Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether

EC Number: -
CAS Number: 179101-81-6

CLH-O-0000006864-64-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 October 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether

EC Number: -

CAS Number: 179101-81-6

Index Number: not allocated

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 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIDALYL (ISO); 2,6-DICHLORO-4-(3,3-DICHLOROALLYLOXY)PHENYL 3-[5-(TRIFLUOROMETHYL)-2-PYRIDYLOXY]PROPYL ETHER

CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIDALYL (ISO); 2,6-DICHLORO-4-(3,3-DICHLOROALLYLOXY)PHENYL 3-[5-(TRIFLUOROMETHYL)-2-PYRIDYLOXY]PROPYL ETHER

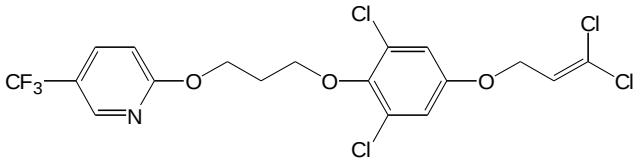
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIDALYL (ISO); 2,6-DICHLORO-4-(3,3-DICHLOROALLYLOXY)PHENYL 3-[5-(TRIFLUOROMETHYL)-2-PYRIDYLOXY]PROPYL ETHER

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIDALYL (ISO); 2,6-DICHLORO-4-(3,3-DICHLOROALLYLOXY)PHENYL 3-[5-(TRIFLUOROMETHYL)-2-PYRIDYLOXY]PROPYL ETHER

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propylether
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	Pyridalyl
EC number (if available and appropriate)	-
EC name (if available and appropriate)	2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl
CAS number (if available)	179101-81-6
Other identity code (if available)	CIPAC no. 792
Molecular formula	$C_{18}H_{14}Cl_4F_3NO_3$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	491.12
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 91%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Pyridalyl CAS no. 179101-81-6	≥ 91%	None	Skin sens. 1 (H317) STOT RE 2 (H373) Aquatic acute 1 (H400) Aquatich chronic 1 (H410)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No impurities present which contributes to the classification of the substance				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal		pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether	-	179101-81-6	Skin. Sens. 1 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H317 H361d H400 H410	GHS07 GHS08 GHS09 Wng	H317 H361d H410	-	M-factor = 1000 (acute) M-factor = 100 (chronic)	-
Resulting Annex VI entry if agreed by RAC and COM		pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether	-	179101-81-6	Skin. Sens. 1 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H317 H361d H400 H410	GHS07 GHS08 GHS09 Wng	H317 H361d H410	-	M-factor = 1000 (acute) M-factor = 100 (chronic)	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification.	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable.	No
Gases under pressure	Hazard class not applicable.	No
Flammable liquids	Data conclusive but not sufficient for classification.	Yes
Flammable solids	Hazard class not applicable.	No
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Data conclusive but not sufficient for classification.	Yes
Pyrophoric solids	Hazard class not applicable.	No
Self-heating substances	Hazard class not applicable.	No
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification.	Yes
Oxidising liquids	Data conclusive but not sufficient for classification	Yes
Oxidising solids	Hazard class not applicable.	No
Organic peroxides	Hazard class not applicable.	No
Corrosive to metals	Data lacking	No
Acute toxicity via oral route	Data conclusive but not sufficient for classification.	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification.	Yes
Acute toxicity via inhalation route	Data inconclusive	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification.	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification.	Yes
Respiratory sensitisation	Data lacking.	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification.	Yes
Carcinogenicity	Data conclusive but not sufficient for classification.	Yes
Reproductive toxicity	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification.	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification.	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes

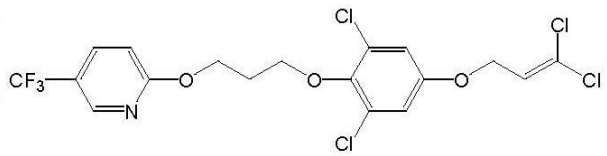
Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Pyridalyl has not previously been assessed for harmonised classification by RAC or TC C&L.
Pyridalyl is not registered under REACH (September 2017).
According to the data presented in the DAR (2013), the classification of pyridalyl is: Skin Sens. 1 (H317), Aquatic Acute 1 and Aquatic Chronic 1.
The conclusions on the peer review of pesticide risk assessment of pyridalyl was published as an EFSA scientific report (2013;11(8):3240). The classification was unchanged. The DAR can be requested via: http://dar.efsa.europa.eu/dar-web/provision. EFSAs peer review is available via the EFSA website (http://www.efsa.europa.eu/en/efsajournal/pub/3240).

RAC general comment

Pyridalyl is an active substance in the meaning of Regulation EC 1107/2009 not registered under REACH. Pyridalyl is intended as an insecticide for agricultural use on fruit, vegetables and cotton against noctuidae, in particular the larval stages of the moths. The chemical structure of pyridalyl is shown below:



According to the dossier submitter (DS) the CLH report has been prepared based on the data on pyridalyl submitted and considered valid (reliability score 1 or 2) in the Draft Assessment Report. All studies were carried out under GLP unless otherwise indicated. The non-GLP studies were range-finding or mechanistic studies. All non-mechanistic studies were carried out in accordance with OECD guidelines. Minor deviations were noted in some cases but these did not affect the overall reliability of the studies. The deviations are included in the summaries when relevant.

The degree of purity of pyridalyl is > 91% with most studies performed with the technical active substance (a.s.) at 93.7%.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pyridalyl is an active substance in the meaning of Regulation EC 1107/2009 and therefore no justification is required.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIDALYL (ISO); 2,6-DICHLORO-4-(3,3-DICHLOROALLYLOXY)PHENYL 3-[5-(TRIFLUOROMETHYL)-2-PYRIDYLOXY]PROPYL ETHER

5 IDENTIFIED USES

Pyridalyl is intended as an insecticide for agricultural use on tomato (field, glasshouse), eggplant (field, glasshouse), sweet pepper (field, glasshouse), chilli-pepper (field, glasshouse), cucurbits (field), lettuce (field) and cotton (field) against noctuidae.

6 DATA SOURCES

Within the context of Regulation EC 1107/2009, a dossier was received by RMS the Netherlands from Sumitomo Chemical Agro Europe S.A.S. This CLH report has been prepared based on the data on pyridalyl that was submitted and evaluated in the DAR (2013).

There is no REACH registration dossier for Pyridalyl (January 4th, 2019)

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101.3 kPa	liquid	Hirose, 2002a	Measured
Melting/freezing point	< -17 °C (approximate value: -26 °C)	Takashima, 2002	Measured, the thermometer used for measurements below -17°C was not certified.
Boiling point	> 227 °C (decomposition)	Sweetapple, 2002a	Measured
Relative density	1.445 g/cm^3 at 20°C (99.5%)	Ishihara, 2002	Measured
	1.442 g/cm^3 at 20°C (93.7%)	Reitz, 2002	Measured
Vapour pressure	6.24 x 10^{-8} Pa at 20°C	Lorence, 2000	Extrapolated from vapour pressure curve
Surface tension	Not required		Water solubility below 1 mg/L.
Water solubility	0.15 µg/L at 20°C, pH 8.0-8.3	Lorence, 2000	Effect of pH was not investigated and is not required in view of the absence of dissociation in the relevant pH range.
Partition coefficient n-octanol/water	Log Pow at 20°C: 8.1	Lorence, 2000	Extrapolated from regression line (HPLC method)
Flash point	111°C	Reitz, 2002	Measured
Flammability	Not required.		
Explosive properties	Not explosive.	Sweetapple, 2002b	Measured
Self-ignition temperature	> 400°C	Greenwood and Liney, 2005	Measured
Oxidising properties	Not oxidising	Mak, 2003	Estimated.
Granulometry	No data		

Property	Value	Reference	Comment (e.g. measured or estimated)
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	pKa: -3.85 and -4.88	Doc MII, Section 1, 2.9.5	Model estimation
Viscosity	No data		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
OPPTS 830.6316 Thermal and impact explodability (Bureau of Explosives impact apparatus).	Pyridalyl is not explosive	Tech.a.s. 93.7%	Sweetapple, 2002b

8.1.1 Short summary and overall relevance of the information provided on explosive properties

One study was carried out in accordance with OPPTS 830.6316. S-1812 (pyridalyl) did not exhibit impact explodability. No exothermic reaction was observed up to 200°C (Sweetapple 2002b).

8.1.2 Comparison with the CLP criteria

Based on experimental data, pyridalyl is considered not explosive. Further, pyridalyl does not contain any chemical groups associated with explosive properties as given in section 2.1.4.2 of the CLP Guidance and therefore no classification is required.

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (pyridalyl is not a gas).

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not relevant.

8.2.2 Comparison with the CLP criteria

Not relevant.

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable.

8.3 Oxidising gases

Hazard class not applicable (pyridalyl is not a gas).

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant.

8.3.2 Comparison with the CLP criteria

Not relevant.

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable.

8.4 Gases under pressure

Hazard class not applicable (pyridalyl is not a gas).

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant.

8.4.2 Comparison with the CLP criteria

Not relevant.

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable.

8.5 Flammable liquids

Table 9: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EEC A.9	flash point 111°C	Tech.a.s. 93.7%	Reitz, 2002
EEC A.2	No boiling point, decomposition occurred at 227°C	Tech.a.s. 99.1%	Sweetapple, 2002a

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

In a study to determine the flash point in accordance with EEC A.9 (closed cup) the flash point was found to be 111°C at 759 mmHg (Reitz, 2002).

A study on the boiling point was carried out in accordance with EEC A.2, capillary method. No boiling point was found, decomposition occurred at 227°C (Sweetapple, 2002a).

8.5.2 Comparison with the CLP criteria

As the flash point is >60 °C classification as flammable liquid is not required.

8.5.3 Conclusion on classification and labelling for flammable liquids

No classification is proposed.

8.6 Flammable solids

Hazard class not applicable (pyridalyl is not a solid).

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Not relevant.

8.6.2 Comparison with the CLP criteria

Not relevant.

8.6.3 Conclusion on classification and labelling for flammable solids

Hazard class not applicable

8.7 Self-reactive substances

No data.

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No data.

8.7.2 Comparison with the CLP criteria

No specific data has been provided. However, pyridalyl does not contain any chemical groups associated with explosive or self-reactive properties in accordance with Table 2.8.4.2 of the CLP Guidance. Therefore, pyridalyl is concluded to not self-reactive.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed.

8.8 Pyrophoric liquids

No specific study was carried out.

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

No specific data derived in accordance with the recommended test method in CLP has been provided. However, pyridalyl has been handled extensively in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections).

8.8.2 Comparison with the CLP criteria

No experimental data are available. However, based on experience in handling of pyridalyl, it is not considered a pyrophoric liquid.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

No classification is proposed.

8.9 Pyrophoric solids

Hazard class not applicable (pyridalyl is not a solid).

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Not relevant.

8.9.2 Comparison with the CLP criteria

Not relevant.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Hazard class not applicable.

8.10 Self-heating substances

Hazard class not applicable (pyridalyl is a liquid).

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

Not relevant.

8.10.2 Comparison with the CLP criteria

Not relevant.

8.10.3 Conclusion on classification and labelling for self-heating substances

Hazard class not applicable

8.11 Substances which in contact with water emit flammable gases

No data.

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No specific data derived in accordance with the recommended test method in CLP has been provided. However, pyridalyl has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas.

8.11.2 Comparison with the CLP criteria

Based on experience in handling of pyridalyl, it is considered not a substance which in contact with water emit flammable gases.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification is proposed.

8.12 Oxidising liquids

Table 10: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
OPPTS 830.6314	No apparent reaction with water, $(NH_4)H_2PO_4$, $KMnO_4$ and granular zinc	Study design and performance does not enable any conclusions with regard to the oxidising properties as defined in EC A.21.	Reitz, 2002
None	Not oxidising (Pyridalyl pure)	Expert statement.	Mak, 2003

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

The oxidising properties were evaluated in a study in accordance with OPPTS 830.6314. In the study there was no apparent reaction with the test reagents (Reitz, 2002). However, the study design does not enable any conclusion with regard to the oxidising properties as defined in EC A.21 or UN Test O.2. In evaluating the oxidising properties of pyridalyl an expert statement on the molecular structure was also provided (Mak, 2003). In general, oxydizing properties are not expected if the molecule does not contain oxygen, chlorine or fluorine, or if the molecule does contain oxygen, chlorine or fluorine and these elements are chemically bonded to carbon or hydrogen only. Pyridalyl does not contain such groups. Therefore, considering the molecular structure of the substance oxydizing properties are not expected.

8.12.2 Comparison with the CLP criteria

Considering the molecular structure pyridalyl is concluded to be not oxidising.

8.12.3 Conclusion on classification and labelling for oxidising liquids

No classification is proposed.

8.13 Oxidising solids

Hazard class not applicable (pyridalyl is not a solid)

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

Not relevant.

8.13.2 Comparison with the CLP criteria

Not relevant

8.13.3 Conclusion on classification and labelling for oxidising solids

Hazard class not applicable.

8.14 Organic peroxides

Hazard class not applicable (pyridalyl is not an organic peroxide).

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Not relevant.

8.14.2 Comparison with the CLP criteria

Not relevant.

8.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable.

8.15 Corrosive to metals

No data.

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data has been provided that addresses this property.

8.15.2 Comparison with the CLP criteria

No data has been provided that addresses this property.

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS concluded that none of the reported physico-chemical properties of pyridalyl result in a requirement for classification using the criteria set out in the CLP Regulation

Comments received during consultation

The following endpoints were open for comment during the consultation: explosive, flammable liquid, self-reactive substance or mixture, pyrophoric liquid, oxidising liquid and substance or mixture that in contact with water emits flammable gas. None received comments during the consultation.

Assessment and comparison with the classification criteria

Comparison with the criteria

Pure pyridalyl is a liquid at room temperature and for the application of the CLP criteria, it is considered as a liquid. RAC supports the DS that pyridalyl should not be classified for the hazard classes pyrophoric liquid and substance or mixture that in contact with water emits flammable gas. The other hazard classes are discussed by RAC.

Explosives

According to CLP and UN TGD (Table A6.1), there is a structural alert based on one C-C unsaturated bond. However, this structural alert is not associated with oxygen. The oxygen balance does not need to be calculated. One study was carried out in accordance with the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency (OPPTS) test 830.6316 where pyridalyl did not exhibit impact explosivity. The results also reported that no exothermic reaction was observed up to 200 °C (Sweetapple, 2002b). However, decomposition occurs at > 227 °C and no data are available regarding the exothermic decomposition energy of pyridalyl at or between 200 and 500 °C.

Overall, although pyridalyl is unlikely to be explosive, RAC is of the opinion that **no classification is warranted for this hazard class due to lack of data**.

Flammable liquids

One closed cup study (method EC A.9) was described in the CLH report. The results indicated that the flash point of liquid pyridalyl is 111 °C. In addition, a study on the boiling point was carried out in accordance with EC A.2 where no boiling point was found, decomposition occurred at 227 °C (Sweetapple, 2002a). RAC concludes that **no classification is warranted for this hazard class.**

Self-reactive substance or mixture

The exothermic decomposition of pyridalyl is unknown. According to CLP criteria, a self-reactive substance is regarded as possessing explosive properties when in laboratory testing

the substance is liable to detonate, to deflagrate rapidly or to show a violent effect when heated under confinement. There is a structural alert for explosivity but the heat of decomposition or the self-accelerating decomposition temperature are not available for pyridalyl. Therefore, RAC is of the opinion that **no classification is warranted for this hazard class due to lack of data.**

Oxidising liquids

In a study on oxidising properties of liquids carried out in accordance with EC A.21, pyridalyl was negative. Therefore, RAC is of the opinion that **no classification is warranted for oxidising liquids**.

Overall, RAC supports the DS's proposal for **no classification of pyridalyl for physical hazards,** noting the lack of data for explosive and self-reactive substance or mixture.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 11: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
US EPA (1989), MAFF	T_{max}: 6-8 hours (phenyl label) 6-24 hours (propenyl label) T½: Phenyl label: 18 hours (5 mg/kg bw), 35 hours (500 mg/kg bw) Propenyl label: 52-60 hours (5 mg/kg bw), 80-91 hours (500 mg/kg bw). C_{max} (phenyl/propenyl label): 0.255/0.412 ppm (males, low dose) 0.212/0.324 ppm (females low dose) 21.04/34.75 ppm (males, high dose) 21.15/32.35 ppm (females, high dose)	Study acceptable	II A 5.1.1/01 Project No. 2144
OPPTS 870.7485	Oral absorption: 3.2-6.8% (phenyl, pyridyl label) 28.3-35.5% (Propenyl label) Distribution: Highest residue found in fat, adrenal gland, pancreas, salivary glands, thyroid, hair/skin, overies and uterus. Metabolism: Major metabolic pathway cleavage of the dichloropropenyl groups and of the methylene bridge between pyridyl and dichlorophenyl rings. Minor pathway oxidation of the pyridyl ring.	Study acceptable, excretion via entero-hepatic circulation not included.	IIA 5.1.1/02 Project No. 807W/1221E-1
OPPTS 870.7485	Distribution:	Study acceptable	IIA 5.1.1/03

Method	Results	Remarks	Reference
Japanese MAFF (12 Nohsan No. 8147)	Highest residue found in GIT contents, small intestine, carcass, stomach, caecum, large intestine, liver, fat, hair/skin, pancreas, muscle, heart and lungs. The following tissues showed an increase of residue concentrations over time, or did not show a decrease after ½ C_{max}: bone marrow, ovaries, uterus, thyroid, pancreas, fat, hair/skin, salivary glands. <u>Metabolism:</u> Metabolite S1812-DP and polar metabolites occurred in all tissues. S-1812-Ph-CH_2CO_2H and HPHM did not occur in fat.		Project No. 986W-1
OPPTS 870.7485	<u>Oral absorption:</u> 11-15% (phenyl label) 36-43% (propenyl label)	Study acceptable. Includes biliary excretion.	IIA 5.1.1/04 Project No. 985W-1
OPPTS 870.7485	<u>Distribution:</u> Highest residue found in fat, liver, skin, thyroids, adrenals, kidneys, thymus and ovaries. Highest accumulation ratios (day 14/day 1) found in fat (ratio 17-23). <u>Elimination:</u> Biphasic decrease, with half-life of 1-5 days (α phase) and 4-24 days (ß phase). Half-life longest in perirenal and testicular fat (10-15 days). In blood and plasma the biological half-life ranged from 1-2 days, in the skin, thymus and uterus the half-life ranged from 4-10 days. <u>Metabolism:</u> Major metabolites: S1812-DP, S-1812-Ph-CH_2CO_2H. Other metabolites identified were S-1812-Py-OH and HPHM.	Study acceptable.	IIA 5.1.3/01 Project No SUM-0009

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

<u>Absorption</u>

In rats receiving ^{14}C-S-1812 at 5 mg/kg bw and 500 mg/kg bw (IIA 5.1.1/01) peak concentrations in blood were observed at 6-8 hours (phenyl label) and 6-24 hours (propenyl label) after a single dose. Maximum concentrations increased dose proportional. In females AUC increased more than dose proportional (propenyl label only).

In rats given a single dose of phenyl (5 mg/kg bw and 500 mg/kg bw) and pyridyl (5 mg/kg bw) labelled (^{14}C)-S-1812 (IIA 5.1.1/02), oral absorption was 3.2-6.8% in 168 hours, based on the amount radiolabel recovered from urine, carcass, expired air and tissues. In rats given a single dose of the propenyl labelled S-1812, oral absorption amounted to 28.3-35.5% at 168 hours, based on radiolabel recovered from urine, carcass, expired air and tissues. Actual absorption might have been higher because of the fact that excretion via entero-hepatic circulation was not taken into account.

After oral repeated dosing of 5 mg/kg bw [phenyl-^{14}C]S-1812 to rats (IIA 5.1.1/03) the main excretion route was the faeces (>90% of the administered dose was found in the faeces). Excretion in urine account for 2.0-4.4% of application rate (AR) within 27 days. These results were in line with the results after a single dose of 5 mg/kg bw [phenyl-^{14}C]S-1812. No difference between sexes was observed.
In bile-cannulated rats given 5 mg/kg bw [phenyl-^{14}C]S-1812 (IIA 5.1.1/04), excretion in bile amounted to 8% at 48 hours. When the radiolabel recovered in bile included, oral absorption amounts to 11-15% for the phenyl and pyridyl labelled S-1812 and 36-43% for the propenyl labelled S-1812. However, as in the bile no parent compound was recovered, it is assumed that only the parent compound recovered in faeces should be considered as not systemic available, which amounts to 31-32% AR for males and females given 5 mg/kg bw. All metabolites found in faeces are considered part of the entero-hepatic cycle and therefore systemically available.
The apparently low absorption of pyridalyl in the bile-cannulated rat can be explained by the very high lypophility of this compound (log Pow 8.1). It is well know that intestinal absorption of such lipophilic compounds requires the presence of bile acids. (Miyazaki et al., 1980, Hacket and Griffits, 1982, Mizuta et al., 1990, and Humberstone et al. 1996). The bile cannulation study with pyridalyl in rats was done without supplementation of bile acids.

Elimination
The half-life in blood (phenyl labelled compound) was approximately 18 hours (5 mg/kg bw dose) and 35 hours (500 mg/kg bw dose). The half-life of the propenyl labelled pyridalyl seemed to be longer, possibly due to incorporation of the radiolabel in endogenous metabolism.
The majority of administered radioactivity was excreted in faeces in all studies, whilst radioactivity in urine ranged from 2-18%. Radioactivity in expired air accounted for 0-12% AR. In bile approximately 8% of the administered radioactivity was recovered in a period of 48 hours.
No differences between sexes were observed.

Distribution
Radioactivity concentrations in tissues were low (0.6-2.1% AR), in the carcass the radioactivity recovered ranged from 0.7-10.5% AR. After a single dose highest residues were found in GIT contents, small intestine, carcass, stomach, caecum, large intestine, liver, fat, adrenal gland, pancreas, salivary glands, thyroid and hair/skin, ovaries and uterus (IIA 5.1.1/02 and IIA 5.1.1/03). After the higher doses (500 mg/kg bw) the recovered radiocarbon concentrations were higher compared to the lower doses. The average radiocarbon concentrations recovered in tissues was higher after dosing with [propenyl-^{14}C]S-1812 compared to [phenyl-^{14}C]S-1812 and [pyridyl-^{14}C]S-1812. For most tissues the residue concentrations decreased after ½ C_{max} (EL). The following tissues showed an increase of residue concentrations over time, or did not show a decrease after ½ C_{max} (EL): bone marrow, ovaries, uterus, thyroid, pancreas, fat, hair/skin, salivary glands.

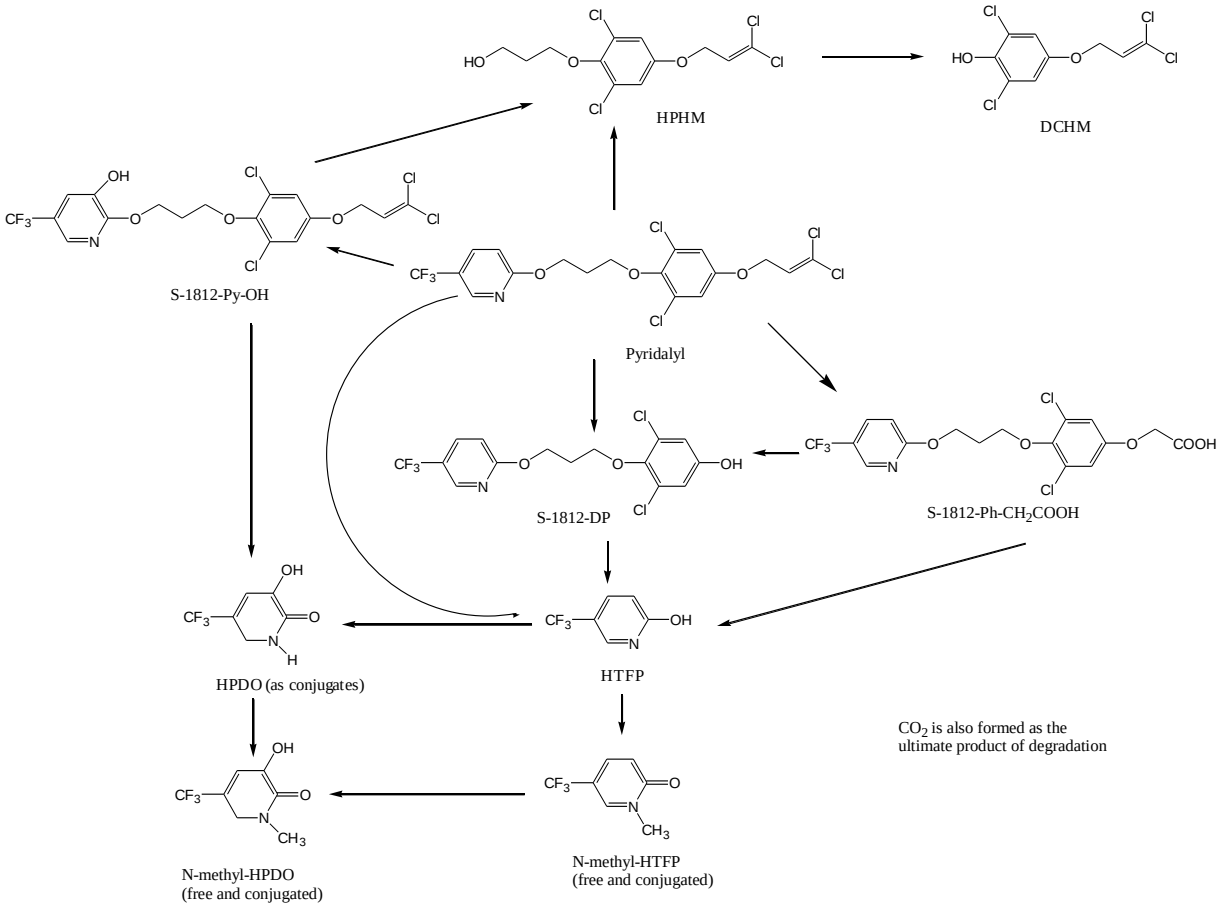
After multiple dosing (14 days) with 5 mg/kg bw ^{14}C-S-1812 highest ^{14}C-concentrations were observed in fat (brown, perirenal and testicular), liver, skin, thyroids, adrenals, kidneys, thymus and ovaries (IIA 5.1.1/05). In perirenal and testicular fat the highest accumulation ratio's (Day 14/Day 1) were found (accumulation ratio: 17-23). In other tissues accumulation ratios were maximally 11 (skin and brown fat). In tissues generally a biphasic decrease of recovered radioactivity was observed (except for blood, perirenal and testicular fat, skin, thymus and uterus), with a biological half-life of 1-5 days (α phase) and 4-24 days (β phase). In perirenal and testicular fat the biological half-life was long and ranged from 10-15 days, in blood and plasma the biological half-life ranged from 1-2 days, in the skin, thymus and uterus the half-life ranged

from 4-10 days. Based on the results of the multiple dosing study, it is concluded that after repeated dosing, pyridalyl accumulates in fatty tissues.

