Committee for Risk Assessment

RAC

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

Adopted

8 October 2020

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

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8 October 2020

CLH-O-0000006864-64-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **-**

CAS Number: **179101-81-6**

The proposal was submitted by **the Netherlands** and received by RAC on **30 January 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **18 March 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24 May 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Žilvinas Uzomeckas**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

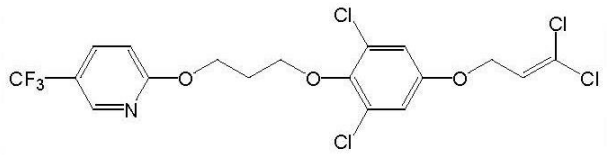
The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 October 2020** by **consensus**.

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	613-RST-VW-Y	pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether	-	179101-81-6	Repr. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H317 H400 H410	GHS07 GHS08 GHS09 Wng	H361d H317 H410		M=1000 M=100	
RAC opinion	613-RST-VW-Y	pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether	-	179101-81-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M=1000 M=100	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	613-RST-VW-Y	pyridalyl (ISO); 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether	-	179101-81-6	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M=1000 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

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%.

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.

HUMAN HEALTH HAZARD EVALUATION

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.**

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.**

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.**

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.**

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**.

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).**

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.**

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 OECD TG 471 No deviations S-1812 (pyridalyl) Batch No. PS 98041G Purity 93.7%	-S9-mix: 9.77, 19.5, 39.1, 78.1, 156, 313 µg/plate +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	**-S9-mix: negative** **+S9-mix: negative** Precipitation: 1250 µg/plate (+S9) and 313 µg/plate (-S9) <u>Cytotoxicity:</u> none	IIA 5.4.1/01 Study no. 3376

Organism/strain: TA100, TA98, TA1535 TA1537, Wp2uvrA			
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	Exp 1: -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 Exp 2: -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 Exp 3: +S9-mix (treatment 6 h and recovery 18 h): 15, 20, 25 µg/mL	**Exp 1:** **-S9-mix: negative** **+S9-mix: positive (within historical control data)** **Exp 2 and 3:** **-S9-mix: negative** **+S9-mic: negative** Cytotoxicity 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 Precipitation ≥ 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% Organism/strain: Chinese hamster ovary (CHO) cells	Exp 1: -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 Exp 2: -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 Positive controls: 5-Bromo-2'-deoxyuridine, 20-methylcholanthrene	**-S9-mix: negative** **+S9-mix: negative** Cytotoxicity -S9-mix: none +S9-mix: 5 µg/ml and above Precipitation 157 µg/ml and above	IIA 5.4.3/02 Project No. 6311-215

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.**

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.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

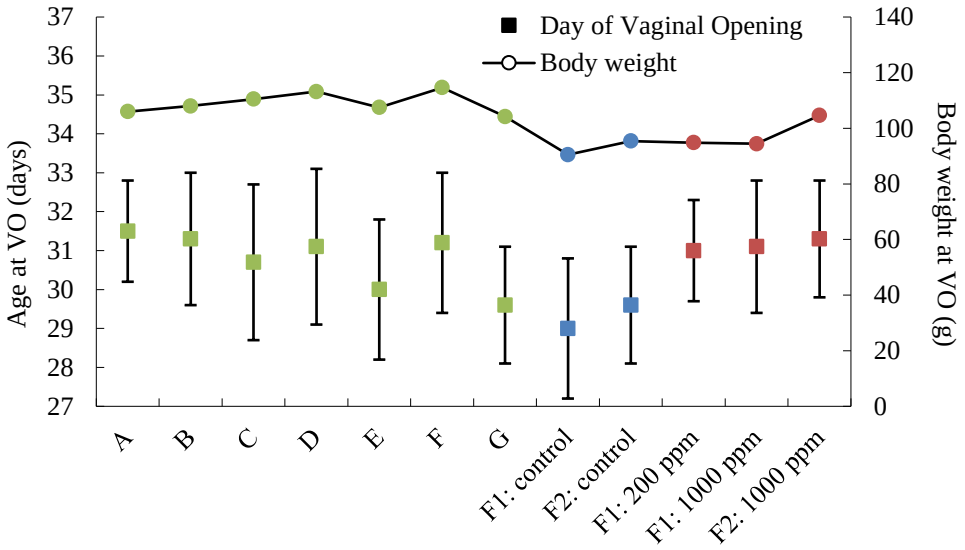
DS proposed classification of pyridalyl as Repr. 2; H361d (may damage the unborn child) based on a delay on vaginal opening supported by a certain hormonal imbalance detected in mechanistic studies. The DS also noted that the effects on pup growth were considered insufficient to support a classification.

