Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

pyridate (ISO); O-(6-chloro-3-phenylpyridazin-4-yl) S-octyl thiocarbonate

EC Number: 259-686-7
CAS Number: 55512-33-9

CLH-O-0000001412-86-186/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

5 December 2017

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Pyridate

EC Number: **259-686-7**

CAS Number: **55512-33-9**

Index Number: **607-232-00-7**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Pyridate***
EC number:	***259-686-7***
CAS number:	***55512-33-9***
Annex VI Index number:	***607-232-00-7***
Degree of purity:	***≥ 900 g/kg***
Impurities:	***Relevant Impurity: -***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Skin Irrit. 2, H315 Skin Sens. 1, H317 Aquatic Acute 1, H400 Aquatic Chronic 1, H410
Current proposal for consideration by RAC	Skin Irrit. 2, H315 Skin Sens. 1B, H317 STOT SE 1, H370 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 M factor = 1 (acute) and 10 (chronic)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Irrit. 2, H315 Skin Sens. 1B, H317 STOT SE 1, H370 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 M factor = 1 (acute) and 10 (chronic)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PYRIDATE (ISO)

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-		-	Conclusive, but not sufficient for classification
2.2.	Flammable gases	-		-	Conclusive, but not sufficient for classification
2.3.	Flammable aerosols	-		-	Conclusive, but not sufficient for classification
2.4.	Oxidising gases	-		-	Conclusive, but not sufficient for classification
2.5.	Gases under pressure	-		-	Conclusive, but not sufficient for classification
2.6.	Flammable liquids	-		-	Conclusive, but not sufficient for classification
2.7.	Flammable solids	-		-	Conclusive, but not sufficient for classification
2.8.	Self-reactive substances and mixtures	-		-	Conclusive, but not sufficient for classification
2.9.	Pyrophoric liquids	-		-	Conclusive, but not sufficient for classification
2.10.	Pyrophoric solids	-		-	Conclusive, but not sufficient for classification
2.11.	Self-heating substances and mixtures	-		-	Conclusive, but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	-		-	Conclusive, but not sufficient for classification
2.13.	Oxidising liquids	-		-	Conclusive, but not sufficient for classification
2.14.	Oxidising solids	-		-	Conclusive, but not sufficient for classification
2.15.	Organic peroxides	-		-	Conclusive, but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	-		-	Data lacking

3.1.	Acute toxicity - oral	-	-	-	Conclusive but not sufficient for classification
	Acute toxicity - dermal	-	-	-	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Skin Irrit. 2, H315	-	Skin Irrit. 2, H315	
3.3.	Serious eye damage / eye irritation	-	-	-	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	Data lacking
3.4.	Skin sensitisation	Skin Sens. 1B, H317	-	Skin Sens. 1, H317	
3.5.	Germ cell mutagenicity	-	-	-	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	-	-	-	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	-	-	-	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	STOT-SE 1, H370	-	-	
3.9.	Specific target organ toxicity – repeated exposure	-	-	-	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	-	-	-	Data lacking
4.1.	Hazardous to the aquatic environment	aquatic acute 1, H400 aquatic chronic 1, H410	Acute: M-factor 1 Chronic: M-factor 10	aquatic acute 1, H400 aquatic chronic 1, H410	
5.1.	Hazardous to the ozone layer				Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word:
Danger

Hazard statements:
Skin Irrit. 2, **H315**: Causes skin irritation
Skin Sens. 1B, **H317**: May cause an allergic skin reaction

STOT SE 1, **H370**: Causes damage to organs
Aquatic Chronic 1, **H410**: Very toxic to aquatic life with long lasting effects

Proposed notes assigned to an entry: -

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Pyridate is a contact herbicide for use in agriculture and horticulture. Pyridate was evaluated for Annex I inclusion as part of the first stage of the work program referred to in Article 8(2) of Council Directive 91/414/EEC. The active substance was included on Annex I by Commission Directive 2001/12/EC of 5 March 2001. Inclusion entered into force on 01 January 2002. Commission Directive 2010/77/EU of 10 November 2010 extended the Annex I expiry date to 31 December 2015. An Annex I renewal of pyridate under Regulation (EU) No 1141/2010 was applied for to the Rapporteur Member State Austria in March 2011 which was accepted to be in compliance with Article 6 of Regulation (EC) 1141/2010, a dossier has been submitted in May 2012 and Austria confirmed completeness in July 2012. The RMS provided its initial evaluation of the dossier on pyridate in the Renewal Assessment Report (RAR), which was received by the EFSA on 11 June 2013. The peer review was initiated on 14 June 2013 by dispatching the RAR for consultation of the Member States and the applicant Belchim Crop Protection nv/sa. Experts consultations in the areas of physical-chemical properties, mammalian toxicology, residues, environmental fate and behaviour, and ecotoxicology were conducted in January - February 2014. A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in June - July 2014. The EFSA Conclusion on the peer review of the pesticide risk assessment of the active substance pyridate was published in August 2014, EFSA Journal 2014;12(8):3801. Pyridate was decided to be approved according to Regulation 1107/2009 by Commission Regulation (EU) 2015/1115 on 9th of July 2015. The entry into force date is the 1st of January 2016.

In accordance with Article 36(2) of the CLP Regulation, pyridate should now be considered for additional harmonised classification and labelling. Therefore, this proposal considers all human health and new environmental end points relevant for the additional harmonised classification and labelling. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of pyridate under Regulation (EU) No 1141/2010. The assessment made under that Directive is attached to the IUCLID 5 dossier.

Pyridate is already listed in Annex VI of the CLP Regulation (it was inserted into Annex I of Directive 67/548/EEC in the 28th ATP with the classifications as Skin Irrit. 2, H315; Skin Sens. 1, H317; Aquatic Acute 1, H400 and Aquatic Chronic 1, H410.

This proposal seeks to update this classification and additionally, to include classification for STOT single dose toxicity as well as a M-factor. During the peer review for Annex I Renewal of pyridate Member States and EFSA agreed that Austria should flag the new proposal for classification and labelling to ECHA, including STOT single and repeated dose toxicity.

2.2 Short summary of the scientific justification for the CLH proposal

Current classification according to Annex VI, Table 3.1 in the CLP Regulation for pyridate is Skin Irrit. 2, H315; Skin Sens. 1, H317; Aquatic Acute 1, H400 and Aquatic Chronic 1, H410.

Regarding human health following classification and labelling will be proposed:

Classification:
Skin Irrit. 2, H315
Skin Sens. 1, H317

STOT SE 1, H370

Labelling:
Pictograms: GHS07, GHS08
Signal word: Danger
H315, H317, H370

Pyridate is already listed in Annex VI of the CLP Regulation (it was inserted into Annex I of Directive 67/548/EEC in the 28th ATP) with the classifications related to human health according to Directive 67/548/EEC: Xi; R38, R43; and according to Regulation (EC) No 1272/2008: Skin Irritation Category 2, H315 and Skin Sensitisation Category 1, H317.

During the peer review for Annex I renewal of pyridate Member States and EFSA agreed that Austria should flag the new proposal for classification and labelling to ECHA, including specific target organ toxicity after single and if not considered overruled by single exposure also by repeated exposure. STOT-SE Category 1, H370 is required based on clinical signs related to neurotoxicity in dogs. According to the ECHA Guidance Document on the Application of the CLP Criteria "Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate." After single and repeated dose clinical signs related to neurotoxicity in dogs in the same dose range are the most severe effect. Therefore the dossier submitter (Austria) considers that classification for STOT-SE solely is more appropriate than classification for both STOT-SE and STOT-RE.

Regarding environment following classification will be proposed:

Pictogram: GHS 09

Signal word: Warning!

Aquatic Acute 1 – H400 'Very toxic to aquatic life'

Aquatic Chronic 1 - H410 'Very toxic to aquatic life with long lasting effects'

M factor = 1 (acute) and 10 (chronic)

Justification for the proposal

H400 follows from the toxicity of the active substance pyridate to aquatic invertebrates (*Daphnia magna*, LC50 = 0.49 mg/L, Egeler, Goth and Seck, 2011).

H410 follows from the long-term toxicity of the active substance pyridate to aquatic invertebrates (Daphnia magna, NOEC = 0.01 mg/L, Wüthrich, 1992a) and the fact that the active substance is not readily biodegradable (Dietschy, 2001). Though in the water sediment study a DT50 of 0.45 days (geomean) was determined for the whole system, the persistent metabolite CL9673 fulfils the classification criteria (NOEC = 0.1 mg/L; Gilberg and Seck, 2011). Further, the log Pow of pyridate is 4.01 (Zohner et al., 1982). Pyridate is therefore not indicated to be rapidly degradable.

Based on the fish bioconcentration study (Lentz, N.R., 2010) with *L. macrochirus* a steady-state BCF (whole fish) of 116 was determined (Ellgehausen and Wüthrich, 1984). The substance Mandestrobin does not meet the CLP criteria (BCF ≥ 500) based on the measured fish BCF.

Pyridate fulfils the criteria for classification as aquatic environmental hazard based on the CLP Regulation and should be classified

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Classification:
Skin Irrit. 2, H315
Skin Sens. 1, H317
Aquatic Acute 1, H400
Aquatic Chronic 1, H410

Labelling:
pictograms: GHS07, GHS09
signal word: Wng
hazard statement codes: H315, H317, H410

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification:
Xi; R38, R43
N; R50-53

Labelling:
Xi, N
R: 38-43-50/53
S: (2-)24-37-60-61

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification:
Skin Irrit. 2, H315
Skin Sens. 1B, H317
STOT SE 1, H370
Aquatic Acute 1, H400
Aquatic Chronic 1, H410

Labelling:
pictograms: GHS07, GHS08, GHS09
signal word: Dgr
hazard statement codes: H315, H317, H370, H410

Justification for the proposal:

Human health effects: Skin Irrit. 2, H315 follows from the skin irritating properties shown in two studies conducted with rabbits (Kynoch, Liggett, 1976 and Shults, Brock, Laveglia, 1995a). Skin Sens. 1B, H317 follows the potential for skin sensitisation shown in guinea pigs in a M&K test (Kynoch, 1976) and a Bühler test (Ullmann, Kups, 1988). STOT-SE 1, H370 follows clinical signs in dog studies (Tomkins, 1987 and Vandaele 1990) indicating a neurotoxic potential after single exposure.

Environmental effects: No justification was provided

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pyridate is used as a pesticide. For pesticides there is no need for justification (cf. Article 36(3) CLP Regulation).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

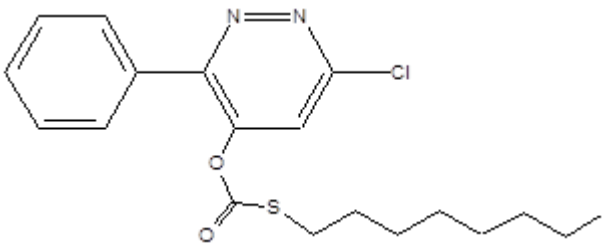
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	259-686-7
EC name:	O-(6-chloro-3-phenylpyridazin-4-yl) S-octyl thiocarbonate
CAS number (EC inventory):	55512-33-9
CAS number:	55512-33-9
CAS name:	Carbonothioic acid, O-(6-chloro-3-phenyl-4-pyridazinyl) S-octyl ester
IUPAC name:	*O*-6-chloro-3-phenylpyridazin-4-yl S-octyl thiocarbonate
CLP Annex VI Index number:	607-232-00-7
Molecular formula:	$C_{19}H_{23}ClN_2O_2S$
Molecular weight range:	378.9 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Pyridate	Min. purity: 900 g/kg	-	-

Current Annex VI entry:

Table 3.1

List of harmonised classification and labelling of hazardous substances

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)		
607-232-00-7	pyridate *O*-(6-chloro-3-phenylpyridazin-4-yl) *S*-octyl thiocarbonate	259-686-7	55512-33-9	Skin Irrit. 2, Skin Sens. 1, Aquatic Acute 1, Aquatic Chronic 1	H315 H317 H400 H410	GHS07 GHS09 Wng	H315 H317 H410			

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Data gap for impurity 10	confidential	confidential	Draft EFSA conclusion

1.2.1 Composition of test material

Human health hazard assessment: purity of tested technical material in the range from 90.3% to 91.5%

Environmental hazard assessment: purity of tested technical material in the range from 91.4% to 92.0%

1.3 Physico-chemical properties

Table 7: Summary of physico - chemical properties

Study	Method	Material / Batch	Results	Conclusion/Comment	Reference
B.2.1.1a Melting point, freezing point or solidification point (IIA 2.1.1)	EEC A1 and OECD Guideline no. 102 (Capillary method with hot block heating) GLP	PGAI 98.9%	Melting Range 26.5 °C – 27.8 °C	EU agreed endpoint	Bates, M. 1996
B.2.1.2a Boiling point (IIA 2.1.2)	EEC A1 and OECD Guideline no. 102 (Capillary method with hot block heating) GLP	PGAI 98.9%	Decomposes from 250 °C without boiling	EU agreed endpoint	Bates, M. 1996
B.2.1.3a Temperature of decomposition or sublimation (IIA 2.1.3)	EEC A1 and OECD Guideline no. 102 (Capillary method with hot block heating) GLP	PGAI 98.9%	Decomposes from 250 °C without boiling	EU agreed endpoint	Bates, M. 1996
B.2.1.4a Relative density (IIA 2.2)	OECD Guideline No 109 GLP	PGAI 98.9%	1.28	EU agreed endpoint	Füldner, H.H, 1998

Study	Method	Material / Batch	Results	Conclusion/Comment	Reference
B.2.1.5a Vapour pressure (IIA 2.3.1)	EPA: 163-2 not GLP (study before 1993) Knudsen sublimation pressure method and calculation using the Clausius –Clapeyron equation	Purity not stated in the study	9.98 x 10^{-7} Pa at 25 °C 4.8 x 10^{-7} Pa at 20 °C	The result was reported in the DAR and cannot be considered acceptable according to the current state of science due to the missing purity. However, this endpoint was considered acceptable in the peer review of 1997. Since the metabolite CL9673 is the biological active substance and environmental relevant component the vapour pressure study was repeated using CL9673, reported in Table B.2-1b.	Landvoigt, W., Creeger, S.M. 1988
B.2.1.6a Volatility, Henry's law constant (IIA 2.3.2)	Calculation		1.21 x 10^{-4} Pa m^3 mol^{-1} at 20 °C <u>Parameters used for calculation:</u> 4.8x10^{-7} Pa at 20 °C and 1.49 mg/L at pH 7, 20 °C	EU agreed endpoint. However, the result is not reliable due to the vapour pressure (see B.2.1.5) used for calculation. Since the metabolite CL9673 is the biological active substance and environmental relevant component the volatility calculation was repeated using CL9673, reported in Table B.2-1b.	Krüger, B. 1995
B.2.1.7a Appearance: physical state and colour (IIA 2.4.1)	Visual examination	PGAI 98.9%	solid at room temperature, colourless	Reported in the DAR 1996	Kettner, R. 1995a
		TGAI 90.5%	dark-brown oily liquid, at room temperature	Reported in the DAR 1996	Schneider, R. 1988 b,c,d
B.2.1.8a Appearance: odour (IIA 2.4.2)	olfactoric assessment	PGAI 98.9%	slightly aromatic odour	Reported in the DAR 1996	Kettner, R. 1995a

Study	Method	Material / Batch	Results	Conclusion/Comment	Reference
		TGAI 90.5%	characteristic odour, which is typical for mercaptans and sulphur containing compounds	Reported in the DAR 1996	Schneider, R. 1988 b,c,d
B.2.1.9.1a Spectra of the active substance [UV/VIS] (IIA 2.5.1.1)	OECD guidelines GLP	PGAI 98.9%	UV/VIS maxima acidic: 247 nm, 204nm neutral: 246nm, 204 nm alkaline: 307, 260, 227, 204 nm (pyridate not stable under alkaline conditions)	Reported in the DAR 1996	Bates, M. 1996
B.2.1.9.2a Spectra of the active substance [IR] (IIA 2.5.1.2)		PGAI 98.9%	FTIR spectrum between 4000 and 650 nm: Absorption bands are consistent with molecular structure	Reported in the DAR 1996	Bates, M. 1996
B.2.1.9.3a Spectra of the active substance [NMR] (IIA 2.5.1.3)		PGAI 98.6%	^{1}H-NMR spectrum Spectrum is consistent with molecular structure	Reported in the DAR 1996	Herling, H. 1996
B.2.1.9.4a Spectra of the active substance [MS] (IIA 2.5.1.4)		PGAI 98.9%	Mass spectrum (EI) [M^+] at m/z 378.3 amu Spectrum is consistent with molecular structure	Reported in the DAR 1996	Bates, M. 1996
B.2.1.9.5a Wavelengths at which UV/VIS molecular extinction occurs, where appropriate, to include a wavelength at the highest absorption above 290 nm (IIA 2.5.1.5)		PGAI 98.9%	**neutral methanol**: 295 nm (2533); 246 nm (14415); 204 nm (22393) **acidic methanol**: 295 nm (2790); 247 nm (14275); 204 nm (24257) **alkaline methanol**: 307 nm (8809); 295 nm (7491); 260 nm (8581); 227 nm (21374); 204 nm (33893) (λ_{max} / Ɛ [L x·cm^{-1} x mol^{-1}])	Results of neutral measurements are reported in the DAR 1996. In addition the acidic and alkaline absorptions determined in this study are added. Ɛ > 1000 at 290 nm in all media	Bates, M. 1996
B.2.1.9.6a Optical purity (IIA 2.5.1.6)			Not relevant (no enantiomers in pyridate)		
B.2.1.10a Spectra of relevant impurities (IIA 2.5.2)			No impurities of toxicological, ecotoxicological or environmental relevance are contained in pyridate.		

