg a.s./L, nominal) for confirmation of lower than expected recoveries obtained in this treatment level on day 9. Mean measured concentrations ranged from 57 to 75% of the nominal levels and defined the treatment levels tested as 1.7, 23 and 210 µg a.s./L.

Mortality and Biological Observations

Following 21 days of exposure, no mortality was observed in either the control, solvent control, or any treatment level. Beginning on test day 6, tadpoles exposed to the 210 µg a.s./L treatment level were observed to be smaller in size compared to the control. The tadpoles at the 210 µg a.s./L treatment level also exhibited reduced food consumption throughout the exposure. Beginning on test day 13, two tadpoles exposed to the 1.7 µg a.s./L treatment level were observed to be deformed (e.g., spinal curvature).

Whole Body Wet Weight

The median day 7 wet weight for tadpoles exposed to the control and solvent control averaged 0.5615 and 0.5212 g, respectively (not significantly different, pooled control = 0.5413 g). The median day 7 wet weight for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 0.5874, 0.4976 and 0.1557 g, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 210 µg a.s./L. The median day 21 wet weight for tadpoles exposed to the control and solvent control averaged 1.4979 and 1.5894 g, respectively (not significantly different, pooled control = 1.5436 g). The median day 21 wet weight for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 1.7206, 1.4406 and 0.3994 g, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 210 µg a.s./L.

Snout-Vent Length (SVL)

The median day 7 snout-vent length for tadpoles exposed to the control and solvent control averaged 20.72 and 19.47 mm, respectively (not significantly different, pooled control = 20.09 mm). The median day 7 snout-vent length for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 20.69, 19.72 and 13.35 mm, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 210 µg a.s./L. The median day 21 snout-vent length for tadpoles exposed to the control and solvent control averaged 27.02 and 27.56 mm, respectively (not significantly different, pooled control = 27.29 mm). The median day 21 snout-vent length for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 28.27, 26.47

and 18.75 mm, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 210 µg a.s./L.

Hind Limb Length

The median day 7 hind limb length for tadpoles exposed to the control and solvent control averaged 3.06 and 2.95 mm, respectively (not significantly different, pooled control = 3.00 mm). The median day 7 hind limb length for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 2.74, 2.67 and 1.43 mm, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 23 and 210 µg a.s./L. Day 21 hind limb length for tadpoles exposed to the control and solvent control averaged 22.68 and 23.21 mm, respectively (not significantly different, pooled control = 22.94 mm). Day 21 hind limb length for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 22.54, 21.10 and 3.44 mm, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 23 and 210 µg a.s./L.

Hind Limb Length Normalized by Snout-Vent Length

The median day 7 hind limb length, normalized by SVL, for tadpoles exposed to the control and solvent control averaged 0.15 and 0.15 mm, respectively (not significantly different, pooled control = 0.15 mm). Day 7 hind limb length, normalized by SVL, for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 0.13, 0.14 and 0.11 mm, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 1.7, 23 and 210 µg a.s./L. Day 21 hind limb length, normalized by SVL, for tadpoles exposed to the control and solvent control averaged 0.82 and 0.83 mm, respectively (not significantly different, pooled control = 0.82 mm). The median day 21 hind limb length, normalized by SVL, for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 0.78, 0.77 and 0.18 mm, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 210 µg a.s./L.

Developmental Stage

The median day 7 developmental stage for tadpoles exposed to the control and solvent control both averaged 54. The median day 7 developmental stage for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 54, 54 and 52, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 210 µg a.s./L. The median day 21 developmental stage for tadpoles exposed to the control and solvent control both averaged 60. The median day 21 developmental stage for tadpoles exposed to 1.7, 23 and 210 µg a.s./L averaged 60, 60 and 55, respectively and this was significantly different compared to the pooled control (Jonckheere-Terpstra Step-Down Test, 95% level) at 210 µg a.s./L.

Thyroid Histology

All tissues submitted from this exposure were examined via histopathology and no clear treatment related lesions were identified.