Metabolism

The main metabolite detected in faeces was S-1812-DP, other metabolites detected in faeces were S-1812-Py-OH, HPHM, DCHM and polar metabolites. In urine no parent compound was detected, identified metabolites were HTFP, HPDO, HPDO glucuronide, HPDO sulphate. In expired air only CO_2 was detected.
In tissues the identified metabolites were S-1812-Ph-CH_2CO_2H, S-1812-DP, S-1812-Py-OH, polar metabolites and HPHM were detected after a single dose;
The formation of major metabolite (S-1812-DP) involves oxidative cleavage of the dichloropropenyl group, yielding S-1812-DP from the phenyl and pyridyl labels and $^{14}CO_2$ and minor polar metabolites from the propenyl label. Other minor path ways are: oxidation of the inter-ring methylene groups and hydroxylation.

Figure 1 Proposed metabolic pathway of pyridalyl in rat



10 EVALUATION OF HEALTH HAZARDS

The mammalian toxicity studies of pyridalyl were assessed in the Draft Assessment Report (April 2003), addenda and Proposed Decision of the Netherlands prepared in the context of the approval (Reg. (EU) No. 143/2014), under Reg. (EC) 1107/2009. Studies considered valid in the DAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. All studies were carried out under GLP unless indicated underwise. The non-GLP studies were range-finding studies or mechanistic studies. Other than the mechanistic studies all studies reported in this section were carried out in accordance with OECD guidelines. Minor deviations were noted in some cases but these did not affect the overall reliability of the studies. The deviations are included in the summaries were relevant.

Acute toxicity

10.1 Acute toxicity - oral route

Table 12: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 (1987) Deviations: none	Rat, Crl:CD (SD), Both sexes 5/sex/dose	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7%	5000 mg/kg bw, single exposure	>5000 mg/kg bw	IIA 5.2.1/01 Report No. 6311-217

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

An acute oral toxicity study was carried out in accordance with OECD 401 (IIA 5.2.1/01). A dose level of 5000 mg/kg bw was applied to males and females (5/sex). No mortality occurred and there were no clinical signs of toxicity. One female showed weight loss during the first week. Gross pathology did not reveal any treatment related findings. The acute oral LD_{50} of S-1812 was found to be greater than 5000 mg/kg bw in male and female rats.

10.1.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance does not have to be classified for acute oral toxicity when the LD_{50} is >2000 mg/kg bw. The LD_{50} of pyridalyl was >5000 mg/kg bw and therefore no classification is required.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification is proposed.

10.2 Acute toxicity - dermal route

Table 13: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402 Deviations: none	Rat, Crl: CD(SD) Both sexes 5/sex/dose	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7%	5000 mg/kg bw, 24 hours on a skin area of 24 cm^2 (occlusive exposure)	>5000 mg/kg bw	IIA 5.2.2/01 Project No. 6311-218

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

An acute dermal toxicity study was carried out in accordance with OECD 402 (IIA 5.2.2/01). A dose level of 5000 mg/kg bw was applied to males and females (5/sex). No mortality occurred and there were no clinical signs of toxicity. One female showed weight loss during the first week. Gross pathology did not reveal any treatment related findings. The acute dermal LD_{50} of S-1812 was found to greater than 5000 mg/kg bw in male and female rats.

10.2.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance does not have to be classified for acute dermal toxicity when the LD_{50} is >2000 mg/kg bw. The LD_{50} of pyridalyl was >5000 mg/kg bw and therefore no classification is required.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed.

10.3 Acute toxicity - inhalation route

Table 14: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference	Remarks
OECD 403 Deviations: none	Rat, Crl: CD(SD) Both sexes 5/sex/dose	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7% MMAD: 2.7 µm GSD: 1.98 aerosol	8.3 mg/L (nominal conc.) 2.01 mg/L (actual conc.) 4 hours, nose-only	> 2.01 mg/L	IIA 5.2.3/01 Project No SMO-568	Tested concentration below 5 mg/L.(or the maximum attainable dose)

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

An acute inhalation toxicity study was carried out in accordance with OECD 403 at a dose level of 2.01 mg/L (IIA 5.2.3/01). No mortality occurred. All animals showed decreased breathing rate and exaggerate breathing during exposure. After exposure all animals showed these clinical signs for 2 hours and 2 days after exposure respectively. Lethargy, whole body cold, and wet fur were observed for all animals after exposure until 2 hours following exposure. Brown staining around snout was observed in one male rat following exposure until 2 hours post exposure. Mean body weight gain of both sexes decreased after the first week following exposure, and increased thereafter. Gross pathology did not reveal any treatment related findings.

It is not clear from the study report that maximum effort was taken to attain higher test substance concentration than 2.01 mg/L. In absence of data it is concluded that the maximum concentration tested is not the highest attainable concentration.

10.3.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance does not have to be classified for acute inhalation toxicity when the LC_{50} is >5.0 mg/L. The LC_{50} of pyridalyl was >2.01 mg/L with no higher concentrations being tested in the available acute inhalation toxicity study.

It is not clear from the study report that maximum effort was taken to attain higher test substance concentration than 2.01 mg/L. In absence of data it is therefore concluded that the maximum concentration tested is not the highest attainable concentration. Since the limit of 5 mg/L was not reached, no conclusion can be drawn on the need for classification.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification is proposed.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for acute oral and dermal toxicity based on OECD Guideline tests performed in accordance with GLP and showing LD_{50} values higher than 5000 mg/kg bw by both routes. The DS also proposed no classification for acute inhalation toxicity based on the inconclusive data provided by an OECD Guideline test showing an LC_{50} higher than 2.01 mg/L.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table below summarises the available studies for acute toxicity of pyridalyl.

***Table**: Summary of animal studies on acute toxicity with pyridalyl.*

Study	Dose level	Results	Reference
OECD TG 401 No deviations Crl:CD (SD) rats 5 animals/sex/dose S-1812 (pyridalyl) Lot No. PS 98041G Purity 93.7%	5000 mg/kg bw Single exposure	No mortalities No clinical signs of toxicity No gross pathology alterations **LD_{50} > 5000 mg/kg bw**	IIA 5.2.1/01 Report No. 6311-217
OECD TG 402 No deviations Crl: CD(SD) rats 5 animals/sex/dose S-1812 (pyridalyl) Lot No. PS 98041G Purity 93.7%	5000 mg/kg bw Occlusive exposure during 24 hours on a skin area of 24 cm^2	No mortalities No clinical signs of toxicity No gross pathology alterations **LD_{50} > 5000 mg/kg bw**	IIA 5.2.2/01 Project No. 6311-218
OECD TG 403 No deviations Crl: CD(SD) rats 5 animals/sex/dose S-1812 (pyridalyl) Lot No. PS 98041G Purity 93.7%	8.3 mg/L (nominal concentration) 2.01 mg/L (actual concentration) 4 hours Nose-only MMAD: 2.7 µm	No mortalities All animals showed decreased breathing rate and exaggerate breathing during exposure. After exposure all animals showed these clinical signs for 2 hours and 2 days after exposure respectively. Lethargy, whole body cold, and wet fur were observed for all animals after exposure until 2 hours following exposure. Brown staining around snout was observed in one male rat following exposure until 2 hours post exposure. Mean body weight gain of both sexes decreased after the first week following exposure and increased thereafter. Gross pathology did not reveal any treatment related findings. **LC_{50} > 2.01 mg/L**	IIA 5.2.3/01 Project No SMO-568

Comparison with the criteria

According to Regulation EC No 1272/2008 a substance does not have to be classified for acute oral and dermal toxicity when LD_{50}s by respective routes of exposure are higher than 2000 mg/kg bw. Pyridalyl at 5000 mg/kg bw did not cause clinical signs or mortality after dosage by both routes and therefore, the classification for acute oral and dermal toxicity is not supported. In conclusion, **no classification of pyridalyl for acute and dermal toxicity.**

According to Regulation EC No 1272/2008 a substance does not have to be classified for acute inhalation toxicity when the LD_{50} is higher than 5 mg/L. Pyridalyl at 2.01 mg/L caused certain respiratory alterations and no mortalities and therefore, the conclusion is that LC_{50} must necessarily be higher than 2.01 mg/L. However, RAC notes that there is no information that allows to conclude whether 2.01 mg/L is the maximum attainable concentration and/or whether the LC_{50} is higher or lower than the cut-off point of 5 mg/L for triggering classification. Therefore, **no classification of pyridalyl for acute inhalation toxicity due to inconclusive data.**

10.4 Skin corrosion/irritation

Table 15: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404 Deviations: none	Rabbit, New Zealand White, 2 males, 4 females	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7%	0.5 ml, 4 hours, semi-occlusive	Observations made at 1, 24, 48 and 72 hours Erythema: 0 Oedema: 0 Reversiblity: not applicable.	IIA 5.2.4/01, Project No. 6311-219

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Pyridalyl technical was evaluated for its primary dermal irritation potential in male and female New Zealand White rabbits when administered undiluted as a single topical application of 0.5 ml under 4 hours semi occluded conditions (IIA 5.2.4/01). The study was carried out in accordance with OECD 404. Dermal irritation readings were taken 30 minutes – 1 hour after removal of test material and subsequently at 24, 48 and 72 hours. Application of the test material did not results in any dermal irritation.

10.4.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.2.2 a substance should be classified for skin irritation Category 2 in the case where:

(1) Mean value of ≥2,3 - ≤4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Pyridalyl does not fulfil the criteria for skin irritation as as no signs of dermal irritation were observed.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification for skin irritation and corrosion based on an OECD TG 404 test showing no dermal irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table below summarises the only available skin/corrosion study.

Table**: Summary of the animal study on skin corrosion/irritation with pyriladyl.**

Study	Dose level	Results	Reference
OECD TG 404 No deviations New Zealand White rabbits 2 males 4 females S-1812 (pyridalyl) Lot No. PS 98041G Purity 93.7%	0.5 mL 4 hours (semi-occlusive)	Observations made at 1, 24, 48 and 72 hours Erythema: 0 (all animals) Oedema: 0 (all animals)	IIA 5.2.4/01 Project No. 6311-219

Comparison with the criteria

RAC notes that the OECD TG 404 test performed observing GLP did not showed any dermal irritation and therefore pyridalyl does not fulfil the criteria for skin irritation classification. Thus, RAC supports the DS's proposal for **no classification of pyridalyl for skin irritation/corrosion.**

10.5 Serious eye damage/eye irritation

Table 16: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 Deviations: none	Rabbit, New Zeland White, males, 6	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7%	0.1 ml, no wash step, single instillation	Observations made at 1, 24, 48 and 72 hours Cornea opacity: 0 Iris: 0 Conjunctiva chemosis: 0 Conjunctival redness: 0.33; 0.33; 0.33; 0; 0; 0 Reversible: yes	IIA 5.2.5/01 Project No 6311-220

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Pyridalyl technical was evaluated for its primary eye irritation potential in six male albino New Zealand White rabbits when administered as a single ocular application of 0.1 ml into the verted lower lid of the right eye with the left eye serving as control (IIA 5.2.5/01). The eyes were not flushed after treatment. Pyridalyl only produced conjunctival redness (score 1) at 1 hour after treatment in 5 out of 6 animals, and in 3 out of 6 animals at 24 hours. All treated had returned to normal appearance by 48 hours after treatment. No corneal opacity, iritis or chemosis was observed at any time point.

10.5.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.3.2.1.2 a substance should be classified for eye irritation Category 2 in the case where:
Substances that produce in at least in 2 of 3 tested animals, a positive response of:
(a) corneal opacity ≥ 1 and/or
(b) iritis ≥ 1, and/or
(c) conjunctival redness ≥ 2 and/or
(d) conjunctival oedema (chemosis) ≥ 2
calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

Pyridalyl does not fulfil the criteria for eye irritation as the mean scores were below these criteria in all animals.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

No classification is proposed.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification of pyridalyl for eye damage/irritation based on an OECD TG 405 study showing only reversible conjunctival redness scored with a maximum value of 1 (in three animals at 24 hours only) and absence of corneal opacity, iritis and chemosis.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table below summarises the only available eye damage/irritation study.

***Table**: Summary of the animal study on eye damage/irritation with pyridalyl.*

Study	Dose level	Results	Reference
OECD 405 No deviations New Zeeland White rabbits 6 males S-1812 (pyridalyl) Lot No. PS 98041G Purity 93.7%	0.1 ml No wash step Single instillation	Observations made at 1, 24, 48 and 72 hours Mean cornea opacity: 0 (all animals) Mean iris: 0 (all animals) Mean conjunctiva chemosis: 0 (all animals) Mean conjunctival redness: 0.33; 0.33; 0.33; 0; 0; 0 Reversible: yes	IIA 5.2.5/01 Project No 6311-220

Comparison with the criteria

According to the CLH-report pyridalyl only produced conjunctival redness (maximum score 1) at 1 hour after treatment in 5 out of 6 animals, and in 3 out of 6 animals at 24 hours. All treated eyes had returned to normal appearance by 48 hours after treatment. Therefore, the scores for conjunctival redness were below the value of 2 that would trigger classification. Moreover, no corneal opacity, iritis or chemosis was observed at any time point. Therefore, RAC supports the DS's proposal for **no classification of pyridalyl for eye damage/irritation.**

10.6 Respiratory sensitisation

No data.

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data.

10.6.2 Comparison with the CLP criteria

Not relevant.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification is proposed.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

DS proposed no classification of pyridalyl for respiratory sensitisation based on lack of data.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC supports the DS's proposal for no **classification of pyridalyl for respiratory sensitisation due to lack of data**.

10.7 Skin sensitisation

Table 17: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 406 Deviations: none	Guinea-pig, Hartley, females 10 controls	S-1812 (pyridalyl), Lot No. PS 98041G,	2% intradermal induction 100% topical induction	24 hours: Slight erythema 8/20 Moderate erythema 2/20 Slight oedema 5/20	IIA 5.2.6/01 Project No 3650

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
	20 test animals (females)	purity 93.7%	10% challenge	48 hours: Slight erythema 16/20 Slight oedema 4/20	

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

A Magnusson and Kligman study was carried out in accordance with OECD 406 (IIA 5.2.6/01). Dose levels were based on the results of a range-finding study using 0.1, 0.2, 0.5, 1, 2, and 5% for intradermal injections and 10, 25, 50 and 100% for topical applications. Intradermal injection with 2% induced slight erythema and no or slight oedema. Topical application with 100% induced slight erythema and no or slight oedema.

In the main study after intradermal induction with 2% S-1812 and after topical induction with 100% S-1812, no observation of irritation was made. After topical challenge with 10% S-1812, slightly patch erythema in 8/20 and 16/20 females was observed after 24 hours and 48 hours respectively. Moderate erythema was observed in 2 females only after 24 hours. Slight oedema was observed in 5/20 and 4/20 females after 24 and 48 hours respectively. Topical challenge in control animals did not induce any dermal reaction.

10.7.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.4.2.2.3.2 substance should be classified for skin sensitisation when ≥ 30% of the animals respond at >1% intradermal induction dose. In the study with pyridalyl there was a positive response in 80% of the animals and therefore concluded to be a skin sensitiser. Since the study did not evaluate an intradermal induction dose below 1% no sub-category can be assigned.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the results of the study pryidalyl should be classified as skin sensitiser, cat. 1 (hazard statement H317 - May cause an allergic skin reaction).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

DS proposed classification of pyridalyl as skin sensitiser category 1 based on an OECD TG 406 study supporting category 1B (80% of sensitisation in Guinea pig after 2% intradermal induction) but that do not allow discarding category 1A since the effect of intradermal 1% or lower induction was not assessed.

Comments received during consultation

One member state competent authority (MSCA) agreed with the rationale behind the proposal for classification as skin sensitiser 1 without sub-categorisation.

Assessment and comparison with the classification criteria

The table below summarises the only available skin sensitisation study.

***Table**: Summary of the animal studies on skin sensitisation with pyridalyl.*

Study	Dose level	Results	Reference
OECD TG 406 No deviations Hartley females Guinea pig 10 controls 20 test animals S-1812 (pyridalyl) Lot No. PS 98041G Purity 93.7%	2% intradermal induction 100% topical induction 10% challenge	<u>24 hours</u> Slight erythema 8/20 Moderate erythema 2/20 Slight oedema 5/20 <u>48 hours</u> Slight erythema 16/20 Slight oedema 4/20	IIA 5.2.6/01 Project No 3650

Comparison with the criteria

The Guinea pig maximisation test showed up to 80% of sensitisation 48 hours after the challenge and therefore the results of this test fulfil the requirements for classification as skin sensitiser category 1B. However, RAC notes that induction with 1% or less (2% induction resulted in 80% positive sensitisation rate) should have been also tested in order to determine whether the requirements for category 1A (≥ 60% at ≤ 1%) are also fulfilled and therefore the sub-categorisation is not supported. In conclusion, RAC supports the DS's proposal for **classification of pyridalyl as skin sensitiser category 1 H317 (may cause an allergic skin reaction).**

10.8 Germ cell mutagenicity

Table 18: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro gene mutation in bacteria OECD 471	S-1812 (pyridalyl), Batch No. PS 98041G,	<u>Organism/strain:</u> TA100, TA98, TA1535 TA1537, Wp2uvrA	-S9: negative +S9: negative <u>Precipitation</u>:	IIA 5.4.1/01 Study no. 3376

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Deviations: none	purity 93.7%	Concentrations tested: -S9: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate +S9: 39.1, 78.1, 156, 313, 625, 1250 µg/plate Positive controls: Sodium azide; 9-Aminoacridine; 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; 2-Aminoanthracene	1250 µg/plate (+S9) and 313 µg/plate (-S9) Cytotoxicity: None	
In vitro chromosomal aberration OECD 473 Deviations: none	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7%	Organism/strain: Chinese hamster lung (CHL/IU) cells Concentrations tested: Exp 1: -S9 (treatment 6 hr and recovery 18 hr): 20, 40, 80 µg/ml +S9 (treatment 6 hr and recovery 18 hr): 15, 20, 25 µg/ml Exp 2: -S9 (treatment and harvest 24 hr): 625, 938, 1250 µg/ml -S9 (treatment and harvest 48 hr): 39.1, 78.1, 156 µg/ml Exp 3: +S9 (treatment 6 hr and recovery 18 hr): 15, 20, 25 µg/ml	-S9: negative +S9: positive Cytotoxicity: Without S9-mix, treatment 6 hr and recovery 18 hr: no With S9-mix, treatment 6 hr and recovery 18 hr: ≥ 20µg/ml Without S9-mix, treatment and harvest 24 hr: ≥ 938 µg/ml Without S9-mix, treatment and harvest 48 hr: ≥ 78.1 µg/ml Precipitation: ≥ 78.1 µg/ml	IIA 5.4.3/02 Project No. 6311-215
In vitro gene mutation in mammalian cells (Chinese hamster ovary) OECD 476 Deviations: none	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7%	Organism/strain: Chinese hamster ovary (CHO) cells Concentration tested: Exp 1: (-S9) 9.4, 18.8, 37.5, 75, 150 and 300 µg/ml (+S9) 2, 4, 5, 6, 7 and 8 µg/ml Exp 2: (-S9) 9.4, 18.8, 37.5, 75, 150 and 300 µg/ml (+S9) 2, 4, 5, 6, 7, 8 and 10	-S9: negative +S9: negative Cytotoxicity: -S9: none +S9: 5 µg/ml and above Precipitation: 157 µg/ml and above.	IIA 5.4.3/02 Project No. 6311-215

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		µg/ml Positive controls: 5-Bromo-2'-deoxyuridine 20-methylcholanthrene		

Table 19: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mammalian erythrocyte micronucleus test OECD 474 Deviations: none	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7%	Organism: Mouse, Crj:CD-1 (ICR) 5 male/dose Dose tested: 500, 1000 and 2000 mg/kg bw 24 hour and 48 hour treatment Positive control: Positive control: Cylophosphamide	Negative Cytotoxicity: - dose range finding test: 1000 and 2000 mg/kg - main test: 2000 mg/kg Toxicity: Clinical sign of soft stool observed at 1000 and 2000 mg/kg bw.	IIA 5.4.4/01 Study No. 3421
UDS test OECD 486 Deviations: none	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7%	Organism: Sprague Dawley rats(Crl: CD (SD)IGS BR) 4/male/dose Dose tested: 500, 1000 and 2000 mg/kg bw Positive control: Dimethylnitrosamine	Negative Cytotoxicity: none	IIA 5.4.5/01 Study No. 6311-214

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

S-1812 (pyridalyl) was evaluated for its mutagenic potential by the reverse mutation test with four strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and one strain of *E. coli* (Wp2uvrA) (IIA 5.4.1/01). The test was conducted using preincubation in the presence and absence of S9 mix. In the main assays, pyridalyl was tested in triplicate at doses ranging from 39.1 to 1250 µg/plate in the

presence of S9 mix and 9.77 to 313 µg/plate in the absence of S9 mix. A confirmatory assay was also performed in triplicate. The tested concentration were based on a range finding study.
S-1812 did not show any dose-dependent increase in the number of revertant colonies. Positive control chemicals showed marked increases. Based on these results, it was concluded that S-1812 is not mutagenic under the test conditions.

S-1812 was evaluated for its mutagenic potential in an *in vitro* mammalian cell gene mutation test using Chinese hamster ovary cells (HGPRT) (IIA 5.4.3/02). Cytotoxicity assays were performed to determine concentrations for the mutation assay. Cell growth was not inhibited by treatment with pyridalyl up to 5000 µg/mL in the absence of metabolic activation (S9). In the presence of S9, cytotoxicity was tested from 0.0740 µg/mL to 37.5 µg/mL and found to be highly cytotoxic above 4.70 µg/mL. Mutagenicity assays were conducted in duplicate and confirmatory assays were conducted with the same doses and protocols.
In one test without S9 a significant increase in mutant frequency was observed at 18.8 µg/ml. However, there was no dose dependent increase since no significant increase was observed at higher concentrations. In trial 1 with metabolic activation a significant increase was observed sporadically. However, again no dose response relationship was observed and all mutation frequencies were within the historical control range. In the confirmatory assay no significant increases were observed. Therefore, the test article was considered negative both with and without metabolic activation.

The clastogenic potential of S-1812 was examined by an *in vitro* chromosomal aberration test using Chinese hamster lung cells (CHL/IU) (IIA 5.4.3/02). Pyridalyl did not induce structural chromosomal aberrations in the CHL/IU cells treated for 6 h without S9 mix. Marginal increases in aberrations and polyploidy were observed in the cells treated with pyridalyl in the presence of S9. No increase in incidence of chromosomal aberration was seen in the 24 – and 48-hour continuous treatments in the absence of S9. A confirmatory 6-hour assay in the presence of S9 found marginal increases in structural aberrations (maximal induction was 9.5%). Based on the results it was concluded that S-1812 has a weak potential to induce chromosome aberrations in this *in vitro* assay.

S-1812 was examined fot its potential to induce micronuclei in bone marrow cells of CD01 mice in an *in vivo* micronucleus test (IIA 5.4.4/01). Dose levels of 500, 1000 and 2000 mg/kg bw (based on a preliminary range finding test) were administered to 5 male mice/group via gavage. Bone marrow smear were prepared 24 (all groups) and 48 (top dose only) hours after administration.
There was no significant decrease in the ratio of polychromatic erythrocytes to whole erythrocytes. The test material induced no significant increase in the incidence of micronucleated polychromatic erythrocytes. The positive control induced marked increases in the incidence of micronuclei. Although the study itself did not show if the bone marrow was reached results from the ADME studies (see point 9) indicate that pyridalyl reaches the bone marrow. Based on the results it was concluded that S-1812 has no potential to induce micronuclei in mouse bone marrow cells.

The unscheduled DNA synthesis (UDS) test was performed with pyridalyl in Sprague Dawley male rats (IIA 5.4.5/01). Dose levels of 500, 1000, and 2000 mg/kg bw (based on a preliminary range finding test) were administered to 4 male rats/group via gavage. The hepatocytes collected 2 to 4 and 15 to 16 hours after administration were cultured with labelled thymidine for 4 hours to assess UDS.
No significant increases in net nuclear grain count or average percent of cells containing five or more net nuclear grains was seen at the 2- to 4-hour time point. At the 15- to 16-hour timepoint, the average percent of cells containing five or more net nuclear grains was slightly elevated in the 1000 mg/kg bw group. However, there were no other indications of increased UDS and the top dose group was not elevated, thus this response was judged to be irrelevant.
It was concluded that pyridalyl does not induce unscheduled DNA synthesis under the conditions of this study.

10.8.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.5.2.2, classification in Category 2 mutagen is based on:
- Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
- Somatic cell mutagenicity tests in vivo, in mammals; or
- Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays

Pyridalyl was tested negative for gene mutation in a bacterial gene mutation study and an *in vitro* mammalian gene mutation studies. Pyridalyl tested positive for clastogenicity *in vitro*, but the *in vivo* micronucleus study gave negative results. It is concluded that pyridalyl does not fulfil the criteria for classification for germ cell mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification is proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of pyridalyl for germ cell mutagenicity based on:

- Negative result in one *in vitro* gene mutation test in bacteria;
- Positive result in one *in vitro* chromosomal aberration test that could not be confirmed in a second assay;
- Negative result in one *in vitro* gene mutation in mammalian cells;
- Negative result in one *in vivo* mammalian erythrocyte micronucleus tests;
- Negative result in one *in vivo* unscheduled DNA Synthesis test with mammalian liver cells.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table below summarises the *in vitro* mutagenicity/genotoxicity tests.

***Table**: Summary of mutagenicity/genotoxicity in vitro studies with pyridalyl.*

Method	Tested concentrations	Results	Reference
In vitro gene mutation in bacteria	-S9-mix: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate	**-S9-mix: negative** **+S9-mix: negative**	IIA 5.4.1/01

			Study no. 3376
OECD TG 471 No deviations S-1812 (pyridalyl) Batch No. PS 98041G Purity 93.7% Organism/strain: TA100, TA98, TA1535 TA1537, Wp2uvrA	+S9-mix: 39.1, 78.1, 156, 313, 625, 1250 µg/plate <u>Positive controls:</u> Sodium azide; 9-aminoacridine; 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; 2-aminoanthracene	Precipitation: 1250 µg/plate (+S9) and 313 µg/plate (-S9) <u>Cytotoxicity:</u> none	
In vitro chromosomal aberration OECD TG 473 No deviations S-1812 (pyridalyl) Batch No. PS 98041G Purity 93.7% Organism/strain: Chinese hamster lung cells	<u>Exp 1:</u> -S9-mix (treatment 6 h and recovery 18 h): 20, 40, 80 µg/mL +S9-mix (treatment 6 h and recovery 18 h): 15, 20, 25 µg/mL <u>Exp 2:</u> -S9-mix (treatment and harvest 24 h): 625, 938, 1250 µg/mL -S9-mix (treatment and harvest 48 h): 39.1, 78.1, 156 µg/mL <u>Exp 3:</u> +S9-mix (treatment 6 h and recovery 18 h): 15, 20, 25 µg/mL	**<u>Exp 1:</u>** **-S9-mix: negative** **+S9-mix: positive (within historical control data)** **<u>Exp 2 and 3:</u>** **-S9-mix: negative** **+S9-mic: negative** <u>Cytotoxicity</u> Without S9-mix, treatment 6 h and recovery 18 h: none With S9-mix, treatment 6 h and recovery 18 h: ≥ 20 µg/mL Without S9-mix, treatment and harvest 24 h: ≥ 938 µg/ml Without S9-mix, treatment and harvest 48 h: ≥ 78.1 µg/ml <u>Precipitation</u> ≥ 78.1 µg/ml	IIA 5.4.3/02 Project No. 6311-215
In vitro gene mutation in mammalian cells OECD TG 476 No deviations S-1812 (pyridalyl) Batch No. PS 98041G Purity 93.7%	<u>Exp 1:</u> -S9-mix: 9.4, 18.8, 37.5, 75, 150 and 300 µg/ml +S9-mix: 2, 4, 5, 6, 7 and 8 µg/ml <u>Exp 2:</u> -S9-mix: 9.4, 18.8, 37.5, 75, 150 and 300 µg/ml +S9-mix: 2, 4, 5, 6, 7, 8 and 10 µg/ml <u>Positive controls:</u> 5-Bromo-2'-deoxyuridine, 20-methylcholanthrene	**-S9-mix: negative** **+S9-mix: negative** <u>Cytotoxicity</u> -S9-mix: none +S9-mix: 5 µg/ml and above <u>Precipitation</u> 157 µg/ml and above	IIA 5.4.3/02 Project No. 6311-215

Organism/strain: Chinese hamster ovary (CHO) cells			

RAC notes that in experiment number 1 of the *in vitro* chromosomal aberration test (IIA 5.4.3/02) the mutation frequencies were significantly higher than concurrent control but within the historical control data and that this positive result could not be replicated in two additional experiments (one of the with longest exposure period).