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</td><td><u>Dosing:</u>
<table>
<tr><th></th><th colspan="2">40 ppm</th><th colspan="2">200 ppm</th><th colspan="2">1000 ppm</th></tr>
<tr><th></th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th></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></td><td>IIA 5.6.1/02

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

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)

1000 ppm (always statistically significant)

	P0		F1	
	M	F	M	F
Body weight	↓ 10% (week 9)	↓ 6% (week 9)	↓ 10% (week 10)	-
Food consumption (week 5)	↓ 12%	-	↓ 10%	-
Ovary weight	-	↑ 11%	-	↑ 22%
Ovary cell vacuolation	-	-	-	8/24
Increased thyroid follicles	-	7/24	-	7/24
Testis weight	-	-	↑ 8%	-

200 ppm (always statistically significant)

	P0		F1	
	M	F	M	F
Body weight (week 9)	↓ 10%	-	-	-
Food consumption (week 5)	↓ 12%	-	-	-
Ovary weight	-	-	-	↑ 12%
Testis weight	-	-	↑ 5%	-

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	<u>Leydig cells:</u> Increased androstenedione. Increased 17α-OH-progesterone (non-significant).	IIA 5.6.9/02

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	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.**	Study No. X0091
Reporter gene assay Non-GLP 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	No effect on human oestrogen, androgen or thyroid receptors. **Conclusion: Pyridalyl did not show a direct effect on human oestrogen, androgen or thyroid receptors.**	IIA 5.6.9/03 Study No. 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**.

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.**

ENVIRONMENTAL HAZARD EVALUATION

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 °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".

<u>Aquatic Acute</u>

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 < $L(E)C_{50}$ ≤ 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 ≤ 0.001 mg/L range, the **chronic M-factor is 100.**

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 Summary for repeated dose toxicity studies in animals with pyridalyl.

Annex 3

***Table**: Summary for repeated dose toxicity studies in animals with pyridalyl.* In all cases, the showed effects were statistically different from the corresponding controls.