Study	Method	Material / Batch	Results	Conclusion/Comment	Reference
B.2.1.11a Solubility in water (IIA 2.6)	OECD-test guideline A 80/6 equivalent to EEC/A.6 flask method not GLP (study before 1993)	^{14}C Pyridate: 99.4 %	1.49 mg/L at pH 7, 20 °C (hydrolysis)	EU agreed endpoint However, due to hydrolysis the result is considered questionable. Value for CL9673 is reported in Table B.2-1b.	Zohner, A et al. 1980
	EEC method A6 OECD 105 GLP	^{14}C-Pyridate: 99.9%	at 20 ± 0.2 °C pH 3: 0.33 ± 0.05 mg/L pH 5: 1.67 ± 0.16 mg/L pH 7: 0.32 ± 0.17 mg/L At pH 5 and pH 7: the solubility was affected by hydrolysis, not possible to give an exact value for water solubility of pyridate	Results are not reliable due to hydrolysis. Values for CL9673 are reported in Table B.2-1b.	Wisson, M. 1996
B.2.1.12a Solubility in organic solvents (IIA 2.7)	OECD 105 GLP	TGAI 91.4%	at 20 °C Heptane: >250 g/L Dichloromethane: >250g/L Methanol: >250g/L Acetone: >250g/L Xylene: >250 g/L Ethyl Acetate: >250g/L	Acceptable	Seck, C., Jein, M. 2011
B.2.1.13a Partition coefficient *n*-octanol/water (IIA 2.8.1)	OECD A 80/8 equivalent to EEC/A.8 not GLP (study before 1993)	^{14}C Pyridate (purity > 98%, estimated) TLC of test substance confirms high	Log Pow = 4.01 ± 0.16 at room temperature	EU agreed endpoint. However, as stated in the DAR 1996 is the result due to rapid hydrolysation not reliable. Value for CL9673 is reported in Table B.2-1b.	Zohner, A. et al. 1982
Effect of pH (4-10) on the n-octanol/water partition co-efficient (IIA 2.8.2)			Effect of pH not relevant since pyridate does not dissociate.		
B.2.1.14a Hydrolysis rate (IIA 2.9.1)	OECD 1-111 GLP	^{14}C Pyridate, > 97.9 %	DT_{50} at 25 °C pH 4: 117h pH 5: 89h pH 7: 58.5h pH 9: 6.2h Pyridate was found to be hydrolytically unstable under acidic, neutral and basic conditions. Hydrolyses proceeded via cleavage of the ester bond leading to CL9673.	Acceptable	Lutringer, C. 1997

Study	Method	Material / Batch	Results	Conclusion/Comment	Reference
B.2.1.15a Direct phototrans-formation (IIA 2.9.2)	US EPA 540/9-82-021, Subdivision N, Section161-2 GLP	^{14}C Pyridate, purity > 98%	Pyridate DT_{50} 3.5 d at pH 5 1.8 d at pH 7.3 2.2 d at pH 9.2 Study lacks sufficient metabolite identification. DT_{50} values indicate initial degradation is through hydrolysis to CL9673.	EU agreed endpoint A new study was conducted using CL9673. See Table B.2-1b.	Van Dijk, A. 1992
B.2.1.16a Quantum yield (IIA 2.9.3)			In the environment pyridate degrades through hydrolysis rather than through photolysis. The quantum yield of the hydrolysis product CL9673 is provided in Table B.2-1b.		
B.2.1.17a Lifetime in the top layer of aqueous systems (calculated and real) (IIA 2.9.4)			In the environment pyridate degrades through hydrolysis rather than through photolysis. The lifetime of the hydrolysis product CL9673 in the top layer of aqueous systems is provided in Table B.2-1b.		
B.2.1.18a Dissociation constant (pKa) (IIA 2.9.5)	Statement according to OECD 112		Dissociation constant cannot be measured since pyridate has a very low water solubility and is not a ionic substance	EU agreed endpoint	Schneider, R. 1989
	VCCLAB ALOGPS 2.1 Pharma Algorithms applet		No pKa value could be determined.	The result of software estimation for the dissociation constant seems questionable. RMS follows the statement Schneider, R. 1989. A new study was conducted using CL9673. See Table B.2-1b.	Weidenauer, M. 2012
	ACD I-lab 2.0 pKa alogarithm version 12.1.0.50374		pKa_1 : -0.84 ± 0.10 pKa_2 : -22.19 ± 0.32		
B.2.1.19a Stability in air, photochemical oxidative degradation (IIA 2.10)	Theoretical calculation according to Atkinson.	Atmospheric Oxidation Programm version 1.5	K_{OH}:15.82 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1} DT_{50}: 8.1 h (0.676 day)	Acceptable	Glänzel, A. 1996
		Estimation	K_{OH}:2.11 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1} DT_{50}: 18.2 h	EU agreed endpoint However, the study is only available in German. Therefore a new study was performed (see below).	Ewald, G. 1993

Study	Method	Material / Batch	Results	Conclusion/Comment	Reference
		AOPWIN model v1.92	K_{OH}:16.707 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1} DT_{50}: 7.68 h (0.64 day)	Acceptable	Buntain, I. 2011
B.2.1.20a Flammability (IIA 2.11.1)			Not relevant. Technical pyridate is a viscous oily liquid		
B.2.1.21a Auto-flammability (IIA 2.11.2)	EEC A.15 GLP	TGAI 91%	Auto-ignition temperature: 245 °C	EU agreed endpoint	Krips, H.J. 1995
B.2.1.22a Flash point (IIA 2.12)	EEC A.9 Pensky-Martens closed cup GLP	TGAI 91.4%*	No flash point was observed before boiling/decomposition.	Acceptable *The purity of batch H1012008 is not reported in this study, however, under IIA 2.7 Seck, C., Jein, M. 2011 a certificate of analysis of this batch number is attached.	Shajad, Y. 2012
B.2.1.23a Explosive properties (IIA 2.13)	EEC A.14 GLP	TGAI 92.1%	Not explosive Negative in thermal sensitivity, shock tests. Friction test not suitable for liquids	Acceptable	Angly H., 1997
B.2.1.24a Surface tension (IIA 2.14)			The notifier argues that the test would not give reproducible results due to the very low water solubility and the hydrolytic instability.	Acceptable Was not required in the DAR 1996 as well.	
B.2.1.25a Oxidizing properties (IIA 2.15)	Statement		No oxidising properties based on consideration of the chemical structure.	Acceptable (The case provided in the DAR 1996 concerns reducing and/or oxidising properties and does not cover this annex point.)	Kaul P., 2012
B.2.1.2.26a pH (IIA 2.16)			No EU requirement		
B.2.1.2.27a Storage stability (IIA 2.17.1)			No EU requirement		
B.2.1.2.28a Stability (temperature, metals) (IIA 2.17.2)			No EU requirement		

Study	Method	Material / Batch	Results	Conclusion/Comment	Reference
B.2.1.2.29a Other/special studies (IIA 2.18)			None		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Pyridate is a contact herbicide for use in agriculture and horticulture.

3 HUMAN HEALTH HAZARD ASSESSMENT

3.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

3.1.1 Non-human information

Pharmacokinetic and metabolism studies with ^{14}C-pyridate were investigated in rats and dogs.

Rats:

Toxicokinetics:

No significant differences were seen between male and female rats. The compound was rapidly absorbed, followed by fast elimination (97 and 99% within 96 hours following the single and multiple dose of 20 mg/kg pyridate, approx. 94% following the single dose of 200 mg/kg pyridate). Based on urinary excretion more than 80% of the applied dose was absorbed and eliminated within 24 hours after treatment with a single dose of 20 mg/kg body weight. Multiple daily treatments with pyridate did not influence the distribution pattern and the excretion rates. Plasma levels reached a maximum within 1 to 2 hours after application of 20 or 200 mg/kg pyridate and decreased rapidly thereafter. Mean plasma peak levels at 200 mg/kg body weight after 1 hour were about 10 times those of 20 mg/kg. At a dose level of 600 mg/kg absorption and urinary excretion were apparently saturated and consequently prolonged.

An evaluation of bile cannulated rats (3 males, 3 females) after a single administration of 20 mg/kg pyridate was concluded to be of limited scientific validity (Cameron, 1988). Three male and three female rats were used for bile duct cannulated studies with application of a single oral dose of 20 mg radiolabelled pyridate/kg bw. 24 hours after treatment 3 - 72 % of the applied radioactivity was found in the gastrointestinal tract and 2 - 35 % in the carcass. These results indicate a reduced absorption with great variabilities. As an explanation concerning these findings the author of the study mentioned, that the variation might be a result of postoperative effects and of the restraint position of the animals. As a consequence out of these findings and their possible cause, it can be stated that this part of the study is of limited scientific validity. On the other hand, it is clear from the excretion of radiolabelled material in the urine of non-surgically treated rats, that at mean 80 % of the administered radioactivity is absorbed, which was also supported by the experts in the PRAS Meeting 109 (January 2014).

The additional studies performed for the renewal of approval with pyridate and its major metabolite pyridafol confirmed the major findings already presented in the DAR, namely that pyridate is rapidly and quantitatively absorbed with maximum blood levels within few hours after administration, undergoes metabolism and the metabolites are rapidly excreted with half-live times around 12 – 19 hours and accordingly only minimal amounts of residues are left in organs/carcass after 7 days. This applies to both investigated molecules, namely pyridate as active ingredient or pyridafol. These findings are independent of sex and dose. Low dose groups pretreated for 14 days showed a faster elimination than single dose groups.

The comparative study with pyridate and pyridafol when administered intravenously again showed no difference between both molecules. Kinetic and mass balance parameters were very similar to those obtained with oral administration, molecule, sex or dose had no influence. The majority of the radioactivity was excreted via the urine and the remaining activity via the faeces.

The radioactivity was distributed rapidly. There was no indication of accumulation in any tissue.

At one and six hours post dosing (200 mg/kg), the highest levels of radioactivity were found in the GI tract, plasma, liver and kidney. Concentrations in fat and brain were one order of magnitude lower than plasma levels. At 24 hours post dose (20 mg/kg) residues were < 0.2 µg/g in all tissues except GI tract and kidneys. The higher residues in kidneys are consistent with the preferred urinary excretion of the radioactivity. At 96 hours post dose the radioactivity in plasma and tissues was at or near the background levels.

Metabolism:

After oral administration pyridate was metabolized rapidly and extensively. Pyridate was hydrolysed to pyridafol (CL 9673 or SAN 1367H), which is the major component in plasma. There was some evidence of the presence of small portions of unchanged pyridate only in the one hour plasma samples. In the rat urine pyridafol and its O-glucuronide conjugate represented approx. 12-19 and 23-50% respectively of the total urinary radioactivity. A third significant urinary component represented approx. 26-37% and was tentatively identified as phenylring hydroxylated pyridafol. Compounds in faeces were pyridate (10-35%) and pyridafol (19-59%).

One of the weaknesses of the ADME data of the DAR is the identification of metabolites in mammals. In order to further consolidate the data additional studies were performed, namely two comparative toxicokinetic studies with pyridate and the main metabolite applied via the oral route and via the intravenous route. Specimens of the study via the oral route were used then to further investigate the metabolic behaviour of pyridate.

The metabolic pattern of pyridate has been further elucidated and a total of 8 metabolites were identified which allowed to establish a comprehensive metabolic pathway. After hydrolyzation to pyridafol glucuronic or sulfate acid conjugation is the major pathway and leads primarily to urinary excretion, which accounts for more than 44% of the radioactivity. Additional minor pathways are proposed. About 10% of the radioactivity is excreted in the faeces, where only pyridate, pyridafol and M1 (after oxidation) were identified. The hydroxyl group of pyridafol is not methylated.

The extent of degradation and the metabolic pattern was independent from dose level and test substance (pyridate or pyridafol). Repeated oral dosing resulted in an enhanced formation of M1 and M2 (after glucuronidation) when compared to the single doses. Generally the relative abundance of the unchanged test substance in faeces was higher for male rats than for female rats.

A further effort was made to elucidate the potential metabolic pathway of the thiocarbonic acid S-octyl ester which is formed after hydrolysation of pyridate to pyridafol. The S-octyl thiocarboxylate is unstable and is expected to undergo decarboxylation, resulting in octan-1-thiol (a mercaptan) and CO_2. Three general pathways for the intermediate octan-1-thiol moiety are proposed, namely thiol methylation as major route and glucuronic acid conjugation or oxidation to sulfinic acid as minor routes.

Dogs:

In dogs there was a difference in the absorption and elimination pattern between males and females. Higher plasma levels and an increased urinary excretion rate (75% in females versus 40-45% in males) at 32 and 80 mg/kg body weight indicate a higher absorption rate in female dogs. This data were derived from only one dog/sex and dose. Therefore the experts in the PRAS Meeting 109 (January 2014) concluded that no oral absorption value, with sufficient validity, can be derived based on this study. At 200 mg/kg absorption was lower and retarded, probably due to saturation of the absorption at this high dose level. Peak plasma levels were obtained between 2 and 12 hours in

the mid and high dose animals. Thereafter the radioactivity decreased rapidly with plasma levels less than 2% of the peak plasma concentration after 48 hours (exception low dose females: 8%). After 96 hours, the plasma radioactivity was in the range of the background level. In contrast to the rat, N-glucuronisation of pyridafol (approx. 70%) is the main conjugate found in the dog urine. Pyridafol represented approx. 18-23% of the total urinary radioactivity.

3.1.2 Human information

Not available.

3.1.3 Summary and discussion on toxicokinetics

Absorption, distribution, excretion and metabolism (toxicokinetics)

Rate and extent of oral absorption	Extensive (> 80%), rapidly and extensively metabolized after administration of single oral low and high dose
Distribution	Evenly distributed, highest amount in GI-tract, plasma, liver and kidney
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	> 90% within 96 hours, > 80% via urine in males and females
Metabolism in animals	Extensively metabolized, hydrolyzation to pyridafol (main metabolite); glucuronic and sulfate acid conjugation

3.2 Acute toxicity

Table 8: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity in the rat (OECD guideline 401)	LD_{50} = 5993 mg/kg bw (M) LD_{50} = 3544 mg/kg bw (F)	-	Ullmann, 1984a
Acute oral toxicity in the rat (OECD guideline 401)	LD_{50} = 4174 mg/kg bw (M) LD_{50} = 2961 mg/kg bw (F)	-	Ullmann, 1988c
Acute oral toxicity in the mouse (OECD guideline 401)	LD_{50} > 10000 mg/kg bw (M+F)	-	Ullmann, 1987b
Acute oral toxicity in the rat (OECD guideline 401)	LD_{50} > 2800 mg/kg bw (M) LD_{50} = 2092 mg/kg bw (F)	Findings also relevant for STOT-SE	Pels Rijcken, 1996a
Acute oral toxicity in the rat (OECD guideline 401)	LD_{50} > 2800 mg/kg bw (M) LD_{50} = 2371 mg/kg bw (F)	Findings also relevant for STOT-SE	Pels Rijcken, 1996b
Acute oral toxicity in the rat (OECD guideline 401)	LD_{50} > 2000 mg/kg bw (M+F)	Findings also relevant for STOT-SE	Pels Rijcken, 1996c
Acute inhalation toxicity in the rat (OECD guideline 403)	LC_{50} > 4.37 mg/kg bw (M+F)	-	Ullmann, 1983
Acute percutaneous toxicity in rabbit (OECD guideline 402)	LD_{50} > 2000 mg/kg bw (M+F)	-	Ullmann, 1984b

3.2.1 Non-human information

3.2.1.1 Acute toxicity: oral

The following studies were evaluated in support of Annex I listing of pyridate and are no longer granted data protection:

IIA 5.2.1/01 Ullmann L. and Sacher R. (1984a) Acute oral (LD_{50}) study with Pyridate technical in rats. RCC Report No.: 036990

IIA 5.2.1/02 Ullmann L. et al (1988c) Acute oral toxicity study with Pyridate technical in rats. RCC Report No.: 202623

IIA 5.2.1/03 Ullmann L. et al (1987b) Acute oral toxicity study with Pyridate technical in mice. RCC Report No.: 088323

A full set of acute oral toxicity studies was provided in the original dossier for first Annex I inclusion. The set of acute oral toxicity studies was performed with different vehicles, namely PEG 400 in the rat studies and gummi arabicum 5% in distilled water in the mouse study.

The results of the studies submitted for the DAR of pyridate are summarized in the following table.

Table 9: Summary of acute oral toxicity studies from the DAR

Animal species	Sex	OECD method	LD_{50} (mg/kg bw)	Toxicological effects	Reference
Rat	male	401	5993		Ullmann, 1984a

Animal species	Sex	OECD method	LD_{50} (mg/kg bw)	Toxicological effects	Reference
	female		3544	≥ 1000 mg/kg bw: Dyspnea (reversible after the 1st day) ≥ 3000 mg/kg bw: Sedation, ataxia, ventral body position, latero-abnominal position, curved body position, ruffeled fur (reversible after 2-4 days) ≥ 5000 mg/kg bw: Loss of weight (reversible after 6-8 days) Pathological changes were observed only in animals found dead ≥ 3000 mg/kg bw and included: Reddened intestine/stomach with corrosion (≥ 5000 mg/kg bw), mottled lung	
Rat	male	401	4174	≥ 1000 mg/kg bw: Slight Sedation, hunched posture, ruffled fur ≥ 2300 mg/kg bw: Dyspnea, ventral body position Clinical signs (except ruffled fur) were reversible after the 1st day ≥ 5000 mg/kg bw: Spasms Clinical signs (except ruffled fur) were reversible after the 2nd day Pathological changes were observed mainly in animals found dead and included: Reddish discoloration of the lung and the intestine, red foci in the lung (at 5000 mg/kg bw)	Ullmann, 1988c
	female		2961		
Mouse	male	401	>10000	≥ 5000 mg/kg bw: Sedation, dyspnea, ventral body position, hunched posture No macroscopic organ changes were observed.	Ullmann, 1987b
	female		>10000		

Based on the studies submitted for the DAR no classification for acute oral toxicity is necessary according to Regulation (EC) No 1272/2008.

There are 3 further acute oral toxicity studies reported here, which were not summarized in the DAR but submitted at the time of the ECCO reviews and included into the Review Report for the active substance pyrdiate (7576/VI/97-final).

Technical pyridate was tested comparatively in three classical OECD 401 tests with 3 different vehicles, namely

- Aqueous 1% carboxymethyl cellulose (CMC)
- Corn oil
- PEG 200

The results showed that the choice of vehicle had no influence on the oral toxicity of pyridate.

Reference:	**Pyridate TC (in 1 % CMC) Assessment of acute oral toxicity in the rat**
Author(s), year:	Pels Rijcken, W.R., 1996a
Guideline(s):	EEC 92/69 EEC, Part B.1, (1992), OECD GL 401, (1987)
GLP:	Yes
Deviations:	None
Validity:	Yes

Material and methods:

Test Material: Pyridate TC
Lot/Batch: KL 10
Purity: 90.4%

Test animals:
Species: Rat
Strain: Wistar strain Crl:(WI) BR (outbred SPF-quality)
Age: 8-9 weeks
Weight at dosing: 280 g ± 20% (males), 204 g ± 20% (females)
Source: Charles River, Germany

Diet: standard pelleted animal diet from Carfil Quality BVBA, Oud-Turnhout, Belgium; *ad libitum*
Vehicle: 1% aqueous carboxymethyl cellulose (1% CMC)
Dose levels: 1000 and 1400 mg/kg bw, females only
2000 and 2800 mg/kg bw, males and females
Group size: 5 per group
Dosing volume: 10 mL/kg bw

Initially, a group of 5 males and 5 females was dosed with 2000 mg pyridate/kg bw by gavage. Due to the number of deaths additional groups of 5 males and/or 5 females were dosed by gavage: 1400 mg/kg bw (females), 2800 mg/kg bw (males and females) and 1000 mg/kg bw (females).

Animals were observed for clinical signs periodically on the day of dosing followed by once daily observations up to 14 days.

Body weights were measured on day 1, 8 and 15 and at death.

Animals were necropsied immediately after death; surviving animals were euthanized at the end of the 14 day observation period and submitted to gross necropsy.

Findings:

There was mortality in the females at doses of 1400 mg/kg bw and higher and in males at the dose of 2800 mg/kg bw, see table below.

Table 9: Acute oral toxicity study (Pels Rijcken, 1996a)

Dose [mg/kg bw]	**Males**		**Females**	
	Mortality	**Time of death**	**Mortality**	**Time of death**
1000	-	-	0/5	-
1400	-	-	3/5	4 h
2000	0/5	-	2/5	2 h
2800	1/5	4 h	3/5	2-4 h
LD_{50} (mg/kg bw)	> 2800		2092	

The body weight development of the surviving animals was not affected by treatment.

Clinical signs observed during the first day were:

≥ 1000 mg/kg bw: Hunched posture

≥ 1400 mg/kg bw: Lethargy, uncoordinated movements
≥ 2800 mg/kg bw: Paddling movements, ventro-lateral recumbency, deep or laboured respiration, piloerection

Table 10: Clinical signs observed ≤ 2000 mg/kg bw (Pels Rijcken, 1996a)

Clinical sign	1000 mg/kg bw		1400 mg/kg bw		2000 mg/kg bw	
	Males	Females	Males	Females	Males	Females
hunched posture	Not tested	4/4 (grade 1)	Not tested	2/5 (grade 1)	1/5 (grade 1)	3/5 (grade 1)
Gait/motility (uncoordinated movements)		0/4		5/5 (grade 1-3)	5/5 (grade 1)	3/5 (grade 1)
Lethargy				5/5 (grade 1-2)	1/5 (grade 1)	

All signs were reversible and had disappeared by day 2.

Haemorrhages in the thymus were found in two females (2800 mg/kg bw) that died on day 1. Pelvic dilatation of the right kidney was found in one male (2000 mg/kg bw). According to the study author this finding is commonly noted in rats of this age and strain and was therefore not considered to be related to treatment.

Conclusion:

The oral LD_{50} for pyridate was calculated to be 2092 mg/kg bw in females and > 2800 mg/kg bw in males. No classification for acute oral toxicity is necessary according to Regulation (EC) No 1272/2008.

Reference:	**Pyridate TC (in corn oil) Assessment of acute oral toxicity in the rat**
Author(s), year:	Pels Rijcken, W.R., 1996b
Guideline(s):	EEC 92/69 EEC, Part B.1, (1992), OECD GL 401, (1987)
GLP:	Yes
Deviations:	None
Validity:	Yes

Material and methods:

Test Material: Pyridate TC
Lot/Batch: KL 10
Purity: 90.4%

Test animals:
Species: Rat
Strain: Wistar strain Crl:(WI) BR (outbred SPF-quality)
Age: 8-9 weeks
Weight at dosing: 284 g ± 20% (males), 198 g ± 20% (females)
Source: Charles River, Germany

Diet: standard pelleted animal diet from Carfil Quality BVBA, Oud-Turnhout, Belgium; *ad libitum*

Vehicle: corn oil
Dose levels: 1400, 2000 and 2800 mg/kg bw, males and females
Group size: 5 per group
Dosing volume: 10 mL/kg bw

Initially, a group of 5 males and 5 females was dosed with 2000 mg pyridate/kg bw by gavage. Due to the number of deaths additional groups of 5 males and 5 females were dosed with 1400 mg/kg bw or 2800 mg/kg bw via gavage.

Animals were observed for clinical signs periodically on the day of dosing followed by once daily observations up to 14 days.

Body weights were measured on day 1, 8 and 15 and at death.

Animals were necropsied immediately after death; surviving animals were euthanized at the end of the 14 day observation period and submitted to gross necropsy.