The table below summarises the mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*.

***Table**: Summary of mutagenicity/genotoxicity in vivo studies with pyridalyl.*

Method	Tested concentrations	Results	Reference
Mammalian erythrocyte micronucleus OECD TG 474 No deviations S-1812 (pyridalyl) Batch No. PS 98041G Purity: 93.7% Organism: Crj:CD-1 (ICR) mouse 5 males/dose	500, 1000 and 2000 mg/kg bw 24 hours and 48 hours treatment <u>Positive control:</u> Cylophosphamide	**Negative** <u>Cytotoxicity:</u> - dose range finding test: 1000 and 2000 mg/kg - main test: 2000 mg/kg <u>Toxicity:</u> Clinical sign of soft stool observed at 1000 and 2000 mg/kg bw	IIA 5.4.4/01 Study No. 3421
UDS test OECD TG 486 No deviations S-1812 (pyridalyl) Batch No. PS 98041G Purity 93.7% Organism: Sprague Dawley rats (Crl: CD (SD)IGS BR) 4 males/dose	500, 1000 and 2000 mg/kg bw 24 hours and 48 hours treatment <u>Positive control:</u> dimethylnitrosamine	**Negative** <u>Cytotoxicity:</u> none	IIA 5.4.5/01 Study No. 6311-214

Pyridalyl induced no significant decrease in the ratio of polychromatic erythrocytes to whole erythrocytes in the *in vivo* micronucleus test. The toxicokinetic studies showed that pyridalyl is able to reach the bone marrow. Therefore, RAC concludes that pyridalyl has no potential to induce micronuclei in mouse bone marrow cells. RAC also notes that pyridalyl also showed no capability to induce unscheduled DNA synthesis in rats.

Comparison with the criteria

Pyridalyl tested negative for gene mutation in a bacterial gene mutation study and in an *in vitro* mammalian gene mutation study. In an *in vitro* study, a weak positive response for clastrogenicity was detected, which alone is considered an inconclusive result. This positive result was not confirmed in two *in vivo* (micronucleus and unscheduled DNA synthesis) tests. Overall, it is concluded that pyridalyl does not fulfil the criteria for classification and therefore RAC supports the DS's proposal for **no classification of pyridalyl for germ cell mutagenicity.**

10.9 Carcinogenicity

Table 20: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 453 Sprague-Dawley rats, Crj:CD(SD) 50/sex/dose (main) 20/sec/dose (satellite) Deviations: none	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7% 0, 30, 100, 500, 1000 mg/kg food (Equal to 0, 1.01, 3.40, 17.1 and 34.3 mg/kg bw/day in males and 0, 1.23, 4.10, 21.1 and 42.8 mg/kg bw/day in females.) Duration: -Main: 104 weeks -Satellite: 52 weeks	No treatment related increase in tumor formation NOAEL: 3.4 mg/kg bw/day males and 4.1 mg/kg bw/day females LOAEL: 17.1 mg/kg bw/day males and 21.1 mg/kg bw/day females based on increased motor activity, reduced body weight (gain), reduced food consumption and brown pigment deposition in the spleen.	IIA 5.5.2/01 Study no: IET 99-0011
OECD 451 ICR mice, Crj:CD-1 52/sec/dose (main) 12/sec/dose	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7% 0, 15, 50, 1000 and 2500 mg/kg food	No treatment related increase in tumor formation NOAEL: 5.0 mg/kg bw/day males and 4.8 mg/kg bw/day females LOAEL: 99 mg/kg bw/day males and females based on decreased body weight and body weight gains.	IIA 5.5.3/01 Study no: IET 99-0012

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
(satellite) Deviations: none	(Equal to 0, 1.53, 5.04, 99 and 267 mg/kg bw/day in males and 0, 1.46, 4.78, 99 and 264 mg/kg bw/day in females) Duration: Main: 78 weeks Satellite: 52 weeks		

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

In a 104-week combined chronic/carcinogenicity study in rats, dose levels of 0, 30, 100, 500 and 1000 mg/kg food (equal to 0, 1.01, 3.40, 17.15 and 34.3 mg/kg bw/day in males and 0, 1.23, 4.10, 21.1and 42.8 mg/kg bw/day in females) were tested (IIA 5.5.2/01).

At functional observations an increase in motor activity was observed in males and females at 1000 mg/kg food and females at 500 mg/kg food. Decreased body weight gains and food consumption were noted in females and males at 500 and 1000 mg/kg food. At haematology, males at 1000 mg/kg food showed statistically significant decreases in haematocrit, haemoglobin and erythrocyte count and an increase in prothrombin time after 52 weeks.

Non-neoplastic observations included histopathological changes in the spleen and liver. Findings in liver included an increased incidence in peliosis in females at 1000 mg/kg food. Findings in spleen included an increased incidence of brown pigment deposition in males at 1000 mg/kg food and females at 500 and 1000 mg/kg food.

Necropsy revealed an increased incidence of hypertrophy of the mammary gland at 30, 100 and 500 mg/kg food in the females killed in extremis or found dead during the study. As no dose-response was observed, these findings were not considered to be related to treatment (see Table 21). At 1000 mg/kg food, an increased incidence of thickened area in the auricles and spots in the liver were observed in females.

In females at 1000 mg/kg food, a significantly increased incidence of adenocarcinoma of the mammary gland in the animals killed in extremis or found dead was noted. A similar increase, but not statistically significant, was noted in all other treatment groups when compared to controls, without a dose response (see Table 21). An increased incidence of adenocarcinoma of the mammary gland was not apparent in the animals killed at 52 of 104 weeks. Historical control data (2 studies, Crj:CD (SD) rats, same lab, dated 1997-2001, study carried out from 1999-2001) indicated that in the present study, the incidence of adenocarcinoma of the mammary gland in control animals was rather low. The incidence of adenocarcinoma of the mammary gland in the historical control studies was 20% (10/50)

and 20.4% (10/49). Since no dose-response was noted and since the observed incidence in treatment groups was equivalent to the incidence in historical control data, the observed increase in adenocarcinoma of the mammary gland was not considered treatment related.

Based on increased motor activity, reduced body weight (gain), reduced food consumption and brown pigment deposition in the spleen at 500 and 1000 mg/kg food, the NOAEL is established at 100 mg/kg food (equal to 3.4 mg/kg bw/day in males and 4.1 mg/kg bw/day in females). No oncogenic potential of the test substance in rats was observed.

Table 21: Summary table of neoplastic and non-neoplastic findings in the rat carcinogenicity study

Dose (mg/kg food)	**0**		**30**		**100**		**500**		**1000**	
Dose (mg/kg bw/day)	**0**	**0**	**1.01**	**1.23**	**3.40**	**4.10**	**17.15**	**21.1**	**34.3**	**42.8**
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality - main - satallite	22/50 0/20	33/50 1/20	21/50 1/20	31/50 0/20	20/50 0/20	23/50 0/20	13/50 0/20	29/50 0/20	25/50 1/20	22/50 2/20
Liver sports	6/50	6/50	2/50	6/50	2/50	3/50	3/50	3/50	2/50	12/50
Liver peliosis - week 52 - week 104 - KIE - Total (main study)	 1/20 3/26 1/22 4/50	 3/18 0/17 3/33 3/50	 0/19 1/29 1/21 2/50	 0/20 0/19 2/31 2/50	 1/20 0/30 1/20 1/50	 0/20 2/27 0/23 2/50	 0/20 6/37 1/13 7/50	 0/20 2/20 2/30 4/50	 0/19 2/25 0/25 2/50	 0/18 7/28* 2/22 9/50
Spleen, brown pigment deposition - week 52 - week 104 - KIE - Total (main study)	 4/20 2/28 7/22 9/50	 4/18 2/17 20/33 22/50	 1/19 0/29 9/21 9/50	 4/20 3/19 17/31 20/50	 2/20 0/30 4/20 4/50	 5/20 9/27 15/23 24/50	 4/20 3/37 7/13 10/50	 3/20 10/20* 17/30 27/50	 3/19 5/25 13/25 18/50*	 11/18* 16/28* 15/22 31/50
Mammary gland hypertrophy - week 52 - week 104 - KIE - Total (main study)		 4/18 4/17 5/33 9/50		 3/20 5/19 14/31* 19/50*		 3/20 4/27 9/23* 13/50		 2/20 5/20 11/30* 16/50		 0/18 4/28 8/22 12/50
Mammary gland adenomas - week 52 - week 104 - KIE - Total (main study)		 0/18 0/17 2/33 2/50		 0/5 0/9 3/31 3/40		 0/4 0/18 3/23 3/41		 1/2 1/14 2/29 4/43		 0/18 1/28 1/22 2/50
Mammary gland fibroadenomas - week 52 - week 104 - KIE - Total (main study)		 0/18 9/17 8/33 17/50		 1/5 5/9 7/31 12/40		 0/4 15/18 6/23 21/41		 0/2 10/14 8/29 18/43		 0/18 11/28 5/22 16/50

Dose (mg/kg food)	**0**		**30**		**100**		**500**		**1000**	
Dose (mg/kg bw/day)	**0**	**0**	**1.01**	**1.23**	**3.40**	**4.10**	**17.15**	**21.1**	**34.3**	**42.8**
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality										
- main	22/50	33/50	21/50	31/50	20/50	23/50	13/50	29/50	25/50	22/50
- satallite	0/20	1/20	1/20	0/20	0/20	0/20	0/20	0/20	1/20	2/20
Liver sports	6/50	6/50	2/50	6/50	2/50	3/50	3/50	3/50	2/50	12/50
Liver peliosis										
- week 52	1/20	3/18	0/19	0/20	1/20	0/20	0/20	0/20	0/19	0/18
- week 104	3/26	0/17	1/29	0/19	0/30	2/27	6/37	2/20	2/25	7/28*
- KIE	1/22	3/33	1/21	2/31	1/20	0/23	1/13	2/30	0/25	2/22
- Total (main study)	4/50	3/50	2/50	2/50	1/50	2/50	7/50	4/50	2/50	9/50
Spleen, brown pigment deposition										
- week 52	4/20	4/18	1/19	4/20	2/20	5/20	4/20	3/20	3/19	11/18*
- week 104	2/28	2/17	0/29	3/19	0/30	9/27	3/37	10/20*	5/25	16/28*
- KIE	7/22	20/33	9/21	17/31	4/20	15/23	7/13	17/30	13/25	15/22
- Total (main study)	9/50	22/50	9/50	20/50	4/50	24/50	10/50	27/50	18/50*	31/50
Mammary gland adenocarcinoma		0/18		2/5		1/4		0/2		0/18
- week 52		3/17		3/9		4/18		6/14		4/28
- week 104		2/33		7/31		5/23		6/29		6/22*
- KIE		5/50		10/40		9/41		12/43		10/50
- Total (main study)										

KIE killed in extremis or found dead
* statistically significant

In a 78-week oral carcinogenicity study, mice were given 0, 15, 50, 1000 and 2500 mg/kg food (IIA 5.5.3/01). Decreased body weight gains were noted at 1000 and 2500 mg/kg food, and a decrease in food consumption at 2500 mg/kg food. At haematology, males at 2500 mg/kg food showed statistically significant decrease in lymphocyte count at week 78. No further treatment-related changes in haematology were observed. Changes in organ weight were noted at interim and terminal necropsy. At 52 weeks, females showed increased absolute and relative liver and kidney weights at 2500 mg/kg food. Changes in liver and kidney weight were not observed in week 78, and were not accompanied by histopathological changes. Males at 2500 mg/kg food showed in week 52 increased relative weights of brain, lung and epididymes, but these changes were considered to be due to the decrease in body weight. In week 78, at 2500 mg/kg food a decrease in absolute weight of the heart was noted in males, an increase in the relative weight of the brain was noted in males and females and an decrease in absolute spleen weight was noted in females. These changes were considered to be due to the decrease in body weight.

Necropsy revealed no toxicologically relevant changes (Table 22). At histopathology, an increased incidence of lung tumours (adenoma and adenocarcinoma) was noted in females at 2500 mg/kg food. However, historical control data (same strain, same labe, 9 studies, dated within 10 years (1992-2001)) indicated, that the observed incidence at 2500 mg/kg food was well within the historical control range (adenoma: range 3.8 – 26.8% animals, average 13.85%; adenocarcinoma: range 2-15.4%, average 9.31% and adenoma plus adenocarcinoma: range 15.4-42.3%, average 23.2%). Furthermore, no neoplastic changes in lungs were observed in males. It is therefore, concluded that the increased incidence of lung tumours in females is considered to be incidental and not related to treatment. No

treatment-related changes in non-neoplastic observations were observed. The only significant changes observed were decreases in the number of animals with increased extramedullary hematopoiesis of the mesenteric lymph node in males, mucosal epithelial cell hyperplasia of the glandular stomach in females and arteritis of the overay in females. Since the changes were decreases instead of increases they were not considered treatment-releated.

Based on the lower body weights and body weight gains at 1000 and 2500 mg/kg food, the NOAEL is established at 50 mg/kg food (equal to 5.0 mg/kg bw/day in males and 4.8 mg/kg bw/day in females). There is no evidence of an oncogenic potential of the test substance in mice.

Table 22: Summary table of neoplastic findings in the mouse carcinogenicity study

Dose (mg/kg food)	**0**		**30**		**100**		**500**		**1000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Dose mg/kg bw/day	**0**	**0**	**1.53**	**1.46**	**5.04**	**4.78**	**99**	**99**	**267**	**264**
Lung adenoma										
- week 52	1/9	1/11	1/10	1/11	0/10	1/12	0/11	1/12	0/12	1/11
- week 78	5/33	5/38	3/37	2/41	6/32	3/41	4/32	6/39	3/36	8/45
- KIE	2/19	1/14	2/15	1/11	3/20	1/11	4/20	0/13	2/16	2/7
- Total (main study)	7/52	6/52	5/52	3/52	9/52	4/52	8/52	6/52	5/52	10/52
Lung adenocarcinoma										
- week 52	0/9	0/11	0/10	0/11	0/10	1/12	0/11	1/12	1/12	0/11
- week 78	4/33	3/38	3/37	4/41	7/32	4/41	7/32	3/39	6/36	5/45
- KIE	2/19	1/14	0/15	0/11	1/20	0/11	4/20	3/13	1/16	2/7
- Total (main study)	6/52	4/52	3/52	4/52	8/52	4/52	11/52	6/52	7/52	7/52

KIE killed in extremis or found dead
* statistically significant

10.9.2 Comparison with the CLP criteria

No information is available regarding carcinogenicity in humans. Therefore category 1A is not applicable.

Classification in category 1B requires "*a causal relationship between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites*".

Classification in category 2 requires "*the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs*".

As there were no treatment related effects on tumor formation in the available experimental animal studies (rat and mouse), classification for carcinogenicity is not considered.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification is proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of pyridalyl based on two carcinogenicity studies (one in rats and one in mice) showing no evidences of treatment-related effects on tumour formation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

104-week combined chronic/carcinogenicity study in rats (IIA 5.5.2/01, study no: IET 99-0011)

The table above in the STOT RE section summarised this study and the main non-neoplastic findings. The table below summarises the main neoplastic findings in females. At 42.8 mg/kg bw/day, a significantly increased incidence of adenocarcinoma of the mammary gland in the animals killed *in extremis* or found dead was noted. A similar increase, but not statistically significant, was noted in all other treatment groups when compared to controls, but without a dose response. Historical control data (2 studies, Crj:CD (SD) rats, same laboratory, dated 1997-2001, study carried out from 1999-2001) indicated that in the present study, the incidence of adenocarcinoma of the mammary gland in control animals was rather low. The incidence of adenocarcinoma of the mammary gland in the historical control studies was 20% (10/50) and 20.4% (10/49). Since no dose-response was noted and the observed incidence in treatment groups was equivalent to the incidence in historical control data, RAC does not consider the observed increase in adenocarcinoma of the mammary gland in females as treatment related. A summary of the neoplastic findings is presented in the table below.

***Table**: Summary of neoplastic findings in females in the rat carcinogenicity study*. KIE killed in extremis or found dead, * statistically significant ($p < 0.05$)

	DOSE (mg/kg bw/day)				
	0	**1.23**	**4.10**	**21.1**	**42.8**
Mortality:					
-main	33/50	31/50	23/50	29/50	22/50
-satellite	1/20	0/20	0/20	0/20	2/20
Mammary gland hypertrophy:					
-week 52	4/18	3/20	3/20	2/20	0/18
-week 104	4/17	5/19	4/27	5/20	4/28
-KIE	5/33	14/31*	9/23*	11/30*	8/22
-Total	9/50	19/50*	13/50	16/50	12/50
Mammary gland adenomas:					
- week 52	0/18	0/5	0/4	1/2	0/18
- week 104	0/17	0/9	0/18	1/14	1/28

- KIE	2/33	3/31	3/23	2/29	1/22
- Total	2/50	3/40	3/41	4/43	2/50
Mammary gland fibroadenomas:					
- week 52	0/18	1/5	0/4	0/2	0/18
- week 104	9/17	5/9	15/18	10/14	11/28
- KIE	8/33	7/31	6/23	8/29	5/22
- Total	17/50	12/40	21/41	18/43	10/50
Mammary gland adenocarcinoma					
- week 52	0/18	2/5	1/4	0/2	0/18
- week 104	3/17	3/9	4/18	6/14	4/28
- KIE	2/33	7/31	5/23	6/29	6/22*
- Total	5/50	10/40	9/41	12/43	10/50

78-week oral carcinogenicity study (IIA 5.5.3/01, study no: IET 99-0012)

The table above in the STOT RE section summarised this study and the main non-neoplastic findings. The table below summarises the main neoplastic findings. An increased incidence of lung tumours (adenoma and adenocarcinoma) was noted in females at 264 mg/kg bw/day. However, historical control data (same strain, same laboratory, 9 studies, dated within 10 years (1992-2001)) indicated that the observed incidence was well within the historical control range (adenoma: range 3.8–26.8% animals, average 13.85%; adenocarcinoma: range 2-15.4%, average 9.31% and adenoma plus adenocarcinoma: range 15.4-42.3%, average 23.2%). No neoplastic changes in lungs were observed in males. RAC concludes that the increased incidence of lung tumours in females is incidental and not related to treatment.

Table**: Summary of neoplastic findings in the mouse carcinogenicity study. KIE killed in extremis or found dead, * statistically significant

	DOSE (mg/kg bw/day)									
	0	**0**	**1.53**	**1.46**	**5.04**	**4.78**	**99**	**99**	**267**	**264**
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Lung adenoma										
- week 52										
- week 78	1/9	1/11	1/10	1/11	0/10	1/12	0/11	1/12	0/12	1/11
- KIE	5/33	5/38	3/37	2/41	6/32	3/41	4/32	6/39	3/36	8/45
- Total (main study)	2/19 7/52	1/14 6/52	2/15 5/52	1/11 3/52	3/20 9/52	1/11 4/52	4/20 8/52	0/13 6/52	2/16 5/52	2/7 10/52
Lung adenocarcinoma-ma										
- week 52	0/9	0/11	0/10	0/11	0/10	1/12	0/11	1/12	1/12	0/11
- week 78	4/33	3/38	3/37	4/41	7/32	4/41	7/32	3/39	6/36	5/45
- KIE	2/19	1/14	0/15	0/11	1/20	0/11	4/20	3/13	1/16	2/7
- Total (main study)	6/52	4/52	3/52	4/52	8/52	4/52	11/52	6/52	7/52	7/52

Comparison with the criteria

RAC notes no treatment related tumours could be detected in one study in rats and one study in mice. Thus, RAC supports the DS's proposal for **no classification of pyridalyl for carcinogenicity.**

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 23: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
None (preliminary study) Rat, Crj:CD(SD) 8/sex/dose	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7% 0, 100, 500 or 1000 mg/kg food	<u>Parental:</u> 500 ppm: Decreased body weight gain and food consumption early in the study (week 0-3) 1000 ppm: decreased body weight (gain), reduced food consumption, single cell necrosis of hepathocytes <u>Fetal findings:</u> 1000 ppm: decreased pup weight No effect on sexual development <u>Reproductive:</u> No treatment related findings	IIA 5.6.1/01 Study No. 99-0076
OECD 416 Rat, Crj:CD(SD) 24/sex/dose Deviations: None	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7% 0, 40, 200 and 1000 mg/kg food (equivalent to 0, 2.8, 13.3 and 66.7 mg/kg bw/day)	Parental NOAEL: 2.8 mg/kg bw/day and LOAEL 13.8 mg/kg bw/day, based on decreased body weight and increased testes and ovary weights. Developmental NOAEL: 2.8 mg/kg bw/day and LOAEL 13.8 mg/kg bw/day, based on decreased pup weights and delay in completion of vaginal opening in F1 pups Reproductive NOAEL: >66.7 mg/kg bw/day, highest dose tested. No LOAEL.	IIA 5.6.1/02 Study No. 99-0077

Table 24: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Investigation of	S-1812	28-dietary study	No effect on testosterone,	IIA 5.6.9/01

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
hormonal activity Non-GLP, none guideline mechanistic study	(pyridalyl) Batch No. PS 98041G, purity 93.7%	Rat Crj:CD(SD) 8/males/dose; 16/females/dose 0, 100, 500, 1000, and 2000 mg/kg bw food (equivalent to 0, 5.5, 25.5, 49.9 or 94.9 mg/kg bw/day in males and 0, 6.1, 29.5, 54.9 or 102.2 mg/kg bw/d for females)	oestradiol and progestrone. Increased corticosterone in females. Decreased dorso-laterel prostate and seminal vesicle weight in males.	Study No. S0998
Sex steroid hormone biosynthesis Non-GLP, none guideline mechanistic study	S-1812 (pyridalyl) Batch No. KOBE-95006, purity 98.4%	Leydig and ovary cells obtained from Crj:CD(SD) rats. Tested concentrations: 0, 1, 3, 10, 20 µM. 5-48 hours incubation period.	<u>Leydig cells:</u> Increased androstenedione Increased 17α-OH-progesterone (non-signifcant). No effect on testosterone <u>Ovary cells:</u> No effect on 17α-OH-progesterone, androstenedione, testosterone and oestradiol. Decrease in androstenedione metabolite production but no dose response observed (86.2, 86.0 and 89%).	IIA 5.6.9/02 Study No. X0091
Reporter gene assay non-GLP, none guideline mechanistic study	S-1812, Batch No. 1980202-1, purity 94.2%	HeLa cells transfected with human oestrogen receptor alpha (hERα), androgen receptor (hAR) and thyroid hormone receptor alpha (hTRα). 40 hours incubation	No effect on human oestrogen, androgen or thyroid receptors.	IIA 5.6.9/03 Study No. RGA-002

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In a preliminary study, Sprague-Dawley rats, 8/sex/group, were given diets containing pyridalyl at dose levels of 0, 100, 500 and 1000 ppm (IIA 5.6.1/01). P animals were fed the diet for approximately 9 or 12 weeks (males, 9 weeks including the mating period; females, 4 weeks prior to the mating period and 8 weeks during the subsequent breeding period) and F1 animals for 10 weeks after weaning.

In the 100 ppm group no adverse effects of the test substance were found. In the 500 ppm group, body weight gains of P males were suppressed during early phase of the study period (week 1-3). In addition, mean food consumption was significantly decreased in P males at treatment week 1. In the 1000 ppm group, decreased mean body weight were found in P males and F1 females, body weight gains of P animal of both sexes and food consumption of P males as well as F1 pup weight of both sexes were affected. Significant increases were found in absolute and relative lung weight of P females and single cell necrosis of hepatocytes was observed in both P and F1 females. No effect on fertility parameters or sexual development markers (preputial separation, vaginal opening) was observed. Based on the results, dose of levels of 100 or less and 1000 ppm were considered suitable for low and high dose levels, respectively.

In an oral 2-generation reproduction study rats were given 0, 40, 200 and 1000 mg/kg food (equivalent to 0, 2.8, 13.8 and 68.7 mg/kg bw/day) (IIA 5.6.1/02).

No adverse effects of the test substance treatment were found in P and F1 parental animals of the 40 ppm group. Changes in parental body weights and food consumption were noted at 200 and 1000 mg/kg food. There were no changes observed between P and F1 parental animals of the treated and control groups in mating indices, fertility indices, gestation indices, implantation sites, oestrus cycle and sperm evaluation. F1 males showed increased absolute testes weights at 200 and 1000 mg/kg food, relative testes weights were increased at 1000 mg/kg food. Increased absolute ovary weights were noted in P and F1 females at 1000 mg/kg food and in F1 females at 200 mg/kg food. Increased relative ovary weights were also seen in P and F1 females at 1000 mg/kg food and in F1 females at 200 mg/kg food. Histopathological examination revealed an increased incidence of vacuolation of ovarian interstitial gland cells and of increased small-sized follicles in the thyroid in the P and F1 females at 1000 mg/kg food.

No treatment-related changes were detected in litter size, viability index or sex ratio or clinical signs of the F1 and F2 pups with values comparable to control animals. Changes in pup weight were noted at 200 and 1000 mg/kg food. Changes in sexual development of F1 and F2 females were noted. In F1 females at 200 and 1000 mg/kg food and in F2 females at 1000 mg/kg food a delay in completion of vaginal opening was noted. In F1 females body weight at vaginal opening was not significantly affected. In F2 females there was a significant increase in body weight at vaginal opening in the high dose group (see table 25).

Statistically significant decreased absolute and/or relative thymus weights were noted in F1 and F2 pups at 200 and/or 1000 mg/kg food. At necropsy no treatment-related abnormalities were observed in F1 and F2 pups. Organs of F1 and F2 pups were not investigated histopathologically.