Conclusions

Pyriproxyfen was found to significantly decrease 7-day and 21-day hind limb length at 23 and 210 µg a.s./L; 7-day hind limb length (normalized by SVL) at 1.7, 23 and 210 µg a.s./L; and 7-day developmental stage, snout-vent length and wet weight at 210 µg a.s./L. Significant reductions were also observed in 21-day developmental stage, 21-day wet weight, 21-day snout-vent length, 21-day hind-limb length (normalized by SVL).

Effects on thyroid gland histopathology were not observed at any treatment level. Mild follicular cell hypertrophy or mild follicular cell hyperplasia was diagnosed in 15 – 30% of the animals from the control and at 1.7 and 23 µg a.s./L. No animals in the 210 µg a.s./L exhibited follicular cell hypertrophy. Given the consistency in histologic appearance between the 210 µg a.s./L treatment group and stage-matched controls, the discrepancy in the incidence of follicular cell hyperplasia was attributed to differences in growth patterns at different developmental stages.

According to the guidelines, endpoints associated with advanced development, asynchronous development, and thyroid histopathology are weighted heavily because they are clearly linked with interactions with the hypothalamus-pituitary-thyroid (HPT) axis. However, delayed development endpoints, which are characterized as decreases in development stage, hind limb length, snout-vent length and wet body weight, are parameters that can potentially be affected by general toxicity, and are weighted less heavily especially when they are correlated with observations of overt morbidity. Based on the reduction in developmental stage, and hind-limb length (normalized by SVL), pyriproxyfen did not result in advanced development of the African clawed frog. Additionally, asynchronous development was not observed during this exposure, based on developmental stage criteria. Effects on thyroid gland histopathology were not observed at any treatment level. Delayed development, based on developmental stage, hind-limb length, wet weight and snout-vent length was indicated and together with observations of reduced food consumption in the high treatment level, indicated overt morbidity or general toxicity of pyriproxyfen to the African clawed frog. It was concluded that pyriproxyfen may not interact with the thyroid hormonal system.

Comments by RMS

The summary above was prepared by the applicant. Some additions need to be made for clarification. The evaluation by the RMS is also presented below.

The test was started with 4 replicates per treatment, each containing 20 tadpoles. For histological assessment, tadpoles were selected as described in the OECD 231 test guideline. For the highest test concentration, an additional group of five control animals that were stage matched to those of the treatment were established. The study was conducted in agreement with the OECD 231 guideline, and the validity criteria were all met. The test guideline contains a list of performance criteria, which can be used as guidance for determining the quality of the test performed and the performance of the control organisms. Three out of ten criteria were not met, but these are not considered to invalidate the test:

1. Test concentrations were not maintained at ≤20% CV over the test period. This was attributed to the increasing biomass by the study author. The concentration gradient was however maintained and therefore the excess of the CV at the two lowest test concentrations (30 and 26%, respectively) is not considered to affect the outcome of the study.
2. The inter-replicate/inter-treatment differentials in pH should not exceed 0.5. The report did not contain detailed information on pH levels per measurement, but only ranges for the entire test period (7.2-8.2 for the control, the solvent control and the lowest test concentration; 7.2-8.0 for the second concentration and 7.3-7.9 for the highest concentration). The differentials in pH within treatments thus exceeded 0.5, but pH levels were very similar across treatments and are therefore not considered to have affected the outcome of the study.
3. Inter-replicate/inter-treatment water temperature differentials should not exceed 0.5°C. The report did not contain detailed information on temperature per measurement, but only ranges for the entire test period (22-23°C for all treatments). The differentials within replicates may thus have exceeded 0.5°C, but as temperatures were similar across treatments, this is not considered to have affected the outcome of the study.

The study is considered valid and reliable.

Results are shown in the Table below. The reported statistical analysis was based on comparison of median values per replicate for all apical endpoints, whereas the test guideline indicates that mean values should be used for body wet weight, SVL, HLL and HLL normalized by SVL. Therefore, statistical analysis based on means per replicate was conducted by the RMS, using the step-down Jonckheere-Terpstra test procedure as indicated in the OECD guideline, since the data were all consistent with a monotone dose-response. As there were no statistically significant differences between the control and the solvent control for any of the tested parameters, the treatments were compared to the pooled control. The resulting NOECs were similar to those found by the author of the report.