Findings:

There was mortality males and females at doses of ≥ 2000 mg/kg bw, see table below.

Table 11: Acute oral toxicity study (Pels Rijcken, 1996b)

Dose [mg/kg bw]	Males		Females	
	Mortality	**Time of death**	**Mortality**	**Time of death**
1400	0/5	--	0/5	--
2000	1/5	4 h	1/5	2 h
2800	0/5	--	4/5	2-4 h
LD_{50} (mg/kg bw)	> 2800		2371	

The body weight development of the surviving animals was not affected by treatment.

Clinical signs observed were:

≥ 1400 mg/kg bw: Lethargy, uncoordinated movements, red staining of the snout, hunched posture, piloerection
≥ 2000 mg/kg bw: Diarrhea, red staining of the head or back
≥ 2800 mg/kg bw: Ventro-lateral recumbency, slow breathing, laboured respiration, uncoordinated movements

The symptoms had disappeared in all surviving animals between days 2 and 3 in the males and between days 3 and 6 in the females.

Table 12: Clinical signs observed ≤ 2000 mg/kg bw (Pels Rijcken, 1996b)

Clinical sign	1400 mg/kg bw		2000 mg/kg bw	
	Males	**Females**	**Males**	**Females**
hunched posture	0/5	4/5 (grade 1)	2/5 (grade 1)	4/5 (grade 1)
Gait/motility (uncoordinated movements)	5/5 (grade 1)	5/5 (grade 1-2)	5/5 (grade 1)	5/5 (grade 1-2)
Lethargy	5/5 (grade 1)	2/5 (grade 1-2)	5/5 (grade 1)	2/5 (grade 1-2)

All clinical signs ≤ 2000 mg/kg bw were reversible at the latest after the 3rd day.

Haemorrhages in the thymus were found in one female (2800 mg/kg bw) that died on day 1. No further abnormalities were found at macroscopic post mortem examination of the animals.

Conclusion:

The oral LD_{50} for pyridate was calculated to be 2371 mg/kg bw in females and > 2800 mg/kg bw in males. No classification for acute oral toxicity is necessary according to Regulation (EC) No 1272/2008.

Reference:	**Pyridate TC (in PEG 200) Assessment of acute oral toxicity in the rat**
Author(s), year:	Pels Rijcken, W.R., 1996c
Guideline(s):	EEC 92/69 EEC, Part B.1, (1992), OECD GL 401, (1987)
GLP:	Yes
Deviations:	None
Validity:	Yes

Material and methods:

Test Material:	Pyridate TC
Lot/Batch:	KL 10
Purity:	90.4%
Test animals:	
Species:	Rat
Strain:	Wistar strain Crl:(WI) BR (outbred SPF-quality)
Age:	approx. 8 weeks
Weight at dosing:	283 g ± 20% (males), 194 g ± 20% (females)
Source:	Charles River, Germany
Diet:	standard pelleted animal diet from Carfil Quality BVBA, Oud-Turnhout, Belgium; *ad libitum*
Vehicle:	PEG 200
Dose levels:	2000 mg/kg bw, males and females
Group size:	5 per group
Dosing volume:	10 mL/kg bw

A group of 5 males and 5 females was dosed with 2000 mg pyridate/kg bw via gavage. As there were no mortalities, no further animals were dosed.

Animals were observed for clinical signs periodically on the day of dosing followed by once daily observations up to 14 days.

Body weights were measured on day 1, 8 and 15 and at death.

Animals were necropsied immediately after death; surviving animals were euthanized at the end of the 14 day observation period and submitted to gross necropsy.

Findings:

No mortality occurred in males and females after dosing of 2000 mg/kg bw pyridate.

The body weight development of the animals was not affected by treatment.

Clinical signs observed during the first day were lethargy, uncoordinated movements, and hunched posture.

Table 13: Clinical signs observed ≤ 2000 mg/kg bw (Pels Rijcken, 1996c)

Clinical sign	2000 mg/kg bw	
	Males	**Females**
hunched posture	0/5	2/5 (grade 1)
Gait/motility (uncoordinated movements)	5/5 (grade 1-2)	5/5 (grade 1)
Lethargy	4/5 (grade 1-2)	0/5

All clinical signs ≤ 2000 mg/kg bw were reversible at the latest after the 3rd day.

No abnormalities were found at macroscopic post mortem examination of the animals.

Conclusion:

The oral LD_{50} for pyridate was calculated to be > 2000 mg/kg bw. No classification for acute oral toxicity is necessary according to Regulation (EC) No 1272/2008.

3.2.1.2 Acute toxicity: inhalation

The following study was evaluated in support of Annex I listing of pyridate and is no longer granted data protection:

IIA 5.2.3/01 Ullmann et al. (1983) 4-hour aerosol inhalation toxicity study (LC_{50}) with pyridate techn. in rats. RCC Project 016255

The results of the study submitted for the DAR of pyridate are summarized in the following table.

Table 14: Summary of the acute inhalation toxicity study from the DAR (Ullmann, 1983)

Animal species	Sex	OECD method	LC_{50} (mg/l air)	Toxicological effects	Reference
Rat	male	403	> 4.37	≥ 2.7 mg/l: Sedation, dyspnea, curved body position and ruffled fur, red discoloration of the lungs, mottled lungs	Ullmann, 1983
	female		> 4.37		

During day 1 and 8, body weight gain was slightly reduced in the rats in both concentration groups (2.7 and 4.37 mg/l air).

Based on the study submitted for the DAR no classification for acute inhalation toxicity is necessary according to Regulation (EC) No 1272/2008.

3.2.1.3 Acute toxicity: dermal

The following study was evaluated in support of Annex I listing of pyridate and is no longer granted data protection:

IIA 5.2.2/01 Ullmann et al (1984b) Acute dermal toxicity (LD_{50}) study with Pyridate techn. in rabbits. RCC Project 037001

The results of the study submitted for the DAR of pyridate are summarized in the following table.

Table 15: Summary of the acute dermal toxicity study from the DAR (Ullmann, 1984b)

Animal species	Sex	OECD method	LD_{50} (mg/kg bw)	Toxicological effects	Reference
Rabbit	male	402	> 2000	Slight to moderate erythema and edema	Ullmann, 1984b
	female		> 2000		

In 2/10 rabbits (male/female) loss of weight was observed during 3 days after the test article application.

Based on the study submitted for the DAR no classification for acute dermal toxicity is necessary according to Regulation (EC) No 1272/2008.

3.2.1.4 Acute toxicity: other routes

No data available.

3.2.2 Human information

No information is available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

3.2.3 Summary and discussion of acute toxicity

Pyridate is of low acute toxicity when tested by the oral route (rats and mice), the inhalative (rats) and the dermal route (rabbits).

3.2.4 Comparison with criteria

All estimated LD_{50} and LC_{50} values are above the criteria for triggering classification and labelling. However, in the OECD testing guidelines for acute toxicity it is stated that “females should be nulliparous and non-pregnant”. Therefore, mortality after a single gavage dose to pregnant animals was not considered for classification for acute toxicity. The dossier submitter (Austria) would like to highlight that in the rat developmental toxicity study overt maternal toxicity resulting in 13/25 deaths > 465 mg/kg bw after application of a single dose (via gavage, vehicle distilled water with 4% carboxy methylcellulose sodium salt) was observed (see section 3.11.2.1). No other factors possibly influencing survival in the two highest dose groups are described in the study report. No specific guidance in the CLP regulation and associated guidance on the relevance of this effect is known to the dossier submitter except for a short note in the “Guidance on the application of CLP

criteria, Version 4.1, June 2015, section 3.7.2.3.1, stating that for repeat dose tests, extrapolation form non-pregnant to pregnant animals cannot easily be performed.

3.2.5 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: No classification proposed

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Proposal before public consultation

Pyridate is of low acute toxicity when tested by the oral route (in rats and mice), the inhalative route (in rats) and the dermal route (rabbits).

All estimated LD_{50} and LC_{50} values are above the criteria for triggering classification and labelling.

In the OECD testing guidelines for acute toxicity (oral) it is stated that "females should be nulliparous and non-pregnant". Therefore, mortality after a single gavage dose to pregnant animals was not considered for classification for acute toxicity. The dossier submitter (Austria) would like to highlight that in the rat developmental toxicity study overt maternal toxicity resulting in 13/25 deaths > 465 mg/kg bw after application of a single dose (*via* gavage, vehicle distilled water with 4% carboxy methylcellulose sodium salt) was observed. No other factors possibly influencing survival in the two highest dose groups are described in the study report. No specific guidance in the CLP regulation and associated guidance on the relevance of this effect is known to the dossier submitter except for a short note in the Guidance on the application of CLP criteria, version 4.1, June 2015, section 3.7.2.3.1, stating that for repeat dose tests, extrapolation from non-pregnant to pregnant animals cannot easily be performed.

Proposal after public consultation

In light of information from a new acute oral toxicity study (Diehl, 2016) that showed mortality in male and female rats at doses of 500 mg/kg bw and above (see additional key elements), classification with acute oral toxicity category 4 was proposed.

Comments received during public consultation

During the public consultation, one MSCA agreed in general with the Dossier Submitter's proposal, but two MSCA disagreed with the non-classification for acute toxicity by the oral route. The two MSCA in disagreement believed that the deaths occurring in 13/25 pregnant female rats following a single dose in the teratogenicity study should be considered relevant to the acute toxicity classification. However, these MSCA had not seen the additional data (Diehl, 2016) that supports classification of pyridate for acute oral toxicity.

Additional data was provided by the applicant during the public consultation. This was in the form of a well-performed single-dose oral neurotoxicity study in rats (Diehl, 2016). The

results of this study are summarised below in the additional key elements section and have been taken into account in RAC's assessment of this endpoint.

Additional key elements

During the public consultation, the applicant disagreed with the proposed classification for STOT SE and STOT RE on the basis of the results of the following acute neurotoxicity study in rats (Diehl, 2016).

Diehl, (2016). Pyridate. Acute single-dose oral gavage neurotoxicity screening study in rats. Report 200971881

In this recent study, performed according to EPA-test guideline OPPTS 870.6200, a preliminary study and a main study were carried out.

In the preliminary phase, Sprague Dawley rats (5/sex/dose) were administered a single dose of pyridate by oral gavage of 0, 500 or 1000 mg/kg bw in corn oil. Animals were then analysed for neurological effects.

There were no mortalities in the control groups. At the mid dose, 1 female was found dead on day 1 and in the top dose group, 2 males and 2 females were sacrificed in moribund condition and 3 females were found dead (all day 1).

For the main phase of the study, Sprague Dawley rats (10/sex/dose) were administered a single dose of pyridate by oral gavage of 0, 62.5, 177 or 500 mg/kg bw in corn oil. Animals were then analysed for neurological effects.

No animals died in the control, low or mid dose groups. At the top dose of 500 mg/kg bw on day 1, one male was found dead and one female was sacrificed in moribund condition.

Clinical signs were observed from a dose of 500 mg/kg bw. These included decreased activity, incoordination, weakness, abnormal breathing, lateral positioning, non-sustained convulsions, tremors and locomotor stereotypy were noted prior to death. Based on these results it can be assumed that doses of 500 and 1000 mg/kg bw were clearly lethal doses, beyond the MTD. Similar signs were observed in the surviving animals and thus are regarded as signs of acute unspecific toxicity. They began approximately 1h post-dose with recovery in almost all cases by the following day.

Parameter	**Doses (mg/kg bw)**				
	0	**62.5**	**177**	**500**	**1000**
Preliminary study					
Mortality	-	./.	./.	+	+
Clinical signs	-	./.	./.	+	+
Main study					
Mortality	-	-	-	+	./.
Clinical signs	-	-	-	+	./.
FOB effects	-	-	-	+	./.

./. = dose not used in the study

+ = the parameter was observed
- = the parameter was not observed

To summarise, the signs of toxicity observed in this study are regarded as unspecific and reversible clinical signs of animals under stress after exposure to lethal doses and not of a specific neurotoxic potential.

Assessment and comparison with the classification criteria

Acute toxicity – oral route

The CLH report for pyridate includes 6 acute oral toxicity studies, 5 in rats and one in mice, all carried out according to OECD TG 401 and some to GLP (the three studies carried out in the 1980s pre-dated GLP). Also available is an acute single-dose neurotoxicity study in rats, provided by the applicant during public consultation. Supporting information was provided by a developmental toxicity study in rats.

Acute Neurotoxicity study in rats (2016)

This study consists of a preliminary phase and a main study. In the preliminary phase, Sprague-Dawley rats (5/sex/dose) received an oral dose of pyridate of 0, 500 or 1000 mg/kg bw by gavage. There were 1/10 mortalities (female) at 500 mg/kg bw and 7/10 (2 males, 5 female) mortalities at 1000 mg/kg bw. The LD_{50} was not provided but from this data it appears to fall between 500 and 1000 mg/kg bw. In the main study, SD rats (10/sex/dose) received pyridate at doses of 62.5, 177 and 500 mg/kg bw. There were no mortalities in the low and mid dose groups and 2/20 rats died at the top dose (1 male, 1 female). Clinical signs were observed only at doses at which mortality occurred (≥ 500 mg/kg bw). These included decreased activity, incoordination, weakness, abnormal breathing, lateral position, non-sustained convulsions, tremors and locomotor stereotypy and were noted prior to death. In animals that survived, the toxic signs were reversible by day 2 post treatment.

Acute toxicity studies in rats (1984 – 1996

Five acute toxicity studies are available, carried out in rats. The results of these studies indicated that the LD_{50} in rats ranged from 2092 – 5993 mg/kg bw, which is above the guidance values for classification for acute toxicity. Clinical signs observed in these studies included sedation, ataxia, altered body positions, piloerection, deep/laboured respiration and ruffled fur.

Acute toxicity study in mice (1987)

The LD_{50} in this study was > 10000 mg/kg bw for both males and females. Clinical signs included sedation, dyspnoea, ventral body position and hunching.

Developmental toxicity study in rats (1986) - Supporting information

Pregnant female Wistar rats received an oral dose of pyridate, by gavage, of 0, 55, 165, 400 or 495 mg/kg bw/d from day 6 to 15 of pregnancy. After one dose of 400 mg/kg bw/d, 1/25 rats died and after a single dose of 495 mg/kg bw/d 13/25 rats died.
Clinical signs were noted in animals from a dose of 400 mg/kg bw/d and above. These included ventral body position, dyspnoea, sedation, somnolence, lack of response to external stimuli, tonic and clonic muscle spasms and ruffled fur.

The results of this study support the recent acute neurotoxicity study, indicating that the LD_{50} of pyridate in rats, following an oral dose, is much lower than previously shown. In the neurotoxicity study, the LD_{50} is between 500 - 1000 mg/kg bw and in pregnant rats, the LD_{50} appears closer to 495 mg/kg bw.

Acute toxicity – inhalation and dermal routes

In a single guideline study (OECD TG 403) in rats (Ullmann, 1983), the LC_{50} was > 4.37 mg/L air in both males and females. Clinical signs occurred at doses ≥ 2.7 mg/l and included sedation, dyspnoea, curved body position and ruffled fur. Red discolouration and mottling of the lungs were also observed.

Acute toxicity by the dermal route was assessed in rabbits in a study carried out according to OECD TG 402 (1984). The LD_{50} was > 2000 mg/kg bw. Slight to moderate erythema and oedema were observed.

RAC assessment of acute toxicity

In 6 guideline oral studies carried out in rats and mice, performed between the years of 1984 – 1996, the LD_{50} for pyridate was > 2000 mg/kg bw day and falling above the cut-off value for classification in category 4 for acute toxicity by the oral route (300 < ATE ≤ 2000).

However, in a new, well-performed study in rats, the LD_{50} was found to be between 500 – 1000 mg/kg bw, which clearly meets the criteria for classification with acute oral toxicity, category 4. This LD_{50} range was supported by the results from a developmental toxicity study in pregnant rats where deaths occurred after a single dose of pyridate from a dose of 400 mg/kg bw/d, indicating that the LD_{50} was approximately 500 mg/kg bw. It is recognised that pregnant rats may be more sensitive to the effects of pyridate; however the results of this study give support to the lower LD_{50} obtained in the acute neurotoxicity study.

There is no clear reason as to why the LD_{50} appears to be so different in the new study compared to the older studies. It is known that older standard studies were designed to determine lethality and estimate LD_{50}, whilst in contrast, contemporary study protocols use signs of evident toxicity rather than lethality as indications of acute toxicity (See Guidance on the application of the CLP criteria, Section 3.1.2.1.2). It is possible that this is true in this case. The guidance on the application of the CLP criteria states that in general, classification should be based on the lowest ATE value available. Therefore, RAC believes that the more recent data provided by the applicant, supported by the results following a single dose of pyridate in pregnant rats should be used for classification purposes. In order to be classified in category 4 for acute toxicity, the LD_{50} should fall between 300 – 2000 mg/kg bw. As the LD_{50} for these two studies lies between 500 and 1000 mg/kg bw, pyridate should be classified for acute toxicity by the oral route, category 4; H302. An ATE value of 500 would be appropriate, given the toxicity seen at 500 mg/kg.

RAC is in agreement with the DS that for both acute inhalation and dermal toxicity, no classification is required, based on the available data.

Overall, RAC considers pyridate should be classified as **Acute Tox. 4; H302** (Harmful if swallowed).

3.3 Specific target organ toxicity – single exposure (STOT SE)

3.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Table 16: Summary of effects observed in rats, mice, dogs and rabbits in comparison to cut off vales

Species- Route (Reference)	Maximum applications	Cut off value Cat 1 STOT SE (1272/2008) [mg/kg bw]	Cut off value Cat 2 STOT SE (1272/2008) [mg/kg bw]	Effects below cut off value	Significance of toxicological effect (1272/2008) below cut off value
Rat- acute oral (Ullmann, 1984a)	1	300	2000	≥ 1000 mg/kg bw: Dyspnea (reversible after the 1st day) ≥ 3000 mg/kg bw: Sedation, ataxia, ventral body position, latero-abnominal position, curved body position, ruffeled fur (reversible after 2-4 days)	Absence of significant toxicity at 1000 mg/kg bw No dose near 2000 mg/kg bw tested
Rat- acute oral (Ullmann, 1988c)	1	300	2000	≥ 1000 mg/kg bw: Slight Sedation, hunched posture, ruffled fur ≥ 2300 mg/kg bw: Dyspnea, ventral body position Clinical signs (except ruffled fur) were reversible after the 1st day	Absence of significant toxicity < 2000 mg/kg bw
Mouse- acute oral (Ullmann, 1987b)	1	300	2000	< 2000 mg/kg bw: Not tested ≥ 5000 mg/kg bw: Sedation, dyspnea, ventral body position, hunched posture	Absence of significant toxicity < 2000 mg/kg bw
Rat- acute oral (Pels Rijcken, 1996a)	1	300	2000	≥ 1000 mg/kg bw: Hunched posture ≥ 1400 mg/kg bw: Lethargy, uncoordinated movements ≥ 2800 mg/kg bw: Paddling movements, ventro-lateral recumbency, deep or	Clinical signs < 2000 mg/kg bw indicating significant impairment of the neurological function

Species- Route (Reference)	**Maximum applications**	**Cut off value Cat 1 STOT SE (1272/2008) [mg/kg bw]**	**Cut off value Cat 2 STOT SE (1272/2008) [mg/kg bw]**	**Effects below cut off value**	**Significance of toxicological effect (1272/2008) below cut off value**
				laboured respiration, piloerection	
Rat- acute oral (Pels Rijcken, 1996b)	1	300	2000	≥ 1400 mg/kg bw: Lethargy, uncoordinated movements, red staining of the snout, hunched posture, piloerection ≥ 2000 mg/kg bw: Diarrhea, red staining of the head or back	Clinical signs < 2000 mg/kg bw indicating significant impairment of the neurological function
Rat- acute oral (Pels Rijcken, 1996c)	1	300	2000	≥ 2000 mg/kg bw: lethargy, uncoordinated movements, and hunched posture	Clinical signs at 2000 mg/kg bw indicating significant impairment of the neurological function
Rat- acute inhalation (Ullmann, 1983)	1	1 (mg/l)	5 (mg/l)	≥ 2.7 mg/l: Sedation, dyspnea, curved body position and ruffled fur, red discoloration of the lungs, mottled lungs	Absence of significant toxicity < 4.37 mg/l
Rabbit- acute percutaneous (Ullmann, 1984b)	1	1000	2000	≥ 2000 mg/kg bw: Slight to moderate erythema and edema	Absence of significant toxicity < 2000 mg/kg bw
Albino CD rat 90 days oral (gavage) with 28 day recovery period (Henck, 1987)	1	300	2000	≥ 177 mg/kg bw: Hypoactivity ≥ 500 mg/kg bw: Ataxia, ptosis, lateral roll See Table 27 for a detailed listing of incidence and severity of findings	Clinical signs < 2000 mg/kg bw indicating significant impairment of the neurological function
Dog- 90 days oral (gavage) (Tomkins, 1987)	1	300	2000	≥ 60 mg/kg bw: Emesis ≥ 200 mg/kg bw: Ataxia, hypoactivity, opisthotonus, muscle fasciculations, head	Clinical signs < 300 mg/kg bw indicating significant impairment of the neurological function

Species- Route (Reference)	**Maximum applications**	**Cut off value Cat 1 STOT SE (1272/2008) [mg/kg bw]**	**Cut off value Cat 2 STOT SE (1272/2008) [mg/kg bw]**	**Effects below cut off value**	**Significance of toxicological effect (1272/2008) below cut off value**
				swing, nystagmus, mydriasis, salivation See Table 35 for a detailed listing of incidence and severity of findings	
Dog- 90 days oral (gavage) (Vandaele, 1990)	1	300	2000	≥ 80 mg/kg bw: Underactivity (F) ≥ 120 mg/kg bw: Ataxia, emesis, opisthotonus See Table 37 for a detailed listing of incidence and severity of findings	Clinical signs < 300 mg/kg bw indicating significant impairment of the neurological function
Dog- 52 weeks oral (gavage) (Bailey, 1989)	1-7	300	2000	≥ 5 mg/kg bw: emesis (M)	Absence of significant toxicity < 2000 mg/kg bw
Rat- developmental (Becker, 1986)	1	300	2000	Dams: ≥ 400 mg/kg bw: 5/25 death, Clinical signs (i.e. ventral body position, dyspnea, sedation, somnolence, lack of response to external stimuli, tonic and clonic muscle spasms, ruffled fur) ≥ 465 mg/kg bw: 13/25 death	Lethal effect, not covered by acute toxicity classification

Position papers and expert statements were submitted regarding neurotoxic effects and their impact on classification and labelling for renewal of approval of pyridate (please refer to Annex).