In order to elucidate the changes in thymus weight of F1 and F2 pups, histopathology of the thymus was performed (IIA 5.6.1/03, separate report). Ten F2 male pups with significantly decreased thymus weight and ten control male F2 pups were selected for histopathological examination. No abnormalities were observed in any of the pups examined.

As no effects on fertility were noted the NOAEL for reproductive effects is set at 1000 mg/kg food (equivalent to 68.7 mg/kg bw/d). Based on decreased body weight (gain) in P parental animals and increased testes and ovary weights in F1 parental animals at 200 and 1000 mg/kg food, the NOAEL for parental effects is set at 40 mg/kg food (equivalent to 2.8 mg/kg bw/day). The NOAEL for developmental toxicity is set at 40 mg/kg food (equivalent to 2.8 mg/kg bw/day), based on the decreased F1 and F2 pups weights and delay in the completion of vaginal opening in F1 pups.

Table 25: Age at vaginal opening in the 2-generation studies

Preliminary study (F1)				
Dose (mg/kg food)	**0**	**100**	**500**	**1000**
Completion of vaginal opening (days)	30.1	29.6	30.9	30.8
Weight at vaginal opening (g)	108	104	104	101
F1 (main study)				
Dose (mg/kg food)	**0**	**40**	**200**	**1000**
Completion of vaginal opening (days)	29.0	29.8	**31.0***	**31.1***
Weight at vaginal opening (g)	90.5	93.8	94.8	94.4
F2 (main study)				
Dose (mg/kg food)	**0**	**40**	**200**	**1000**
Completion of vaginal opening (days)	29.6	29.5	30.3	**31.3***
Weight at vaginal opening (g)	95.4	98.2	97.5	**104.6***

Data from the rat reproduction toxicity and oral subchronic toxicity studies indicated that pyridalyl may affect lipid metabolism and consequently hormone levels. Vacuolization was observed in endocrine organs such as the ovary and adrenal glands in rats, and decreases in testosterone and oestradiol were noted in the 90-day rat study at 233 mg/kg bw/day in males and 256 mg/kg bw/day for females. Three mechanistic studies were performed to further investigate the effect of pyridalyl on steroid hormone biosynthesis (see section 3.10.3 of Annex 1).

Rats were administered 100, 500, 1000 or 2000 mg/kg food of S-1812 via the diet for 28 days in order to investigate changes in steroid hormone status (IIA 5.6.9/01). Diet levels were chosen to explore effect seen at a dose, which was twice as high as the top dose level of the multigeneration study. S-1812, did not affect testosterone, oestradiol and progesterone levels in rats after 4 week dietary exposure. In females higher values for corticosterone were noted at 500, 1000 and 2000 mg/kg food, due to high individual values in each group, but not attaining statistical significance. The observed change in corticosterone in females is of unknown toxicological significance. In addition, decreased dorso-lateral prostate and seminal vesicle weights were noted in males at 500, 1000 and 2000 mg/kg food. Furthermore, histopathology revealed an increased incidence of vacuolation of ovarian interstitial gland cells in females at 2000 mg/kg food. It was concluded that an effect on the endocrine system cannot complete ruled out, however, equivocal effects occur at dose levels equal or higher than dose levels showing general toxicity (e.g. decreased body weight).

Fresh cultures of Leydig or ovarian cells were cultured in medium containing pyridalyl, in order to examine differences in steroid hormone production as a result of exposure to pyridalyl (IIA 5.6.9/02). This study was not conducted to GLP and there is no guideline appropriate for this kind of assay. Leydig and ovarian cells were incubated in medium containing different concentrations of test material which, after 5 and 48 hours, were sampled for analysis of the following hormone levels: progesterone, 17α-OH-PG, androstenedione, testosterone and oestradiol.
In Leydig cells S-1812 caused an increase in androstenedione and 17 α-OH-progesterone levels. In addition, S-1812 caused a slight decrease in androstenedione metabolite production, indicating an inhibition of 17β-hydroxysteroid dehydrogenase activity. However, the decrease was only slight,

93% and 94% of controls at 3 and 30 µM, respectively, and no decrease was noted at 10 µM. No change was noted in testosterone levels.
In cultures of rat ovary cells, S-1812 caused did not affect the concentrations of progesterone, 17α-OH-progesterone, androstenedione and oestradiol. After incubation of [^{14}C]androstenedione, S-1812 caused a decrease in androstenedione metabolite production in ovary cells. However, the effect was only statistically significant at 10 µM and no clear dose response was observed (86.2, 86.0 and 89% of control). Incubation of the ovarian cells with [^{14}C]testosterone and S-1812, did not change [^{14}C]oestradiol production.
In conclusion, slight changes in the steroid hormone biosynthesis pathway were noted after exposure of rat Leydig and ovarian cells to S-1812, however, the changes did not result in alterations in testosterone of oestradiol levels.

In a gene reporter assay with Hela cells from human cervical carcinoma, the effects of S-1812 on transactivation by human oestrogen receptor alpha (hERα), androgen receptor (hAR) and thyroid hormone receptor alpha (hTRα) was investigated (IIA 5.6.9/03). S-1812 had no direct agonistic effects on the oestrogen receptor alpha, the androgen receptor and the thyroid hormone receptor alpha. S-1812 had also no effect on the expression due to the agonists, therefore also had no antagonistic response. It was concluded that S-1812 did not show a direct effect on human oestrogen, androgen or thyroid receptors.

Overall, pyridalyl showed slight changes in steroid biosynthesis pathways at high dose levels only after exposure of Leydig and ovarian cells. However, these changes did not result in alterations in testosterone of oestradiol levels. The 2-generation study showed no effects on mating indices, fertility indices, gestation indices, implantation sites, oestrus cycle and sperm evaluation indicating that pyridalyl has no effect on fertility. .

10.10.3 Adverse effects on development

Table 26: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 414 Deviations: none Rat, Crj:CD(SD) 24/females/dose	0, 10, 50 and 250 mg/kg bw/day GD 6-19	Maternal NOAEL 10 mg/kg bw/day and LOAEL of 50 mg/kg bw/day, based on decreased body weight gain. Developmental NOAEL >250 mg/kg bw/day, highest dose tested. A LOAEL was not determined.	IIA 5.6.10/02 Study No 00-0094
OECD 414	0, 15, 50 and 150 mg/kg	Maternal NOAEL: 50 mg/kg bw/day and a LOAEL of 150 mg/kg bw/day, based on decreased body weight gain and mortality.	IIA 5.6.10/01

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Deviations: none Rabbit, Japanese White (Kbl:JW) 25/females/dose (30 in high dose)	bw/day GD 6-27	Developmental NOAEL: 50 mg/kg bw/day and a LOAEL of 150 mg/kg bw/day,based on decreased foetal weight.	Study No 00-0095

10.10.4 Short summary and overall relevance of the provided information on adverse effects on development

A teratogenicity study was conducted to evaluate the potential maternal and developmental toxicity of pyridalyl in rats (IIA 5.6.10/02). The test substance was dissolved in corn oil and administered orally, via gavage, to pregnant Crj:CD (SD) female rats (24 per group) once per day from days 6 to 19 of gestation at dose level of 0, 10, 50 or 250 mg/kg. The dose levels were based on a preliminary study.

In the 10 mg/kg group no adverse effects were observed in maternal rats in such parameters as clinical findings, body weight, body weight gains, food consumption and pathological findings. In the 50 mg/kg and 250 mg/kg bw groups, mean body weight gains were significantly lower than those in the control group.In the 250 mg/kg bw group reduced food consumption was also observed. No other effects were observed in maternal animals. As for foetuses, the number of live foetuses, percent resorptions and foetal deaths, foetal body weights, placental weights, and sex ratio were comparable to those in the control group. There were no treatment-related abnormalities in external, visceral and skeletal examinations of live foetuses up to and including the highest dose of 250 mg/kg. External examination of live foetuses revealed mandibular micrognathia in one fetus in the 10 and 250 mg/kg bw group and omphalocele in one fetus in the 10 mg/kg bw group. No visceral malformations were observed in any of the treatment groups. Regarding visceral and skeletal variations the incidences were comparable to the control group.

The NOAEL for maternal effects was set at 10 mg/kg bw/day, based on the decrease in body weight gain (>10%) in maternal females at 50 and 250 mg/kg bw. Based on the absence of treatment-related findings on foetuses the NOAEL for developmental toxicity was set at 250 mg/kg bw/day.

A teratogenicity study was conducted to evaluate the potential maternal and developmental toxicity of pyridalyl in rabbits (IIA 5.6.10/01). Potential effects of the test substance on maternal animals and their foetuses were examined by treating artificially inseminated female rabbits (Kbl:JW), 25-30 females per group, with pyridalyl from days 6 through 27 of gestation at daily oral doses of 0, 15, 50 and 150 mg/kg. The dose levels were based on a preliminary study.

No adverse effects of the test substance treatment on maternal rabbits were observed in the 15 and 50 mg/kg groups. In the 150 mg/kg group, mean maternal body weight gains and food consumption after day 15 of gestation were lower than those in the control group. In this dose group, 1 dam was found dead on day 26 of gestation, 3 dams aborted on days 24-27 of gestation, and 1 dam prematurely delivered pups on day 28 of gestation. Neither observations at caesarean sectioning nor external, visceral and skeletal examinations of foetuses revealed adverse effects of the test substance treatment in the treated groups with the exception of the foetal weights in the 150 mg/kg group. Mean foetal weights in the 150 mg/kg group were lower than those in the control group, and the difference from the control in females was statistically significant.

The NOAEL for maternal effects was set at 50 mg/kg bw/day, based on decreased body weight gain and mortality. Based on decreased foetal weight at 150 mg/kg bw/day the NOAEL for developmental toxicity was set at 50 mg/kg bw/d.

10.10.5 Comparison with the CLP criteria

10.10.5.1 Effects on fertility

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2, classification as for effects on fertility is based on:

Category 1A:
Known human reproductive toxicant
Category 1B:
Presumed human reproductive toxicant largely based on data from animal studies
— clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or
— the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects
Category 2:
Suspected human reproductive toxicant
— some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and
— where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study).
— the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

According to the CLP criteria classification as Repr. 1A is based on human data. No human data are available for pyridalyl and therefore, classification as Repr 1A is not justified.

Since no effect on fertility or sexual function was found in the experimental animal studies the criteria for classification for cat. 1B and cat 2 are not met.

10.10.5.2 Effects on development

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2, classification as for effects on development is based on:

Category 1A:

Known human reproductive toxicant

Category 1B:

Presumed human reproductive toxicant largely based on data from animal studies

— clear evidence of an adverse effect on development in the absence of other toxic effects, or

— the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects

Category 2:

Suspected human reproductive toxicant

— some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and

— the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study).

— the adverse effect on development is considered not to be a secondary non-specific consequence of the other toxic effects

According to the CLP criteria classification as Repr. 1A is based on human data. No human data are available for pyridalyl and therefore, classification as Repr 1A is not justified.

In the developmental toxicity studies in rats and rabbits no relevant adverse effects on development were observed.

In the multigeneration study (IIA 5.6.1/02), a delay in completion of vaginal opening was observed. This adverse effect was consistently observed in two generations, i.e. in the F1 (a delay of 2.1 days compared with controls) and F2 females (1.7 days).
It is however noticed that also a decreased pup growth was observed in the preceding period (reflected by the reduced body weight at postnatal day 21 in the F1/F2 male and female pups) together with maternal toxicity (i.e. reduced body weight). Therefore, it cannot be firmly concluded that the delayed completion of vaginal opening is a direct effect of pyridalyl. However, feed restriction studies in rats focussing on postnatal development and puberty have shown that feed restriction not only induces delayed vaginal opening, but this marker of puberty is also acquired at a much lower body weight than in control animals (Carney et al., 2004; Kennedy and Mitra, 1963). In the current 2-generation study of pyridalyl, it was shown that in the F1 females weight at vaginal opening was similar as in control animal. In the F2 high dose females there was even a significant increase in weight at vaginal opening. This may provide some support for considering the observed adverse effect on development as a direct effect of pyridalyl, though it is acknowledged that there may still be some uncertainties concerning this issue.

In the 90-day study, decreased oestradiol was observed which could be an underlying mode of action causing the delay in sexual maturation. However, in the 90-day study the decrease in oestradiol was observed at a dose level of 256 mg/kg bw/day, which is higher than the highest dose tested in the 2-generation study (66.7 mg/kg bw/day). In a 28-dietary study investigating the hormonal activity of

pyridalyl no effect on oestradiol was observed up to 102.2 mg/kg bw/d. Higher corticosterone values were however noted in females due to high individual values in each groups.

Overall, taking all the information into account, the adverse effect of delayed completion of vaginal opening should be considered for classification for effects on development. However, it is acknowledged that there is still some uncertainty as to whether the observed adverse effect (i.e. delayed vaginal opening) is a direct effect. Therefore, it is considered that there is some evidence of adverse effects on the reproduction. This effect warrants classification in category 2.

10.10.5.3 Adverse effects on or via lactation

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2.2, classification for lactation effects is based on:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

In the 2-generation rat study, some effects on pup development were observed which might be relevant for this endpoint. At postnatal day 0, the pup weights were similar to the control animals, whereas at the end of the lactation period (postnatal day 21) pup weight was significantly reduced (F1 and F2, males and females). However, it is noted that this effect was observed in the presence of maternal toxicity (i.e. reduced parental bw). Therefore, these effects on pup growth are considered insufficient and are not further taking into account for classification for adverse effects on or via lactation.

10.10.6 Conclusion on classification and labelling for reproductive toxicity

Classification of pyridalyl for reproductive toxicity (effects on development) as Repr. 2 (H361d: May damage the unborn child) is required.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

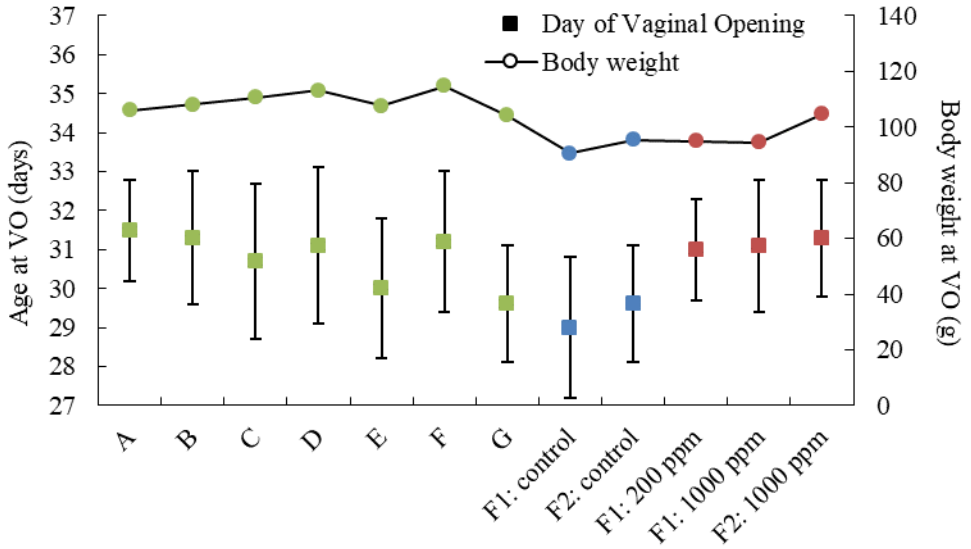
DS proposed classification of pyridalyl as Repr. 2; H361d (may damage the unborn child) based on a delay on vaginal opening supported by a certain hormonal imbalance detected in mechanistic studies. The DS also noted that the effects on pup growth were considered insufficient to support a classification.

Comments received during consultation

One MSCA agreed with the proposed classification of pyridalyl as Repr. 2; H361d based the observed delay in vaginal opening in both F1 and F2 generations in the 2-generation reproductive toxicity study. According to this MSCA there was a general delay in the development of offspring rather than maternal toxicity; and, since at the time of vaginal opening there were no significant decreases in female offspring body weight, the delayed in sexual maturation of female offspring is not a attributable to maternal toxicity. In the response to comments, the DS replied that this effect was observed in only one of the two generations; which raises doubts about whether the effect is dose-related or not and therefore, the most appropriate classification would be category 2.

The Industry released a position paper against the proposal of classification as Repr. 2; H361d. The arguments raised were based on the following points:

1 The age and weight of control animals at vaginal opening in the F1 and F2 generations were at the lower end or below the range of historical controls (A to G are study IDs); and age and weight of putatively affected offspring remained within the historical control data range of the performing facility (The Institute of Environmental Toxicology), as shown in the figure below.



2 In the U.S. EPA Standard Evaluation Procedure of Pubertal Development and Thyroid Function in Intact Juvenile-Peripubertal Female Rats (OCSPP 890.1450, U.S. EPA) the performance criteria for age at vaginal opening in SD rats are as follows; mean: 33.5 days, acceptable range: 30.67 to 35.62 days.

3 There was no effect on anogenital distance measured in F2 offspring (see data on the table below).

***Table**: Anogenital distance in F2 pups in the two-generation toxicity study*.

Dose level (mg/kg bw/day)	0	2.8	13.3	66.7
F2 AGD (mm)	0.93	0.95	0.94	0.95
AGD/(bw)$^{1/3}$	0.503	0.518	0.514	0.530
Body weight (g)	6.3	6.1	6.1	5.9

AGD/(Bw)$^{1/3}$: AGD relative to cube root of bodyweight

4 The preliminary study (Study No. IET 99-0076, Report No. SUT-0018) showed no differences on both the age at vaginal opening and the body weight at vaginal opening in F1 females between the control and 1000 ppm (equivalence to mg/kg bw/day not stated in the CLH report) group.

5 There were no effects on mating indices, fertility indices, gestation indices, implantation sites, oestrus cycle, anogenital distance and uterine weight in the two-generation toxicity study with pyridalyl. These findings support that slight delayed vaginal opening in F1 females is not based on the inhibition of oestradiol production or anti-estrogenic activity.

6 Increased ovary weight and/or increased incidence of vacuolation of ovarian interstitial gland cells were observed in P and F1 females in the two-generation toxicity study. In the metabolism study in rats (Study Nos. 807W/1221E-1 and 3515, Report Nos. SUM-0008 and SUM-0005, respectively), pyridalyl was widely distributed, with high and persistent residues occurring in fatty tissues. Pyridalyl was predominantly found in fat, but ovaries also showed relatively high residues. As lipid is abundant in ovarian interstitial cells, high residues of pyridalyl in ovaries are considered to have caused accumulation of lipid followed by the changes in lipid metabolism. Therefore, the effects on ovaries are attributed to high affinity of pyridalyl for lipid, but not to changes in steroidogenesis. RAC, in absence of other supplementary information, does not support this view.

7 Industry provided an *in silico* reprotoxicity and developmental toxicity assessment of pyridalyl using Derek Nexus. Derek Nexus is a rule-based expert system using structure activity relationships to make predictions on 5α-Reductase inhibition, androgen receptor modulation, developmental toxicity, oestrogen receptor modulation, oestrogenicity, teratogenicity, testicular toxicity and thyroid toxicity. Derek Nexus returned a negative prediction for all stated endpoints. However, it is not clear from the assessment report whether the negative prediction was caused by the Pyridalyl structure being outside the applicability domains of the models, and thus that the predictions are not valid, or represented true negative predictions.

In conclusion, according to the Industry, the differences in age at vaginal opening were marginal and there were not caused by pyridalyl exposure.

Assessment and comparison with the classification criteria

Adverse effect on fertility and sexual function

Table below summarises the available animal studies on fertility and sexual function.

***Table**: Summary for animal studies on adverse effects on sexual function and fertility with pyridalyl.* In all cases, the showed effects were statistically different form the corresponding controls.

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Preliminary study

No Guideline

No GLP

Crj:CD(SD) rats

8/sex/dose

S-1812 (pyridalyl)

Batch No. PS 98041G

Purity 93.7%

0, 100, 500 or 1000 mg/kg food (equivalence to mg/kg bw/day not stated in the CLH report)</td><td><u>Parental:</u>
500 ppm (males): Decreased body weight gain and food consumption early in the study (week 0-3)

1000 ppm: decreased body weight (gain), reduced food consumption, single cell necrosis of hepatocytes

<u>Foetal findings:</u>
1000 ppm (females): decreased pup weight (gain), single cell necrosis of hepatocytes

No effect on sexual development

<u>Reproductive:</u>

No treatment related findings</td><td>IIA 5.6.1/01

Study No. 99-0076</td></tr>
<tr><td>OECD TG 416

Deviations: F1 and F2 organs not histopathologically investigated

Crj:CD(SD) rats

24/sex/dose

S-1812 (pyridalyl)

Batch No. PS 98041G

Purity 93.7%

0, 40, 200 and 1000 ppm in food (0, 2.8, 13.8 and 68.7 mg/kg bw/day for males and 0, 3.11, 15.7 and 79.1 mg/kg bw/day for females)</td><td><u>Dosing:</u>
<table>
<tr><td></td><td colspan="2">40 ppm</td><td colspan="2">200 ppm</td><td colspan="2">1000 ppm</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td>P premating</td><td>2.8</td><td>3.1</td><td>14</td><td>16</td><td>69</td><td>79</td></tr>
<tr><td>P gestation/lactation</td><td>-</td><td>4.3</td><td>-</td><td>22</td><td>-</td><td>111</td></tr>
<tr><td>F1 premating</td><td>3.4</td><td>3.6</td><td>17</td><td>18</td><td>84</td><td>91</td></tr>
<tr><td>F1 gestation/lactation</td><td>-</td><td>4.6</td><td>-</td><td>23</td><td>-</td><td>117</td></tr>
</table>
<u>Parental:</u>
1000 ppm (always statistically significant)
<table>
<tr><td></td><td colspan="2">P0</td><td colspan="2">F1</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td>Body weight</td><td>↓ 10% (week 9)</td><td>↓ 6% (week 9)</td><td>↓ 10% (week 10)</td><td>-</td></tr>
<tr><td>Food consumption (week 5)</td><td>↓ 12%</td><td>-</td><td>↓ 10%</td><td>-</td></tr>
<tr><td>Ovary weight</td><td>-</td><td>↑ 11%</td><td>-</td><td>↑ 22%</td></tr>
<tr><td>Ovary cell vacuolation</td><td>-</td><td>-</td><td>-</td><td>8/24</td></tr>
<tr><td>Increased thyroid follicles</td><td>-</td><td>7/24</td><td>-</td><td>7/24</td></tr>
<tr><td>Testis weight</td><td>-</td><td>-</td><td>↑ 8%</td><td>-</td></tr>
</table>
200 ppm (always statistically significant)
<table>
<tr><td></td><td colspan="2">P0</td><td colspan="2">F1</td></tr>
<tr><td></td><td>M</td><td>F</td><td>M</td><td>F</td></tr>
<tr><td>Body weight (week 9)</td><td>↓ 10%</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Food consumption (week 5)</td><td>↓ 12%</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Ovary weight</td><td>-</td><td>-</td><td>-</td><td>↑ 12%</td></tr>
<tr><td>Testis weight</td><td>-</td><td>-</td><td>↑ 5%</td><td>-</td></tr>
</table></td><td>IIA 5.6.1/02

Study No. 99-0077</td></tr>
</table>

Foetal findings:
1000 ppm (always statistically significant)

	F1		F2	
	M	F	M	F
Body weight by day 21	↓ 10%	↓ 10%	↓ 10%	↓ 8%
Vaginal opening (day)		31.1 vs 29.0 in controls	-	31.3 vs 29.6 in controls
Weanling body weight	↓ 13%	↓ 13%	-	-
Weanling thymus weight	↓ 20%	↓ 23%	-	-

200 ppm (always statistically significant)

	F1		F2	
	M	F	M	F
Body weight by day 21	↓ 9%	↓ 8%	↓ 7%	↓ 7%
Vaginal opening (day)	-	31.0 vs 29.0 in controls	-	-
Weanling body weight	↓ 11%	↓ 16%	-	-
Weanling thymus weight	↓ 8%	↓ 3%	-	-

Reproductive:
No treatment related findings

Changes in pup weight and sexual development were noted at 13.3 and 66.7 mg/kg bw/day in F1 and F2. In F1 females at 13.3 and 66.7 mg/kg bw/day and in F2 females at 66.7 mg/kg bw/day a delay in completion of vaginal opening was noted (table below). In F1 females body weight at vaginal opening was not significantly affected. In F2 females there was a significant increase in body weight at vaginal opening in the high dose group (table below).

***Table**: Age at vaginal opening in the 2-generation studies (IIA 5.6.1/02 and IIA 5.6.1/02).* Other data of these studies is summarised in table above. According to the position paper provided by the Industry the historical control data of the performing facility (The Institute of Environmental Toxicology) were: i) for vaginal opening: 30.8 (29.6-31.5) days; ii) for weight at the vaginal opening day: 109.1 (104.2-114.6) g.

Preliminary study (F1)				
Dose (mg/kg food)	**0**	**40**	**200**	**1000**
Dose [mg/kg bw/day]	**[0]**	**[2.8]**	**[13.3]**	**[66.7]**
Completion of vaginal opening (days)	30.1	29.6	30.9	30.8
Weight at vaginal opening (g)	108	104	104	101
F1 (main study)				
Dose (mg/kg food)	**0**	**40**	**200**	**1000**
Dose [mg/kg bw/day]	**[0]**	**[2.8]**	**[13.3]**	**[66.7]**
Completion of vaginal opening (days)	29.0	29.8	**31.0***	**31.1***
Weight at vaginal opening (g)	90.5	93.8	94.8	94.4
F2 (main study)				
Dose (mg/kg food)	**0**	**40**	**200**	**1000**
Dose [mg/kg bw/day]	**[0]**	**[2.8]**	**[13.3]**	**[66.7]**
Completion of vaginal opening (days)	29.6	29.5	30.3	**31.3***
Weight at vaginal opening (g)	95.4	98.2	97.5	**104.6***

Three mechanistic studies (see table below) were provided in the CLH report for investigating the effects of pyridalyl on steroid hormone biosynthesis. Overall, it is concluded that pyridalyl caused slight changes in steroid biosynthesis pathways at high dose levels only after exposure of Leydig and ovarian cells. However, these changes did not result in alterations in testosterone of oestradiol levels.

***Table**: Summary of other studies relevant for assessment of the toxicity on sexual function and fertility caused by pyridalyl.*

Method	Results	Reference
28-dietary study Non-GLP No guideline S-1812 (pyridalyl) Batch No. PS 98041G Purity 93.7% Crj:CD(SD) rats 8/males/dose 16/females/dose 0, 5.5, 25.5, 49.9 or 94.9 mg/kg bw/day in males 0, 6.1, 29.5, 54.9 or 102.2 mg/kg bw/day in females	No effect on testosterone, oestradiol and progesterone. Increased corticosterone in females (not statistically significant). Decreased dorsolateral prostate and seminal vesicle weight in males. Vacuolation of ovarian. **Conclusion: An effect on endocrine system cannot completely ruled out.**	IIA 5.6.9/01 Study No. S0998
Sex steroid hormone biosynthesis Non-GLP No guideline S-1812 (pyridalyl) Batch No. KOBE-95006 Purity 98.4% Leydig and ovary cells obtained from Crj:CD(SD) rats Tested concentrations: 0, 1, 3, 10, 20 µM. 5-48 hours incubation	<u>Leydig cells:</u> Increased androstenedione. Increased 17α-OH-progesterone (non-significant). No effect on testosterone. <u>Ovary cells:</u> No effect on 17α-OH-progesterone, androstenedione, testosterone and oestradiol. Decrease in androstenedione metabolite production but no dose response observed (86.2, 86.0 and 89%). **Conclusion: Slight changes in the steroid hormone biosynthesis pathway without changes in testosterone and oestradiol levels.**	IIA 5.6.9/02 Study No. X0091
Reporter gene assay Non-GLP	No effect on human oestrogen, androgen or thyroid receptors. **Conclusion: Pyridalyl did not show a direct effect on**	IIA 5.6.9/03 Study No.