Method	Results	Reference
OECD TG 407 4-week oral Deviations: no FOB, weight of epididymides not measured Non-GLP Crj:CD(SD) rats 6/sex/dose S-1812 (pyridalyl) Batch NSA-950525 Purity 98.7% 0, 7.05, 19.5, 64.6 and 182 mg/kg bw/day for males 0, 7.21, 19.6, 66.4 and 188 mg/kg bw/day for females	<u>182/188 (m/f) mg/kg bw/day</u> ↓ 10% body weight ↓ 16% bodyweight gain ↓ 13% food consumption ↑ cholesterol (62%) and phospholipids (42%) ↑ 18% liver weight ↑ 33% absolute ovary weight Adrenal cortex and ovary vacuolation (6 females) Degeneration of interstitial glands cells and persisted corpus luteum (females) <u>64.6/66.4 (m/f) mg/kg bw/day</u> ↑ 8.3% increased relative liver weight (males and females) Ovary vacuolation (1 female) <u>19.5/19.6 (m/f) mg/kg bw/day</u> ↑ 7.2% liver weight in females <u>7.05/7.21 (m/f) mg/kg bw/day</u> No treatment related effects	IIA 5.3.1/01 Study no. S0418 Category 1 ≤ 30 mg/kg bw/day 30 mg/kg bw/day ≤ Category 2 ≤ 300 mg/kg bw/day
OECD TG 408 13-week rat oral Deviations: no FOB non-GLP Crj:CD(SD) rats 10 animals/sex/dose (+6/sex/dose for hormone analysis) S-1812 (pyridalyl) Batch no. KOBE951006 Purity 98.4% 0, 4.68, 47.4, 133 and 233	<u>233/256 (m/f) mg/kg bw/day</u> ↓ 13% reduced body weight and body weight gain ↓ food consumption ↑5% haemoglobin and haematocrit ↑ lymphocytes, white blood cell counts, APTT, total protein and A/G ratio ↓ 9% testes weight ↓11% epididymides weight ↓ 18% pituitary weight in both sexes ↑ 49% increased ovary weight ↑ 67% increased lung weight ↑ 18% increased liver weight ↑ 28% increased relative adrenal weight ↓ 54% testosterone ↓ 73% oestradiol Adrenal vacuolation (6 males + 10 females), liver hypertrophy (8 males + 7 females), foamy eosinophilic cells in lung alveoli (7 females), interstitial cell vacuolation in the ovaries (10 females), single cell necrosis in liver in females (grade: 4 slight + 6 mild vs 4 slight in controls) <u>133/153 (m/f) mg/kg bw/day</u> ↓ 11% body weight and body weight gain ↓ food consumption	IIA 5.3.2/01 Study No. S0450 Category 1 ≤ 10 mg/kg bw/day 10 mg/kg bw/day ≤ Category 2 ≤ 100 mg/kg bw/day

mg/kg bw/day for males 0, 5.37, 55.5, 153 and 256 mg/kg bw/day for females	↑ 3% haemoglobin ↑ 5% haematocrit ↑ lymphocytes, white blood cell counts, APTT, total protein, A/G ratio, cholesterol, phospholipids and gamma-GTP ↓ 15% testes weight ↓ 10% epididymides weight ↓ 18% pituitary weight ↑ 8.4% increased liver weight Liver hypertrophy (6 males + 3 females) Single cell necrosis in liver in females (grade: 2 slight + 8 mild vs 4 slight in controls) Interstitial cell vacuolation in the ovaries (10 females) <u>47.4/55.5 (m/f) mg/kg bw/day</u> ↓ body weight gain ↑ 3% haemoglobin ↑ 4% haematocrit ↑ lymphocytes, white blood cell, total protein, A/G ratio, cholesterol, phospholipids and gamma-GTP Single cell necrosis in liver in females (grade: 6 slight + 2 mild vs 4 slight in controls) <u>4.68/5.37 (m/f) mg/kg bw/day</u> No treatment related adverse effects	
OECD TG 408 13-week oral No deviations Crj:CD(SD) rats 10/sex/dose S-1812 (pyridalyl) Batch no. PS-98041G Purity 93.7% 0, 5.56. 56.0, 111.3 mg/kg bw/day for males 0, 6.45, 64.0 and 128.6 mg/kg bw/day for females	<u>111.3/128.6 (m/f) mg/kg bw/day</u> 1 mortality (female) related to hepatic necrosis ↓ 11% body weight decrease ↓ 14% reduced body weight gain ↓ food consumption. ↑ 55% cholesterol increased ↑ 40% gamma-GTP ↓ 32% creatine phosphokinase ↑ 9% albumin/globulin ratio ↑ 15% liver weight Hepatocytes: centrilobular hypertrophy of hepatocytes (8/9 females + 8/10 males), single cell necrosis in 9 females (grade: 5 slight + 4 moderate vs 4 slight in controls). Vacuolation of interstitial gland cells in ovaries (8/9 females) <u>56.0/64.0 (m/f) mg/kg bw/day</u> ↓ 8% body weight decrease ↓ 12% body weight gain ↓ food consumption ↑ 6% liver weight <u>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 Category 1 ≤ 10 mg/kg bw/day 10 mg/kg bw/day ≤ Category 2 ≤ 100 mg/kg bw/day
OECD TG 408 13-week oral Deviations: no FOB and ophthalmoscopy ICR (Crj:CD-1) mouse 12/sex/dose	<u>721/879 (m/f) mg/kg bw/day</u> ↓ 9% reduced boy weight ↓ 28% body weight gain ↓ 8% haematocrit and haemoglobin ↓ 6% red blood cell count ↑ 78% ALP increased ↑ 7% total protein ↑ 17% albumin increased and AG ratio ↑ 75% cholesterol ↓ 64% triglycerides ↑ 22% creatine increased ↑ 4% calcium	IIA 5.3.2/03 Study No. SUT-0004 Category 1 ≤ 10 mg/kg bw/day 10 mg/kg bw/day ≤