Table CA 8.1.4/01-1 Results for apical endpoints

	Developmental stage [a)]	**Snout-vent length (mm)** [b)]	**Hindlimb length (mm)** [b)]	**Normalized hindlimb length** [b)]	**Whole body wet weight (g)** [b)]
Day 7					
Control	54	20.32	2.96	0.14	0.5442
Solvent control	54	19.37	2.84	0.15	0.5014
Pooled control	54	19.84	2.95	0.15	0.5228
1.7 µg a.s./L	54	20.74	2.75	0.13 *	0.5945
23 µg a.s./L	54	19.47	2.62 *	0.13 *	0.5070
210 µg a.s./L	52 *	13.49 *	1.48 *	0.11 *	0.1698 *
NOEC	23 µg a.s./L	23 µg a.s./L	1.7 µg a.s./L	<1.7 µg a.s./L	23 µg a.s./L
Day 21					
Control	60	26.59	21.36	0.84	1.5078
Solvent control	60	27.23	22.20	0.85	1.5921
Pooled control	60	26.91	21.78	0.84	1.5499
1.7 µg a.s./L	60	27.42	21.74	0.82	1.6330
23 µg a.s./L	60	26.16	20.33 *	0.80	1.4509
210 µg a.s./L	55 *	18.96 *	3.700 *	0.19 *	0.4271 *
NOEC	23 µg a.s./L	23 µg a.s./L	1.7 µg a.s./L	23 µg a.s./L	23 µg a.s./L

* Significantly different from pooled control at 5% level

a) Mean of medians per replicate

b) Mean of means per replicate

Effects on thyroid gland histopathology were not observed at any treatment level. Mild follicular cell hypertrophy or mild follicular cell hyperplasia was diagnosed in 15 – 30% of the animals from the control, the solvent control and at 1.7 and 23 µg a.s./L. No animals in the 210 µg a.s./L exhibited follicular cell hypertrophy and only two animals exhibited follicular cell hyperplasia (one from the 210 µg a.s./L group and one from the stage-matched controls). Given the consistency in histologic appearance between the 210 µg a.s./L treatment group and stage-matched controls, the discrepancy in the incidence of follicular cell hyperplasia was attributed to differences in growth patterns at different developmental stages. The histological results are summarised in the table below. There were no indications of pyriproxyfen-induced changes in thyroid histology.

Table CA 8.1.4/01-02 Histological findings in thyroid (number of animals in which the finding was recorded)

Finding	Control	Solvent control	1.7 µg a.s./L	23 µg a.s./L	210 µg a.s./L	Stage-matched control
Number of animals assessed (*n*)	20	20	20	20	20	5
Thyroid gland hypertrophy	1	2	1	0	0	1
Thyroid gland atrophy	0	1	0	0	0	0
Follicular cell hypertrophy	5	4	5	6	0	0
Follicular cell hyperplasia	5	4	6	3	1	1
Increased follicular lumen area	2	1	1	2	1	0
Decreased follicular lumen area	0	0	0	0	0	1
Changes in colloid quality	1	0	0	0	0	0
Lymphocytic inflammation	1	0	0	0	0	0
Pharyngeal epithelial necrosis	7	10	10	10	0	0

Based on the lack of advanced development, asynchronous development or changes in thyroid histology, it is concluded that pyriproxyfen was thyroid inactive. The NOEC from the present study was 1.7 µg a.s./L, based on reduced hindlimb length at and above 23 µg a.s./L.

Reliability of endpoints

The current study does not allow for the calculation of ECx values. According to Regulation (EU) No 283/2013, the statistical power of the NOEC shall be assessed in case no ECx values can be derived. However, this is not possible as long as default type I and II errors have not been fixed, default variance for any response variable have not been established and typical detectable effect sizes of each parameter have not been calculated (EFSA Supporting publication 2015:EN-924). Considering the level of effect on which the NOEC was based (i.e. a 7% reduction in hindlimb length, compared to the pooled control at the next higher level) and the complete absence of effects at the level of the NOEC, the NOEC is considered sufficiently protective.

The NOEC of pyriproxyfen in an amphibian metamorphosis assay was 1.7 µg a.s./L and may be used for risk assessment. Pyriproxyfen was concluded to be thyroid inactive.