No guideline S-1812 (pyridalyl) Batch No. 1980202-1 Purity 94.2% HeLa cells transfected with human oestrogen receptor alpha (hERα), androgen receptor (hAR) and thyroid hormone receptor alpha (hTRα) 40 hours incubation	**human oestrogen, androgen or thyroid receptors.**	RGA-002

RAC notes that the limit of solubility of pyridalyl is 0.00015 mg/L; which corresponds to sub nanomolar concentration. Thus, RAC doubts of the reliability of the *in vitro* studies summarised in the table of above due to the difficulties of achieve concentrations of 20 µM even when DMSO was present in the media. Thus, RAC considers these two *in vitro* studies rather inconclusive.

Adverse effect on development

The table below summarises the animal studies on adverse effects on development.

***Table**: Summary for animal studies on developmental toxicity with pyridalyl.*

Method	Results	Reference
OECD TG 414 No deviations Crj:CD(SD) rats 24/females/dose 0, 10, 50 and 250 mg/kg bw/day GD 6-19	<u>Maternal effects:</u> 250 mg/kg bw/day: ↓ 33% body weight gain and 13% food consumption 50 mg/kg bw/day: ↓ 11% body weight gain <u>Developmental effects:</u> 250 mg/kg bw/day: mandibular micrognathia (1 foetus) 10 mg/kg bw/day: mandibular micrognathia (1 foetus), omphalocele (1 foetus)	IIA 5.6.10/02 Study No 00-0094
OECD 414 No deviations Japanese White (Kbl:JW) rabbits 25/females/dose (30 in high dose) 0, 15, 50 and 150 mg/kg bw/day GD 6-27	<u>Maternal effects:</u> 150 mg/kg bw/day: 0% mean body weight gain, ↓ 7% food consumption. 1 dam dead on GD 26, 3 abortions on GD 24-27, 1 premature delivering (GD 28). <u>Developmental effects:</u> 150 mg/kg bw/day: ↓ 12% mean female foetal weight	IIA 5.6.10/01 Study No 00-0095

<u>Comparison with the criteria</u>

Fertility and sexual function

According to repeated dose toxicity studies hormone synthesis was disrupted in adrenals and ovaries at relatively well tolerated doses of 256 mg/kg bw/day. The top dose of the 2-generation toxicity study was 67 mg/kg bw/day and no issues of concern as regard to fertility and sexual performance were reported at that dose. However, RAC notes that this dose seems well below of the dose apparently causing hormonal disruption. Thus, RAC cannot conclude on fertility due to lack of data at doses with the capability to disrupt hormonal homeostasis.

RAC notes that according to CLP Regulation, Annex I 3.7.1.3. Adverse effects on sexual function and fertility are *"any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems"*. Thus, the reported effects on sexual maturation, if any, should be considered fertility effects rather than developmental effects, as the DS considered.

A delay in the vaginal opening in female offspring in F1 and F2 of the 2-generation reproductive toxicity study was reported. At this respect, RAC notes that:

- The values of vaginal opening and weight at vaginal opening of affected rats were within the historical control data of the performing facility, while records of the control animals were below such controls (see above section "Comments received during consultation"). It suggests that the alterations in vaginal opening might be due to an incidental reduction in record of controls rather than treatment-related issue.
- The records of vaginal opening of controls are below performance criteria for age at vaginal opening of the U.S. EPA Standard Evaluation Procedure of Pubertal Development and Thyroid Function in Intact Juvenile-Peripubertal Female Rats (OCSPP 890.1450, U.S. EPA). However, the values of the animals treated at the two highest doses were within such values; suggesting again a statistical artefact related to lower records in control animals rather than a pyridalyl exposure related cause. However, RAC also doubts about the relevance of these control data because they do not correspond to the same performing facility and were not within the temporal framework usually considered as valid.
- Pyridalyl does not affect *in vitro* sex hormone biosynthesis (hormones involved in vaginal opening).
- No other alterations in reproductive performance suggest that pyridalyl is altering the sex hormone biosynthesis, although a concern about whether the highest tolerable dose was indeed reached was raised by RAC (see comments above).
- Decreases in oestradiol could provide an underlying mode of action causing the delay in sexual maturation. This decrease was observed in the 13-week study in rat (IIA 5.3.2/01, Study No. S0450) at 256 mg/kg bw/day, while a second study (IIA 5.3.2/02, Study No. 98-0075) did not showed this reduction at 128.6 mg/kg bw/day. The delays in vaginal opening were observed at 66.7 mg/kg bw/day; which is a dose that,

according to the 13-week toxicity studies, should not induce oestradiol reductions.

All the above stated considerations suggest that the delay in female sexual maturation is probably not caused by pyridalyl exposure. Moreover, the delay in vaginal opening at these very low exposure levels might be considered too small to warrant classification.

RAC notes that repeated dose toxicity studies have demonstrated that the substance is able to induce severe reductions in testosterone concentration at well-tolerated doses of 233 mg/kg bw/day and that these studies also consistently report ovarian alterations starting at 130 mg/kg bw/day. However, the highest dose tested in the 2-generation toxicity study was 84 mg/kg bw/day in males during premating period and 117 mg/kg bw/day in females during gestation/lactation period. Thus, the doses employed for assessing alterations in sexual function and fertility were clearly below those able to alter the sexual hormone balance and the ovarian histology. Consequently, RAC proposes **no classification of pyridalyl for sexual function and fertility due to inconclusive data.**

Development

No issues of concern were found in the developmental toxicity studies. Indeed, the effects reported in the rat studies (single incidences of mandibular micrognathia at two different doses and one incidence of omphalocele at the lowest dose) were not dose related, while the effects in rabbits (3 abortions and one premature delivering) were reported in presence of severe maternal toxicity (total suppression of body weight gain).

Overall, RAC supports **no classification of pyridalyl for developmental toxicity.**

Lactation

In the 2-generation rat study, some effects on pup development were observed which might be relevant for this endpoint. At postnatal day 0, the pup weights were similar to the control animals, whereas at the end of the lactation period (postnatal day 21) pup weight was significantly reduced (F1 and F2, males and females). However, it is noted that this effect was observed in the presence of maternal toxicity (i.e. reduced parental bw) and that the most severe effects were a reduction of up to 13% of bodyweight. RAC considers these effects **insufficient to support classification** and therefore supports the DS's proposal for **no classification of pyridalyl for lactation.**

10.11 Specific target organ toxicity-single exposure

Table 27: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference

OECD 401 Deviations: none Rat, Crl:CD (SD), Both sexes 5/sex/dose	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7% 5000 mg/kg bw, single oral exposure	No clinical signs of toxicity. Weight loss (-3.5%) in one female during week 1. One female showed an enlarged kidney during pathology which was not considered treatment related. No further abnormalities were seen.	IIA 5.2.1/01 Report No. 6311-217
OECD 402 Deviations: none Rat, Crl:CD (SD), Both sexes 5/sex/dose	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7% 5000 mg/kg bw, single dermal exposure	No clinical signs of toxicity. Weight loss (-1.3%) in one female during week 1.	IIA 5.2.2/01 Project No. 6311-218
OECD 403 Deviations: none Rat, Crl:CD (SD), Both sexes 5/sex/dose	S-1812 (pyridalyl), Lot No. PS 98041G, purity 93.7% 2.01 mg/L (actual conc.) 4 hours, nose-only	No mortality Decreased breathing rate, exaggerated breathing during exposure. Lethargy, whole body cold and wet fur until 2 hours after exposure Brown staining around snout in one male until 2 hours of exposure Decreased body weight after week 1.	IIA 5.2.3/01 Project No SMO-568

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

An acute oral toxicity study was carried out in accordance with OECD 401 (IIA 5.2.1/01). No mortality occurred and there were no clinical signs of toxicity. One female showed weight loss during the first week. Gross pathology did not reveal any treatment related findings.

An acute dermal toxicity study was carried out in accordance with OECD 402 (IIA 5.2.2/01). No mortality occurred and there were no clinical signs of toxicity. One female showed weight loss during the first week. Gross pathology did not reveal any treatment related findings.

An acute inhalation toxicity study was carried out in accordance with OECD 403 (IIA 5.2.3/01). No mortality occurred. All animals showed decreased breathing rate and exaggerated breathing during exposure. After exposure all animals showed these clinical signs for 2 hours and 2 days after exposure, respectively. Lethargy, whole body cold, and wet fur were observed for all animals after

exposure until 2 hours following exposure. Brown staining around snout was observed in one male rat following exposure until 2 hours post exposure. Mean body weight gain of both sexes decreased after the first week following exposure, and increased thereafter. Gross pathology did not reveal any treatment related findings. It is not clear from the study report that maximum effort was taken to attain higher test substance concentration than 2.01 mg/L. In absence of data it is concluded that the maximum concentration tested is not the highest attainable concentration.

10.11.2 Comparison with the CLP criteria

Based on the results of the acute oral and dermal toxicity study no classification for STOT-SE cat 1 or 2 is required as only reduced body weight was observed in one animal at a dose level above the limit for category 2 (>2000 mg/kg bw).

The acute inhalation study did not reveal any specific target organ toxicity and therefore classification with cat 1 or 2 is not required. It is noted that the acute inhalation was carried out with an exposure concentration below the upper guidance value for category 2 of 5 mg/L.

With regard to category 3, there was no sign of respiratory tract irritation. Lethargy was observed until 2 hours after exposure. As there were no clear indications of a narcotic effect, it is concluded that there is no clear evidence that pyridalyl can cause narcotic effects and no classification is proposed.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification is proposed.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

DS proposed no classification of pyridalyl for STOT-SE based on the results of acute toxicity studies (see the table in the section above).

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Comparison with the criteria

Acute oral and dermal toxicity studies showed no clinical effects and were performed at a single dose above the limit dose of 2000 mg/kg bw for warranting classification as STOT SE category 2. The acute inhalation toxicity was performed using a concentration within the range for classification as STOT SE category 2. However, the study did not reveal any

specific target organ toxicity.

In addition, no signs of respiratory tract irritation were reported in the acute inhalation toxicity study and only minor reversible lethargy was found in this acute inhalation toxicity study; which is not considered enough for supporting a classification as STOT RE category 3. Thus, no target organ toxicity could be detected at doses for warranting category 1 or 2 and no effects for warranting category 3 could neither be detected. In conclusion, RAC supports the DS's proposal **not to classify pyridalyl for STOT SE.**

10.12 Specific target organ toxicity-repeated exposure

Table 28: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
OECD 407 Deviations: no FOB, weight of epididymides not measured non-GLP Rat Crj:CD(SD) 6/sex/dose	4-week rat, oral (control and highest dose 14 days) S-1812, Batch NSA-950525 purity 98.7% 0, 70, 200, 700 and 2000 ppm (equal to 0, 7.05, 19.5, 64.6 and 182 mg/kg bw/d for males and 0, 7.21, 19.6, 66.4 and 188 mg/kg bw/d for females)	<u>2000 ppm (182/188 (m/f) mg/kg bw/day):</u> Decreased body weight (-10%), body weight gain (-16%) and food consumption (-13%) in males. Increased cholesterol and phospholipids in both sexes. Increased liver weight (+18%) in males and females. Increased absolute ovary weight (+33%). Adrenal cortex vacuolation, ovary vacuolation and degeneration of interstitial glands cells and persisted corpus luteum. <u>700 ppm (64.6/66.4 (m/f) mg/kg bw/day):</u> Increased relative liver weight (+8.3%) in males and females. Ovary vacuolation. <u>200 ppm (19.5/19.6 (m/f) mg/kg bw/day):</u> Increased liver weight in females (+7.2%) <u>70 ppm (7.05/7.21 (m/f) mg/kg bw/day):</u> No treatment related effects.	IIA 5.3.1/01 Study no. S0418

OECD 408 Deviations: no FOB non-GLP Rat Crj:CD(SD) 10/sex/dose (+6/sex/dose for hormone analysis)	13-week rat, oral S-1812, batch no. KOBE951006, purity 98.4% 0, 70, 700, 2000 and 3500 mg/kg food (equal to 0, 4.68, 47.4, 133 and 233 mg/kg bw/d for males and 0, 5.37, 55.5, 153 and 256 mg/kg bw/d for females)	<u>3500 ppm (233/256 (m/f) mg/kg bw/day):</u> Reduced body weight (-13%) and body weight gain. Decreased food consumption. Increased haemoglobin (+5%), haemotocryt (+5%) in males. Lymphocytes and white blood cell counts increased in both sexes. APTT increased in males and decreased in females. Total protein (females) and A/G ratio (males) increased. Decreased testes weight (-9%) , decreased epididymides weight (-11%), decreased pituitary weight in both sexes (-18%), increased ovary weight (+49%), increased lung weight (+67%), increased liver weight (+18%), increased relative adrenal weight (+28%). Decreased testosterone in males and oestradiol in females. Adrenal vacuolation, liver hypertrophy, foamy, eosinophilic cells in alveoli, interstitial cell vacuolation in the ovaries. <u>2000 ppm (133/153 (m/f) mg/kg bw/day):</u> Reduced body weight (-11%) and body weight gain. Decreased food consumption. Increased haemoglobin (+3%), haemotocryt (+5%) in males. Lymphocytes increased in both sexes and white blood cell counts increased in females. APTT increased in males and decreased in females. Total protein (females) and A/G ratio (males) increased. Cholesterol increased in both sexes. Phospholipids increased in males and gamma-GTP in both sexes. Decreased testes weight (-15%), decreased epididymides weight (-10%), decreased pituitary weight in both sexes (-18%), increased liver weight (+8.4%). Liver hypertrophy, interstitial cell vacuolation in the ovaries. <u>700 ppm (47.4/55.5 (m/f) mg/kg bw/day):</u> Reduced body weight gain. Increased haemoglobin (+3%), haemotocryt (+4%) in males. Lymphocytes and white blood cell counts increased in females. Total protein (females) and A/G ratio (males) increased. Cholesterol increased in males. Increased phospholipids increased and gamma-GTP in males. Vacuolation of interstitial gland cells in the ovaries. <u>70 ppm (4.68/5.37 (m/f) mg/kg bw/d):</u> No treatment related adverse effects.	IIA 5.3.2/01 Study No. S0450

OECD 408 Deviations: none Rat Crj:CD(SD) 10/sex/dose	13-week rat, oral S-1812, batch no. PS-98041G, purity 93.7% 0, 100, 1000 and 2000 mg/kg food (equal to 0, 5.56. 56.0, 111.3 mg/kg bw/day for males and 0, 6.45, 64.0 and 128.6 mg/kg bw/d for females	<u>2000 ppm (111.3/128.6 (m/f) mg/kg bw/day):</u> Mortality (1/10 females) related to hepatic necrosis. Body weight decrease (-11%), reduced body weight gain (-14%), reduced food consumption. Cholesterol increased (+55%), gamma-GTP increased in females (+40%), creatine phosphokinase decreased (-32%) and albumin/globulin ratio increased (+9%) in males. Increased liver weight (+15%) Centrilobular hypertrophy of hepatocytes, single cell necrosis of hepatocytes, foamy cell accumulation of the alveolar space, vacuolation of the adrenal zona reticularis, vacuolation of interstitial gland cells in ovaries. <u>1000 ppm (56.0/64.0 (m/f) mg/kg bw/day):</u> Body weight decrease (-8%), reduced body weight gain (12%), reduced food consumption. Increased liver weight (+6%) in females Single cell necrosis of hepatocytes, foamy cell accumulation of the alveolar space, vacuolation of interstitial gland cells in ovaries. <u>100 ppm (5.56/6.45 (m/f) mg/kg bw/day):</u> No treatment related adverse effects)	IIA 5.3.2/02 Study No 98-0075

OECD 408 Deviations: no FOB and ophthalmoscopy Mouse, ICR (Crj:CD-1) 12/sex/dose	13-week, mouse oral S-1812, batch no. PS-98041G, purity 93.7% 0, 70, 700, 3500 and 7000 mg/kg food (equal to 0, 8.17, 81.7, 379 and 721 mg/kg bw/d for males and 0, 9.50, 86.8, 415 and 879 mg/kg bw/d for females)	<u>7000 ppm (721/879 (m/f) mg/kg bw/day):</u> Reduced boy weight (-9%) and body weight gain (-28%) Haematocrit (-8%), haemoglobin (-8%) and red blood cell count (-6%) decreased. ALP increased (+78%), total protein increased (7%), albumin increased, AG ratio increased (+17%), cholesterol increased (+75%), triglycerides decreased (-64%), creatine increased (+22%), calcium increased (+4%) Increased absolute and relative liver weight (+32%; +45%), relative kidney weight decreased (-11%), ovary weight decreased (-29%) Centrilobular vacuolation and centrilobular hypertrophy of hepatocytes, increased incidence of basophilic change in tubular cells, brown pigment deposition in adrenals, ovary atrophy <u>3500 ppm (379/415 (m/f) mg/kg bw/day):</u> Reduced boy weight (-10%) and body weight gain (-32%) Haematocrit (-7%), haemoglobin (-6%) and red blood cell count (-6%) decreased ALP increased (+43%), total protein increased (6%), albumin increased, AG ratio increased (+11%), cholesterol increased (+69%), creatine increased (+22%). Increased absolute and relative liver weight (+15%; +25%), ovary weight decreased (-21%) Centrilobular hypertrophy of hepatocytes, ovary atrophy <u>700 ppm (81.7/86.8 (m/f) mg/kg bw/day):</u> Increased albumin (+8%) and total cholesterol (+28%) <u>70 ppm (8.17/9.50 (m/f) mg/kg bw/day):</u> No treatment related adverse effects.	IIA 5.3.2/03 Study No. SUT-0004

OECD 409 Dog, Beagle 4/sex/dose Deviations: high dose animals initially received 1000 mg/kg bw/day. On day 2 and 3 one male and one femalie died. Dose reduced to 300 mg/kg bw/day from day 15 in males and 8 in females. Two additional animals were assigned to the high dose group.	13-week dog oral S-1812, batch no. PS-98041G, purity 93.7% 0, 10, 100 and 300 mg/kg bw/day	<u>300 mg/kg bw/day:</u> Mortality in 1 female, clinical signs included tachypnea, wheezing, abdominal repiration and/or dyspnea. Decreased body weight (-8%) Deceased erythrocytes (-14%), haemoglobin (-15%) and haematocrit (-14%) Increased ALP and cholesterol in one female. Slight decreased in calcium (-4%) Increased absolute and relative lung weight, increased absolute and relative liver weight (+22% and +38%), increased relative kidney weights in females (+26%) Vacuolation of hepatocytes, hypertrophy of the centrilobular hepatocytes, vacuolation of the cortical cells in the adrenals. In lungs, thickening of the arterial and arteriolar wall and cellular infiltration of lymphocytes. Brown pigment in the proximal tubules in kidneys. <u>100 mg/kg bw/day:</u> Clinical signs included tachypnea, wheezing, abdominal repiration and/or dyspnea Decreased body weight (-6%) Slight decreased in calcium (-4%) Increased absolute and relative lung weight Vacuolation of hepatocytes, vacuolation of the cortical cells in the adrenals. In lungs, thickening of the arterial and arteriolar wall and cellular infiltration of lymphocytes. Brown pigment in the proximal tubules in kidneys. <u>10 mg/kg bw/day:</u> No treatement related adverse effects.	IIA 5.3.3/01 Study No. 29814
OECD 409 Dog, Beagle 4/sex/dose Deviations: none	1-year dog oral 0, 1.5, 5, 20 and 80 mg/kg bw/day	<u>80 mg/kg bw/day:</u> Decrease in MCH (-7%) Increase in ALP (218% of control) Increased absolute and relative liver weight (+30% and +32%). Increased absolute and relative lung weight (+17% and +20%). Decreased absolute and relative epididymis weight (-7% and -8%) <u>1.5, 5 and 20 mg/kg bw/day:</u> No treatment related adverser effects.	IIA 5.3.4/01 Study No. 29917

OECD 410 Rats, Crj:CD(SD) 10/sex/dose Deviations: none	4-week rat dermal S-1812, batch no PS-98041G, purity 93.7% 0, 30, 100 and 1000 mg/kg bw/day	<u>1000 mg/kg bw/day:</u> Increased cholesterol (+22%), decreased food consumption <u>30 and 100 mg/kg bw/day:</u> No treatment related adverse effects.	IIA 5.3.7/02 Study No. 20047
OECD 453 Sprague-Dawley rats, Crj:CD(SD) 50/sex/dose (main) 20/sec/dose (satellite) Deviations: none	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7% 0, 30, 100, 500, 1000 mg/kg food (equal to 0, 1.01, 3.40, 17.1 and 34.3 mg/kg bw/day in males and 0, 1.23, 4.10, 21.1 and 42.8 mg/kg bw/day in females. Main: 104 weeks Satellite: 52 weeks	<u>1000 ppm (34.3 mg/kg bw/day):</u> Increased motor activity. Decreased body weight (-16%) and body weight gain (-11%), decreased food consumption. Decrease in haematocrit (-10%), haemoglobin (-8%), RBC count (-10%) and prothrombin time, all at week 52 only. Increased incidence of thickened area in the auricles and spots in the liver. Increased incidence in peliosis in the liver, brown pigment deposition in the spleen <u>500 ppm (17.1 mg/kg bw/day):</u> Increased motor activity. Decreased body weight (-20%) and body weight gain (-10%), decreased food consumption. <u>30 and 100 ppm (1.0 and 3.4 mg/kg bw/day):</u> No treatment related adverse effects.	IIA 5.5.2/01 Study no: IET 99-0011

OECD 451 ICR mice, Crj:CD-1 52/sec/dose (main) 12/sec/dose (satellite) Deviations: none	S-1812 (pyridalyl), Batch No. PS 98041G, purity 93.7% 0, 15, 50, 1000 and 2500 mg/kg food (equal to to 0, 1.53, 5.04, 99 and 267 mg/kg bw/day in males and 0, 1.46, 4.78, 99 and 264 mg/kg bw/day in females Main: 78 weeks Satellite: 52 weeks	<u>2500 ppm (264 mg/kg bw/day):</u> Reduced body weight (-18%) and body weight gain (-39%), reduced food consumption (-8%). Decreased lymphocyte count. Increased absolute and relative liver weight (+17% and +30%), increased absolute and relative kidney weight (+28% and +15%). <u>1000 ppm (99 mg/kg bw/day):</u> Reduced body weight (-10%) and body weight gain (-19%) <u>15 and 50 ppm (1.46 abd 4.8 mg/kg bw/day):</u> No treatment related adverse effects.	IIA 5.5.3/01 Study no: IET 99-0012

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

<u>Oral exposure</u>

Four weeks of oral exposure of rats to 70, 200, 700 or 2000 mg/kg food of pyridalyl via the diet (equal to 0, 7.05, 19.5, 64.6 and 182 mg/kg bw/d for males and 0, 7.21, 19.6, 66.4 and 188 mg/kg bw/d for females), resulted in changes in body weight, food consumption, clinical biochemistry (changes in cholesterol, phospholipids, triglycerides, bilirubin and gamma-GTP), increased liver, lung and ovary weights and histopathological changes in adrenals and ovary at 2000 mg/kg food (IIA 5.3.1/01). Changes in cholesterol and liver weight were noted at 700 mg/kg food.

Dietary exposure of rats to 0, 70, 700, 2000 and 3500 mg/kg food (equal to 0, 8.17, 81.7, 379 and 721 mg/kg bw/d for males and 0, 9.50, 86.8, 415 and 879 mg/kg bw/d for females) for 13 weeks resulted in decreased body weight gain and food consumption at 2000 and 3500 mg/kg food (IIA 5.3.2/01). Haemoglobin and haematocrit were minimally increased at 700, 2000 and 3500 mg/kg food in males. Mean cell volume was minimally increased at 2000 and 3500 mg/kg food and mean cell haemoglobin was minimally increased at 3500 mg/kg food in males. Changes in white blood cell parameters were noted at 700, 2000 and/or 3500 mg/kg food (white blood cells, lymphocytes, monocytes, basophils) and may be related to abnormal fatty metabolism. Platelets were increased at 2000 and 3500 mg/kg food in females. Elongation of coagulation time was noted at 2000 and 3500 mg/kg food and may reflect disturbed liver function as a number of coagulation proteins are synthesized in the liver.

Changes in glucose, total cholesterol, phospholipids and gamma-GTP at 700, 2000 and/or 3500 mg/kg food may reflect disturbance of liver function; A/G ratio and gamma-GTP increase may indicate enzyme induction and increased cholesterol and phospholipids in specific may point to affected lipid synthesis/metabolism. At 3500 mg/kg food, testosterone was decreased in males and oestradiol was

decreased in females. An abnormal oestrous cycle was noted in one female at 3500 mg/kg food (dioestrous period lasted 7 days).
Increases in ovary, lung, liver weights were noted at 2000 and 3500 mg/kg food and increased adrenal weights were noted at 3500 mg/kg food. Histopathology showed an increased incidence of centrilobular hypertrophy of hepatocytes in both sexes at 2000 and 3500 mg/kg food, which was due to proliferation of the smooth endoplasmatic reticulum (SER) as determined by electron microscopy. An increased incidence of mononuclear cell infiltration and single cell necrosis (mild severity grade) of hepatocytes was noted at 700, 2000 and 3500 mg/kg food in females. Adrenals were noted to be enlarged in females at 3500 mg/kg food and pale in females at 2000 and 3500 mg/kg food. Vacuolation of the zona reticularis was seen in males and females at 3500 mg/kg food and in females at 2000 mg/kg food. In females at 3500 mg/kg food an increased incidence of vacuolation of the zona fasciculata was noted, while decreased vacuolation of the zona glomerulosa was seen. Adrenal vacuolation was confirmed to be fatty in nature by electron microscopy. An enlarged ovary was observed in females at 3500 mg/kg food and vacuolation of interstitial gland cells was increased in incidence at 700 mg/kg food and above. In the alveoli of the lung an increased incidence of accumulation of foamy/eosinophilic cells was seen in both sexes at 3500 mg/kg food.
Effects seen on the liver, i.e. increased total cholesterol, phospholipids and gamma-GTP, and increased relative weights and centrilobular hypertrophy caused by SER proliferation point to disturbance of lipid synthesis/metabolism and induction of enzymes. Hormone synthesis/excretion seems to be disrupted in adrenals as well as ovaries with concomitant vacuolation of the zona reticularis in the adrenal and the interstitial gland cells in the ovaries. The NOAEL is set at 70 mg/kg food (equivalent to 4.68 mg/kg bw/d for males and 5.37 mg/kg bw/d for females) based on decreased body weight gain, changes on white blood cell parameters and effects on the liver and ovaries.