S-1812 (pyridalyl) Batch no. PS-98041G Purity 93.7% 0, 8.17, 81.7, 379 and 721 mg/kg bw/day for males 0, 9.50, 86.8, 415 and 879 mg/kg bw/day for females	↑ 32% absolute liver weight ↑ 45% relative absolute liver weight ↓ 11% relative kidney weight ↓ 29% ovary weight Hepatocytes: centrilobular vacuolation (12 males) and centrilobular hypertrophy (12 males + 10 females) Kidney: increased incidence of basophilic change in tubular cells (6 males + 8 females) Adrenal: brown pigment deposition (7 males) Ovary atrophy (7 females) <u>379/415 (m/f) mg/kg bw/day</u> ↓ 10% reduced boy weight ↓ 32% body weight gain ↓ 7% haematocrit ↓ 6% haemoglobin and red blood cell count ↑ 43% ALP increased ↑ 6% total protein increased ↑ 11% albumin increased, AG ratio increased ↑ 69% cholesterol increased ↑ 22% creatine increased ↑ 15% absolute liver weight ↑ 25% relative liver weight ↓ 21% ovary weight Hepatocytes: centrilobular vacuolation (9 males) Ovary atrophy (4 females) <u>81.7/86.8 (m/f) mg/kg bw/day</u> ↑ 8% increased albumin ↑ 28% total cholesterol <u>8.17/9.50 (m/f) mg/kg bw/day</u> No treatment related adverse effects	Category 2 ≤ 100 mg/kg bw/day
OECD TG 409 13-week oral Beagle dogs 4/sex/dose Deviations: high dose animals initially received 1000 mg/kg bw/day. On day 2 and 3 one male and one female died. Dose reduced to 300 mg/kg bw/day from day 15 in males and 8 in females. Two additional	<u>300 (1000) mg/kg bw/day</u> Mortality in 1 female and 1 male Clinical signs: tachypnea, wheezing, abdominal respiration and/or dyspnoea ↓ 8% body weight ↓ 14% erythrocytes and haematocrit ↓ 15% haemoglobin ↑ ALP and cholesterol in one female ↓ 4% calcium ↑ 33% absolute lung weight ↑ 22% absolute liver weight ↑ 38% relative liver ↑ 26% relative kidney weights <u>100 mg/kg bw/day</u> Clinical signs: tachypnea, wheezing, abdominal respiration and/or dyspnoea ↓ 6% body weight ↓ 4% calcium	IIA 5.3.3/01 Study No. 29814 Category 1 ≤ 10 mg/kg bw/day 10 mg/kg bw/day ≤ Category 2 ≤ 100 mg/kg bw/day