Reference list

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 5.1.1/01		1998a	Metabolism of S-31183 in rats Company Report No: NNM-80-0001 Sumitomo Chemical Co Ltd GLP, Unpublished.	Y	SUM
CA 5.1.1/02		1988b	Metabolism of S-31183 in rats (Tissue distribution study) Company Report No: NNM-80-0002 Sumitomo Chemical Co Ltd., Not GLP, Unpublished	Y	SUM
CA 5.1.1/03		1993a	Metabolism of phenoxyphenyl-^{14}C-pyriproxyfen in rats (high-dose, ^{14}C-concentrations in tissues) Company Report No: NNM-30-0028 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.1.1/04		1993b	Metabolism of [pyridyl-2,6-^{14}C]pyriproxyfen in rats (pyridyl-^{14}C-labeled test compound, single oral administration at low- and high doses) Company Report No: NNM-30-0025 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.1.1/05		1995	Metabolism of pyriproxyfen 2. Comparison of *in vivo* metabolism between rats and mice J. Agric. Food Chem. 43, 2681-2686 Not GLP, Published	Y	
CA 5.2.1/01		1987a	Acute oral toxicity of S-31183 in rats Company Report No: NNT-70-0005 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.2.1/02		1987b	Acute oral toxicity of S-31183 in mice Company Report No: NNT-70-0014 Sumitomo Chemical Co., Ltd GLP unpublished	Y	SUM
		1993	Addendum to the final report: Acute oral toxicity of S-31183 in mice Company Report No: NNT-70-0110	Y	SUM

			Sumitomo Chemical Co., Ltd GLP unpublished		
CA 5.2.1/03		1986	Acute oral toxicity of S-31183 in dogs Company Report No: NNT-60-0012 Sumitomo Chemical Co., Ltd. GLP, Unpublished	Y	SUM
CA 5.2.2/01		1987c	Acute dermal toxicity of S-31183 in rats Company Report No: NNT 70-0006 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.2.2/02		1987d	Acute dermal toxicity of S-31183 in mice Company Report No: NNT-70-0015 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
		1994	Amendment to the final report: Acute dermal toxicity of S-31183 in mice Company Report No: NNT-70-0111 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.2.3/01		1987	Acute inhalation toxicity of S-31183 in rats Company Report No: NNT-70-0022 Sumitomo Chemical Co Ltd., GLP, Unpublished	Y	SUM
CA 5.2.3/02		1987e	Acute inhalation toxicity of S-31183 in mice Company Report No: NNT-70-0023 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
		1995	Addendum to report: Acute inhalation toxicity of S-31183 in mice Company Report No: NNT-50-0131 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.2.4/01, CA 5.2.5/01		1987f	Primary eye and skin irritation tests with S-31183 in rabbits Company Report No: NNT-70-0004 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.2.6/01		1987g	Skin sensitization test with S-31183 in guinea pigs Company Report No: NNT-70-0003 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
		1995	Amendment of Report: Skin sensitization test with S-31183 in guinea pigs Company Report No: NNT-50-0130 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM

CA 5.3.1/01		1988a	One-month oral toxicity study of S-31183 in rats Company Report No: NNT-80-0038 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.3.1/02		1987	Four-week oral toxicity study of S-31183 in dogs Company Report No: NNT-70-0013 Sumitomo Chemical Co., Ltd GLP, Unpublished	Y	SUM
CA 5.3.2/01		1989	Sub-chronic toxicity study with S-31183 in rats Company Report No: NNT-91-0045 Sumitomo Chemical Co Ltd., GLP Unpublished	Y	SUM
CA 5.3.2/02		1988b	Six-month chronic oral toxicity study of S-31183 in rats Company Report No: NNT-80-0039 Sumitomo Chemical Co Ltd., GLP, Unpublished	Y	SUM
CA 5.3.2/03		1988	Three-month oral toxicity study of S-31183 in dogs Company Report No NNT-80-0037 Sumitomo Chemical Co., Ltd. GLP, Unpublished	Y	SUM
CA 5.3.2/04		1991	S-31183: Toxicity study by oral (capsule) administration to Beagle dogs for 52 weeks. Amended final report Company Report No. NNT-11-0081 LSR report no.: 91/0776 Life Science Research Limited, UK GLP, Unpublished	Y	SUM
CA 5.3.2/05		1993	S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks (additional investigation) Company Report No: NNT-31-0102 Sumitomo Chemical Co., Ltd. Report No. NNT-31-0102 GLP, Unpublished	Y	SUM
CA 5.3.3/01		1993	21-day dermal toxicity study in rats with S-31183 Company Report No: NNT-31-0094 Sumitomo Chemical Co Ltd., GLP, Unpublished	Y	SUM
CA 5.3.3/02		1988	Sub-acute inhalation toxicity study of S-31183 in rats Company Report No: NNT 80-0031 Sumitomo Chemical Co Ltd., GLP, Unpublished	Y	SUM