In the Pesticide Peer Review Meeting 109 (January 2014) the relevance of clinical signs for assessing neurotoxicity potential of pyridate was discussed.

After single or repeated exposure the dog seems to be the most sensitive species. In rats, neurotoxic effects were also observed but at higher dose levels than in dogs.

Two 90-day studies were available in dogs (i.e. Tomkins, 1987 and Vandaele, 1990). After single exposure (during the 90-day dog studies), effects were observed below 300 mg/kg bw per day. These effects included clinical signs (e.g. ataxia and opisthotonus in both studies; nystagmus in the

first study) at 200 mg/kg bw per day (first dog study) and 120 mg/kg bw per day (second dog study). In the first dog study, emesis was only observed in 2 animals at 60 mg/kg bw per day, but was not considered as neurotoxic effect in dogs, and the NOAEL for single exposure is 60 mg/kg bw. In the second dog study, lower activity was observed in one animal at 80 mg/kg bw per day and is considered as a first indication of neurotoxicity (after single dose administration), with a NOAEL for single exposure of 40 mg/kg bw.

The experts in the Pesticide Peer Review Meeting 109 agreed on a combined NOAEL of 60 mg/kg bw per day for acute effects in the 90-day dog studies.

The proposal STOT SE category 1 was supported by the experts of the Pesticide Peer Review Meeting 109.

3.3.2 Comparison with criteria

Signs of impairment of the neurological function were consistently observed in oral gavage studies in rats (acute studies) and in repeated dose studies in rats and dogs with onset of signs 1-3 hours after dosing. The signs were specific (ataxia, sedation, dyspnea, uncoordinated movements, tremor) and for the dog studies within the guidance value range for STOT SE Category 1 classification of ≤ 300 mg/kg. Therefore classification as STOT SE Category 1 is proposed.

Clinical signs indicating significant impairment of the neurological function were more pronounced after a single application. According to the Guidance on the Application of the CLP Criteria (ECHA, Jun 2015) "Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate." No accumulation or exacerbation of the toxicity is seen after repeated application. Therefore Austria, as the dossier submitter, considers that effects after repeated application are already covered by STOT-SE.

3.3.3 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: STOT-SE Category 1, H370

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

Summary of the Dossier Submitter's proposal

Proposal before public consultation

Signs of impairment of the neurological function following a single dose were consistently observed in oral gavage studies in rats (acute studies) and in repeated dose studies in rats and dogs with onset of signs 1-3 hours after dosing. The signs were specific (ataxia, sedation, dyspnoea, uncoordinated movements, tremor) and for the dog studies within the guidance value range for STOT SE Category 1 classification of ≤ 300 mg/kg bw. Therefore classification with STOT SE Category 1 without specifying target organs is proposed.

Proposal after public consultation

The new study on acute neurotoxicity indicates that the effects caused by pyridate administration are signs of unspecific, systemic toxicity rather than specific neurotoxicity, occurring at doses leading to mortality. Therefore, the DS concluded that classification for acute oral toxicity (Category 4; H302) seemd more appropriate than classification for STOT SE.

Comments received during public consultation

Three MSCAs specifically agreed with the STOT SE classification, however this was before the additional study data became available to classify for acute oral toxicity.

Additional key elements

Please see Diehl (2016) study provided in the acute toxicity section.

Assessment and comparison with the classification criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance. STOT SE should be considered where there is clear evidence of toxicity to a specific organ in the absence of lethality.

In a recent study provided by the applicant during the public consultation (Diehl, 2016), groups of animals were given a single dose of either 0, 500, 1000 mg/kg bw pyridate or 0, 62.5, 177, 500 mg/kg bw pyridate. Mortality was observed from a dose of 500 mg/kg bw. Clinical signs were observed prior to death and included decreased activity, incoordination, weakness, abnormal breathing, lateral positioning, non-sustained convulsions, tremors and locomotor stereotypy. These signs were only observed in groups dosed with 500 mg/kg bw and above. Where motor activity signs occurred in animals that did not die, full recovery was noted in most cases by day 2 of cessation of dosing.

In three of the acute toxicity studies, carried out by the oral route in rats (Pels Rijcken, 1996 a, b and c), clinical signs such as hunching, lateral positioning and uncoordinated movements, indicating potential impairment of neurological function, were observed at doses of 1000 – 2000 mg/kg bw (see table below).

Study	Clinical signs	Mortality
Acute oral study rats (1% CMC) (Pels Rijcken, 1996a)	≥ 1000 mg/kg bw – hunched posture	0/5
	≥ 1400 mg/kg bw – lethargy, uncoordinated movements	3/5
	≥ 2800 mg/kg bw – paddling movements, ventro-lateral recumbency, deep or laboured respiration, piloerection	4/5
Acute oral study rats (corn oil) (Pels Rijcken, 1996b)	≥ 1400 mg/kg bw – lethargy, uncoordinated movements, hunched posture	0/5

	≥ 2000 mg/kg bw – diarrhoea, red staining of the head and back	2/5
Acute oral study rats (PEG200) (Pels Rijcken, 1996c)	≥ 2000 mg/kg bw – lethargy, uncoordinated movements, hunched posture	0/5

In these studies, significant mortality was noted from a dose of 1400 mg/kg bw and, therefore, the effects observed cannot be considered as specific toxicity occurring in the absence of lethality.

Furthermore, in a developmental study in rats, significant mortality was observed after a single dose of 495 mg/kg bw/d (13/25 pregnant rats died). Rats treated with this dose suffered clinical signs indicative of neurological toxicity. These symptoms were said to be more pronounced on the first day of dosing and decreased in severity as the study progressed. Whilst data from pregnant rats cannot be used for acute toxicity classification, it does add to the weight of evidence that the neurological effects observed with pyridate occurred at doses leading to mortality.

Conclusion

RAC considers that the clinical signs observed in the acute toxicity studies and the repeated dose studies in the dossier occurred at doses that clearly exceeded the MTD and are considered signs of impending death. The neurotoxic effects observed cannot be considered as clear evidence of toxicity to a specific organ as in general, lethality always occurred or the clinical signs occurred at a dose within the numeric classification criteria for an acute toxicity classification (Category 4: 300 – 2000 mg/kg bw). According to the Guidance on the Application of the CLP Criteria (Version 5.0 – June 2017), older acute toxicity studies which tended to only measure lethality as an observational endpoint will generally not provide useful information for STOT SE. As well as newer toxicity test protocols, valuable information can be provided by other standard studies such as neurotoxicity tests.

The recent and well-performed neurotoxicity screening study submitted by the applicant provides clear data indicating an oral LD_{50} between 500 – 1000 mg/kg bw in rats. Clinical signs occurred only at doses causing mortality and were regarded as non-specific and reversible clinical signs of stress following exposure to lethal doses.

Therefore, RAC agrees that the observed toxicity would best be covered by classification for acute toxicity by the oral route rather than an additional classification with STOT SE 1 or 2.

As there was no evidence of respiratory tract irritation or of a narcotic effect following administration of pyridate, classification with STOT SE 3 is also considered to be not necessary.

3.4 Irritation

3.4.1 Skin irritation

No change is proposed regarding classification and labelling compared with the current entry in Annex VI of Regulation (EC) No. 1272/2008.

3.4.2 Eye irritation

No change is proposed regarding classification and labelling compared with the current entry in Annex VI of Regulation (EC) No. 1272/2008.

3.4.3 Respiratory tract irritation

No change is proposed regarding classification and labelling compared with the current entry in Annex VI of Regulation (EC) No. 1272/2008.

3.5 Corrosivity

No change is proposed regarding classification and labelling compared with the current entry in Annex VI of Regulation (EC) No. 1272/2008.

3.6 Sensitisation

3.6.1 Skin sensititsation

Table 17: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Magnusson and Kligman test	Sensitising	No control group included	Kynoch R.; 1976
Buehler test (guinea pig)	Sensitising		Ullmann L., Kups A.; 1988

3.6.1.1 Non-human information

The following studies were evaluated in support of Annex I listing of pyridate and are no longer granted data protection:

Magnusson and Kligman test:

IIA 5.2.6/01 Kynoch R. (1976) Screening test for delayed contact hypersensitivity with CL 11.344 (Standard) in the Albino Guinea-pig. Huntingdon Project No.: 6498/D13/76

Despite the age and lack of GLP (not formally existing at the time of study) this study is still considered to be valid.

The test was carried out on 10 male Duncan Hartley guinea pigs. 0.1 ml (1% v/v) of the test substance with Freund's complete adjuvant was injected intradermally. No control group was included in the study. The highest non- irritating concentration was 1% for intradermal induction

and undiluted pyridate for topical application. Topical application was done one week after intradermal injection with 0.4 ml pyridate covered for 48 hours. After two weeks the animals were challenged with 0.1 ml pyridate (50% v/v). All the animals showed positive results (grade 1-4 oedema and erythema) up to 72 hours after removal of the patch.

Modified Bühler test (OECD 406 1981):

IIA 5.2.6/02 Ullmann L., Kups A. (1988) Determination of skin irritation and capacity of allergenic sensitization by the open epicutaneous test on guinea pigs (OET) with pyridate tech. RCC project 098144

Different dose levels of pyridate were used (0.5%, 1%, 3% and 10% in ethanol) in the induction period (6 animals per dose group).

3% pyridate was the highest non-irritating concentration. Reactions of animals after the first and second challenge can be found in the following table.

Table 18: Summary of the Buehler test from the DAR (Ullmann, Kups, 1988)

Group	**Induction concentration (%)**	**Challenge concentration (%) Positive/total animals**			
		0.5%	**1%**	**3%**	**10%**
1st challenge					
1 (control)	-	0/6	0/6	0/6	1/6
2	0.5	0/6	0/6	0/6	2/6
3	1	0/6	0/6	0/6	2/6
4	3	0/6	0/6	0/6	1/6
5	10	0/6	0/6	1/6	6/6
2nd challenge					
1 (control)	-	0/6	0/6	0/6	1/6
2	0.5	0/6	0/6	0/6	0/6
3	1	0/6	0/6	0/6	3/6
4	3	0/6	0/6	0/6	2/6
5	10	0/6	0/6	1/6	5/6

3.6.1.2 Human information

Two pertinent reports have been submitted in the original data package.

IIA 5.9.1/01 Eberhartinger, C. (1977) Sensitivity test with pyridate on humans, Linz Municipal Hospital

IIA 5.9.1/02 Hiebler M.R. (1980) Statement of the medical group in Chemie Linz, Chemie Linz AG

In these reports there were neither indication for skin sensitisation nor were there indications for any other adverse effects as a result of potential exposure to pyridate.

A new and up to date report from the actual production site was provided by the notifier:

Reference:	**Pyridate: Medical surveillance on plant personnel -**
Author(s), year:	Fuqiang S., 2012
Report No.	Changzhou Watson Fine Chemical Co., Ltd, PR China
Guidelines:	Not applicable
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Yes

Pyridate has been produced in the Changzhou Watson Fine Chemical facility since 2006. The yearly production amounted to approx 100 tons and involved a total of 100 workers. They were involved in production up to 56 hours per week and 6 months per year. The workers were checked medically at least yearly. Checks included ECG, Type B ultrasonic, urine sediment, X-ray, blood pressure, haematology and blood biochemistry.

Over the entire observation period no abnormal behaviour and no health complaints of the workers had been noticed and no changes of the worker health status were recorded which could be linked to the production of pyridate. Especially no indications of sensitisation or other allergenic effects have been noticed since the start of the pyridate production.

3.6.1.3 Summary and discussion of skin sensitisation

Pyridate was sensitising to the skin in a Magnusson and Kligman Test and in a modified Buehler test.

3.6.1.4 Comparison with criteria

Pyridate was sensitising to the skin in the Magnusson and Kligman Test for 100% of the animals responding to 1% intradermal induction dose and to 33.3% of the animals responding to 3% topical induction dose (highest non-irritating dose) in the Buehler Test. No control group was included in the Magnusson and Kligman Test.

According to "ECHAs Guidance on the Application of the CLP Criteria", Version 4.1 – June 2015, section 3.4.2.2.3.2, sub-categorization into Category 1A and 1B can be considered.

In the available database, there are no indications for sensitisation in humans.

According to OECD TG 406 (1992) for Magnusson and Kligman tests, the induction concentration should be mildly to moderately irritant, challenge exposures should be done with the highest non-irritating dose (1%, Kynoch R. (1976)). At 1% induction concentration, 100% of animals showed a positive response, which is consistent with –sub-category 1A (≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose). No control group was included in this study.

According to OECD TG 406 (1992) for Buehler tests, the induction concentration should be mildly irritating, challenge exposures should be done with the highest non-irritating dose (3%; Ullmann L., Kups A. (1988)). At 3% challenge concentration, 16% of animals showed a positive response, which is consistent with –sub-category 1B (≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % induction concentration).

Peer reviewed proposal of EFSA is classification as Skin sensitiser 1B, H317.

3.6.1.5 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: Skin sensitiser 1B, H317

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Pyridate was sensitising to the skin in the Magnusson and Kligman Test (Kynoch, 1976) with 100% of the animals responding to a 1% intradermal induction dose. In a Buehler test (Ullmann and Kups, 1988), 33.3% of the animals responded to a 3% topical induction concentration (highest non-irritating concentration).

In the available database, there are no indications for sensitisation in humans.

According to OECD TG 406 (1992) for Magnusson and Kligman tests, the induction concentration should be mildly to moderately irritant, challenge exposures should be done with the highest non-irritating dose. At 1% induction concentration, 100% of animals showed a positive response, which is consistent with sub-category 1A (≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose). No control group was included in this study.

According to OECD TG 406 (1992) for Buehler tests, the induction concentration should be mildly irritating and challenge exposures should be done with the highest non-irritating dose. At 3% challenge concentration, 33% of animals showed a positive response, which is consistent with sub-category 1B (≥ 15% to < 60% responding at > 0.2% to ≤ 20% induction concentration).

The DS concluded that the most appropriate classification is Skin Sensitisation Category 1B: H317 and noted that the peer reviewed proposal of EFSA is classification as Skin Sensitiser 1B: H317.

Comments received during public consultation

Skin sensitisation was not open for comments in the public consultation. However, 5 MSCAs submitted comments relating to this endpoint.

One MSCA made a general comment agreeing with the classification proposal. A second MSCA specifically supported the proposal to classify pyridate in Category 1B for skin sensitisation. A third MSCA commented on the lack of detail in the reporting of the animal studies in the CLH report. The fourth MSCA considered that the current classification as Skin Sensitiser 1: H317, should be maintained because Category 1A cannot be excluded taking into account the results of the maximisation study. The final MSCA commented that in line with example 8 of the CLP guidance (chapter 3.4.6.1.8), these data would lead to Cat. 1A.

Assessment and comparison with the classification criteria

Pyridate is currently classified in Category 1 for skin sensitisation in Annex VI of the CLP Regulation.

Magnusson and Kligman test (1976, non-GLP)

Ten male guinea pigs were exposed to the test substance (0.1 ml, 1% v/v) with Freund's complete adjuvant by intraperitoneal injection. No control group was included in this study.

The highest non-irritating concentration was 1%. One week after intradermal injection, 0.4 ml pyridate was applied topically and covered for 48 hours. Two weeks later, the animals were challenged with 0.1 ml pyridate (50% v/v). Grade 1-4 oedema and erythema was observed in all animals up to 72 hours after removal of the patch.

According to Table 3.4.3 in Annex I of the CLP Regulation, Category 1A is warranted when ≥ 60% of animals respond at > 0.1% to ≤ 1% intradermal induction dose in a Guinea Pig Maximisation Test (GPMT). In the available study, 100% of animals showed a positive response at 1% induction concentration, indicating that Category 1A may be appropriate. However, no control group was included in this study. Furthermore, RAC concurs with a comment submitted during the public consultation, describing the possibility that the vehicle used for the challenge resulted in irritancy reactions in the animals which were mistaken for sensitisation. However, the vehicle used in the test is unknown. For these reasons, it is not possible to draw a firm conclusion on the results of this study.

Modified Buehler test (OECD TG 406, 1981)

In the induction period, guinea pigs (6/dose group) were exposed to 0.5%, 1%, 3% or 10% pyridate in ethanol. The highest non-irritating concentration was 3%. The results are summarised in the table below.

Group	**Induction**	**Challenge concentration (5) positive/total animals**			
	Concentration (%)	0.5%	1%	3%	10%
1st challenge					
1 (control)	-	0/6	0/6	0/6	1/6
2	0.5	0/6	0/6	0/6	2/6
3	1	0/6	0/6	0/6	2/6
4	3	0/6	0/6	0/6	1/6
5	10	0/6	0/6	1/6	6/6
2nd challenge					
1 (control)	-	0/6	0/6	0/6	1/6
2	0.5	0/6	0/6	0/6	0/6
3	1	0/6	0/6	0/6	3/6
4	3	0/6	0/6	0/6	2/6
5	10	0/6	0/6	1/6	5/6

According to Table 3.4.4 of Annex I of the CLP Regulation, category 1B is warranted when ≥ 15 % to < 60% of animals respond at > 0.2 % to ≤ 20 % topical induction dose in a

Buehler assay. In the available Buehler assay, 33% of animals responded at 3% topical induction dose (the highest non-irritating concentration) and therefore the substance meets the criteria for classification in Category 1B.

Human information

Three reports were covered briefly in the CLH report. It was described that there were no indications of skin sensitisation as a result of potential exposure to pyridate in the 100 workers employed in a facility manufacturing approximately 100 tonnes of pyridate per year in China. However, as stated in Annex I (section 3.4.2.2.4.2) of the CLP regulation, evidence from animal studies is usually much more reliable than evidence from human exposure. These studies are not considered to invalidate the positive results of the animal studies.

Conclusion

Pyridate is a skin sensitiser. According to Annex I of the CLP regulation (section 3.4.2.2.1), "*Skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation. Where data are sufficient a refined evaluation according to section 3.4.2.2.1.3 allows the allocation of skin sensitisers into sub-category 1A, strong sensitisers, or sub-category 1B for other skin sensitisers.*"

The results of the Magnusson and Kligman test and the Buehler tests indicate that the most appropriate category would be 1A or 1B, respectively. However, it is noted that no control group was included in the Magnusson and Kligman test and therefore it is not considered appropriate to base a classification on the results of this study. Classification in category 1B would be a possibility based on the results of the Buehler test.

However, according to the Guidance on the Application of the CLP criteria, "*Classification into sub-categories is only allowed if data are sufficient (CLP Annex I 3.4.2.2.1.1). Therefore care should be taken when classifying substances into Category 1B when Category 1A cannot be excluded.*" Although the results of the Magnusson Kligman test are not sufficient for classification (due to lack of controls), they indicate that classification in Category 1A for skin sensitisation could be a possibility. Since Category 1A cannot be excluded, RAC considers that the most appropriate approach would be to maintain the existing classification – **Skin Sensitisation; Category 1: H317**.