animals were assigned to the high dose group. S-1812 (pyridalyl) Batch no. PS-98041G Purity 93.7% 0, 10, 100 and 300 (1000) mg/kg bw/day	<u>10 mg/kg bw/day</u> No treatment related adverse effects	
OECD TG 409 1-year oral Beagle dogs 4/sex/dose No deviations 0, 1.5, 5, 20 and 80 mg/kg bw/day	<u>80 mg/kg bw/day</u> ↓ 7% decrease MCH ↑ 218% ALP ↑ 30% absolute liver weight ↑ 32% relative liver weight ↑ 17% absolute lung weight ↑ 20% relative lung weight ↓ 7% absolute epididymis weight ↓ 8% relative epididymis weight <u>1.5, 5 and 20 mg/kg bw/day</u> No treatment related adverse effects	IIA 5.3.4/01 Study No. 29917 Category 1 ≤ 2.5 mg/kg bw/day 2.5 mg/kg bw/day ≤ Category 2 ≤ 25 mg/kg bw/day
OECD TG 410 4-week dermal Crj:CD(SD) rats 10/sex/dose No deviations S-1812 (pyridalyl) Batch no PS-98041G Purity 93.7% 0, 30, 100 and 1000 mg/kg bw/day	<u>1000 mg/kg bw/day</u> ↑ 22% cholesterol ↓ food consumption <u>30 and 100 mg/kg bw/day</u> No treatment related adverse effects	IIA 5.3.7/02 Study No. 20047 Category 1 ≤ 60 mg/kg bw/day 60 mg/kg bw/day ≤ Category 2 ≤ 600 mg/kg bw/day
OECD TG 453 Main: 104 weeks oral Satellite: 52 weeks oral Sprague-Dawley rats	<u>34.3/42.8 (m/f) mg/kg bw/day</u> ↑ motor activity ↓ 16% body weight ↓ 11% body weight gain ↓ food consumption ↓ 10% haematocrit ↓ 8% haemoglobin ↓ 10% RBC count and prothrombin time	IIA 5.5.2/01 Study no: IET 99-0011 Category 1 ≤ 1.25

	Liver peliosis (7/28 females)	mg/kg
50/sex/dose (main)	Brown pigment deposition in the spleen (16/28 females)	bw/day
		1.25
20/sex/dose (satellite)	Auricle thick area (6/50 females)	mg/kg bw/day ≤
	<u>17.1 mg/kg bw/day</u>	Category 2
No deviations		≤ 12.5
	↑ motor activity	mg/kg
S-1812 (pyridalyl)	↓ 20% body weight ↓ 10% body weight gain ↓ food consumption.	bw/day
Batch No. PS 98041G	<u>1.0 and 3.4 mg/kg bw/day</u>	
Purity 93.7%	No treatment related adverse effects	
0, 1.01, 3.40, 17.1 and 34.3 mg/kg bw/day in males		
0, 1.23, 4.10, 21.1 and 42.8 mg/kg bw/day in females		
OECD TG 451	<u>264 mg/kg bw/day</u>	IIA 5.5.3/01
Main: 78 weeks oral	↓ 18% reduced body weight ↓ 39% body weight gain ↓ 8% food consumption (-8%)	Study no: IET 99-0012
Satellite: 52 weeks oral	↓ lymphocyte count ↑ 17% absolute liver weight ↑ 30% relative liver weight	Category 1 ≤ 2.5 mg/kg bw/day
ICR mice	↑ 28% absolute kidney weight ↑ 30% relative kidney weight	
52/sex/dose (main)	<u>99 mg/kg bw/day</u>	
12/sex/dose (satellite)	↓ 10% body weight ↓ 19% body weight gain	2.5 mg/kg bw/day ≤ Category 2 ≤ 25 mg/kg bw/day
No deviations	<u>1.46 and 4.8 mg/kg bw/day</u>	
S-1812 (pyridalyl)	No treatment related adverse effects	
Batch No. PS 98041G		
Purity 93.7%		
0, 1.53, 5.04, 99 and 267 mg/kg bw/day in males		
0, 1.46, 4.78, 99 and 264 mg/kg bw/day in females		