Dietary exposure of rats to pyridalyl at 0, 100, 1000 or 2000 mg/kg food (equal to 0, 5.56, 56.0 and 111.3 mg/kg bw/d for males and 0, 6.45, 64.0 and 128.6 mg/kg bw/d for females) for 13 weeks, resulted in decreased body weight gain and food consumption at 1000 and 2000 mg/kg food (IIA 5.3.2/02). Clinical biochemistry revealed changes indicative of liver toxicity and included increases in total cholesterol and gamma glutamyltranspeptidase at 2000 mg/kg food. An increased relative liver weight was noted at 1000 and 2000 mg/kg food. Macroscopic examination showed a dark and enlarged liver in half of males and females at 2000 mg/kg food. Histopathology showed centrilobular hypertrophy of hepatocytes in both sexes at 2000 mg/kg food. In females at 1000 and 2000 mg/kg food an increased incidence of single cell necrosis of hepatocytes (slight to moderate) was noted. In both sexes an increased incidence of foamy cell accumulation of the alveolar space was found at 1000 and 2000 mg/kg food compared to the controls. Vacuolation of the adrenal zona reticularis was seen in females at 2000 mg/kg food. In ovary, vacuolation of interstitial gland cells was observed in females at 1000 and 2000 mg/kg food.
The NOAEL is set at 100 mg/kg food (equal to 5.56 mg/kg bw/d for males and 6.45 mg/kg bw/d for females) based on decreased body weight gain, decreased food consumption, increased liver weights and histopathological changes seen in liver and ovary.

Dietary exposure of mice to 0, 70, 700, 3500 or 7000 mg/kg food of pyridalyl (0, 8.17, 81.7, 379 and 721 mg/kg bw/d for males and 0, 9.50, 86.8, 415 and 879 mg/kg bw/d for females mg/kg bw/day) resulted in decreased body weight gain at 3500 and 7000 mg/kg food (IIA 5.3.2/03). Changes in liver enzymes, total cholesterol and triglycerides were noted at 700 mg/kg food and above and are considered to be related to liver injury and/or changes in lipid metabolism. At 3500 and 7000 mg/kg food, increased

absolute and relative liver weights were noted in males and females. Absolute kidney weight was decreased at 3500 and 7000 mg/kg food in males and relative kidney weight was decreased at 7000 mg/kg food in males. Ovary weight was decreased in females at 3500 and 7000 mg/kg food. Macroscopic examination revealed an accentuated lobular liver pattern in most males at 7000 mg/kg food. Histopathology showed centrilobular vacuolation in all males at 7000 mg/kg food and centrilobular hypertrophy of hepatocytes in all males and females at 7000 mg/kg food and males at 3500 mg/kg food. In the kidneys of both sexes at 7000 mg/kg food an increased incidence of basophilic change in tubular cells was noted. In the adrenals a brown pigment deposition in the cortico-medullary junction was seen with increased incidence at 7000 mg/kg food in males. The decreased ovary weight correlated with the atrophy noted in females at 3500 and 7000 mg/kg food.
The NOAEL is set at 70 mg/kg food (equal to 8.17 mg/kg bw/d for males) based on increased albumin and total cholesterol in males at 700 mg/kg food and histopathological effects found in the liver at 3500 mg/kg food and higher.

In a semichronic oral toxicity study in dogs, animals were given doses of 0, 10, 100 and 300/1000 mg/kg bw/d pyridalyl in gelatin capsules for 13 weeks (IIA 5.3.3/01). One male and one female receiving 1000 mg/kg bw/day died on days 2 and 3, respectively. Therefore, the high dose level was decreased to 300 mg/kg bw/day. Abnormal respiration as tachypnea, wheezing, abdominal respiration and/or dyspnea were observed in high dose males and in females at 100 mg/kg bw/day and above. Decreased body weight gain was observed 100 and 300 mg/kg bw/day.
Slight changes indicative of liver toxicity were noted in males and females at 300 mg/kg bw/day.
Absolute and relative lung weights were increased at 100 and 300 mg/kg bw/day. Absolute and relative liver weights were increased at 300 mg/kg bw/day. Relative kidney weights were increased at 300 mg/kg bw/day in females. Histopathology showed changes in liver, adrenals, lungs and kidneys. In liver, vacuolation of hepatocytes was noted in females at 100 and 300 mg/kg bw/day, and hypertrophy of the centrilobular hepatocytes was noted in females at 300 mg/kg bw/day. Hepatocyte inclusion was noted in females at 100 and 300 mg/kg bw/day. In adrenals, vacuolation of the cortical cells in the zona fasciculate was observed in the dead and surviving females at 100 and 300 mg/kg bw/day, and surviving males at 300 mg/kg bw/day. In lungs, thickening of the arterial and arteriolar wall and cellular infiltration of lymphocytes were noted females at 100 and 300 mg/kg bw/day and males at 300 mg/kg bw/day. Changes in lungs are related to the abnormal respiration seen in females at 100 and 300 mg/kg bw/day and males at 300 mg/kg bw/day. In kidneys, deposition of brown pigment in the proximal tubules was observed in the surviving females at 100 and 300 mg/kg bw/day.
Based on changes in body weight and histopathological changes in adrenals, liver and lungs at 100 and 300 mg/kg bw/d, the NOAEL is set at 10 mg/kg bw/day .

In an 1-year oral toxicity study in dogs, animals were given doses of 0, 1.5, 5, 20 or 80 mg/kg bw/day of pyridalyl in gelatin capsules (IIA 5.3.4/01). A decrease in MCH was noted in at 80 mg/kg bw/day. Changes indicative of liver toxicity were noted at clinical biochemistry and included an increase in alkaline phosphatase at 80 mg/kg bw/day. Non statistically significant changes in organ weights were noted in liver (except for relative weight in females), lungs and epididymes at 80 mg/kg bw/day. Changes in organ weights were not accompanied by macroscopic or histopathological changes. Based on changes in MCH, liver (organ weight, clinical biochemistry) and lungs (organ weight) at 80 mg/kg bw/d, the NOAEL is set at 20 mg/kg bw/day.

Detailed summaries of the chronic toxicity studies are included in section 10.9.1.

Dermal exposure:

Four weeks of dermal exposure of rats to 0, 30, 100 and 1000 mg/kg bw/day, resulted in an increased total cholesterol in males at 1000 mg/kg bw/day (122% of control) (IIA 5.3.7/02). Females at 1000 mg/kg bw/day showed decreased food consumption. Based on these effects, the NOAEL for systemic effects was set at 100 mg/kg bw/day. As no local effects were observed the NOAEL for local effects was established at >1000 mg/kg bw/day

Overall, the subacute and semichronic studies in rats, mice and dogs, indicate that the main target organs of pyridalyl are liver, lungs, adrenals and ovary.

10.12.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) the following criteria apply for STOT RE:

Category 1 (H372):
Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.
Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:
reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.
Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case):
Rat:
28-day: ≤ 30 mg/kg bw/d
90-day: ≤ 10 mg/kg bw/d

Category 2 (H373)
Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be Harmful to human health following repeated exposure.
Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.
Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case):
Rat:
28-day: ≤ 300 mg/kg bw/d
90-day: ≤ 100 mg/kg bw/d

Regarding classification with Category 1 no adverse findings were observed at the dose levels relevant for classification with Category 1 other than a slight significant increase in liver weight (+7.2%) in females in the 28-day oral study in rat at 19.5 mg/kg bw/day. Since the effect is very slight and not supported by histopathological findings pyridalyl does not fit the criteria for classification with Category 1.

Regarding classification with Category 2 no morbidity or death was observed at the dose levels relevant for classification. In addition, no changes in central or peripheral nervous systems was observed.
There were slight changes in haematological parameters but these do not fit the criteria for classification in accordance with the CLP guidance (e.g. reduction in Hb at ≥20%).
Increased liver weight combined with clinical chemistry changes related to disturbance of liver function was observed in several of the studies. These liver findings are not considered to reflect significant or severe organ damage or a significant or severe adverse effect in clinical biochemistry.
Overall it is concluded that pyridalyl does not meet the criteria for classification with STOT-RE Category 2.

10.12.3 Conclusion on classification and labelling for STOT RE

No classification is proposed.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The repeated dose toxicity studies with pyridalyl reported, at doses warranting classification as STOT RE, the following effects: slight increases (7.2%) in liver weight without histopathological support; slight haematological changes (variations always lower than 20%); and, increased liver weight combined with clinical chemistry changes related to disturbance off liver function. Overall, DS did not consider these changes robust enough for proposing a classification of pyridalyl for STOT RE.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The table in Annex 3 summarises the available dose repeated toxicity studies.

The data allow the identification of liver, ovary and adrenal cortex as the main target organs after repeated administration of pyridalyl. In addition, effects on body weight are also compared with the criteria.

Body weight and body weight gain

The reported effects were:

- Reductions up to 16% in the 4-week oral study in rats at doses that would warrant category 2;
- Reductions up to 12% in the 13-week oral study in rats at doses that would warrant category 2;
- Reductions up to 6% in the 13-week oral study in dogs at limit dose (100 mg/kg bw/day) that would warrant category 2.

RAC does not consider these body weight changes severe enough for supporting a classification as STOT RE.

Liver

The reported effects were:

- Increases up to 18% liver weight with support of minor clinical chemistry alterations (potentially warranting category 2) and up to 7% (potentially warranting category 1) in the 4-week oral study in rats;
- Increase of liver weight up to 6%, slight/mile single cell necrosis and foamy cell

accumulation in the 13-week oral study in female rats that would warrant category 2;

- Vacuolation in the 13-week oral study in dogs at limit dose (100 mg/kg bw/day) that would warrant category 2.

The effects reported at doses that would warrant classification as category 1 were not supported by histopathological findings. They are not considered by RAC severe enough for supporting classification. Some effects reported at doses that would warrant classification as category 2 were observed in some cases at the limit dose (100 mg/kg bw/day). RAC considers that they cannot be considered significant or severe organ damage because they were not accompanied by clear changes in clinical chemistry. Therefore, they should not be taken into consideration for classification as STOT RE.

Ovary

The reported effects were:

- Vacuolation and increase up to 33% of weight in the 4-week oral study in rats at doses that would warrant category 2;
- Vacuolation in the 13-week oral study in rats at doses that would warrant category 2.

RAC does not consider these effects severe enough for supporting a classification as STOT RE.

Adrenal cortex

The reported effects were:

- Vacuolation in the 13-week oral study in rats at doses that would warrant category 2;
- Vacuolation in the 13-week oral study in dogs at limit dose (100 mg/kg bw/day) that would warrant category 2.

RAC does not consider these effects to be robust enough to support a classification as STOT RE.

Lung

The effects on lungs included changes in weigh, increased foamy/eosinophilic cells in alveoli, and histopathological changes. In dogs, histopathological changes were accompanied with abnormal respiration. However, since lung weights were in most studies not associated with histopathological changes, they were correlated with changes in body weights and they all appeared well above the guideline values, RAC does not consider these effects on lung would trigger a classification as STOT RE.

Overall, RAC supports the DS's proposal for **no classification of pyridalyl for STOT RE.**

10.13 Aspiration hazard

No data available.

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

No data available.

10.13.2 Comparison with the CLP criteria

Not relevant

10.13.3 Conclusion on classification and labelling for aspiration hazard

No classification is proposed.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of pyridalyl for aspiration toxicity based on lack of data.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC supports the DS's proposal for **no classification of pyridalyl for aspiration toxicity due to lack of data.**

11 EVALUATION OF ENVIRONMENTAL HAZARDS

The environmental hazards of pyridalyl were assessed in the Draft Assessment Report (April 2003), addenda and Proposed Decision of the Netherlands prepared in the context of the approval (Reg. (EU) No. 143/2014), under Reg. (EC) 1107/2009. Studies considered valid in the and DAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. All studies were carried out under GLP unless indicated underwise. Studies were carried out in accordance with relevant test guidelines. Minor deviations were noted in some cases which have been included in the study summaries bellow. The deviations did not affect the overall acceptability of the studies.

11.1 Rapid degradability of organic substances

Table 29: Summary of relevant information on rapid degradability

Method	Test material	Results	Remarks	Reference
Ready biodegradability OECD 301F Deviations: none	Pyridalyl, batch no PS-98041G, purity 93.7%	No degradation was observed after 28 days. Pyridalyl was not readily biodegradable	-	IIA 7.7/01 Study No. 850273
Hydrolysis US EPA OPPTS 835.2110	Pyridalyl, batch no. RI97020, purity 98.6%	No hydrolytic degradation after 30 days incubation at 25°C in 0.01M buffer of pH 5, pH 7 and pH 9 containing 10% acetonitrile.	According to the author, the high amount of cosolvent was required to prepare homogeneous aqueous solutions with sufficient radioactivity for measurement. The study is acceptable since acetonitrile at 10% v/v is not expected to affect hydrolysis.	IIA 7.5/01, study No VP-22605
Field dissipation/accumulation Incl. SETAC (1995) Deviations: none	Pyridalyl 10 EW, batch SBM03/002/006, purity 10.4%	DT50: 2.3-39 days DT90: 176-350 days No indication of accumulation.	-	IIA 7.3.1/01, Study No 0333/ 210-D2149
Aerobic degradation in water- sediment OECD 308 Deviations: none	Pyridalyl, batch RIS2003-001, radiochemical purity 99.9% and IRS2003-003, radiochemical purity 99.2%	Half-life total water/sediment: 129-366 days (persistence and modelling) Dissipation from water phase with half-lives of 6.5-11 days. Dissipation from sediment 121-244 days.	-	IIA 7.8.3/01, Study No. 0333/212-D2149
Aerobic degradation in soil OECD 307	Pyridalyl, batch RIS2003-001, radiochemical purity 99.3% and IRS2003-003, radiochemical purity 100.0%	DT50 (persistence, 20°C): 53-272 days DT90 (persistence, 20°C): 465-150302 days DT50 (modelling, 20°C, non-normalised): 75.1-163 days	Deviations from guideline: Microbial activity of soil SK 912091 and SK 15556090 were slightly below 1% of organic carbon as recommended by OECD 307, but during incubation the microbial activity in soil SK 912091 increased to 1.0% of organic carbon, and that of SK 15556090	IIA 7.1.1/01, Study No. 0333/211-D2149

Method	Test material	Results	Remarks	Reference
			remained at levels comparable to that at the start. The results for these two soils were considered acceptable.	
Aerobic degradation in soil US-EPA 162-1	Pyridalyl, batch RIS98018, purity 98.3%	DT50 (normalized): 290-507 days.	Deviations from guideline: Information on history of the field site did not include information on treatment with chemicals. However, this did not impact the acceptability of the study.	IIA 7.1.1/02, Study No. 12152
Photochemical degradation in water EPA N:161-2 Deviations: none	Pyridalyl, batch 980302G, purity 99.7%	DT50 (photolysis, 25°C): 3.5 days under test conditions.	-	IIA 7.6/01, Study No. 885W-2
Soil absorption study OPPTS 835.1220	Pyridalyl, batch RIS98015, purity 99.1%	Koc 402000-2060000 L/kg	Deviations from guideline: History of test soils was not reported. The pH was not measured after adsorption, but pyridalyl is not an ionisable substance. This did not impact the acceptability of the study.	IIA 7.4.1/01, Study No VP-12140
Fate in outdoor mesocosms EWOFFT (1992), Hill *et al.* (1994), OECD (1996), SETAC (1991), WWF/RESOLVE (1992), HARAP (1998).	Pyridalyl, batch RIS2003-001, chemical purity not reported.	DT50 1.0-3.5 days and DT90 3.7-12 days under field conditions.	Total recovery in run-off enclosures showed high variability (22-96%) and therefore this information was not considered to be accurate.	IIA 7.8.3/02, Study No. 1043.014.310

11.1.1 Ready biodegradability

The ready biodegradability of pyridalyl (batch PS-98041G, purity 93.7%) was studied in a 28-day biodegradation test by following the Biological Oxygen Demand (BOD) using manometric methods according to OECD 301F (IIA 7.7/01). There were no deviations from the test guideline.

BOD in the inoculum controls (8 and 9 mg/L after 28 days) satisfied the validity criterion of OECD 301F (≤60 mg/L). The pass level for the reference substance (60% degradation) was reached within 4 days. After 28 days, the BOD in the flasks with pyridalyl was 7 and 9 mg/L, indicating that pyridalyl was not readily biodegradable in this test. This was not due to inhibitory effects of pyridalyl since the time course of the BOD in the toxicity control and the procedural control was similar during the test, with comparable levels after 28 days (157 mg/L in toxicity control, 157-159 mg/L in the procedural control).

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Pyridalyl (batch no. RI97020, purity 98.6%) was found to be stable to hydrolysis in a study carried out in accordance with US EPA OPPTS 835.2110 (IIA 7.5/01). The conduct of the study does not differ significantly from EEC method C.7. No hydrolytic degradation was observed after 30 days incubation at 25°C in 0.01M buffer of pH5, pH 7 and pH 9 containing 10% acetonitrile. The high amount of acetronitirle was required to prepare homogeneous aqueous solutions with sufficient radioactivity for measurement. Since acetronitrile at 10% is not expected to affect hydrolysis the study was considered acceptable. Pyridalyl represented an average of 96.8, 96.3 and 95.8% of the dose for the pH 5, pH 7 and pH9 buffer systems.

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

A field dissipation/accumulation study was carried out following single spring treatment of bare soil with the formulation pyridalyl 10 EW at two locations in Southern France and two in Italy at 600 g a.s./ha, residues of pyridalyl dissipated with DT50 values of 2.6, 39, 2.6 and 24.3 days, and DT90 values were 176, 350, 207 and 282.6 days (IIA 7.3.1/01). The maximum occurrence of the metabolites S-1812-DP, S-1812-DP-Me and HTFP in any trial represented 9.7%, <5.3% and 12.9% of the applied amount of pyridalyl. Following three consecutive annual spring treatments of bare soil at one location in Southern France and one in Italy at 600 g a.s./ha, there was no indication of accumulation of pyridalyl.. Metabolite S-1812-DP-Me was not detected above 0.01 mg/kg soil. The residue of <0.01 mg/kg represented <5.3% of the highest measured residue of pyridalyl (including metabolite levels, corrected for differences in molecular mass), which was the highest level of formation of the metabolite in all 4 trails. Based on the results from the field study it is concluded that metabolite S-1812-DP-Me is regarded of no concern.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Water-Sediment

In a water/sediment systems (OECD 308), treated with [pyridyl-2,6-14C]-pyridalyl or [dichlorophenyl-U-14C]- pyridalyl at a concentration of 70 µg/L and incubated at 20°C in the dark for 100 days, pyridalyl degraded in the total water/sediment system with half-lives of 129-366 days (persistence and modelling) (IIA 7.8.3/01). All studies indicate slow degradation of pyridalyl.

Pyridalyl dissipated from the water phase with half-lives of 6.5-11 days. The levels of parent pyridalyl reached a maximum in sediment of 78-85% AR on day 14-49, and pyridalyl dissipated from the sediment with half-lives of 121-244 days. The non-extractable fraction in sediment increased to a maximum of 10-15% AR, and CO2 increased to 1-9% AR. The main metabolite was S-1812-DP, which

reached maximum levels in water and sediment of 0.4-1.7% AR and 11-18% AR respectively. Besides pyridalyl and S-1812-DP, the following metabolites were found at low levels: S-1812-Ph-CH2COOH (max. 1.9% AR in water and 4.2% AR in sediment), HTFP (max. 4.4% AR in water and 1.6% AR in sediment) and S-1812-DP-Me (max. 2.0% AR in water and 3.5% AR in sediment).

Soil

In laboratory studies (OECD 307) the aerobic incubation of pyridalyl at 20°C was evaluated in four soils (pH in water 5.3-8.0, 1 .2-4.2% oc) (IIA 7.1.1/01). The microbial activity (determined by fumigation/extraction) of soil SK 912091 and SK 15556090 at the start of incubation represented 0.6% and 0.7%, respectively, of the organic carbon content of these soils. This is slightly below the level of 1% of organic carbon recommended by OECD 307, but during incubation the microbial activity in soil SK 912091 increased to 1.0% of organic carbon, and that of SK 15556090 remained at levels comparable to that at the start. The results for these two soils are considered to be acceptable.
Pyridalyl degraded with DT50 values of 53-272 days (persistence) and 135-2117 days (modelling, non-normalised). DT90 (persistence) values were 465-150302 days.
Metabolites S-1812-DP, S-1812-DP-Me and HTFP accounted for up to 12.2% AR, 12.4% AR and 14.7% AR, respectively. No other metabolites were detected at >10% AR, or at >5% AR during at least two successive samplings. The rate of degradation of S-1812-DP, S-1812-DP-Me and HTFP was investigated in three soils (pH in water 5.0-8.1, 1.3-3.0% oc). S-1812-DP degraded with DT50 values of 46-112 days (persistence) and 46-109 days (modelling, non-normalised), and the DT90 (persistence) values were 151-3289 days. S-1812-DP-Me degraded with DT50 values of 72-407 days (persistence) and 83-1000 days (modelling, non-normalised), and the DT90 (persistence) values were >2 years.
HTFP degraded with DT50 values of 24-46 days (persistence) and 40-76 days (modelling, nonnormalised), and the DT90 (persistence) values were 152-326 days.

Overall, the laboratory data suggest that the substance will degrade slowly in water sediment systems. Acceptable mesocosm data suggest a rapid to moderate degradation of the substance in water sediment systems. Based on these studies, the main metabolite S-1812-DP showed a moderate degradation in water sediment systems.
With regard to degradation in soil, the laboratory data suggest a very slow degradation of the substance in soil. The results of acceptable field studies at four representative sites indicate a moderate to slow degradation in soil. Based on these studies, the relevant metabolites S-1812-DP and HTFP degrade slowly and slowly to moderately respectively.

11.1.4.4 Photochemical degradation

Photolysis in water

A photolysis study was carried out with pyridalyl (batch 980302G, purity 99.7%) in accordance with EPA N:161-2 guideline (IIA 7.6/01). The mean DT50 (photolysis, 25°C) for [pyridyl-2,6-^{14}C]-pyridalyl and [dichlorophenyl-U-^{14}C]-pyridalyl is 3.5 days under test conditions (Xenon light, 12 h light, 531 W/m2 for the 300-800 nm range). The photo-metabolites HTFP (max. 17.5% AR at the end of incubation) and S-1812-PYP (max. 63% AR on day 14, 57% AR on day 21 and 30) are stable to photolysis under the test conditions.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.2.1 Summary of data/information on environmental transformation

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

Vapor pressure:

Pyridaly has a low vapour pressure of 6.24 x 10^{-8} Pa at 20°C (see Table 7) and therefore concluded to be non-volatile.

Adsorption/desorption from soil:

A batch equilibrium adsorption study with four soils was conducted on pyridalyl (IIA 7.4.1/01). Test solutions were prepared at concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1 µg/L by adding [dichlorophenyl-U-^{14}C] pyridalyl in acetonitrile to 0.01M $CaCl_2$ solution (final concentration acetonitrile 0.01% v/v). In a pre-test at 0.075 µg/L in all 4 soils, the minimum time required to reach equilibrium was determined to be 16 hours. In a screening test at 0.075 µg/L, two tubes with soil were equilibrated for 16 hours to check adsorption of pyridalyl on glass walls, which was found to be insignificant (not detectable in 7 tubes, 4.3% in remaining tube). Mass balances were determined in the main adsorption test at 0.075 µg/L by determining the radioactivity in the centrifuged supernatants (LSC) and in the soil pellet (combustion/LSC). In this mass balance test, the supernatants were extracted with hexane and the radioactivity in both phases was quantified by LSC. To verify the results from the adsorption study, Koc values were estimated using a HPLC method (determination of retention factor for 16 reference compounds with published Koc values) and using two Molecular Fragment Constant Methods (method 1: Molecular Connectivity Indices by Meylan *et al.* (1992); method 2: Fragment Constant Method by Tao *et al.* (1999)). In addition, soil thin layer chromatography was performed (determination of mobility of pyridalyl in water on thin layers of all 4 soils).

Mass balances ranged from 96-98% AR. Radioactivity in supernatants in the mass balance test represented 10-15% AR and that adsorbed to soil 82-88% AR. The extracted aqueous phases in the mass balance test contained no radioactivity. Adsorption Kf values were 3270-29900 L/kg (1/n values 0.99-1.18), and corresponding adsorption Koc values 402000-2060000 L/kg. Sorption in these four soils showed a relationship to organic carbon content (linear regression analysis of the Kf values of these 4 soil versus % oc gave $r^2 = 0.64$), where no relationship was observed with pH (r^2 0.10) or clay content (r^2 0.01). Pyridalyl is therefore rapidly absorpted to soil.

Fate in outdoor microcosms:

The fate of pyridalyl was investigated in outdoor microcosms in Switzerland (1 m deep) treated with [dichlorophenyl-U-14C]-pyridalyl formulated as 35 WP, either via simulated spray-drift (nominal concentration 1.4-1.5 µg/L) or run-off (nominal concentration 6.5 µg/L) (IIA 7.8.3/02). Pyridalyl dissipated from the water treated via spray-drift and run-off with SFO DT50 values of 1.1 and 3.5 days, respectively, and SFO DT90 values of 3.7 and 12 days, respectively. After 21 days, the level of pyridalyl in sediment had increased to 10-12% AR (spray-drift) or 29-64% AR (run-off). S-1812-DP was found at maximum levels of 2.2% AR in water and 1.8% AR in sediment. S-1812-DP dissipated from the water treated via spray-drift and run-off with SFO DT50 values of 11 and 5.5 days, respectively, and SFO DT90 values of 37 and 18 days,

respectively. The level of S-1812-PhCH2COOH (not detected in sediment) increased in water up to 6.1% AR (spray-drift) or 1.3% AR (run-off).

11.4 Bioaccumulation

Table 30: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioaccumulation in fish *Lepomis macrochirus* OPPTTS 850.1730	BCF: 26858 and 22352 L/kg wwt at 0.05 and 0.15 µg a.s./L.	The study was in accordance with OECD 305. The study report did not provide information to evaluate the accuracy of the kinetic parameters (e.g. plot of fitted and experimental values, confidence intervals). Kinetic BCF values were therefore estimated by the RMS in the DAR based on the raw data according to OECD 305.	IIA 8.2.6.1/01 Study No 013648-1
Bioaccumulation in oligochaetae *Lumbriculus variegatus* Test method 100.3, OECD 305	BCF 1.19 kg sediment dwt/kg worm wwt (15.8% OC) CT50: 46 days	As the concentration in worms increased throughout exposure a steady state BCF was not accepted. Kinetic BCF values were therefore re-evaluated in the DAR by the RMS in accordance with the methods outlined in Annex 6 of OECD 305.	IIA 8.2.7/02 Study No SUM-0041

11.4.1 Estimated bioaccumulation

No data available.

11.4.2 Measured partition coefficient and bioaccumulation test data

The Log Kow for Pyridalyl was determined according to the OPPTS 830.7570 method (Lorence, 2000). It was extrapolated from the regression line with 6.1 as the highest Log Kow value. The study was considered to be acceptable and the Log Kow was determined to be 8.1 at 20°C.