3.6.2 Respiratory sensitisation

No data available.

3.7 Repeated dose toxicity

Table 19: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Wistar rat 4 weeks oral (dietary)	0, 1000, 3000 and 10000 ppm/diet (equivalent to 0, 118, 354, and 1180 mg/kg bw/d for males; 0, 117, 351 and 1170 mg/kg bw/d for females) NOAEL 3000 ppm LOAEL = 10000 ppm based on: ↓ bw, ↓ HB, changes in organ weight and haematology	-	Til, 1979
Mouse (Swiss Random strain) 4 weeks oral (dietary)	0, 1000, 3000 and 10000 ppm/diet (equivalent to 0, 188, 564 and 1880 mg/kg bw/d for males; 0, 224, 672 and 2240 mg/kg bw/d for females) NOAEL: 1000 ppm LOAEL = 3000 ppm based on: ↑ relative spleen weight	-	Til, 1980a
Wistar and Sprague Dawley rat 4 weeks oral (dietary)	0, 0.3 and 1% (equivalent to 0, 354 and 1180 mg/kg bw/d for males; 0, 351 and 1170 mg/kg bw/d for females) NOAEL could not be determined LOAEL = 3000 ppm based on: ↓ bw (Wistar and Sprague Dawley rat), ↓ HB (Sprague Dawley rat)	Supporting information only	Til, 1980b
Albino CD rat 90 days oral (gavage) with 28 day recovery period	0, 62.5, 177 and 500 mg/kg bw/d (gavage in corn oil) 10 males and females were used in an ascending dose group (500 mg/kg bw/d, increased to 600 mg/kg bw/d after 2 weeks) NOAEL = 62.5 mg/kg bw/d LOAEL = 177 mg/kg bw/d based on: ↑ relative liver weight, death (one female), clinical signs, minor histopathological effects (basophilic material in germinal follicles of mesenteric lymph nodes)	-	Henck, 1987
CD rat 90 day oral (dietary)	0, 400, 1200 and an ascending dose of 3600 (week 1-6) / 4600 (week 7-8)/ 8000 (week 9-13) ppm (equivalent to 0, 28.11, 86.01 and 397.9 mg/kg bw/d for males; 0, 31.6, 96.01 and 448.8 mg/kg bw/d for females) NOAEL: 1200 ppm LOAEL = 6000 ppm based on: ↓ bw gain, ↓ urinary pH, ↓ HB (females)	-	Danks, 1991

Beagle dog 90 days oral (gavage)	0, 20, 60 and 200 mg/kg bw/d NOAEL: 20 mg/kg bw/d LOAEL = 60 mg/kg bw/d based on: Clinical signs	-	Tomkins, 1987 + Re-evaluation histopathological slides Armstrong, Chevalier, 1997
Beagle dog 90 days oral (gavage)	0, 40, 80 and 120 mg/kg bw/d NOAEL: 40 mg/kg bw/d LOAEL = 80 mg/kg bw/d based on: Clinical signs, ↓ erythrocyte parameters, ↑ Heinz bodies, ↑ relative liver and kidney weight, histopathological changes in the liver (pigmentation in Kupffer cells)	-	Vandaele, 1990 + Re-evaluation histopathological slides Armstrong, Chevalier, 1997
Beagle dog 52 weeks oral (gavage)	ascending doses of 0, 5/10/30 (low), 20/60/80/100 (mid) and 60/100/120/140/150 mg/kg bw/d (high) NOAEL = 30 mg/kg bw/d LOAEL = 60 mg/kg bw/d based on: ↓ bw, clinical signs	-	Bailey, 1989 + Re-evaluation histopathological slides Armstrong, Chevalier, 1997
Sprague Dawley rat 21 day dermal	0 and 1000 mg/kg bw/d NOAEL = 1000 mg/kg bw/d	-	Perry, Duffen, 1988

3.7.1 Non-human information

3.7.1.1 Repeated dose toxicity: oral

28-day toxicity (rat)

The following studies were evaluated in support of Annex I listing of Pyridate and are no longer granted data protection:

IIA 5.3.1/01 Til H.P. et al. (1979) Subacute (4-week) oral toxicity study with pyridate in rats. TNO Report No.: R 6187

Pyridate (batch No. CL 11344) was applied at 0, 1000, 3000 and 10000 ppm to groups of 10 male and female Wistar rats via diet in either standard basal diet or a semi-purified diet for 28 days. According to the "Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data, EFSA Journal 2012;10(3):2579" ppm in feed can be converted to mg/kg bw/d by using a factor of 0.118 for male rats and 0.117 for female rats, leading to a dose of 0, 118, 354 and 1180 mg/kg bw/d in males and 0, 117, 351 and 1170 in females. Haematology and histopathology was performed only on control and top dose animals. In the following table the results obtained with standard basal diet are summarised.

Table 20: Summary of the oral 28 toxicity study in rat from the DAR (Til et al., 1979)

Dose (ppm)	**0**		**1000**		**3000**		**10000**	
	male	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Body weight in g (day 0)	51.9	51.2	52.1	50	52.5	50.1	52.5	51
Body weight in g (day 7)	89.4	80.2	86.6	75.8	82.4*	72.2*	56*	54.7*
Body weight in g (day 14)	125.7	108	125	102.4	116.7*	96.8*	71.4*	67.9*

Dose (ppm)	0		1000		3000		10000	
	male	female	male	female	male	female	male	female
Body weight in g (day 28)	201.4	146.6	200.6	141.8	189.2*	135.2*	108.5*	99*
Mean food intake in g (day 1)	6.5	6	6.5	4.3	4.7	3.5	2.7	2.3
Mean food intake in g (day 14)	15.5	13.6	15.8	13	14.6	12.1	7.9	7.5
Mean food intake in g (day 28)	15.8	12	16.1	12.4	15	11.3	8.3	8
RBC	6.3	6.4	-	-	-	-	6.4	6.4
WBC	18.6	16.2	-	-	-	-	18.5	18.2
HB	8.6	9.2	-	-	-	-	8.3*	8.4*
PCV	0.475	0.491	-	-	-	-	0.443*	0.475
Relative kidney weight	0.725	0.8	0.735	0.827	0.722	0.81	0.806*	0.832
Relative thymus weight	0.261	0.286	0.265	0.26	0.256	0.298	0.278	0.334*
Relative lung weight	0.594	0.649	0.574	0.661	0.598	0.676	0.641*	0.717*
Slight peribronchial and peribronchiolar lymphoid aggregates	0/10	-	-	0/10	3/10	-	-	5/10
Slight focal pneumonia	0/10	-	-	0/10	0/10	-	-	1/10

* statistically significant

No death or clinical signs were observed during the study. In the DAR the NOEL was set at 1000 ppm (118 mg/kg bw/d in males and 117 mg/kg bw/d in females) and the NOAEL at 3000 ppm (354 mg/kg bw/d in males and 351 mg/kg bw/d in females), based on decreased body weight as well as changes in haematology and organ weight which can be confirmed.

IIA 5.3.1/02 Til H.P. et al. (1980a) Subacute (4-week) oral toxicity study with pyridate in mice. TNO report No.: R 6396

Pyridate (batch No. CL 11344) was applied at 0, 1000, 3000 and 10000 ppm to groups of 10 male and female mice from the Swiss Random strain via diet for 28 days. According to the "Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data, EFSA Journal 2012;10(3):2579" ppm in feed can be converted to mg/kg bw/d by using a factor of 0.188 for male mice and 0.224 for female mice, leading to a dose of 0, 188, 564 and 1880 mg/kg bw/d in males and 0, 224, 672 and 2240 in females. Histopathology was performed only on control and top dose animals. In the table below the results are summarised.

Table 21: Summary of the oral 28 toxicity study in mice from the DAR (Til et al., 1980a)

Dose (ppm)	0		1000		3000		10000	
	male	female	male	female	male	female	male	female
Body weight in g (day 0)	15.7	16	16.2	15.8	15.7	15.7	16.8	15.5

Dose (ppm)	0		1000		3000		10000	
	male	female	male	female	male	female	male	female
Body weight in g (day 14)	27.1	20.5	26.4	22.1*	25.8	21.5	25.4	20.5
Body weight in g (day 28)	30.5	23.8	29.9	23.9	29	23.5	28.1	22.5
Mean food intake in g (day 7)	34.7	29.9	35.2	32.4	35.4	32.1	43.6	36.2
RBC	10.4	10.4	10.5	10.3	10.4	10.2	10.9*	10.5
Relative kidney weight	1.61	1.206	1.602	1.271	1.547	1.164	1.648	1.29
Relative spleen weight	0.43	0.521	0.38	0.605	0.404	0.688	0.5	0.699*
Relative liver weight	5.19	4.33	5.24	4.57	5.46	4.46	6.37*	5.72*

* statistically significant

No death or clinical signs were observed during the study. In the DAR the NOEL was set at 1000 ppm (188 mg/kg bw/d in males and 224 mg/kg bw/d in females), based on increased spleen weights in females at 3000 ppm (672 mg/kg bw/d in females), which can be confirmed.

There is one further oral 28 day toxicity study in rat reported here which was not summarized in the DAR and not listed in the updating statement for renewal of approval.

Reference:	**Sub-acute (4-week) oral toxicity study with pyridate in Wistar and Sprague-Dawley rats**
Author(s), year:	Til H.P., 1980b
Guideline(s):	No guideline quoted, the study was conducted as a comparative study to investigate possible strain differences
GLP:	Yes
Deviations:	Not applicable
Validity:	Supporting information

Material and methods:

Test Material: Pyridate tech (CL 11344
Lot/Batch: not specified
Purity: not specified

Test animals:
Species: Rat
Strain: Cpb:Wu Wistar Random (SPF)
Sprague Dawley (SPF)
Age: "weanlings"
Weight at dosing: Wistar: males ca. 60 g, females ca. 53 g
Sprague Dawley: males ca. 78 g, females ca. 70 – 80 g
Source: Wistar: Institute for the Breeding of Laboratory Animals, TNO Zeist (NL)

Sprague-Dawley: Broeckman Institute, Helmond, NL

Diet: The institute's powdered stock diet *ad libitum*

Dose levels: 0, 0.3 and 1% According to the "Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data, EFSA Journal 2012;10(3):2579" ppm in feed can be converted to mg/kg bw/d by using a factor of 0.118 for male rats and 0.117 for female rats, leading to a dose of 0, 354 and 1180 mg/kg bw/d in males and 0, 351 and 1170 in females.

Group size: 10 males and females per group

The 6 groups of rats were dosed with their daily diet for 28 days. The medicated diets were prepared fresh daily (for two days on Saturdays) with the use of a mechanical blender. The high dose diet was prepared first, the low dose by diluting parts of it with stock diet. No data on contents, homogeneity or stability are reported.

All animals were observed daily for mortality and symptoms. Feed consumption was noted daily, body weights weekly. Feed utilization was calculated and expressed as grams weight gain per gram feed consumed.

A limited haematological examination (hemoglobin concentration) was performed after 24 days of dosing.

At the end of the study the animals were killed by decapitation and grossly examined for pathological changes. Absolute and relative to body organ weight was determined for kidney, thymus, spleen, liver and lung.

Findings:

No deaths or abnormalities of behaviour were observed in any of the groups. In both top-dose groups the rats emaciated especially during the first 2 weeks of the administration of pyridate. There were no noticeable differences in condition between the two strains of rats used.

Table 22: Mean body weights in grams

Strain	**Pyridate dose in %**	**Days on study**				
		0	**7**	**14**	**21**	**28**
Males						
Wistar	0	61.5	98.6	141.8	184.9	226.2
Wistar	0.3	60.4	87.4*	124.5*	160.8*	197.1*
Wistar	1	59.8	63.0*	72.4*	86.2*	102.7*
Sprague Dawley	0	77.8	131.2	178.7	222.3	267.3
Sprague Dawley	0.3	79.6	125.0	170.6	211.1	250.7*
Sprague Dawley	1	76.6	72.9*	88.4*	107.3*	125.4*
Females						
Wistar	0	52.7	82.1	113.7	138.4	158.3
Wistar	0.3	56.2	80.0	108.9	130.0	149.3
Wistar	1	52.7	53.0*	64.4*	76.6*	89.3*
Sprague Dawley	0	81.7	121.2	146.5	166.2	185.6
Sprague Dawley	0.3	69.1	103.7*	131.6*	150.5*	165.3*
Sprague Dawley	1	80.2	72.7*	82.6*	94.1*	106.0*

* statistically significant

Table 23: Mean weekly feed intake in grams

Strain	Pyridate dose in %	Days on study			
		7	14	21	28
Males					
Wistar	0	83.1	118.2	134.0	136.8
Wistar	0.3	64.6	92.4	118.7	120.5
Wistar	1	31.0	45.6	54.3	67.5
Sprague Dawley	0	110.9	137.8	144.6	152.2
Sprague Dawley	0.3	100.1	131.6	137.3	143.3
Sprague Dawley	1	31.3	50.3	68.5	74.7
Females					
Wistar	0	66.8	90.8	102.2	98.8
Wistar	0.3	60.0	79.8	97.8	91.4
Wistar	1	24.4	38.6	51.4	54.9
Sprague Dawley	0	96.0	103.8	111.8	109.5
Sprague Dawley	0.3	79.9	98.4	99.6	90.9
Sprague Dawley	1	31.5	45.2	55.9	56.6

Mean body weight and feed intake showed a dose-related decrease in males and females of both strains.

Table 24: Mean haemoglobin values of rats after treatment with pyridate

Strain	Pyridate dose in %	Haemoglobin concentration	
		males	females
Wistar	0	8.2	8.5
Wistar	0.3	8.2	8.6
Wistar	1	8.1	8.2*
Sprague Dawley	0	8.5	8.9
Sprague Dawley	0.3	8.5	8.5*
Sprague Dawley	1	8.3	8.0*

* statistically significant

The haemoglobin content of the blood of female rats of the Sprague-Dawley strain fed 0.3% pyridate diet and of female rats of both strains fed 1.0% pyridate was slightly decreased. Therefore Sprague-Dawley rats seem to be slightly more susceptible to pyridate than Wistar rats in this respect.

Table 25: Mean relative organ weights in grams/100g bw

Strain	Pyridate dose in %	Body weight	Organ				
			Kidney	Thymus	Spleen	Liver	Lung
Males							
Wistar	0	226.2	0.848	0.314	0.244	4.95	0.537
Wistar	0.3	197.1	0.868	0.272*	0.273	5.02	0.522
Wistar	1	102.7	0.925**	0.275	0.276	4.66*	0.588*
Sprague Dawley	0	267.3	0.807	0.267	0.298	4.54	0.579
Sprague Dawley	0.3	250.7	0.822	0.250*	0.298	4.82*	0.587
Sprague Dawley	1	125.5	0.857	0.257	0.397*	4.82	0.646
Females							
Wistar	0	158.3	0.854	0.300	0.240	4.39	0.607
Wistar	0.3	149.3	0.917	0.314	0.238	4.76	0.654
Wistar	1	89.3	0.946	0.328	0.299*	4.50	0.729*
Sprague Dawley	0	185.6	0.800	0.223	0.308	3.88	0.685
Sprague Dawley	0.3	165.3	0.885*	0.256*	0.325	4.45*	0.732
Sprague Dawley	1	106.0	0.818	0.286*	0.371*	4.87*	0.734

* statistically significant

Relative kidney, spleen and lung weight increased with dose. Relative thymus weight in females increased with dose level of pyridate. No appreciable differences were seen between the two strains.

Conclusion:

From the results obtained it is concluded that there are no distinct differences in susceptibility towards pyridate between Wistar and Sprague Dawley rats. No NOAEL could be established. The LOAEL at a dose level of 0.3% ppm (354 mg/kg bw/d in males and 351 mg/kg bw/d in females) pyridate is set due to a decrease in body weight in male Wistar and female Sprague Dawley rat >10%. Furthermore haemoglobin content was significantly decreased in female Sprague Dawley rat at a dose level of 0.3% pyridate.

90-day toxicity (rat)

The following studies were evaluated in support of Annex I listing of pyridate and are no longer granted data protection:

IIA 5.3.2/01 Henck J.W. et al. (1987) 90 day rat oral subchronic toxicity study with a 28 day recovery period of pyridate technical. TRL Study No.: 043-005

Pyridate (batch No. EOA-Knr: 2429966 CEO-Knr: 2556520, purity: 92%) was applied to groups of 35 male and female Albino CD rats via gavage in corn oil at 0, 62.5, 177 and 500 mg/kg bw/d for 90 days. 10 rats per group were used for interim sacrifice after 4 weeks, 10 rats per group for terminal sacrifice and 15 rats were used to assess recovery for 4 weeks after 90 days treatment. In addition, 10 males and females were used in an ascending dose group (500 mg/kg bw/d, increased to 600 mg/kg bw/d after 2 weeks). No clinical chemistry, haematology analysis and detailed histopathology were done for the ascending dose group. However all gross lesions were observed histopathologically. Total and free T3 and T4 as well as plasma and RBC cholinesterase was measured in 5 males, 5 females in the control group; 5 males, 4 females at 62.5 mg/kg bw/d; 4 males, 5 females at 177 mg/kg bw/d; 2 males, 0 females at 500 mg/kg bw/d and all surviving animals in the ascending dose group (9 males and 6 females). Brain Cholinesterase was measured at the final necropsy in 10 males, 5 females in the control group; 10 males, 9 females at 500 mg/kg bw/d and all surviving animals in the ascending dose group (9 males and 6 females). Gross post mortem examinations were performed on all rats, while full histopathology was done only in control and high dose rats at the final necropsy and in those found dead. Furthermore livers, lungs and kidneys from all rats from the 62.5 and 177 mg/kg bw/d dose groups at final necropsy as well as spleen, mesenteric lymph nodes and mammary glands (females) from rats of the 62.5 and 500 mg/kg bw/d dose groups at the end of recovery period were assessed for histopathology. The results are summarised in the following table.

Table 26: Mortality, clinical signs and body weight in the oral 90 day toxicity study in rat with a 28 day recovery period from the DAR (Henck et al., 1987)

Dose (mg/kg)	0		62.5		177		500		500/600	
	male	female	male	female	male	female	male	female	male	female
Mortality	0/45	0/45	0/45	1/451	1/451	1/45	4/45	10/45[1]	1/10	4/10

Dose (mg/kg)	0		62.5		177		500		500/600	
	male	female	male	female	male	female	male	female	male	female
Clinical observations	-	-	-	-	hypo-activity/ salivation	hypo-activity/ salivation	hypo-activity/ salivation/ ataxia	hypo-activity/ salivation/ ataxia	hypo-activity/ salivation/ ataxia	hypo-activity/ salivation/ ataxia
Body weight in g (week 13)	537	281	529	279	514	268	388*	240*	355*	235*
Body weight in g (week 17 after recovery)	535	290	557	286	545	286	482	266	-	-

[1]: one rat died as a result of cage or gavage accident
* statistically significant

Dose dependent mortality and clinical signs occurred from 177 mg/kg bw/d onwards. Body weight was significantly decreased > 10% at 500 mg/kg bw/d, but recovered to < 10% decrease in the recovery period.

Clinical signs were reversible in the recovery period.

Table 27: Clinical signs observed ≤ 2000 mg/kg bw after the first dose (Henck et al., 1987)

Clinical sign	177 mg/kg bw		500 mg/kg bw		500/600 mg/kg bw	
	Males	Females	Males	Females	Males	Females
Hypoactivity- 1	3/45	1/45	36/45	29/45	5/10	5/10
Hypoactivity- 2	0/45	0/45	7/45	7/45	0/10	0/10
Ataxic-1	0/45	0/45	2/45	3/45	0/10	0/10
Ataxic-2	0/45	0/45	1/45	1/45	0/10	0/10
Ptosis	0/45	0/45	1/45	0/45	0/10	0/10
Lateral roll	0/45	0/45	0/45	1/45	0/10	0/10

Table 28: Haematology in the oral 90 day toxicity study in rat with a 28 day recovery period from the DAR (Henck et al., 1987)

Dose (mg/kg)	0		62.5		177		500	
	male	female	male	female	male	female	male	female
Week 5								
PT (sec)	14	14	13.9	14	14.3	13.8	15.7*	13.9
APTT (sec)	21.6	21.6	22.8	21.8	22.9	17.9*	31.6*	21.1
Week 14								
RBC x 106/µl	8.64	8.46	8.65	8.38	8.83	8.65	8.43	7.75*
HGB g/dl	15.7	16.3	15.8	15.9	16.1	16.4	16.3	15.4*
WBC x 103/µl	15.9	8.9	15.2	8.6	16.3	9.6	11.8*	8.5
MCV fl	56.6	57.4	57.6	57.8	58.7*	57.6	60.1*	61*
MCH pg	18.2	19.3	18.3	19.1	18.3	18.9	19.3*	19.9
MCHC g/dl	32.4	33.7	31.8	33.1	31.4*	33.1	32.3	32.7*
Week 18 (recovery)								
MCH pg	19.4	20.8	19.6	20.5	20.2	21.2	20.4*	21.1

* statistically significant

A slight but significant anaemia (RBC -9%) was seen in 500 mg/kg bw/d females after 13 weeks but not at week 5 or after recovery period. APTT was transiently increased in 500 mg/kg bw/d males at week 5.