CA 5.4.1.1/01		1988	Reverse mutation test of S-31183 in bacterial systems Company Report No: NNT-80-0034 Sumitomo Chemical Co Ltd., GLP, Unpublished	N	SUM
CA 5.4.1.2/01		1989	*In vitro* chromosomal aberration test of pyriproxyfen in Chinese hamster ovary cells (CHO-K1) Company Report No. NNT-80-0054 Biochemistry and Toxicology Laboratory, Japan GLP, Unpublished	Y	SUM
CA 5.4.1.3/01		1990	*In vitro* gene mutation test of S-31183 in V79 Chinese hamster cells Company Report No. NNT-90-0067 Biochemistry and Toxicology Laboratory, Japan GLP, Unpublished	N	SUM
CA 5.4.1.3/02		1988	Assessment of unscheduled DNA repair synthesis in mammalian cells after exposure to S-31183 Company Report No: NNT-91-0053 Sumitomo Chemical Co Ltd.,	Y	SUM
CA 5.4.2/01		1991	Mouse micronucleus test on S-31183 Company Report No: NNT-11-0082 Sumitomo Chemical Co Ltd GLP, Unpublished	Y	SUM
CA 5.5.1/01		1991a	Combined chronic toxicity and oncogenicity study in rats with S-31183 Company Report No: NNT-11-0085 Sumitomo Chemical Co Ltd., GLP, Unpublished	Y	SUM
		1994	Addendum to the final report: Combined chronic toxicity and oncogenicity study in rats with S-31183 Company Report No. NNT-41-0112 Hazelton Washington, Inc., USA GLP, Unpublished	Y	SUM
		1994	Amendment 1 & 2 to the final report: Combined chronic toxicity and oncogenicity study in rats with S-31183 Company Report No. NNT-41-0113 Hazelton Washington report no.: 343-214 Hazelton Washington, Inc., USA GLP, Unpublished	Y	SUM
CA 5.5.1/02		1991b	Oncogenicity study in mice with S-31183 Company Report No: NNT-11-0084 Sumitomo Chemical Co Ltd., GLP, Unpublished	Y	SUM

		1994	Amendment to the final report: Oncogenicity study in mice with S-31183 Company Report No. NNT-41-0117 Hazelton Laboratories America, Inc., USA GLP, Unpublished	Y	SUM
		1994	Supplemental data and review of oncogenicity study with S-31183 (Sumilarv) in mice Company Report No. NNT-41-0116 Hazelton Laboratories America, Inc., USA GLP, Unpublished	Y	SUM
CA 5.6.1/01		1991	A dietary 2-generation (1 litter) reproduction study of S-31183 in the rat. Company Report No: NNT-11-0087 Sumitomo Chemical Co Ltd GLP, Unpublished	Y	SUM
CA 5.6.1/02		1988a	Study by orally administration of S-31183 to rats prior to and in the early stages of pregnancy Company Report No: NNT-81-0036 Sumitomo Chemicals Co Ltd., GLP, Unpublished	Y	SUM
CA 5.6.2/01		1988b	Perinatal and postnatal study of S-31183 orally administered to rats Company Report No:NNT-80-0030 Sumitomo Chemical Co., Ltd. GLP, Unpublished	Y	SUM
CA 5.6.2/02		1988c	Study by administration of S-31183 during the period of fetal organogenesis in rats Company Report No NNT 80-0029 Sumitomo Chemical Co., Ltd. GLP, Unpublished	Y	SUM
CA 5.6.2/03		1988	Study of S-31183 by oral administration during the period of fetal organogenesis in rabbits Company Report No: NNT 80-0033 Sumitomo Chemical Co Ltd GLP, Unpublished	Y	SUM
CA 5.7.1/01		2010	An oral (gavage) dose range-finding acute neurotoxicity study of pyriproxyfen T.G. in rats Company Report No. NNT-0181 WIL Research Laboratories, LLC, USA GLP, Unpublished	Y	SUM
CA 5.7.1/02		2011a	An oral (gavage) acute neurotoxicity study of pyriproxyfen T.G. in rats Company Report No. NNT-0194	Y	SUM