Bluegill sunfish (*Lepomis macrochirus*) were exposed to [dichlorophenyl-U-^{14}C]-pyridalyl for 49 days in a flow-through system, followed by 57 days of depuration in clean water (IIA 8.2.6.1/01). Nominal concentrations of 0.05 and 0.15 µg/L (concentrations at/or near the limit of water solubility), plus solvent control (DMF, 0.1 mL/L) were each tested in one replicate aquarium containing 65 fish at test initiation. No undissolved material or emulsion is expected. An additional aquarium was set up at 0.15 µg/L to provide fish samples for metabolite identification. The report did not provide any information to evaluate the accuracy of the kinetic parameters (e.g. plot of fitted and experimental values, confidence intervals, coefficient of correlation). Kinetic BCF values were therefore also estimated by the RMS based on the reported raw data according to the methods outlined in Annex 6 of OECD 305 (1996) using non-linear parameter estimation methods and curve fitting with the computer program Modelmaker V 4.0. The k_2 value was first determined from the depuration curve, and implemented as a constant into the equation describing the uptake of residues (equation 2 in Annex 6 of OECD 305

(1996)). The estimates by the RMS produced BCF values which did not differ significantly from the reported value, and the fit was found to be acceptable in all cases.
BCF values for pyridalyl in whole fish 26858 and 22352 L/kg wwt at 0.05 and 0.15 µg a.s./L respectively (lipid BCF normalised to 1% fat 3671 and 2835 L/kg wwt); CT50 for pyridalyl in whole fish 30-31 days. CT90 values were not estimated (only 60-76% clearance within 57 days of depuration).

A study was conducted to determine the BCF of pyridalyl in oligochaetae (*Lumbriculus variegatus*) exposed for 28 days in sediment/water systems containing spiked sediment (IIA 8.2.7/02). Natural sediment for the test, which was obtained from Strohs Folly Brook, Wareham, Massachusetts, USA and sieved through a 0.50-mm sieve, had the following characteristics: 15.8% oc, 84% sand, 14% silt, 2% clay and pH 5.9 (4 g/kg $CaCO_3$ was added prior to the test to buffer the system). It was reported that no dead oligochaetae or adverse effects were observed in the treatment or control vessels. The radioactivity concentration in sediment was relatively stable during exposure, the mean concentration during exposure was 0.98 mg/kg. The mean radioactivity concentrations in overlying and pore water were low (≤0.19 and ≤1.4 µg/L respectively). The mean radioactivity concentrations in worms increased throughout exposure. A steady state level had not been reached by day 28. As the concentration in worms increased throughout exposure (and part of depuration), it is not accepted that a steady state BCF is calculated (approach 1). Approach 2 is described in OECD 305 (1996), Annex 6, equation 1. This approach assumes that a smooth uptake curve is defined by the experimental data and that the midpoint of the smooth uptake curve can be identified accurately. In the present study a smooth uptake curve was not determined: linear regression of natural log tissue concentration against uptake time performed by author of report on mean values gave a poor fit (coefficient of correlation (r^2) value of 0.42). A justification for Cm = 0.95 mg/kg was not provided, and T = 28 days was reported to be empirically estimated without further explanations. Kinetic BCF values were therefore also estimated by the RMS according to the methods outlined in Annex 6 of OECD 305 (1996) using non-linear parameter estimation methods and curve fitting with the computer program Modelmaker V 4.0. The BCF was found to be low (1.19 kg sediment dwt/kg worm wwt).

11.5 Acute aquatic hazard

Table 31: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks[1]	Reference
Acute toxicity fish FIFRA 72-1 OPPTS 850.1075	Rainbow trout (*Oncorthynchus mykiss)*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	96 hr LC50: 0.5 mg a.s./L (emulsion in water, measured); <0.15 µg/L (dissolved pyridalyl)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl Clinical signs observed in surviving fish included loss of equilibrium, darkened pigmentation and lethargy	IIA 8.2.1.1/01 Study No. 13048.6206
Acute toxicity fish FIFRA 72-1,	Bluegill sunfish (*Lepomis macrochirus*)	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	96 hr LC50: >24 mg a.s./L (emulsion in water, measured)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.2.1.2/01 Study No. 13048.6207

OPPTS 850.1075					
Acute toxicity fish FIFRA 72-3 OPPTS 850.1075	Sheepshead minnow (*Cyprinodon variegatus)*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	96 hr LC50: >32 mg a.s./L (emulsion in water, measured)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.11.1/01 Study No. 12709.6200
Acute toxicity FIFRA 72-2 OPPTS 850.1010	*Daphnia magna*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	48hr LC50: 3.8 µg a.s./L (emulsion in water, measured) <0.15 µg a.s./L (dissolved pyridalyl)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.3.1.1/01 Study No. 13048.6208
Higher tier Acute toxicity OECD 202	*Daphnia magna*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	48 h LC50: 0.346 mg a.s./L (emulsion in water, nominal)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.3.1.1/02 Study No 1043.046.110
Acute toxicity FIFRA 72-3 OPPTS 850.1035	Mysid *(Americamysis bahia)*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	96 hr LC50: 1.0 µg a.s./L (emulsion in water, measured)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.11.1/02 Study No. 12709.6198
Acute toxicity OPPTS 850.1025	Eastern oyster *Crassostrea virginica*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	96 hr LC50: 0.82 mg a.s./L (emulsion in water, measured)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.11.1/03 Study No. 12709.6199.
Acute toxicity ASTM 729	*Chironomus yoshimatsui*	S-1812 (pyridalyl), batch no PK-98062,, 92.4%	48 hr LC50 1.1 mg a.s./L (emulsion in water, measured)	Non-GLP study Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.5.1/01
Acute toxicity OECD 201	*Pseudokirchneriella subcapitata*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72 hr EbC50 & ErC50 > 0.20 mg a.s./L (emulsion in water, nominal)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.4/01 Study No. 12709.6207
Acute toxicity JMAFF No. 12	*Selenastrum capricornutum*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72 hr EbC50 & ErC50 >10 mg a.s. /L(emulsion in water, nominal)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.4/02 Study No 0109EAI
Acute toxicity OECD 201	*Navicula pelliculosa*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72 hr EbC50 & ErC50 > 0.20 mg a.s./L (emulsion in water, nominal)	Deviations from test guideline: - Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.4/04 SUW-0017

				- Average growth factor for control was only 9.1 instead of ≥16. Since test species is known to b a slow grower, the study was accepted.	
Acute toxicity FIFRA 72-3 OPPTS 850.5400	*Skeletonema costatum*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72 hr EbC50 & ErC50 > 0.15 mg a.s./L (emulsion in water, measured)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.11.1/04 Study No. 12709.6205
Acute toxicity OPPTS 850.4400	Duckweed (*Lemna gibba*)	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72 hr ErC50> 0.17 mg a.s./L (emulsion in water, measured)	Deviations from test guideline: Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.6/01 Study No. 12709.6208

[1]As the water solubility of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimized by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this information to US EPA, which subsequently approved the use of DMF:HCO-40 in these toxicity tests. No information about the nature of the micelles (size distribution), any undissolved (i.e. non-micelle) substance is given. Also no assessment of physical effects or genuine toxicity that are responsible for the effects observed, is performed. However, Pyridalyl 10EW is an emulsion in water formulation containing 100 g/L of pure pyridalyl and hence, the use of studies with emulsions is permitted as stated in OECD series on testing and assessment, Number 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures).

11.5.1 Acute (short-term) toxicity to fish

A 96-hour acute toxicity test in rainbow trout (*Oncorhynchus mykiss*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 0.094, 0.19, 0.37, 0.75 and 1.5 mg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control (IIA 8.2.1.1/01). Measured concentrations were 0.11, 0.19, 0.38, 0.74 and 1.7 mg a.s./L (representing 99-117% of nominal) at the start of the test and 0.10, 0.18, 0.37, 0.75 and 1.5 mg a.s./L (representing 95-106% of nominal) after 96 hours. The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C) by at least a factor of 627. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. LC50 for the emulsion in water was 0.5 mg a.s./L. The LC50 for dissolved pyridalyl was <0.15 µg a.s./L.
A 96-hour acute toxicity test in bluegill sunfish (*Lepomis macrochirus*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with S-1812 at nominal test concentrations of 3.9, 6.5, 11, 18 and 30 mg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control (IIA 8.2.1.2/02). Endpoints were based on mean measured concentrations (2.9, 5.6, 10, 16 and 24 mg a.s./L). No clinical signs were observed in any concentration, and mortality after 96 hours was limited to 0, 0, 5, 5, 0, 0 and 0% in the control, the solvent control and at 2.9, 5.6, 10, 16 and 24 mg a.s./L, respectively. The LC50 was >24 mg a.s./L (emulsion in water).

A 96-hour acute toxicity test in the marine fish species sheepshead minnow (*Cyprinodon variegatus*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 3.9, 6.5, 11, 18 and 30 mg a.s./L, with a solvent

(hydrogenated castor oil and DMF, 1:1) and untreated control (IIA 8.11.1/01). Measured concentrations were 74-111% of nominal (74, 100, 100, 111 and 110% at 3.9, 6.5, 11, 18 and 30 mg a.s./L, respectively) at the start and 88-109% of nominal concentrations at the end of the test. LC50: >32 mg a.s./L (emulsion of dehydrogenated castor oil and DMF (1:1) in water).

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

A 48-hour acute toxicity test in *Daphnia magna* (2 replicates of ten daphnids each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 3.2, 5.4, 9.0, 15 and 25 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control (IIA 8.3.1.1/01). Endpoints were based on mean measured concentrations (2.2, 3.8, 6.4, 11 and 17 µg a.s./L). The test was performed at concentrations exceeding the water solubility of pyridalyl. The EC50 was 3.8 µg a.s./L (emulsion in water) and <0.15 µg a.s./L for dissolved pyridalyl.

A 48-hour higher tier acute toxicity test in *Daphnia magna* (4 replicates of five daphnids each per concentration) was conducted in natural water-sediment systems under static conditions with radiolabelled S-1812 (pyridalyl) at nominal test concentrations of 0.88, 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control (IAA 8.3.1.1/02). Endpoints were based on nominal concentrations. EC50 was 34.6 µg a.s./L (emulsion in water).

A 96-hour acute toxicity test in the marine mysid *Americamysis bahia* (2 replicates of ten mysids (<24 hours old) each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 0.41, 0.69, 1.2, 1.9 and 3.2 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control (IIA 8.11.1/02). Endpoints were based on mean measured concentrations. 96-hour LC50: 1.0 µg a.s./L (tested as an emulsion in water).

A 96-hour acute toxicity test in eastern oyster (*Crassostrea virginica*) (2 replicates of twenty oysters each per concentration) was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 mg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control (8.11.1/03). Endpoints were based on mean measured concentrations. 96-hour EC50: 0.82 mg a.s./L (tested as an emulsion in water).

A 48-hour acute toxicity test in *Chironomus yoshimatsui* (1 replicate containing an unspecified number of midges each per concentration) was conducted under static conditions with S-1812 (radiolabelled pyridalyl) at nominal test concentrations of 0.010, 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg/L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control (IIA 8.5.1/01). The study was not conducted under GLP. Endpoints were based on mean measured pyridalyl concentrations. 48-hour LC50: 1.1 mg a.s./L (tested as an emulsion in water).

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

In all acute toxicity to algae and other aquatic plants the tests were performed at a concentration exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C). The tested solution is therefore likely to have been an emulsion rather than a true solution, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

A 96-hour toxicity test on green algae (*Pseudokirchneriella subcapitata*) (3 replicates per test group, each containing 1.0 x 10^4 cells/mL at the start) was conducted with S-1812 TG at a nominal test concentration of 0.20 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control (IIA 8.4/01). The 72-hour endpoints were based on the on the nominal concentration. 72-hour

EbC50 and ErC50 >0.20 mg a.s./L, 72-hour NOEbC and NOErC: 0.20 mg a.s./L (tested as an emulsion in water).

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per test group, each containing 1.0 x 10^4 cells/mL at the start) was conducted with S-1812 TG at a nominal test concentration of 10 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control (IIA 8.4/02). 72-hour EbC50 and ErC50: >10 mg a.s./L, 72-hour NOEbC and NOErC: 10 mg a.s./L (tested as an emulsion in water).

A 96-hour toxicity test on freshwater diatoms (*Navicula pelliculosa*) (3 replicates per test group, each containing 1.0 x 10^4 cells/mL at the start) was conducted with S-1812 TG at a nominal test concentration of 0.20 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control (IIA 8.4/04). The study was carried out in accordance with OECD 201 with the exception tha the average growth factor for the control was 9.1 instead of ≥16 as stated in OECD 201. However, since the tested species is known to be a slow grower, the study result is accepted.72-hour EbC50 and ErC50 >0.20 mg a.s./L, 72-hour NOEbC and NOErC: 0.20 mg a.s./L (tested as an emulsion in water).

A 96-hour toxicity test on marine diatoms (*Skeletonema costatum*) (3 replicates per test group, each containing 7.7 x 10^4 cells/mL at the start) was conducted under static conditions with S-1812 TG at a nominal test concentration of 0.20 mg a.s./L, with untreated and solvent (dehydrogenated castor oil and DMF, 1:1) control (IIA 8.11.1/04). 72-hour EbC50 and ErC50 >0.15 mg a.s./L, 72-hour NOEbC and NOErC: 0.15 mg a.s./L (tested as an emulsion in water).

A 7-day toxicity test on the growth of duckweed (*Lemna gibba*) (3 replicates per concentration, each containing five plants with three fronds each) was conducted with S-1812 TG (pyridalyl) at a nominal test concentration of 0.20 mg a.s./L, with untreated control and solvent (dehydrogenated castor oil and DMF, 1:1) control (IIA 8.6/01). Test solutions were renewed every two days. Measured concentrations were 0.18 mg a.s./L at test initiation (92% of nominal) and 0.16 mg a.s./L (78% of nominal) at the end of the test. The reported 7-day EC- and NOEC-values were based on frond density and growth rate (calculated from frond density). This is in agreement with OPPTS 850.4400, but not with the recommendations in OECD 221 (2006). According to the latter guideline, in addition to frond density, biomass should be measured at the end of the test (i.e. dry or wet weight, or total frond area). This latter parameter should then be used to determine the yield and corresponding EC- and NOEC values. The test was performed before the OECD guideline was published. Furthermore, no effects were observed on frond number or frond development (frond appearance). Therefore, the test is accepted. The 7-day ErC50 is >0.17 mg a.s./L; 7-day NOErC: 0.17 mg a.s./L (tested as an emulsion in water).

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No other studies are available that are relevant for C&L.

11.6 Long-term aquatic hazard

Table 32: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks[1]	Reference
EPA 72-4, OECD 210, OPPTS 850.1400	Rainbow trout *Oncorhynchus mykiss*	S-1812 (pyridalyl), Batch no. PS-98041G, 93.7%	89 d NOEC 0.024 mg a.s./L (emulsion in water, measured) Based on reduced body weight	Tested concentration exceeded the water solubility of pyridalyl	IIA 8.2.4/01 Study No. 13048.6220
FIFRA 72-	*Daphnia*	S-1812 (pyridalyl),	21 d NOEC =0.0014	Tested	IIA 8.3.2.1/01

4, OECD 211 and OPPTS 850.1300	*magna*	Batch no. PS-98041G, 93.7%	mg a.s./L (emulsion in water, measured) Based on parental survival, reproduction and growth	concentration exceeded the water solubility of pyridalyl	Study N0. 13048.6221
FIFRA 72-4	Mysid *(Americamysis bahia)*	S-1812 (pyridalyl), Batch no. PS-98041G, 93.7%	NOEC 0.00045 mg a.s./L (emulsion in water, measured) Based on mortality, reproduction and growth	Tested concentration exceeded the water solubility of pyridalyl	IIA 8.11.1/05 Study No. 12709.6202
OECD 219	*Chironomus riparius*	S-1812 (pyridalyl), Batch no. PS-98041G, 93.7%	28 d NOEC 0.012 mg a.s./L (emulsion in water, nominal) emergence	Tested concentration exceeded the water solubility of pyridalyl	IIA 8.5.2/05 Study No. 13048.6401
OECD 201	*Pseudokirchneriella subcapitata*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72h NOEbC: 0.2 mg a.s./L 72 h NOErC: 0.2 mg a.s./L (emulsion in water, nominal)	Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.4/01 Study No. 12709.6207
JMAFF No. 12	*Selenastrum capricornutum*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72h NOEbC: 10 mg a.s./L 72 h NOErC: 10 mg a.s./L (emulsion in water, nominal)	Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.4/02 Study No 0109EAI
OECD 201	*Navicula pelliculosa*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72h NOEbC: 0.2 mg a.s./L 72 h NOErC: 0.2 mg a.s./L (emulsion in water, nominal)	Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.4/04 SUW-0017
FIFRA 72-3 OPPTS 850.5400	*Skeletonema costatum*	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	72h NOEbC: 0.15 mg a.s./L 72 h NOErC: 0.15 mg a.s./L (emulsion in water, nominal)	Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.11.1/04 Study No. 12709.6205
OPPTS 850.4400	Duckweed (*Lemna gibba*)	S-1812 (pyridalyl), batch no PS-98041G, 93.7%	NOErC: 0.17 mg a.s./L	Tested concentration far exceeded the water solubility of pyridalyl	IIA 8.6/01 Study No. 12709.6208

[1]As the water solubility of S-1812 was extremely low (less than 1 ppb), the Study Sponsor concluded that it should be optimized by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. The Study Sponsor provided the details of this information to US EPA, which subsequently approved the use of DMF:HCO-40 in these toxicity tests. No information about the nature of the micelles (size distribution), any undissoved (i.e. non-micelle) substance is given. Also no assessment of physical effects or genuine toxicity that are responsible for the effects observed, is performed. However, Pyridalyl 10EW is an emulsion in water formulation containing 100 g/L of

pure pyridalyl and hence, the use of studies with emulsions is permitted as stated in OECD series on testing and assessment, Number 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures) .

11.6.1 Chronic toxicity to fish

The chronic toxicity tests in fish were performed at concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20˚C). The tested solutions, especially at the higher test concentrations, are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

A 89-day fish early life stage flow-through study was undertaken with rainbow trout (*Oncorhynchus mykiss*) (IIA 8.2.4/01). Newly fertilised eggs (1 hour post fertilisation, two replicates/concentration, 2 incubation cups/replicate, 50 eggs/incubation cup) were exposed to pyridalyl (93.7% pure) at nominal concentrations of 6.3, 13, 25, 50 and 100 µg a.s./L plus control and solvent control (hydrogenated castor oil and DMF, 1:1). Mean measured concentrations were 6.9, 12, 24, 49 and 96 µg a.s./L, representing 95 to 110% of nominal. Embryo viability and larval survival at hatch were not significantly affected in any of the test concentrations when compared to the pooled control group. Larvae began to exhibit signs of swim-up development and behaviour on day 39 (10 days post-hatch, details per group not reported). By day 46 (17 days post-hatch), all surviving larvae in the test solutions and the controls reached the swim-up stage. At the end of the test, survival was significantly reduced at the highest test concentration. Mean total length was not affected up to 49 µg a.s./L, but mean wet weight and dry weight were significantly reduced at this concentration. Based on reduced body weight, the NOEC was 24 µg a.s./L (tested as an emulsion in water).

11.6.2 Chronic toxicity to aquatic invertebrates

The chronic toxicity of S-1812 (pyridalyl) to *Daphnia magna* was assessed in a 21-day flow-through study (IIA 8.3.2.1/01). The nominal concentrations were 0.98, 2.0, 3.9, 7.8 and 16 µg a.s./L plus an untreated and a solvent control (hydrogenated castor oil and DMF, 1:1). Mean measured concentrations were 0.93, 1.4, 2.7, 5.7 and 11 µg a.s./L (67-95% of nominal concentrations). Survival of adult daphnia was statistically significantly reduced at and above 2.7 µg a.s./L, Concentrations of 2.7 µg a.s./L and above were therefore excluded from statistical analysis of other parameters, which, based on data from a limited number of survivors at these concentrations, appeared to be unaffected by treatment. Reproduction, as measured by the cumulative number of offspring per female daphnid, and time to first brood were not affected up to 1.4 µg a.s./L. Mean total body length after 21 days was significantly reduced at 0.93 and 1.4 µg a.s./L, but a clear dose response was not observed. Furthermore, body length was not affected in the preliminary test preceding the reported test (mean body length in preliminary test: 4.7, 5.0, 4.8, 4.8, 4.9, 4.9 and 4.8 mm for the control, solvent control, at 0.13, 0.25, 0.5, 1.0 and 2.0 µg a.s./L, respectively). In addition, mean body dry weight was not affected at any concentration. Therefore, the apparent effect on body length was considered an anomaly and not biologically relevant, and the NOEC for growth may be set at ≥1.4 µg a.s./L. The NOEC for parental survival, reproduction and growth was 1.4 µg a.s./L (tested as an emulsion in water). The test was performed at concentrations exceeding the water solubility of pyridalyl.

The chronic toxicity of S-1812 TG (pyridalyl) to the marine shrimp *Americamysis bahia* was assessed in a 28-day flow-through study (IIA 8.11.1/05). Mysids (≤24 hours old, 60 per treatment, 30 mysids per

replicate vessel) were exposed to nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 µg a.s./L plus an untreated and a solvent-control (hydrogenated castor oil and DMF, 1:1). Endpoints were based on mean measured concentrations. The overall NOEC for mysid mortality, reproduction and growth was 0.45 µg a.s./L (tested as an emulsion in water).

The chronic toxicity of radiolabelled pyridalyl to *Chironomus riparius* (1 day old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions (IIA 8.5.2/05). Nominal test concentrations were 0.38, 0.75, 1.5, 3.0, 6.0, 12 and 24 µg a.s./L, with untreated and solvent control. The only effect was a decrease of 11% in emergence rate at the highest concentration compared to the pooled controls (statistically significant). Mean development rate was not affected at any concentration. Effect concentrations were based on nominal concentrations. The NOEC for emergence was 12 µg a.s./L, while the NOEC for development rate was 24 µg a.s./L.

11.6.3 Chronic toxicity to algae or other aquatic plants

See study summaries in section 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No other studies are available that are relevant for C&L.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

The criteria for Category Acute 1 in line with Table 4.1.0 (a) from the Guidance on the Application of the CLP Criteria are:

96 hr LC50 (for fish)	≤ 1 mg/l and/or
48 hr EC50 (for crustacea)	≤ 1 mg/l and/or
72 or 96 hr ErC50 (for algae or other aquatic plants)	≤ 1 mg/l.

Pyridalyl is a poorly water-soluble substance, 0.15 µg/L at 20°C, pH 8.0-8.3. The acute toxicity tests were carried out at nominal concentrations far exceeding the water solubility of pyridalyl. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration. Actual dissolved concentrations were not known, since for analysis water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

The CLP Guidance on the Application of CLP criteria provides the following guidance for poorly soluble substances:

a) where the acute toxicity is recorded at levels in excess of the water solubility, the L(E)C50 for classification purposes may be considered to be equal to or below the measured water solubility.
b) where no acute toxicity is recorded at levels in excess of the water solubility, the L(E)C50 for classification purposes may be considered to be greater than the measured water solubility.

Acute toxicity was observed in rainbow trout (LC_{50} 0.5 mg a.s./L, emulsion in water), *Daphnia magna* (EC_{50} 3.8 µg a.s/L, emulsion in water), mysid (LC_{50}: 1.0 µg a.s./L, emulstion in water), eastern oyster (EC_{50} 0.82 mg a.s./L, emulsion in water) and *Chironomus yoshimatsui* (LC_{50} 1.1 mg a.s./L, emulsion in water). No information is available to assess whether physical effects could be possibly the cause of any observed toxicity. The CLP Guidance states that when acute toxicity is recorded at levels in excess of the water solubility, the L(E)C50 for classification purposes may be considered to be equal to or below the measured water solublity. Based on an L(E)C50 of 0.15 µg/L for dissolved pyridalyl it concluded that classification for Category Acute 1 is needed and the M-factor is concluded to be 1000.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Pyridalyl has a log Kow value of >4 (8.1), and the experimentally determined BCF is >500 L/kg in fish.Pyridalyl therefore has a high potential for bioaccumulation in aquatic organisms in line with the CLP Guidance. In a biodegradation study following Biological Oxygen Demand (BOD) pyridalyl was not found to be readily biodegradable. In a hydrolysis study no hydrolytic degradation was observed after 30 days. Aerobic degradataion in water-sediment showed a half-life of 129 to 366 days. Pyridalyl dissipated from the water phase with half-lives of 6.5-11 days and from the sediment with half-lives of 121-244 days. In a photolysis study photochemical degradation in water was 3.5 days under the test conditions. Overall, pyridalyl is not considered as rapidly degradable for classification purposes. The criteria for Category Chronic 1 and 2 in the CLP Guidance for non-rapidly degradable substances for which adequate chronic toxicity data are available are:

Category Chronic 1:

Chronic NOEC or ECx (for fish)	≤0.1 mg/l and/or
Chronic NOEC or ECx (for crustacea)	≤0.1 mg/l and/or
Chronic NOEC or ECx (for algae or other aquatic plants)	≤0.1 mg/l.

Category Chronic 2:

Chronic NOEC or ECx (for fish)	> 0.1 to ≤ 1 mg/l and/or
Chronic NOEC or ECx (for crustacea)	> 0.1 to ≤ 1 mg/l and/or
Chronic NOEC or ECx (for algae or other aquatic plants)	> 0.1 to ≤ 1 mg/l.

Pyridalyl is a poorly water-soluble substance, 0.15 µg/L at 20°C, pH 8.0-8.3. The chronic toxicity tests were carried out at nominal concentrations far exceeding the water solubility of pyridalyl. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration. Actual dissolved concentrations were not known, since for analysis water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl.

Adequate chronic toxicity data is available for fish, invertebrates and algae/aquatic plants. In the tests in which chronic toxicity was observed the NOECs ranged from 1.4 µg a.s./L in *Daphnia magna* to 24 µg a.s./L in fish. These NOECs reflect the emulsion in water concentration since as indicated in the study summaries the tests were performed at nominal concentrations above the water solubility of 0.15 µg a.s./L. No information is available to assess whether physical effects could be possibly the cause of any observed toxicity. The CLP Guidance states that when chronic toxicity is recorded at levels in excess of the water solubility, the NOEC for classification purposes may be considered to be equal to or below the measured water solublity.

Considering a NOEC of 0.15 µg a.s./L for dissolved pyridalyl classification for chronic toxicity Category 1 is required with an M-factor of 100.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 1000.

Long-term aquatic hazard: category Chronic 1, M-factor: 100.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS concluded that pyridalyl is 'not rapidly degradable', has high potential for bioaccumulation and proposed classification based on the measured water solubility of 0.00015 mg/L (at 20 °C, pH 8 – 8.3). Pyridalyl is a poorly water-soluble substance and acute / chronic toxicity is recorded at levels in excess of the water solubility. Therefore, according the CLP guidance the L(E)C_{50} / NOEC (EC_{10}) for classification purposes may be considered to be equal to or below the measured water solubility.