Table 29: Clinical chemistry in the oral 90 day toxicity study in rat with a 28 day recovery period from the DAR (Henck et al., 1987)

Dose (mg/kg)	0		62.5		177		500		500/600	
	male	female	male	female	male	female	male	female	male	female
Week 5										
CREAT mg/dl	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7*		
ALB g/dl	3.58	3.72	3.6	3.69	3.56	3.94	3.79*	3.9		
SGOT U/l	59.2	57	53	59.3	54	57.6	58.5	48.8*		
TOT BILI mg/dl	0.13	0.16	0.15	0.16	0.16	0.22*	0.18*	0.21*		
Week 14										
BUN mg/dl	12.3	15.2	12.8	13.5	12.5	13.7	11.5	11.6*		
ALB g/dl	3.67	4.08	3.73	4.01	3.77	4.13	3.99*	3.91		
ALK PHOS U/l	68.6	33.9	73.7	44.6	68.7	46.5	97.4	75.6*		
SGPT U/l	29.9	27.6	31.4	23.8	37.9	26.8	46*	33.6		
K meq/l	6.34	8.46	6.32	7.74	6.26	8.09	6.68	6.74*		
TOT BILI mg/dl	0.19	0.22	0.2	0.21	0.21	0.22	0.27*	0.25		
GLOB g/dl	3.52	3.43	3.3	3.52	3.35	3.43	3.08*	3.06*		
A/G	1.05	1.2	1.13	1.14	1.13	1.21	1.31*	1.29*		
Urinary pH	7.6	7.9	8.1	8.1	7.8	8.2	8	6.6*		
Week 18 (recovery)										
GLUC mg/dl	250.9	185.6	261.3	208.8	235	202.9	207*	173.8		
CREAT mg/dl	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.6*		
TP g/dl	7.09	7.64	7.15	7.5	7.08	7.67	6.78*	7.31		
Cholinesterase and T3 and T4 thyroxin blood levels (week 14)										
Brain Cholinesterase uM/g/min	9.097	8.445					8.785	7.986	7.768	8.848
Plasma Cholinesterase mM/l/min	0.382	1.39	0.386	1.463	0.413	0.55	0.505		0.31	0.503
RBC Cholinesterase mM/l/min	1.282	0.97	1.376	1.245	1.213	1.4	1.31		1.383	1.578
Total T3 ng/ml	1.1	1.28	1.2	1.02	1.22	1.06	1.21		1.08	1.06
Free T3 pg/ml	6.82	6.26	6.82	5.25	6.48	5.84	5.7		5.61	5.88
Total T4 ng/ml	29.46	22.86	27.82	24.08	29.65	22.82	12.65*		13.9*	16.07*
Free T4 pg/ml	28.16	14.18	26.32	15.25	26.38	15.54	10.95*		11.93*	12.22

* statistically significant

Bilirubin levels increased slightly at 177 (at week 5) and 500 mg/kg bw/d. Plasma cholinesterase in females was inhibited from 177 mg/kg bw onwards; there was no effect on brain or erythrocyte cholinesterase. At 500 and 500/600 mg/kg bw/d thyroxin levels were lower than those of controls. Potassium levels in females were reduced at 500 mg/kg bw/d, as was urinary pH of this group.

Table 30: Organ weights in the oral 90 day toxicity study in rat with a 28 day recovery period from the DAR (Henck et al., 1987)

Dose (mg/kg)	0		62.5		177		500		500/600	
	male	female	male	female	male	female	male	female	male	female
Organ weights absolute										
Brain	2.07	1.87	2.09	1.88	2.07	1.88	1.94*	1.82	1.98	1.82

Dose (mg/kg)	0		62.5		177		500		500/600	
	male	female	male	female	male	female	male	female	male	female
Gonads	3.61	0.074	3.44	0.073	3.51	0.077	3.21*	0.081	3.34	0.094
Adrenal Gland	0.056	0.07	0.056	0.065	0.054	0.062*	0.075*	0.097*	0.074*	0.091
Statistically difference in absolute organ weights was reversible after recovery period										
Organ weights relative to body weight										
Liver	3.33	2.95	3.52	3.05	3.32	3.29*	3.44	3.83*	3.79*	4.2*
Thymus	0.07	0.089	0.066	0.101	0.057	0.086	0.047*	0.05*	0.045*	0.053*
Kidneys	0.717	0.738	0.797 *	0.783	0.826*	0.833*	0.934*	0.944*	0.974*	1.036*
Thyroids (% of bw x 1000)	4.78	7.57	4.45	5.46*	4.52	5.42*	5.95*	7.09	5.4	7.7
Statistically difference in relative organ weights (with the exemption of liver, adrenal and kidney weight in females receiving 500 mg/kg bw/d pyridate) was reversible after recovery period										
Organ weights relative to brainweight										
Adrenal Gland	2.71	3.755	2.717	3.466	2.594	3.283*	3.856*	5.363*	3.753*	4.988

* statistically significant

Increases in liver and kidney weight were seen in females from 177 mg/kg bw/d onwards. Kidney weight was increased in males in all dose groups. Absolute gonad and brain weight in males was significantly decreased at 500 mg/kg bw/d but not at 500/600 mg/kg bw/d. Adrenal gland weight was increased in females from 177 mg/kg bw/d onwards and in males in the 500 and 500/600 mg/kg bw/d group. Relative thyroid weight was significantly decreased in females at 62.5 and 177 mg/kg bw/d but not at 500 mg/kg bw/d. This change was not accompanied by a change in hormones (T3 or T4). In males relative thyroid weight was significantly enhanced at 500 mg/kg bw/d but not at 500/600 mg/kg bw/d.

Table 31: Histopathology in the oral 90 day toxicity study in rat with a 28 day recovery period from the DAR (Henck et al., 1987)

Dose (mg/kg)	0		62.5		177		500		500/600	
	male	female	male	female	male	female	male	female	male	female
Basophilic material in germinal follicles of mesenteric lymph nodes	0/20	0/20	0/20	0/19	2/19	1/20	8/17	6/14	4/9	2/6
Basophilic material in germinal follicles of mesenteric lymph nodes after recovery	2/15	0/15					8/15	0/11		
Increased pigment in the spleen (mean rank for hemosiderin)	41.4	32.7	20.4	52.5	61.5	52.7	90.3	70.9	87.8	90
Increased pigment in the spleen (mean rank for hemosiderin) after recovery	46.9	45					80.9	59.3		

Dose (mg/kg)	0		62.5		177		500		500/600	
	male	female	male	female	male	female	male	female	male	female
Mammary hyperplasia (mean rank)- no lesion found		46.1		38.79		46.68		74.71*		88.33*

* statistically significant

In the DAR the NO(A)EL was set at 62.5 mg/kg bw/d, which can be confirmed. At the next higher dose rate one female rat died, relative liver weight was enhaced > 10% in females and clinical signs could be observed in both sexes as well as minor histopathological effects (basophilic material in germinal follicles of mesenteric lymph nodes) occured.

IIA 5.3.2/02 Danks A.(1991) Pyridate technical: Toxicity study by dietary administration to CD rats for 13 weeks. LSR Report No.: 90/AGL002/0614

Pyridate (batch No. 2759523, 11344/AR22, purity 92.51%) was applied to groups of 10 male and female CD rats via diet at 0, 400, 1200 and an ascending dose of 3600 (week 1-6) / 4600 (week 7-8)/ 8000 (week 9-13) ppm for 13 weeks. Based on the results observed at 8000 ppm an additional group was fed 6000 ppm with a similarly constituted contemporatory control. The nominal dietary concentrations corresponded to an average daily intake of 0, 28.11, 86.01 and 397.9 mg/kg bw/d in males and 0, 31.6, 96.01 and 448.8 mg/kg bw/d in females. Detailed histopathology was performed only for control and top dose (6000 ppm) animals. The results are summarised in the tables below.

Table 32: Summary of the oral 13 weeks toxicity study in male rats from the DAR (Danks, 1991)

Dose (ppm)	0	0 (pair-fed to 6000 ppm)	400	1200	6000	3600/4600/8000
Body weight (g)- week 13	428	428	406	429	375	368
Body weight gain (g)	312	308	290	314	253*	253*
RBC (mil/cmm)	8.07	9.01	8.11	7.96	8.63*	8.24
Urea (mg%)	30	29	28	31	34*	31
Urinary pH	7.1	7	7.3	7	6.2*	6.2*
Reducing substances in urine (incidences)	0	0	0	0	0	9
Kidney weight relative (%)	0.816	0.795	0.831	0.817	0.842	0.886*
Liver weight relative (%)	3.81	3.49	3.6	3.68	3.77*	3.87

Dose (ppm)	**0**	**0 (pair-fed to 6000 ppm)**	**400**	**1200**	**6000**	**3600/4600/8000**
Mesenteric lymph nodes with follicles containing mineral deposits	0	0	0	0	0	7

* statistically significant

Table 33: Summary of the oral 13 weeks toxicity study in female rats from the DAR (Danks, 1991)

Dose (ppm)	**0**	**0 (pair-fed to 6000 ppm)**	**400**	**1200**	**6000**	**3600/4600/8000**
Body weight (g)- week 13	240	229	236	232	202	215
Body weight gain (g)	128	124	125	123	95*	104*
Hb (g%)	15.9	15.6	15.6	15.8	14.6*	15.2*
RBC (mil/cmm)	7.69	8.4	7.54	7.71	7.69*	7.41
Urea (mg%)	31	36	31	33	40	37*
Urinary pH	6.7	6.7	6.8	6.8	6.2*	6.1*
Reducing substances in urine (incidences)	0	0	0	0	0	9
Kidney weight relative (%)	0.862	0.91	0.9	0.914	1.026*	0.907
Liver weight relative (%)	3.67	4.18	3.94	3.92	4.49*	4.12*
Mesenteric lymph nodes with follicles containing mineral deposits	0	0	0	0	0	5

* statistically significant

No clinical signs were observed which were considered to be related to the administration of pyridate. No clinicals signs were observed after the first dose. In the DAR the NO(A)EL was set at 1200 ppm equivalent to 86 mg/kg bw/d in males and 96 mg/kg bw/d in females, which can be confirmed. At the next higher dose body weight gain was decreased > 10%, urinary pH was significantly lower than in the control group and haemoglobin levels were significantly decreased in females.

90-day toxicity (dog)

The following studies were evaluated in support of Annex I listing of pyridate and are no longer granted data protection:

IIA 5.3.3/01 Tomkins E.C. (1987) 90 day dog oral subchronic toxicity study. TRL Study No.: 043-002

Pyridate (batch No. Knr: 2556520, purity 92%) was applied to groups of 4 male and female Beagle dogs via gelatine capsule by oral gavage at 0, 20, 60 and 200 mg/kg bw/d for 90 days. The results are summarised in the tables below:

Table 34: Summary of the 90 day toxicity study in dogs from the DAR (Tomkins, 1987)

Dose (mg/kg)	**0**		**20**		**60**		**200**	
	male	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Clinical signs (emesis, ataxia, opisthotonus, hypoactivity, salivation, nystagmus, mydriasis, muscle fasciculation)					2-3/4	2-3/4	4/4	4/4
Mortality	0/4	0/4	0/4	0/4	0/4	0/4	4/4	3/4
Body weight initial (kg)	7.7	7	7.2	7.2	7.6	7.2	7.3	7.6
Body weight week 1 (kg)	8	8	7.5	8.1	8.3	7.8	6.4	6.8
Body weight week 12 (kg)	10.6	9.5	10.6	9.6	10	9.1	-	9
SGOT (U/l)	24.4	24.7	27.3	30.6	26.2	24.8	-	16.6
SGPT (U/l)	21.3	16.9	14.6	16.7	12.9*	9.2	-	6.2
Plasma cholinesterase (mM/l/min)	1.21	1.44	1.24	1.39	1.6	1.54	-	0.87
Erythrocyte cholinesterase (mM/l/min)	2.32	2.89	3.05	2.7	3.15*	2.91	-	1.8
Relative liver weight (%)	3.16	3.26	4.08*	3.78	3.8	4.16	-	4.62
Minimal to slight degenerative myelopathy of the sciatic nerve	0/4	0/4	0/4	0/4	0/4	0/4	2/4	2/4
(Broncho)pneumonia	0/4	0/4	0/4	1/4	0/4	0/4	2/4	2/4

* statistically significant

Furthermore food consumption was decreased in both sexes in the highest dose group.

Clinical signs included in the 20 mg/kg bw/d group in males: Emesis: 1/4 and in females: Emesis: 2/4.

Clinical signs included in the 60 mg/kg bw/d group in males: Emesis: 4/4, salivation: 2/4, ataxia: 1/4 and mydriasis: 3/4 and in females: Emesis: 4/4, salivation: 2/4, ataxia: 2/4, nystagmus 1/4, mydriasis: 1/4.

Clinical signs included in the 200 mg/kg bw/d group in males: Emesis: 4/4, hypoactivity: 4/4, opisthotonus: 4/4, muscle fasciculations: 4/4, head swing: 1/4, nystagmus: 4/4, salivation: 4/4, ataxia: 4/4 and mydriasis: 4/4 and in females: Emesis: 4/4, salivation: 4/4, ataxia: 4/4, hypoactivity: 4/4, opisthotonus: 4/4, muscle fasciculations: 4/4, head swing: 1/4, nystagmus 4/4, mydriasis: 4/4 and head tilt: 2/4.

Clinical signs at 20 and 60 mg/kg bw/d were reversible. Generally clinical signs were less severe with increasing exposure period.

Table 35: Clinical signs observed ≤ 2000 mg/kg bw after the first dose (Tomkins, 1987)

Clinical sign	60 mg/kg bw		200 mg/kg bw	
	Males	Females	Males	Females
Emesis	1/4 (grade 1)	1/4 (grade 1)	4/4 (grade 2-3)	4/4 (grade 3)
Ataxia	0/4	0/4	4/4 (grade 3)	3/4 (grade 3)
Hypoactivity	0/4	0/4	3/4	2/4
Opisthotonus	0/4	0/4	4/4 (grade 2)	3/4 (grade 2-3)
Muscle fasciculations	0/4	0/4	2/4	0/4
Head swing	0/4	0/4	1/4	0/4
Nystagmus	0/4	0/4	4/4	3/4
Mydriasis	0/4	0/4	4/4	4/4
Salivation	0/4	0/4	0/4	1/4

In the DAR the NO(A)EL was set at 20 mg/kg bw/d which can be confirmed. At the next higher dose clinical signs were observed.

IIA 5.3.3/02 Vandaele E.A.N. (1990) Pyridate technical: Toxicity study by oral (capsule) administration to Beagle dogs for 13 weeks. LSR Report No.: 89/AGL001/0806

Pyridate (batch No. 2759523, 11.344/AR22, purity 92.51%) was applied to groups of 5 male and female Beagle dogs via gelatine capsule by oral gavage at 0, 40, 80 and 120 mg/kg bw/d for 90 days. The results are summarised in the tables below.

Table 36: Body weight and clinical signs in the 90 day toxicity study in dogs from the DAR (Vandaele, 1990)

Dose (mg/kg)	0		40		80		120	
	male	female	male	female	male	female	male	female
Body weight initial (kg)	10	9	9.9	9.2	10	9	10	9.2
Body weight week 13 (kg)	13.1	11.5	13	12.3	13	11.7	12.8	11.3
Clinical signs								
Head shaking	0/5	0/5	1/5	0/5	0/5	1/5	0/5	0/5
Salivation	0/5	0/5	0/5	0/5	2/5	2/5	5/5	5/5
Ataxia	0/5	0/5	0/5	0/5	1/5	4/5	5/5	5/5
Vacant expression	0/5	0/5	0/5	0/5	1/2	0/5	0/5	0/5
Underactivity	0/5	0/5	0/5	0/5	2/5	2/5	2/5	5/5
Hunched posture	0/5	0/5	0/5	0/5	0/5	3/5	5/5	5/5
Emesis	0/5	0/5	0/5	0/5	0/5	1/5	5/5	5/5
Pupils dilated	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5
Prostration	0/5	0/5	0/5	0/5	0/5	0/5	4/5	5/5
Opisthotonus	0/5	0/5	0/5	0/5	0/5	0/5	5/5	5/5
Tremor	0/5	0/5	0/5	0/5	0/5	0/5	1/5	3/5

* statistically significant

Reduced body weight gain (-16%) and reduced food consumption was observed in females of the highest dose group. Clinical signs (i.e. ataxia, prostration, underactivity, opisthotonus, emesis,

salivation, pallor, cold to touch, dry nose, tremor, hunched posture and vacant expression) were observed to a mild to moderate extent in the 80 mg/kg bw/d group and to a marked extent in the highest dose group.

Clinical signs included in the 40 mg/kg bw/d group in males: Head shaking: 1/5; no clinical signs in females.

Clinical signs included in the 80 mg/kg bw/d group in males: Salivation: 2/5, ataxia: 1/5, vacant expression: 1/5 and under activity 2/5 and in females: Salivation: 2/5, ataxia: 4/5, hunched posture: 3/5, emesis: 1/5, pupils dilated: 2/5, head shaking: 1/5 and under activity 2/5.

Clinical signs included in the 120 mg/kg bw/d group in males: Ataxia: 5/5, prostration: 4/5, underactivity: 2/5, opisthotonus: 5/5, emesis: 5/5, salivation: 5/5, tremor: 1/5, hunched posture 5/5 and in females: Ataxia: 5/5, prostration: 5/5, underactivity: 5/5, opisthotonus: 5/5, emesis: 5/5, salivation: 5/5, tremor: 3/5, hunched posture 5/5.

Generally clinical signs were less severe with increasing exposure period.

Table 37: Clinical signs observed ≤ 2000 mg/kg bw after the first dose (Vandaele, 1990)

Clinical sign	80 mg/kg bw		120 mg/kg bw	
	Males	Females	Males	Females
Emesis	0/5	0/5	2/5	3/5
Ataxia	0/5	0/5	2/5	0/5
Underactivity	0/5	1/5	0/5	1/5
Opisthotonus	0/5	0/5	2/5	0/5

Table 38: Haematology in the 90 day toxicity study in dogs from the DAR (Vandaele, 1990)

Dose (mg/kg)	0		40		80		120	
	male	female	male	female	male	female	male	female
Week 6								
PCV (%)	40	42	38	40	35*	35*	36*	35*
Hb (g%)	13.9	14.5	13.4	13.9	12.2*	12.2*	13.1	12.4*
RBC (mil/cmm)	5.91	6.19	5.62	5.82	5.05*	5.1*	5.23*	4.99*
MCV (cμ)	67	67	67	68	68	68	70*	70*
Number of animals with a reticulocyte count > 2%	0	0	1	0	2	1	4	1
Number of animals with Heinz bodies	0	0	0	0	0	1	1	1
Platelets	225	257	240	234	303*	294	282*	289
Week 12								
Hb (g%)	14.4	15.5	14.3	15.7	13.2	13.6*	13.9	14.4
RBC (mil/cmm)	6.27	6.77	6.09	6.67	5.62*	5.81*	5.65*	5.97*
MCV (cμ)	65	65	67	68	66	67	69*	69*
Number of animals with a reticulocyte count > 2%	0	0	1	1	4	1	4	2
Number of animals with Heinz bodies	0	0	0	0	4	1	5	5
Platelets	199	202	237	237	283*	269*	273*	268*

* statistically significant

Reduction in erythrocyte parameters (> 10%) and increase in Heinz bodies was seen at 80 and 120 mg/kg bw/d. Mild reticulocytosis was seen at 120 mg/kg bw/d.