			WIL Research Laboratories, LLC, USA GLP, Unpublished		
CA 5.7.1/03		2011b	An 90-day oral dietary neurotoxicity study of pyriproxyfen T.G. in rats Company Report No. NNT-0202 WIL Research Laboratories, LLC, USA GLP, Unpublished	Y	SUM
CA 5.8.2/01		2011	Pyriproxyfen: 4 week dietary immunotoxicity study in the female CD-1 mouse Company Report No. NNT-0204 Huntingdon Life Sciences, UK GLP, Unpublished	Y	SUM
CA 5.8.3/01		2012a	A pubertal development and thyroid functions assay of pyriproxyfen T.G. administered orally in intact juvenile/peripubertal male rats Company Report No. NNT-0210 WIL Research Laboratories, LLC, USA GLP, Unpublished	Y	SUM
CA 5.8.3/02		2012b	A pubertal development and thyroid function assay of pyriproxyfen T.G. administered orally in intact juvenile/peripubertal female rats Company Report No. NNT-0211 WIL Research Laboratories, LLC, USA GLP, Unpublished	Y	SUM

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study** **Y/N**	**Owner**
CA 7.2.1.1/01	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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 7.2.1.2/03	Ponte, M.	2015	Determination of the quantum yield of [^{14}C]pyriproxyfen in pH 7 buffer solution under artificial sunlight Sumitomo Chemical Co., Ltd, report No.: NNM-0087 PTRL West GLP, Unpublished	N	SUM
CA 7.2.2.1/01	Itoh, K., Tanoue, A., Matsuda, T.	1988	Biotic degradation of 1-(4-phenoxyphenoxy)-2-(2-pylidyloxy)propane (code name: S-31183) by activated sludge Sumitomo Chemical Co., Ltd. Report No. NNM-0064 GLP, Unpublished	N	SUM
CA 7.2.2.2/01	Adam, D.	2015	[^{14}C] Pyriproxyfen – aerobic mineralisation in surface water – simulation biodegradation test Sumitomo Chemical Co., Ltd, report No.: NNM-0086 Innovative Environmental Services (IES) Ltd GLP, Unpublished	N	SUM
CA 7.2.2.3/01	Lewis, C.J.	2000a	(^{14}C)-Pyriproxyfen: Degradation and retention in water-sediment systems Sumitomo Chemical Co., Ltd. Report No. NNM-0076 Covance Laboratories Ltd GLP, Unpublished	N	SUM
CA 7.2.2.4/01	Lewis, C.J.	2003	[^{14}C]Pyriproxyfen: Degradation in a water-sediment system in the light. Sumitomo Chemical Co., Ltd., Unpublished report No.: NNM-0081 Covance Laboratories Ltd GLP, Unpublished **Study provided at the request of the RMS** PREVIOUSLY SUBMITTED		

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 7.2.2.3/03	Cooke, J.	2016b	Pyriproxyfen: kinetic assessment of water/sediment studies Sumitomo Chemical Co., Ltd, report No.: NNM-0096 JSC International Limited Not GLP, Unpublished	N	SUM
CA 7.3/04	Yoshida, M., Kodaka, R., Fujisawa, T.	2013	Stability of pyriproxyfen in air (calculation by Atkinson's method) Sumitomo Chemical Co., Ltd, report No.: NNP-0120 Not GLP, Unpublished	N	SUM