Therefore, the DS proposed classification of pyridalyl as Aquatic Acute 1 based on an L(E)C_{50} of 0.00015 mg/L with M-factor of 1000 and Aquatic Chronic 1 based on NOEC / EC_{10} of 0.00015 mg/L with M-factor of 100 considering that pyridalyl is not rapidly degradable.

Degradation

The results of a hydrolysis study according to US EPA OPPTS 835.2110 (not significantly different from EEC method C.7; GLP) showed that pyridalyl is hydrolytically stable at pH 5, 7 and 9 over a 30-day period at 25 °C. The test systems were made up of buffer plus 10% acetonitrile to prepare the homogenous aqueous solution. Since acetonitrile at 10% is not expected to affect hydrolysis, the study was considered acceptable (IIA 7.5/01, study No. VP-22605)

The results of a photolysis study according to EPA N:161-2 (GLP) showed that DT_{50} of pyridalyl at 25 °C is 3.5 days under test conditions. The photo-metabolites HTFP (max. 17.5% AR at the end of incubation) and S-1812-PYP (max. 63% AR on day 14, 57% AR on day 21 and 30) are stable to photolysis under the test conditions (IIA 7.6/01, Study No. 885W-2).

No readily biodegradability of pyridalyl was observed at 28-day biodegradation test by following the Biological Oxygen Demand (BOD) using manometric methods according to OECD TG 301F (GLP). BOD in the inoculum controls (8 and 9 mg/L after 28 days) satisfied the validity criterion of OECD TG 301F (≤ 60 mg/L). The pass level for the reference substance (60% degradation) was reached within 4 days. After 28 days, the BOD in the flasks with pyridalyl was 7 and 9 mg/L, indicating that pyridalyl was not readily biodegradable in this test (IIA 7.7/01Study No. 850273).

Aerobic degradation study in water/sediment according to OECD TG 308 indicateed slow degradation of pyridalyl. Pyridalyl degraded in the total water/sediment system with half-lives of 129-366 days. From the water phase pyridalyl dissipated with half-lives of 6.5-11 days and from sediment phase with half-lives of 121-244 days (IIA 7.8.3/01, Study No. 0333/212-D2149).

Aerobic degradation study in soil according to OECD TG 307 at 20 °C in laboratory was evaluated in four soils. Pyridalyl degraded with DT_{50} values of 53-272 days (persistence) and DT_{50} 75.1–163 days (modelling, non-normalised). DT_{90} values were 465-150302 days (IIA 7.1.1/01, Study No. 0333/211-D2149). In another aerobic degradation in soil according to US-EPA 162-1 normalized DT_{50} values of pyridalyl were in the range 290–507 days (IIA 7.1.1/02, Study No. 12152).

Overall, due to the results summarised above, the DS concluded that pyridalyl is not ultimately degraded to > 70% within 28 days (equivalent to a half-life < 16 days), or rapidly transformed to non-classifiable products. As a consequence, pyridalyl was considered as not rapidly degradable, according to CLP criteria.

Aquatic Bioaccumulation

The results of available experimental aquatic study according to OECD TG 305 to determine the bioconcentration potential (BCF) of pyridalyl, indicated that BCF values in whole fish is above the CLP trigger criteria of ≥ 500 (IIA 8.2.6.1/01 Study No 013648-1). The study was conducted a flow-through system with Bluegill sunfish (*Lepomis macrochirus*) and exposure to two different treatment levels (i.e. 0.05 and 0.15 µg/L). The study report did not provide information to evaluate the accuracy of the kinetic parameters, so kinetic BCF values were therefore estimated by the RMS in the DAR based on the raw data according to OECD TG 305. The BCF values for pyridalyl in whole fish was 26858 and 22352 L/kg wwt at 0.05 and 0.15 µg a.s./L respectively (lipid BCF normalised to 1% fat 3671 and 2835 L/kg wwt).

Bioaccumulation study in oligochaeta (*Lumbriculus variegatus*) based on test method 100.3, OECD TG 305 was conducted in order to determine BCF as well. However, the concentration in worms increased throughout exposure so steady state BCF was not accepted. Hence, kinetic BCF values were therefore estimated by the RMS according to the methods outlined in Annex 6 of OECD TG 305 using non-linear parameter estimation methods and curve fitting. The BCF was found to be low (1.19 kg sediment dwt/kg worm wwt) (IIA 8.2.7/02 Study No SUM-0041).

Determined log K_{OW} of pyridalyl according to the OPPTS 830.7570 method is 8.1 at 20 ^{o}C and meets the CLP trigger value of ≥ 4 indicating a potential for bioaccumulation.

Consequently, as BCF in fish and log K_{OW} are above the CLP trigger values of 500 and 4, respectively, the DS concluded that pyridalyl has a high potential for bioaccumulation.

Aquatic Toxicity

There are ecotoxicological tests results from available acute and chronic studies for all trophic levels. However, as the water solubility of the test item was extremely low, it was concluded that it should be optimized by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). The use of the 1:1 DMF:HCO-40 mixture yielded a water solubility approximating 30 mg/L. Nevertheless, no information about the nature of the micelles (size distribution), any undissolved (i.e. non-micelle) substance was given. Also, no assessment of physical effects or genuine toxicity that is responsible for the observed effects was performed.

***Table**: Acute aquatic toxicity*

Test organism	Guideline, test method	Short-term result (endpoint)	Reference
Fish			
Rainbow trout *(Oncorhynchus mykiss)*	FIFRA 72-1 OPPTS 850.1075	96 h LC_{50} 0.5 mg/L (m) 96 h LC_{50} < 0.00015 mg/L (dissolved pyridalyl)	IIA 8.2.1.1/01 Study No. 13048.6206
Bluegill sunfish *(Lepomis macrochirus)*	FIFRA 72-1, OPPTS 850.1075	96 h LC_{50} > 24 mg/L (m)	IIA 8.2.1.2/01 Study No. 13048.6207
Sheepshead minnow *(Cyprinodon variegatus)*	FIFRA 72-3 OPPTS 850.1075	96 h LC_{50} > 32 mg/L (m)	IIA 8.11.1/01 Study No. 12709.6200
Aquatic invertebrates			
Daphnia magna	FIFRA 72-2 OPPTS 850.1010	48 h LC_{50} 0.0038 mg/L (m) 48 h LC_{50} < 0.00015 mg/L (dissolved pyridalyl)	IIA 8.3.1.1/01 Study No. 13048.6208
Daphnia magna	OECD TG 202	48 h LC_{50} 0.346 mg/L (n)	IIA 8.3.1.1/02 Study No 1043.046.110
Mysid *(Americamysis bahia)*	FIFRA 72-3 OPPTS 850.1035	96 h LC_{50} 0.001 mg/L (m)	IIA 8.11.1/02 Study No. 12709.6198
Eastern oyster *(Crassostrea virginica)*	OPPTS 850.1025	96 h LC_{50} 0.82 mg/L (m)	IIA 8.11.1/03 Study No. 12709.6199
Chironomus Yoshimatsu	ASTM 729	48 h LC_{50} 1.1 mg/L (m)	IIA 8.5.1/01
Algae			
Pseudokirchneriella subcapitata	OECD TG 201	72 h E_rC_{50} >0.2 mg/L (n)	IIA 8.4/01 Study No. 12709.6207
Selenastrum capricornutum	JMAFF No. 12	72 h E_rC_{50} >10 mg/L (n)	IIA 8.4/02 Study No 0109EAI
Skeletonema costatum	FIFRA 72-3 OPPTS 850.5400	96 h E_rC_{50} >0.15 mg/L (m)	IIA 8.11.1/04 Study No. 12709.6205
Navicula pelliculosa	OECD TG 201	72 h E_rC_{50} >0.2 mg/L (n)	IIA 8.4/04 SUW-0017
Aquatic plants			
Duckweed *(Lemna gibba)*	OPPTS 850.4400	72 h E_rC_{50} >0.17 mg/L (m)	IIA 8.6/01 Study No. 12709.6208

m: measured concentration, n: nominal concentration

Pyridalyl is poorly soluble substance (0.00015 mg/L at 20 °C; pH 8.0-8.3) and acute toxicity tests were carried out at nominal concentrations far exceeding the water solubility. Actual dissolved concentrations were not known. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. As well no information is available to assess whether physical effects could be possibly the cause of any observed toxicity.

Therefore, according to CLP guidance (section poorly soluble substances), for classification purposes DS considered L(E)C_{50} as equal to the measured water solubility. Hence the L(E)C_{50} of 0.00015 mg/L lead to classification Aquatic Acute 1. As the L(E)C_{50} is > 0.0001 mg/L but ≤ 0.001 mg/L an Acute M-factor of 1000 should also be applied.

***Table**: Chronic aquatic toxicity*

Test organism	Guideline, test method	Short-term result (endpoint)	Reference
Fish			
Rainbow trout *(Oncorhynchus mykiss)*	OECD TG 210, EPA 72-4, OPPTS 850.1400	89 d NOEC 0.024 mg/L (m)	IIA 8.2.4/01 Study No. 13048.6220
Aquatic invertebrates			
Daphnia magna	OECD TG 211, FIFRA 72-4, OPPTS 850.1300	21 d NOEC 0.0014 mg/L (m)	IIA 8.3.2.1/01 Study N0. 13048.6221
Mysid (Americamysis bahia)	FIFRA 72-4	28 d NOEC 0.00045 mg/L (m)	IIA 8.11.1/05 Study No. 12709.6202
Chironomus riparius	OECD TG 219	28 d NOEC 0.012 mg/L (n)	IIA 8.5.2/05 Study No. 13048.6401
Algae			
Pseudokirchneriella subcapitata	OECD TG 201	72 h NOE_rC 0.2 mg/L (n)	IIA 8.4/01 Study No. 12709.6207
Selenastrum capricornutum	JMAFF No. 12	72 h NOE_rC 10 mg/L (n)	IIA 8.4/02 Study No 0109EAI
Skeletonema costatum	FIFRA 72-3 OPPTS 850.5400	72 h NOE_rC 0.15 mg/L (n)	IIA 8.11.1/04 Study No. 12709.6205
Navicula pelliculosa	OECD TG 201	72 h NOE_rC 0.2 mg/L (n)	IIA 8.4/04 SUW-0017
Aquatic plants			
Duckweed (Lemna gibba)	OPPTS 850.4400	7 d NOE_rC 0.17 mg/L (m)	IIA 8.6/01 Study No. 12709.6208

m: measured concentration, n: nominal concentration

Pyridalyl is poorly soluble substance (0.00015 mg/L at 20 °C; pH 8.0-8.3) and chronic toxicity tests were carried out at nominal concentrations far exceeding the water solubility. Actual dissolved concentrations were not known. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. As well no information is available to assess whether physical effects could be possibly the cause of any observed toxicity.

Therefore, according to CLP guidance (section poorly soluble substances), for classification purposes the DS considered NOEC as equal to the measured water solubility. Hence, the NOEC of 0.00015 mg/L and being 'not rapidly degradable' lead to classification Aquatic Chronic 1. As NOEC is > 0.0001 mg/L but ≤ 0.001 mg/L a chronic M-factor of 100 should also be applied.

Comments received during consultation

Three MSCAs submitted comments on the environmental part of the DS's proposals. One MSCS agreed with the proposed classification by the DS without further comments. The second MSCA agreed with proposed classification and supported setting of L(E)C_{50} and NOEC based on water solubility because the exposure concentrations of the dissolved fractions of

pyridalyl in the aquatic toxicity studies are unknown and no information is available neither about micelles formation nor about the potential physical effects of the non-dissolved pyridalyl. The third MSCA pointed out that given exposure solution preparation method using DMF solvent and HCO-40, is likely that the analytical measurement of treatments represented dissolved and undissolved test item. Therefore, effects seen at concentrations above the quoted water solubility of 0.00015 mg/L may not have resulted from dissolved test item and could represent physical effects from the emulsion.

As an example for aquatic acute toxicity, the MSCA referred to studies with *Daphnia magna* (IIA 8.3.1.1/02 Study No 1043.046.110) and Mysid (IIA 8.11.1/02 Study No. 12709.6198). MSCA noted that in *Daphnia magna* study no significant immobilisation was observed for treatments up to an including 0.0207 mg/L and 95% immobilisation was observed at the next treatment 0.0455 mg/L. The MSCA noted that it would be useful to include further details of measured concentrations including if samples were filtered before analysis to aid interpretation. In the acute toxicity to Mysid study with the quoted EC_{50} of 0.001 mg/L, the MSCA indicates that there were no observed effects up to the quoted water solubility and effects were only observed in emulsion treatments above the water solubility at 0.0019 mg/L and above. However, the MSCA noted that the solutions were observed to be clear and colourless. Based on that, the MSCA asked for further details of the sample procedure such as filtration to help assess if treatments represented dissolved fractions.

Overall, the MSCA noted that it was not clear if $L(E)C_{50}$ values represented dissolved test item or if quoted $L(E)C_{50}$ reflected physical effects due the emulsion treatment / concentrations above the quoted water solubility. Therefore, the application for acute hazard classification is unclear.

According to aquatic chronic toxicity, the MSCA indicated that NOECs for fish (0.024 mg/L) and *Daphnia magna* (0.0014 mg/L) are significantly above the quoted water solubility. In the chronic toxicity study with Mysid, the MSCA identified significant differences in reproduction compared to the solvent control for all treatments including concentrations below the quoted water solubility i.e. 0.000066 and 0.00012 mg/L treatments based on mean measured concentrations. However, the MSCA noted that for these and further additional treatments above the water solubility, the effect were a positive increase in offspring. It is unclear if this was due to a poor performing solvent control as significant differences were noted between the solvent and procedural controls and it is also unclear if this apparent effect is relevant for hazard classification. A decrease in reproduction was only observed at the highest concentration of 0.0009 mg/L and the quoted NOEC of 0.00045 mg/L is based on a 12% reduction in reproduction at 0.0009 mg/L. This indicates that while an EC_{10} endpoint would exceed the quoted experimental water solubility of 0.00015 mg/L, it is within the same classification range and applicable for hazard classification. MSCA noted that solutions were observed to be clear and colourless and ask further details of the sample procedure such as filtration to help assess if treatments represented dissolved fractions.

However, the DS could not find further details of the sample procedure such as filtration in the case of the mentioned studies. RAC responses are provided in the RCOM document.

Assessment and comparison with the classification criteria

Degradation

No hydrolysis of pyridalyl was observed and substance was stable at pH 5, 7 and 9 over a 30-day period at 25 °C.

No readily biodegradability of pyridalyl was observed at 28-day biodegradation test according to OECD TG 301F.

Aerobic degradation in water/sediment system shows that in total system degradation of pyridalyl was DT_{50} 129–366 days at 20 °C. Dissipation from water phase was DT_{50} 6.5-11. Dissipation from sediment – DT_{50} 121-244 days.

Aerobic degradation in soil studies results shows that DT_{50} (persistence, 20 °C) values of pyridalyl were in the range 53-272 days, DT_{90} (persistence, 20 °C) values were in the range 465-150302 days and DT_{50} (modelling, 20 °C, non-normalised) values were in the range 75.1-163 days. The other study results show DT_{50} values of pyridalyl were in the range of 290-507 days (normalized).

Pyridalyl degrades by photolysis in sterile aqueous pH 7 buffers solutions with half-life of 3.5 days. The indicated photo-metabolites HTFP (max. 17.5% AR on day 30) and S-1812-PYP (max. 63% AR on day 14, 57% AR on day 21 and 30).

Regarding photolysis test results, pyridalyl seems to be primarily degraded with half-life < 16 days however, information on photochemical degradation is difficult to use for classification purposes. The actual degree of photochemical degradation in the aquatic environment depends significantly on local conditions (e.g. water depth, suspended solids, turbidity as well as seasonal influences). In addition, photolytic degradation led to formation at least of two components with unknown toxicity. Therefore, primary degradation via photolysis cannot be used to conclude that pyridalyl is rapidly degradable. Hence, RAC considers that pyridalyl is not readily biodegradable and all degradation information does not provide sufficient information to show that thiamethoxam is ultimately degraded to a level > 70% within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable products.

Consequently, RAC agrees that pyridalyl should be considered as not rapidly degradable under the CLP regulation.

Aquatic Bioaccumulation

In the available experimental study to determine the bioconcentration potential, the determined BCF values for pyridalyl in whole fish was 26858 and 22352 L/kg wwt at 0.05 and 0.15 µg a.s./L respectively (lipid BCF normalised to 1% fat 3671 and 2835 L/kg wwt). That is well above the CLP trigger BCF criteria of ≥ 500. Determined log K_{OW} of pyridalyl of 8.1 as well meets the CLP trigger value for indication of bioaccumulation (log K_{OW} ≥ 4). However, in bioaccumulation study with oligochaeta (*Lumbriculus variegatus*) BCF was found to be low (1.19 kg sediment dwt/kg worm wwt). Following the CLP guidance (section Bioaccumulation) BCF in fish is taken in preference. Therefore, based on the BCF_{fish} above 500, RAC agrees with the DS that pyridalyl has high potential for bioaccumulation according to the CLP criteria.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for all trophic levels. Due to very low solubility in water, the test item was optimised by using a co-solvent solution consisting of a mixture of 1:1 dimethylformamide (DMF) and hydrogenated castor oil (HCO-40). However, all aquatic toxicity tests (acute and chronic) were performed at concentrations far exceeding the water solubility of pyridalyl. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. The dissolved fraction of pyridalyl in lower nominal concentrations may have been even lower than that in the highest test concentration, since these concentrations were prepared by dilution from the highest test concentration. As well according to OECD TG 23 the testing of aqueous dispersions and emulsions is not generally advocated.

For classification, endpoints should be based on the concentration of dissolved pyridalyl. However, actual dissolved concentrations are not known, and measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. No information is available to assess whether physical effects could be possibly the cause of any observed toxicity as well.

The CLP guidance, for poorly soluble substances indicate some practical rules:

"a. w*here the acute toxicity is recorded at levels in excess of the water solubility, the L(E)C$_{50}$ for classification purposes may be considered to be equal to or below the measured water solubility. In such circumstances it is likely that category Chronic 1 and/or category Acute 1 should be applied. In making this decision, due attention should be paid to the possibility that the excess undissolved substance may have given rise to physical effects on the test organisms. Where this is considered the likely cause of the effects observed, the test should be considered as invalid for classification purposes*

…

d. where chronic toxicity data are available, the same general rules should apply. In principle, only data showing no observed effect concentrations at levels above the water solubility limit, or greater than 1 mg/L need be considered. Again, where these data cannot be validated by measuring the concentrations, the techniques used to achieve the maximum dissolved concentrations must be considered as appropriate".

Aquatic Acute

Aquatic Acute toxicity (≤ 1 mg/L) was observed in fish, invertebrates, algae and aquatic plants. The most acutely sensitive trophic group was invertebrates (Mysid and *Daphnia magna*) at range of L(E)C$_{50}$ from 0.001 to 0.0346 mg/L.

A 48-hour acute toxicity test in *Daphnia magna* was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 3.2, 5.4, 9.0, 15 and 25 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. Endpoints were based on mean measured concentrations (2.2, 3.8, 6.4, 11 and 17 µg a.s./L). The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/L at 20 °C) by at least a factor of 21. The author of the report stated that observations of the physical characteristics of the test solutions (e.g. presence of precipitate,

film on the solution's surface) were made and recorded, but the results of these observations were not reported. Actual concentration (as % of nominal) at the start was 76–87 and at the end 50–67%. The 48 h EC_{50} based on mean measured concentration was 0.0038 mg/L (tested as an emulsion in water).

A second 48-hour acute toxicity test in *Daphnia magna* was conducted in natural water-sediment systems under static conditions with radiolabelled S-1812 (pyridalyl) at nominal test concentrations of 0.88, 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. Endpoints were based on nominal concentrations. Actual concentration (as % of nominal) at the start was 87-99 and at the end 66-84%. The test was performed at concentrations exceeding the water solubility of pyridalyl (0.15 µg/litre at 20 °C) by at least a factor of 6. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Observed mortality was 0% at 20.7 µg/L and 95% at 45.5 µg/L concentration. No information on observations of the physical characteristics of the test solutions was available. The 48 h EC_{50} based on nominal concentration was derived 0.0346 mg/L (tested as an emulsion in water).

A 96-hour acute toxicity test in the marine Mysid *Americamysis bahia* was conducted under flow-through conditions with S-1812 (pyridalyl) at nominal test concentrations of 0.41, 0.69, 1.2, 1.9 and 3.2 µg a.s./L, with a solvent (hydrogenated castor oil and DMF, 1:1) and untreated control. Endpoints were based on mean measured concentrations (88-96% and 88-100% of nominal concentrations at the start and the end of the test, respectively). The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/L at 20 °C) by at least a factor of 3. The tested solutions are therefore likely to have been emulsions rather than true solutions, and the truly dissolved fraction of pyridalyl may have been lower than the water solubility. Actual dissolved concentrations are not known, since for analysis, water samples were extracted twice by liquid-liquid partition with methylene chloride. Measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. However, solutions were reported to be clear and colourless throughout the 96-hour exposure period. No information on observations of the physical characteristics of the test solutions was available. The 96 h LC_{50} based on mean measured concentration was derived 0.001 mg/L (tested as an emulsion in water).

Overall, RAC assumes that acute toxicity is recorded at levels in excess of the water solubility. However, no information is available to assess whether physical effects could be possibly the cause of any observed toxicity. Therefore, RAC cannot consider that observed acute effects at concentrations above the quoted water solubility of 0.00015 mg/L represent only physical effects. In support, in the aquatic acute study with Mysid, solutions were reported to be clear and colourless throughout the 96-hour exposure period. Based on that, RAC considers that available acute toxicity tests are valid for classification purposes. Consequently, RAC agrees that L(E)C_{50} for aquatic acute classification purposes shall be based on measured water solubility of 0.00015 mg/L. In addition, results from Mysid study of LC_{50} 0.001 mg/L will be in same order of magnitude for deriving M-factor.

Aquatic Chronic

Aquatic Chronic toxicity (for not rapidly degradable substance ≤ 0.1 mg/L) was observed in fish and invertebrates. The most chronically sensitive trophic group was invertebrates (*Daphnia magna* and Mysid) at range of NOEC from 0.0014 to 0.00045 mg/L.

The chronic toxicity of S-1812 (pyridalyl) to *Daphnia magna* was assessed in a 21-day flow-through study. The nominal concentrations were 0.98, 2.0, 3.9, 7.8 and 16 µg a.s./L plus an untreated and a solvent control (hydrogenated castor oil and DMF, 1:1). The test was performed at nominal concentrations exceeding the water solubility of pyridalyl (0.15 µg/L at 20 °C) by at least a factor of 7. Mean measured concentrations were 0.93, 1.4, 2.7, 5.7 and 11 µg a.s./L (67-95% of nominal concentrations), although actual dissolved concentrations are not known, and measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. No information on observations of the physical characteristics of the test solutions was available. The 21 d NOEC based on mean measured concentration for parental survival, reproduction and growth was 0.0014 mg/L (tested as an emulsion in water).

The chronic toxicity of S-1812 TG (pyridalyl) to the marine Mysid *Americamysis bahia* was assessed in a 28-day flow-through study at nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 µg/L plus an untreated and a solvent-control (hydrogenated castor oil and DMF, 1:1). The nominal test concentration at the level of the NOEC was three times higher than the water solubility limit of pyridalyl. Measured concentrations were 84-120% of nominal concentrations throughout the test period. Endpoints were based on mean measured concentrations. Actual dissolved concentrations are not known, and measured concentrations therefore represented the total of dissolved and non-dissolved pyridalyl. No information on observations of the physical characteristics of the test solutions was available. However, it was indicated that solutions were reported to be free of visible signs of undissolved test substance. The 28 d NOEC based on mean measured concentration for mortality, reproduction and growth was 0.00045 mg/L (tested as an emulsion in water).

Overall, RAC assumes that chronic toxicity is recorded at levels in excess of the water solubility although no information is available to assess whether physical effects could be possibly the cause of any observed toxicity. Therefore, RAC cannot consider that observed chronic effects at concentrations above the quoted water solubility of 0.00015 mg/L represent only physical effects. In support of this, in the aquatic chronic study with Mysid, solutions were reported to be free of visible signs of undissolved test substance. Based on this, RAC considers that available chronic toxicity tests are valid for classification purposes. Consequently, RAC agrees that NOEC for chronic acute classification purposes shall be based on measured water solubility of 0.00015 mg/L. In addition, results from Mysid study of NOEC 0.00045 mg/L will be in same order of magnitude for deriving M-factor.

Conclusion on classification

Pyridalyl is considered as not rapidly degradable and fulfils the criteria for bioaccumulation. Based on the available and reliable information, RAC agrees with the DS that pyridalyl warrants classification as:

Aquatic Acute 1 based on $L(E)C_{50}$ = 0.00015 mg/L. As this acute toxicity value falls within the $0.0001 < L(E)C_{50} \leq 0.001$ mg/L range, the **acute M-factor is 1000**.

Aquatic Chronic 1 based on NOEC =0.00015 mg/L. As this chronic toxicity value falls within the $0.0001 < NOEC \leq 0.001$ mg/L range, the **chronic M-factor is 100.**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No data

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

No data.

12.1.2 Comparison with the CLP criteria

Not relevant.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

No classification proposed. Data lacking.

13 ADDITIONAL LABELLING

None.

14 REFERENCES

A full reference list for all the studies from the DAR are included in Annex I. In addition, the following references were used in this CLH report.

Carney EW, Zablotny CL, Marty MS, Crissman JW, Anderson P, Woolhiser M, Holsapple M. The effects of feed restriction during in utero and postnatal development in rats. Toxicol Sci. 2004 Nov;82(1):237-49.

Hackett, A.M. and L.A. Griffits The metabolism and excretion of 3-palmitoyl-(+)-catechin in the rat. Xenobiotica, 1982; 12, 447-456

Humberstone, A.J., C.J.H. Porter and W.N. Charman A physicochemical basis for the effect of food on the absolute oral bioavailability of halofantrine. J. Pharm. Sci. 1996; 85, 525-529

Kennedy GC, Mitra J. Body weight and food intake as initiating factors for puberty in the rat. J Physiol. 1963 Apr;166:408-18.

Meylan W., Howard P.H., Boethling R.S. Molecular topology/fragment contribution method for predicting soil sorption coefficients. Environ. Sci. Technol. 1992; 26: 1560-1567

Miyazaki, S. , T. Yamahira, H. Inoue and T. Nadai Interaction of drugs with bile components II Effects of bile on absorption of indomethacin and phenylbutazone in rats. Chem. Pharm. Bull. 1980; 28 (1), 323-326

Mizuta, H., Y. Kawazoe, K. Haga and K. Effects of bile and meal on the gastrointestinal absorption of 2-[3-(3,5-di-tert-butyl-4-hydroxypehnyl)-1H-pyrazolo[3,4-b]pyridine-1-yl]ethyl acetate, a new non-steroidal anti-inflammatory agent. Ogawa Yakugaku Zasshi, 1990; 110, 598-603

Tao S. Piao H. Dawson R. Lu X. Hu H. Estimation or organic carbon normalized sorption coefficient (KOC) for soils using the fragment constant method. Environ. Sci. Technol 2000; 33: 2729-2725

15 ANNEXES

The study summaries from the DAR of pyridalyl have been included in Annex I.