Table 39: Clinical Chemistry in the 90 day toxicity study in dogs from the DAR (Vandaele, 1990)

Dose (mg/kg)	0		40		80		120	
	male	female	male	female	male	female	male	female
Week 6								
ALT (iu/l)	29	31	18*	22	10*	11*	9*	8*
AP (iu/l)	79	87	95	95	84	100	87	87
AST (iu/l)	30	27	30	30	26	27	25	22
Concentration of total bilirubin mg%	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.4
Albumin	2.8	2.8	2.9	2.9	3	3	3.1*	3.3*
Week 12								
ALT (iu/l)	31	32	20*	22*	11*	16*	7*	9*
AP (iu/l)	68	66	73	69	68	62	73	68
AST (iu/l)	28	24	25	21	25	23	28	21
Concentration of total bilirubin mg%	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3
Albumin	3.1	3.3	3.2	3.5	3.2	3.5	3.6*	4.1*

* statistically significant

Alanine amino transferase (ALT) was significantly reduced in all treated dose group. The statistically significant decrease in ALT was not accompanied by a change >10% in relative liver weight or histopathological changes at 40 mg/kg bw/d. Furthermore AP and AST activity and concentration of total bilirubin were not affected at any concentration. Therefore the statistically significant decrease in ALT at 40 mg/kg bw/d was not considered adverse. Higher albumin values were seen in top dose animals.

Table 40: Organ weights in the 90 day toxicity study in dogs from the DAR (Vandaele, 1990)

Dose (mg/kg)	0		40		80		120	
	male	female	male	female	male	female	male	female
Absolute organ weight (in g)								
Ovary		0.77		0.9		0.92		0.99*
Relative organ weight (in %)								
Kidneys	0.4	0.39	0.45	0.41	0.46*	0.46*	0.46*	0.49*
Liver	2.98	2.87	3.03	3.01	3.38	3.33*	3.61*	3.73*

* statistically significant

Relative liver and kidney weight was increased > 10% in both sexes at 80 and 120 mg/kg bw/d.

Table 41: Histopathology in the 90 day toxicity study in dogs from the DAR (Vandaele, 1990)

Dose (mg/kg)	0		40		80		120	
	male	female	male	female	male	female	male	female
Brain focal gliosis	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Epididymides aspermia	0/5		0/5		0/5		1/5	
Pigmentation in Kupffer cells	3/5	2/5	1/5	2/5	5/5	5/5	5/5	5/5
Lung bronchopneumonia	0/5	0/5	0/5	0/5	0/5	1/5	1/5	1/5
Left sciatic nerve myelin digestion chambers	1/5	2/5	0/5	1/5	0/5	0/5	2/5	1/5

Dose (mg/kg)	0		40		80		120	
	male	female	male	female	male	female	male	female
Increased cellularity of sternal bone marrow	0/5	0/5	0/5	0/5	0/5	0/5	4/5*	4/5*
Testes immaturity	0/5		0/5		0/5		1/5	
Thyroids parafollicular cell hyperplasia	2/5	1/5	0/5	1/5	1/5	1/5	2/5	3/5

* statistically significant

Histopathological changes were seen in the liver (increased amount of pigment in Kupffer cells) in the 80 and 120 mg/kg bw/d dose group. According to the study author the increased cellularity of sternal bone marrow in top dose animals can be regarded as a normal respone to the decreased red cell parameters. All other histopathological findings were according to the study authors considered to be consistent with the spectrum of changes commonly encountered in dogs of this age at this laboratory (no historical control data in the study report). Historical control data (including 16 studies starting between Feb. 1988 and Oct. 1990) for thyroid and sciatic nerve findings were submitted in Nov. 2013 (Taylor I. 2013: Data requirement 2.1- evaluation table). No myelin digestion chambers in sciatic nerves were detected in the historical control data while parafollicular cell hyperplasia ranged from 0-100% (mean 8.22%).

In the DAR the NOAEL was set at 40 mg/kg bw/d which can be confirmed. At the next higher dose clinical signs, reduction in erythrocyte parameters and increase in Heinz bodies, increases in relative liver and kidney weight and histopathological changes in the liver were observed.

52-weeks toxicity (dog)

The following study was evaluated in support of Annex I listing of pyridate and is no longer granted data protection:

IIA 5.3. 4/01 Bailey D.E. (1989) Chronic toxicity study in dogs with pyridate technical. HLA Study no.: 2495-100

Pyridate (Lot No. 2759523, LH No. 23, 293A&B, purity 91.5%) was applied to groups of 5 male and female Beagle dogs via capsule by oral gavage in ascending doses of 0, 5/10/30 (low), 20/60/80/100 (mid) and 60/100/120/140/150 (high) mg/kg bw/d for 52 weeks.

Table 42: Dose adaptation in the 1 year toxicity study in dogs from the DAR (Bailey, 1989)

Group	Dose Level (mg/kg bw/d)	Dosing duration (weeks)
Control	0	1-53
Low	5	1-34
	10	35
	30	36-53
Mid	20	1-34
	60	35-38
	80	39-42
	100	43-53[a]
High	60	1-34
	100 (males only)	35
	120	36-38
	140	39-42

Group	Dose Level (mg/kg bw/d)	Dosing duration (weeks)
	150	43-53

[a] dosed at 80 mg/kg bw/d for 2 days in week 49

The results are summarised in the following tables:

Table 43: Body weight (changes) in the 1 year toxicity study in dogs from the DAR (Bailey, 1989)

Dose (mg/kg)	0		5/10/30 (low)		20/60/80/100 (mid)		60/100/120/140/150 (high)	
	male	female	male	female	male	female	male	female
Body weight initial (kg)	7.5	7	8.5	6.5	8	6.7	8.1	6.5
Body weight week 35 (kg)	11.7	10	11.9	9.2	12.6	9.6	12.3	8.9
Body weight week 36 (kg)	11.8	10	11.9	9.2	12.6	9.5	12.2	8.8
Body weight week 52 (kg)	12.1	10.5	12	9.3	11.8	9	12.1	8.8
Body weight change week 0-34 (kg)	4.1	2.9	3.2	2.8	4.7	3.1	4.4	2.4
Body weight change week 34-42 (kg)	0.2	0.2	0.1	-0.1	-0.6*	-0.6*	-0.4*	0
Body weight change week 42-52 (kg)	0.3	0.4	0.1	0.2	-0.3	-0.1	-0.1	-0.1

* statistically significant

Body weight loss (-16%) was seen in mid and high dose animals from week 35 onwards. No treatment related effect was noted for food consumption. Clinical signs (i.e. salivation, ataxia, mydriasis, dyspnea, tremors, increased respiration and inability to stand) were observed to a mild to moderate extent in the mid dose group and were marked in the high dose group. No clinical signs, except emesis, were observed below a dose level of 100 mg/kg bw/d. Clinical signs included in the 20/60/80/100 mg/kg bw/d group in males: Emesis: 5/5, salivation: 2/5, ataxia: 4/5, tremors: 1/5, hunched posture: 1/5, and mydriasis: 3/5 and in females: Emesis: 5/5, ataxia: 2/5, unable to stand: 1/5 and mydriasis: 2/5 and up to 100 mg/kg bw/d (week 34/35) in the 60/100/120/140/150 dose group in males: Emesis: 5/5 and in females: Emesis: 4/5, salivation: 1/5. Clinical signs > 100 mg/kg bw/d (> week 35) included in males: Emesis: 5/5, prostrate: 4/5, ataxia: 4/5, salivation: 2/5, mydriasis: 4/5, tremors: 1/5, hunched posture: 2/5 and nystagmus: 1/5 and in females: Emesis: 5/5, prostrate: 2/5, ataxia: 5/5, salivation: 5/5 and mydriasis: 4/5.

Clinical signs observed in week 1 of dosing (the day is not indicated in the study report) included in males: Emesis: 1/5 (5 mg/kg bw).

No treatment related effects were observed for haematological parameters.

Table 44: Clinical chemistry in the 1 year toxicity study in dogs from the DAR (Bailey, 1989)

Dose (mg/kg)	0		5/10/30 (low)		20/60/80/100 (mid)		60/100/120/140/150 (high)	
	male	female	male	female	male	female	male	female
Week 26								
Globulin (G/DL)	3	2.8	2.7	2.7	2.9	2.6	2.7	2.3
ALT (U/L)	45	34	40	43	33	32	33	25
Week 52								

Dose (mg/kg)	0		5/10/30 (low)		20/60/80/100 (mid)		60/100/120/140/150 (high)	
	male	female	male	female	male	female	male	female
Globulin (G/DL)	**3.2**	**2.9**	**3**	**2.8**	**2.8**	**2.6**	**2.6**	**2.3***
ALT (U/L)	40	31	33	34	24	20	34	17

* statistically significant

Decreases serum globulin was seen in top dose females (significantly only at week 52). There was a tendency (not significant) to decreased ALT values in top dose animals.

Table 45: Histopathology in the 1 year toxicity study in dogs from the DAR (Bailey, 1989)

Dose (mg/kg)	0		5/10/30 (low)		20/60/80/100 (mid)		60/100/120/140/150 (high)	
	male	female	male	female	male	female	male	female
Spleen extramedullary hematopoiesis increased	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Nerve sciatic degenerative myelopathy	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5

* statistically significant

No treatment related effects were seen on absolute and relative organ weights. Degenerative myelopathy of the sciatic nerve and extramedullary hematopoiesis in the spleen was seen in one male dog of the highest dose group.

In the DAR the NO(A)EL was set at 30 mg/kg bw/d which can be confirmed. When the dose in the mid dose group was increased from 20 to 60 mg/kg bw/d body weight was impaired (small loss in body weight). Furthermore clinical signs were observed from 100 mg/kg bw/d onwards.

3.7.1.2 Repeated dose toxicity: inhalation

No data available.

3.7.1.3 Repeated dose toxicity: dermal

The following study was evaluated in support of Annex I listing of pyridate and is no longer granted data protection:

IIA 5.3.7/01 Perry C.J. and Duffen J. (1988) Pyridate 3 week toxicity study in rats. IRI Project No.: 437242

Pyridate (batch No. 2759523, CL 11.344/AR 22, purity 91.5%) was applied to groups of 5 male and female Sprague Dawley rat over an area of approximately 10% of the total body surface on 21 consecutive days. The doses used were 0 and 1000 mg/kg bw/d. In the table below the results are summarised.

Table 46: Summary of the dermal 21 toxicity study in rat from the DAR (Perry, Duffen, 1988)

Dose (mg/kg bw/d)	0		1000	
	male	female	male	female
Body weight initial (kg)	313	218	310	223
Body weight week 3 (kg)	397	257	370	254

Dose (mg/kg bw/d)	0		1000	
	male	female	male	female
ALT (U/l)	79	65	91*	75
Liver weight (adjustment for differences in final body weight- covariance analysis)	15.6	9.98	17.47*	10.61*
Liver weight absolute (g)	16.39	10.07	16.68	10.53
Skin encrustration	0/5	0/5	3/5	0/5
Skin dark areas	0/5	0/5	1/5	1/5
Epidermal hyperplasia at treatment site	0/5	0/5	4/5*	4/5*
Scab(s) at treatment site	0/5	0/5	2/5	0/5

* statistically significant

No clinical signs, besides skin effects, were detected.

In the DAR the systemic NO(A)EL was set at 1000 mg/kg bw/d which can be confirmed. No signs of adverse systemic toxic effect were seen. Irritancy signs of the skin were seen at the dosing site.

3.7.1.4 Repeated dose toxicity: other routes

No data available.

3.7.1.5 Human information

No information is available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

3.7.1.6 Other relevant information

A review of the neurotoxic potential of pyridate in light of the findings in the dog studies was provided. Thereby the relevance of the neurotoxicity findings in the dog studies (gavage administration) for the risk assessment and the classification was assessed (please refer to Annex).

Histopathological Re-examination dog-studies:

Reference:	**Re-examination of the sciatic nerves from the three studies on dogs**
Author(s), year:	Armstrong J.M., Chevalier H-J., 1997
Report/Doc. number:	Sandoz Agro Doc TDS 456, ASTOX Archives No 97/106 Expert statement
Guideline(s):	Not applicable
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Yes

The slides of the sciatic nerves of all 3 dog studies were submitted for a peer review in 1997.

The reviewing pathologists disagreed with the diagnostic terminology “Degenerative Myelinopathy” for the changes seen in the first 90 day study (Tompkins, 1987) and the 1-year study (Bailey, 1989) and changed it into “Myelin Digestion Chambers”. There was essential agreement with the grading, although the finding was noted in a few more/other dogs.

According to the reviewing pathologists these changes are commonly observed in sections of sciatic nerves from a number of different species/strains of laboratory animals (e.g. rats, dogs, mice etc.) and tend to be more common and severe in older animals, particularly in mice and rats.

Table 47: Re-evaluation of histopathology of sciatic nerves from the first 90 day dog study (Tompkins, 1987)

Dose (mg/kg)	0		20		60		200	
	male	female	male	female	male	female	male	female
Sciatic nerve-reevaluation: Myelin dig. Chambers (grade)	0/4	0/4	0/3	0/4	0/4	0/4	3/4 (1, one animal grade 2)	3/4 (1)
Sciatic nerve- DAR: Degenerative myelinopathy (grade)	0/4	0/4	0/4	0/4	0/4	0/4	2/4 (1 and 2)	2/4 (1)

Table 48: Re-evaluation of histopathology of sciatic nerves from the sceond 90 day dog study (Vandaele, 1990)

Dose (mg/kg)	0		40		80		120	
	male	female	male	female	male	female	male	female
Sciatic nerve-reevaluation: Myelin dig. Chambers (grade)	1/5 (1)	0/5	0/5	2/5 (1)	0/5	0/5	2/5 (1)	3/5 (1)
Sciatic nerve- DAR: Degenerative myelinopathy (grade)	1/5 (1)	2/5 (1)	0/5	1/5	0/5	0/5	2/5 (1)	1/5 (1)

Table 49: Re-evaluation of histopathology of sciatic nerves from one year dog study (Bailey, 1989)

Dose (mg/kg)	0		5/10/30 (low)		20/60/80/100 (mid)		60/100/120/140/150 (high)	
	male	female	male	female	male	female	male	female
Sciatic nerve-reevaluation: Myelin dig. Chambers (grade)	0/5	0/5	1/5 (1)	0/5	2/5 (1)	0/5	1/5 (2)	1/5 (1)
Sciatic nerve-DAR: Degenerative myelinopathy (grade)	0/5	0/5	0/5	0/5	0/5	0/5	1/5 (2)	0/5

The reviewing pathologists concluded that the changes noted in the three studies are not considered to represent toxic effects of a test article.

3.7.1.7 Summary and discussion of repeated dose toxicity

In short term toxicity experiments in rats, oral administration of high dose levels showed clinical signs like hypoactivity and salivation, decreases in bodyweight or bodyweight gain as well as minor biochemical and haematological changes. In two 90 day experiments in dogs, oral administration (capsules) of 60 and 200 mg/kg bw/day (first study), respectively, 80 and 120 mg/kg bw/day (second study) showed neurotoxic symptoms like emesis, ataxia, opisthotonus and hypoactivity. Minor changes in biochemical and haematological parameters were seen at dose levels above 60 mg/kg bw. In the first study severe mortality occurred in the highest dose group (200 mg/kg

bw/day) with seven of eight dogs dying during the in-life phase. Two males and 2 females at this lethal dose level showed a slight or minimal degenerative myelopathy of the sciatic nerve. Increased bronchopneumonia or pneumonia was noted in 4 of 8 animals of the high dose group. Also in the 12 month study in dogs, oral administration of high dose levels of pyridate (> 80 mg/kg bw/day) produced clinical symptoms like hypoactivity, ataxia and salivation, as well as degenerative myelopathy of the sciatic nerve.

No signs of systemic toxicity were observed when pyridate, at the limit dose of 1000 mg/kg bw, was tested in rats via the dermal route of exposure during 21 days.

3.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

3.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Table 50: Summary of effects observed in rats, mice, dogs and rabbits in comparison to cut off values

Species-Route (Reference)	Study duration	Cut off value Cat 1 STOT RE (1272/2008) [mg/kg bw/d]	Cut off value Cat 2 STOT RE (1272/2008) [mg/kg bw/d]	Effects below cut off value	Significance of toxicological effect (1272/2008) below cut off value
Rat- oral (Til, 1979)	28 days	30	300	≥ 354 (M)- 351 (F) mg/kg bw/d: ↓ bw, ↓ HB, changes in organ weight and haematology.	No evidence of organ dysfunction after 28 days of daily dosing.
Mouse- oral (Til, 1980a)	28 days	30	300	around 300 mg/kg bw: Not tested ≥ 564 (M)- 672 (F) mg/kg bw/d: ↑ relative spleen weigh	No evidence of organ dysfunction after 28 days of daily dosing.
Rat- oral (Til, 1980b)	28 days	30	300	≥ 354 (M)- 351 (F) mg/kg bw/d: ↓ bw (Wistar and Sprague Dawley rat), ↓ HB (Sprague Dawley rat)	No evidence of organ dysfunction after 28 days of daily dosing.
Rat- oral (gavage) with 28 days recovery period (Henck, 1987)	90 days	10	100	≥ 177 mg/kg bw/d: ↑ relative liver weight, death (one female), clinical signs (hypoactivity, salivation), minor histopathological effects (basophilic material in germinal follicles of mesenteric lymph nodes)	No evidence of organ dysfunction after 90 days of daily dosing., no mortality at the NOAEL of 62.5 mg/kg bw/d
Rat- oral (Danks, 1991)	90 days	10	100	None (NOAEL: 86.01 (M)-96.01 (F) mg/kg bw/d)	-

Species-Route (Reference)	**Study duration**	**Cut off value Cat 1 STOT RE (1272/2008) [mg/kg bw/d]**	**Cut off value Cat 2 STOT RE (1272/2008) [mg/kg bw/d]**	**Effects below cut off value**	**Significance of toxicological effect (1272/2008) below cut off value**
Dog- oral (gavage) (Tomkins, 1987)	90 days	10*	100*	≥ 60 mg/kg bw/d: Clinical signs (emesis, salivation, ataxia, mydriasis, nystagmus) See study report in section 3.7.1.1. for details ; Table 34 and text	Clinical signs < 100 mg/kg bw/d indicating significant impairment of the neurological function ; no exacerbation of effects after multiple doses
Dog- oral (gavage) (Vandaele, 1990)	90 days	10*	100*	≥ 80 mg/kg bw/d: Clinical signs (salivation, ataxia, hunched posture, emesis, pupils dilated, head shaking, underactivity), ↓ erythrocyte parameters, ↑ Heinz bodies, ↑ relative liver and kidney weight, histopathological changes in the liver (pigmentation in Kupffer cells) ≥ 120 mg/kg bw/d: ↓ bw gain (F), clinical signs (opisthotonus, tremor, prostration), ↓ HB, changes in organ weight and haematology, myelin digestion chambers See study report in section 3.7.1.1. for details ; Table 36 and text	Clinical signs < 100 mg/kg bw/d indicating significant impairment of the neurological function ; ; no exacerbation of effects after multiple doses
Dog- oral (gavage) (Bailey, 1989)	51 weeks	2.5*	25*	None (NOAEL: 30 mg/kg bw/d)	-
Rat- dermal (Perry and Duffen 1988)	21 days	80	800	≤ 1000 mg/kg bw/d: Skin irritancy signs	No evidence of organ dysfunction after 21 days of daily dosing

* In the CLP guidance there is no advice as to the use of rat-specific guidance values for studies with other experimental species (such as dogs). According to the RAC opinion on cymoxanil (ECHA/RAC/CLH-0-0000002970-73-01/F adopted 14.09.2012) "earlier RAC has considered using the same guidance values for rat and dog studies. In 2006, The Netherlands presented a corresponding thought starter (ECBI/64/06) with considerations on how to translate guidance values for the rat to guidance values to dogs based on allometric scaling and different life spans of species. However, these preliminary discussions on the use of allometric scaling and different life spans of species for RDT classification have not yet been finalized and the corresponding concepts have not yet been integrated into the CLP guidance. Thus for now RAC prefers to generally start with the guidance values for the 90-day oral rat study, to adapt these 90-day rat guidance values for

different durations of exposure to rats according to Haber's rule and then to use the original or duration-adjusted rat guidance values without further changes for test results with other animal species."

Position papers and expert statements were submitted regarding neurotoxic effects and their impact on classification and labelling for renewal of approval of pyridate (please refer to-Annex).

In the Pesticide Peer Review Meeting 109 (January 2014) the relevance of clinical signs for assessing neurotoxicity potential of pyridate was discussed. The proposal STOT RE was also discussed. The RMS (Austria) supported STOT RE category 2 only on the basis of clinical effects (based on reevaluation of histopathological findings). The changed description of degenerative myelopathy of sciatic nerves into myelin digestion chambers is not expected to change the nature and relevance of the findings (triggering clinical signs of neurotoxicity). However, the dose response relationship for the histopathological findings below 100 mg/kg bw/d is not so clear anymore after re-evaluation of the dog studies (including the 1-yr dog study).

The majority of the experts in the Pesticide Peer Review Meeting 109 supported STOT-RE, Cat. 2 classification, if not considered overruled by STOT SE in CLP regulation. The final decision will be taken by RAC (ECHA).

3.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Clinical signs indicating significant impairment of the neurological function were more pronounced after a single application. According to the Guidance on the Application of the CLP Criteria (ECHA, Jun 2015) "Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate." No accumulation or exacerbation of the toxicity is seen after repeated application. Therefore Austria, as the dossier submitter, considers that effects after repeated application are already covered by STOT-SE.

3.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Regulation (EC) No. 1272/2008: no classification proposed

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)
Summary of the Dossier Submitter's proposal In short-term toxicity experiments in rats, oral administration of high dose levels showed clinical signs like hypoactivity and salivation, decreases in bodyweight or bodyweight gain as well as minor biochemical and haematological changes. In two 90 day experiments in dogs, oral administration (capsules) of 60 and 200 mg/kg bw/d (first study), 80 and 120 mg/kg

bw/d (second study) showed neurotoxic symptoms like emesis, ataxia, opisthotonus and hypoactivity. Minor changes in biochemical and haematological parameters were seen at dose levels above 60 mg/kg bw/d. In the first study severe mortality occurred in the highest dose group (200 mg/kg bw/d) with seven of eight dogs dying during the in-life phase. Two males and 2 females at this lethal dose level showed a slight or minimal degenerative myelopathy of the sciatic nerve. Increased bronchopneumonia or pneumonia was noted in 4 of 8 animals of the high dose group. Also in the 12 month study in dogs, oral administration of high dose levels of pyridate (> 80 mg/kg bw/d) produced clinical symptoms like hypoactivity, ataxia and salivation, as well as degenerative myelopathy of the sciatic nerve.

No signs of systemic toxicity were observed when pyridate, at the limit dose of 1000 mg/kg bw/d, was tested in rats via the dermal route of exposure during 21 days.

The DS referred to the Pesticide Peer Review Meeting 109 (January 2014), at which the majority of those present supported STOT RE Cat. 2 classification, although this was provided a STOT SE classification was not found more appropriate.

The DS considered that clinical signs indicating significant impairment of the neurological function were more pronounced after a single application. According to the Guidance on the Application of the CLP Criteria (ECHA, Jun 2015) "Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT SE only would be appropriate." No accumulation or exacerbation of the toxicity is seen after repeated application. Therefore the DS considered that effects after repeated application are already covered by STOT SE* and therefore proposed no classification.

* RAC notes that in light of the additional information submitted during the public consultation, the DS concluded that Acute Tox. 4 was more appropriate than both STOT SE and STOT RE because clear evidence for specific neurotoxic effects in the absence of lethality are lacking.

Comments received during public consultation

Comments were received from five MSCA and one industry stakeholder. All comments were made on the basis the original classification proposal of STOT SE.

Two MSCA made a general comment, supporting the original classification proposal.

A third MSCA considered that after single and repeated dose, clinical signs related to neurotoxicity in dogs in the same dose range were the most severe effect. According to the DS, no accumulation or exacerbation of toxicity was observed after repeated application and these neurological signs were reversible. Therefore the MSCA considered that classification with STOT SE would be more appropriate than classification with STOT RE.

Two MSCA suggested STOT RE 2 for slightly different reasons. The first MSCA considered that the clinical signs of neurotoxicity were more severe at the same dose after repeated dosing in both 90 day dog studies compared to after single dosing. The MSCA also noted that the combined NOAEL for repeated dose effects in the 90 day dog studies (40 mg/kg bw/d) is lower

than the combined NOAEL for acute effects in the 90 day dog studies (60 mg/kg bw/d). Finally, it was noted that recovery was not always complete in the first subchronic study. The second MSCA commented that myelin digestion chambers are now seen as the hallmark of Wallerian degeneration that takes place after axonal injury. The MSCA considered that this might have another cause than the clinical symptoms (basis for STOT SE 1) and therefore could warrant classification with STOT RE 2.

One industry stakeholder considered that there is no evidence of a direct subchronic neurotoxic effect of pyridate and therefore that a STOT RE classification would not be appropriate.

Assessment and comparison with the classification criteria

Ten oral repeated dose studies in rats, mice and dogs are available. These studies range from 28 days to 2 years in duration. In addition, there is one available repeated dose toxicity study via the dermal route. The main concern for pyridate is whether there is an acute or repeated dose neurotoxic effect and whether this is severe enough for classification.

Oral

Rat, 28 days

Two 4-week dietary studies in rats are available. In both studies, pyridate was administered to Wistar rats at doses of up to approximately 1180/1170 mg/kg bw/d in males/females, respectively. Sprague Dawley (SD) rats were also included in the second study. This summary will focus on the effects observed at doses below or close to the guidance value for classification (300 mg/kg bw/d).

There were no deaths or clinical signs reported in either of the studies. Decreased bodyweight and food consumption was observed in both sexes in both studies.

A small but significant decrease in haemoglobin was observed in female SD rats at 354 mg/kg bw/d. In Wistar rats, small effects on the blood were observed at the top dose only.

In both studies, significant changes to organ weights tended to be restricted to the top dose only. No adverse histopathology was reported in the first study, although investigations were limited to controls and top dose animals only. Similarly, organs do not appear to have been examined histopathologically in the second study and therefore it is unclear whether any organ weight changes represent adverse effects.

In the first study, slight peribronchial and peribronchiolar lymphoid aggregates were observed in 3/10 males at 354 mg/kg bw/d and in 5/10 top dose females. Slight focal pneumonia was observed in 1/10 top dose females.

Neither of the subacute studies in rats presents a cause for concern for STOT RE at dose levels relevant for classification.

Rat, 90 days

Two studies are available.

1) Albino CD rats (45/sex/dose) were exposed to 0, 62.5, 177 and 500 mg/kg bw/d (gavage in corn oil) for 90 days. Ten additional males and females were used in an ascending dose group (500/600 mg/kg bw/d). The following summary focuses on effects observed below or close to the guidance value for classification (100 mg/kg bw/d).

There was a dose-dependent increase in liver weight (relative to bodyweight) in females (significant from 177 mg/kg bw/d). A dose-related decrease in relative thymus weight was observed in females from 62.5 mg/kg bw/d.

Clinical signs (tabulated below) were reported to be reversible during the recovery period.

	Males			**Females**		
Dose (mg/kg)	**177**	**500**	**500/600**	**177**	**500**	**500/600**
Hypoactivity-1	3/45	36/45	5/10	1/45	29/45	5/10
Hypoactivity-2	0/45	7/45	0/10	0/45	7/45	0/10
Ataxic-1	0/45	2/45	0/10	0/45	3/45	0/10
Ataxic-2	0/45	1/45	0/10	0/45	1/45	0/10
Ptosis	0/45	1/45	0/10	0/45	0/45	0/10
Lateral roll	0/45	0/45	0/10	0/45	1/45	0/10

There also appears to have been signs of a slight anaemia after 13 weeks (at dose levels above the guidance values for classification), which reversed during the recovery period. Increased pigment in the spleen was observed from 177 mg/kg bw/d in males and from 62.5mg/kg bw/d in females.

Following histopathological examination, an increased incidence of basophilic material in the germinal follicles of mesenteric lymph nodes was observed in both sexes from 177mg/kg bw/d. After recovery, this effect was observed in males only.

At doses relevant for classification, the only effects were small and transient changes in clinical chemistry parameters and increased pigment in the spleen in females.

2) CD rats (10/sex/group) were exposed to 0, 400, 1200, 3600/4600/8000 and 6000ppm pyridate for 90 days (equivalent to approximately 0, 28.11, 86.01, 397.9 and 430 mg/kg bw/d for males; 0, 31.6, 96.01, 448.8 and 480 mg/kg bw/d for females).

No adverse effects were reported at dose levels relevant for classification.

Above the guidance values for classification, decreases in bodyweight, red blood cell concentration and haemoglobin levels, together with significant increases in urea and the presence of reducing substances in the urine were found. Mesenteric lymph nodes with follicles containing mineral deposits were observed at 3600/4600/8000 ppm only.

This study does not support classification of pyridate for specific target organ toxicity following repeated oral administration of pyridate to rats for 90 days at dose levels relevant for classification.

Rat, 2 years

Wistar rats (75/sex/dose) were exposed to pyridate (purity 90.3%) in the diet at doses of 0, 3.6, 18 and 115 mg/kg bw/d for 121 weeks. The following summary focusses on effects observed below or close to the guidance value for classification (12.5mg/kg bw/d).

In the absence of clear dose-response relationships, there is considered to be no clear evidence of the blood being a target organ of toxicity in rats following exposure to pyridate. Significant reductions in haemoglobin were observed in females only at 79 weeks (10.1, 9.2, 10. 9mmol/l) and 120 weeks (9.6, 8.6, 9.2, 9.1mmol/l) at 0, 3.6, 18 and 115 mg/kg bw/d. Similarly, significant reductions in red blood cells were observed in low and top dose females at weeks 79 and 120. Decreases in white blood cells were observed and were significant in top dose females at 79 weeks and in low and top dose females at week 120. Measurements of packed cell volume (PCV) were inconsistent but were significantly lower in treated females than in controls at week 120.

Lactate dehydrogenase (LDH) levels increased in a dose dependent manner in males at week 121 but was significant at the top dose only (495.6, 550.4, 593.1, 755.8 U/l at 0, 3.6, 18 and 115 mg/kg bw/d).

Under the conditions of this study, there was no clear evidence of a treatment-related repeated dose effect at doses relevant for classification.

Summary of findings in rats

In rats, there were only minor and transient changes at doses levels relevant for classification.

Mouse, 28 days

In a 4-week dietary study, Swiss mice (10/sex/group) were exposed to pyridate up to 10000 ppm (1880 and 2240mg/kg bw/d in males and females, respectively). No deaths or clinical signs were reported. Increases in relative weights of the liver and spleen were observed in treated animals. However, in the absence of histopathological data, it is not possible to conclude on the biological significance of these changes in organ weights.

Mouse, 18 months

B6C3F1 mice were exposed to 0, 400, 800, 1200/1400/1600 (ascending dose) and 7000ppm pyridate (91.5% purity) in the diet for 18 months. These doses are equivalent to 0, 48, 98, 170 and 853 mg/kg bw/d for males; 0, 55, 115, 204 and 1045 mg/kg bw/d for females. Neoplastic findings are reported in the carcinogenicity section. All dose levels in this study were above the guidance value for classification (16.7mg/kg bw/d).

Incidences of mortality increased in females only at the top 2 doses. There were no reports of treatment-related clinical signs.

Histopathological effects were observed in the adrenal gland, liver, salivary gland, kidneys, lung, pancreas, thymus and ovary and tended to be restricted to the top dose only. Since all dose levels exceeded the guidance values for classification, no further details are provided here.

Summary of findings in mice

There is no evidence in mice to support classification of pyridate for STOT-RE effects.

Dog, 90 days

In the first of two 90-day studies, beagles were exposed to 0, 20, 60 and 200 mg/kg bw/d in a gelatine capsule. Deaths (4/4 males; 3/4 females) were observed at the top dose only, which is above the guidance value for classification (100mg/kg bw/d). There were no significant bodyweight changes. Decreased food consumption was observed in both sexes at the top dose.

Clinical signs are tabulated below. Onset of symptoms was reported at 1-3 hours post dosing. Clinical signs observed at the low and mid doses were reported to be reversible. Complete recovery occurred within 24 hours for exposure up to 19 days, however for longer exposures it was reported that recovery was not always complete.

Dose (mg/kg bw/d)	**0**	**20**		**60**		**200**	
		males	**females**	**males**	**females**	**males**	**females**
Emesis		1/4	2/4	4/4	4/4	4/4	4/4
Salivation		0/4	0/4	2/4	2/4	4/4	4/4
Ataxia		0/4	0/4	1/4	2/4	4/4	4/4
Mydriasis		0/4	0/4	3/4	1/4	4/4	4/4
Nystagmus		0/4	0/4	0/4	1/4	4/4	4/4
Hypoactivity		0/4	0/4	0/4	0/4	4/4	4/4
Muscle fasciculations		0/4	0/4	0/4	0/4	4/4	4/4
Head swing		0/4	0/4	0/4	0/4	1/4	1/4
Opisthotonus*		0/4	0/4	0/4	0/4	4/4	4/4
Head tilt		0/4	0/4	0/4	0/4	0/4	2/4

*tetanic spasm of the back muscles

For comparison, clinical signs observed in animals after the first dose are tabulated below.

Dose (mg/kg bw)	**0**	**20**	**60**		**200**	
			males	**females**	**males**	**females**
Emesis	0/4	0/4	1/4 (grade 1)	1/4 (grade 1)	4/4 (grade 2-3)	4/4 (grade 3)
Salivation	0/4	0/4	0/4	0/4	0/4	1/4
Ataxia	0/4	0/4	0/4	0/4	4/4 (grade 3)	3/4 (grade 3)
Mydriasis	0/4	0/4	0/4	0/4	4/4	4/4
Nystagmus	0/4	0/4	0/4	0/4	4/4	3/4
Hypoactivity	0/4	0/4	0/4	0/4	3/4	2/4
Muscle fasciculations	0/4	0/4	0/4	0/4	2/4	0/4
Head swing	0/4	0/4	0/4	0/4	1/4	0/4
Opisthotonus	0/4	0/4	0/4	0/4	4/4 (grade 2)	3/4 (grade 2-3)

The same clinical signs were observed in animals exposed to either a single dose or repeated doses of pyridate although the incidences of the clinical signs were greater after repeated exposure, particularly at 60 mg/kg bw/d. It was reported that the clinical signs were generally less severe with increasing exposure period. However, data on the severity of effects after repeated exposure are not available and therefore it is not possible to confirm this assertion.

On this basis, there appears to be a minor repeated dose effect of pyridate exposure on clinical signs.

Minimal to slight degenerative myelinopathy of the sciatic nerve was observed in top dose animals only (2/4 males and 2/4 females). (Broncho)pneumonia was observed in 1/4 low dose females, 2/4 top dose males and 2/4 top dose females compared to 0/4 for both male and female controls.

Serum glutamic-pyruvic transaminase (SGPT) (U/l) decreased in both sexes and was significant in males at 60 mg/kg bw/d. Erythrocyte cholinesterase levels increased in treated males compared to controls (2.32, 3.05 and 3.15 M/l/min at 0, 20 and 60 mg/kg bw/d). No data were presented on brain cholinesterase inhibition.

Under the conditions of this study, there were only observations of various clinical signs at dose levels relevant for classification. Although degenerative myelinopathy was observed, this was above the dose level relevant for classification.

In the second 90-day study, Beagles (5/sex/group) were exposed to 0, 40, 80 and 120 mg/kg bw/d by gavage for 90 days. At the top dose, reduced bodyweight gain (16%) and reduced food consumption was observed in females. There was no treatment-related effect on bodyweight.

No clinical signs were reported in controls. At the low dose, head shaking was observed in 1 male. Clinical signs were described as mild to moderate at the mid dose and marked at the top dose. Onset of symptoms was observed within 1.5 hours of dosing and symptoms were mostly gone after 6 hours. The incidences of clinical signs at the mid and high doses are tabulated below.

	Males		**Females**	
Dose (mg/kg bw/d)	**80**	**120**	**80**	**120**
Head shaking	0/5	0/5	1/5	0/5
Salivation	2/5	5/5	2/5	5/5
Ataxia	1/5	5/5	4/5	5/5
Vacant expression	1/5	0/5	0/5	0/5
Underactivity	2/5	2/5	2/5	5/5
Hunched posture	0/5	5/5	3/5	5/5
Emesis	0/5	5/5	1/5	5/5
Pupils dilated	0/5	0/5	2/5	0/5
Prostration	0/5	4/5	0/5	5/5
Opisthotonus	0/5	5/5	0/5	5/5
Tremor	0/5	1/5	0/5	3/5

For comparison, the following clinical signs were observed after the first dose.

	Males		**Females**	
Dose (mg/kg bw/d)	**80**	**120**	**80**	**120**
Emesis	0/5	2/5	0/5	3/5
Ataxia	0/5	2/5	0/5	0/5
Underactivity	0/5	0/5	1/5	1/5
Opisthotonus	0/5	2/5	0/5	0/5

Evidently, more clinical signs and higher incidences of those clinical signs were observed in animals following repeated exposure compared to after a single dose. There are no available data to inform on whether the duration of exposure had any effect on the severity of the clinical signs.

Brain focal gliosis was observed in one top dose male only. This isolated incidence is not considered to present cause for concern. Left sciatic nerve myelin digestion chambers were observed in 1, 0, 0, 2 males and 2, 1, 0 and 1 females at 0, 40, 80 and 120 mg/kg bw/d, respectively. It is noted that myelin digestion chambers in sciatic nerves were not detected in the historical data. However, in the absence of a clear dose-response, the finding is not clearly treatment-related.

After 12 weeks, haemoglobin levels in both sexes were lower than controls from 80mg/kg bw/d but the effect was significant in mid dose females only. Red blood cell levels were significantly lower in mid and top dose animals compared to controls. Similarly, mean corpuscular volume remained significantly increased in both sexes at the top dose. Heinz bodies were observed at the mid dose (4 males, 1 female) and top dose (5 males, 5 females) vs 0 animals in controls. The reticulocyte count was greater than 2% in 0, 1, 4, and 4 males and 0, 0, 1 and 2 females in at 0, 40, 80 and 120 mg/kg bw/d, respectively. Significant increase in platelets was observed in all dose groups (significant from 80 mg/kg bw/d). Increased cellularity of the sternal bone marrow was observed in 4/5 males and females at the top dose only. The study author considered this to be a normal response to the decreased red cell parameters.

The relative weights of the liver and kidneys increased > 10% in both sexes from 80mg/kg bw/d. Increased incidences of pigmentation of the Kupffer cells occurred from 80 mg/kg bw/d in both sexes. Alanine amino transferase (ALT) was reduced in all treated dose groups. There were no treatment-related effects on AP and AST activity or on bilirubin concentration. Significant increases in albumin were observed in top dose animals (both sexes) at weeks 6 and 12. At 40mg/kg bw/d, the decrease in ALT was not accompanied by a change > 10% in relative liver weight or histopathological changes.

There was a dose-related increase in the absolute weight of the ovary (significant at the top dose) but no corresponding histopathological changes were reported. There were single isolated incidents of epididymides aspermia and testes immaturity in top dose males.

Additional effects observed at histopathological investigation are tabulated below.

	Males				**Females**			
Dose (mg/kg bw/d)	**0**	**40**	**80**	**120**	**0**	**40**	**80**	**120**
Thyroid parafollicular cell hyperplasia	2/5	0/5	1/5	2/5	1/5	1/5	1/5	3/5
Lung bronchopneumonia	0/5	0/5	0/5	1/5	0/5	0/5	1/5	1/5

The study author considered these remaining histopathological findings in dogs to be consistent with the changes one would expect to see for animals of this age under laboratory conditions. According to historical control data, as noted in the CLH report, parafollicular cell hyperplasia was observed in 0-100% of animals (mean 8.22%). Given this information, the findings in this study are not considered to present a cause for concern.

This study is considered to be consistent with the first 90-day study in dogs because there were observations of clinical signs at dose levels relevant for classification. In contrast to the first study, there was also evidence of blood toxicity at dose levels close to the guidance value for classification.

Dog, 52-weeks

Beagles (5/sex/group) were given ascending doses of pyridate of 0, 5/10/30 (low), 20/60/80/100 (mid) and 60/100/120/140/150 mg/kg bw/d (high) by gavage for 52 weeks.

Body weight losses of up to 16% were observed in mid and high dose animals from week 35 onwards.

Clinical signs (tabulated below) were described as mild to moderate at the mid dose and marked in the high dose group. Emesis was the only clinical sign observed below 100mg/kg bw/d. It was reported that the major clinical signs in the mid dose group occurred at 100mg/kg bw/d and in the high dose group at 120mg/kg bw/d and higher.

	Males		**Females**	
Dose group	**Mid**	**Top**	**Mid**	**Top**
Emesis	5/5	5/5	5/5	5/5
Salivation	2/5	2/5		5/5
Ataxia	4/5	4/5	2/5	5/5
Tremors	1/5	1/5		
Hunched posture	1/5	2/5		
Mydriasis	3/5	4/5	2/5	4/5
Unable to stand			1/5	
Prostrate		4/5		2/5
Nystagmus		1/5		

Nerve sciatic degenerative myelopathy were observed in one top dose male.

There were no treatment-related haematological effects. Decreased serum globulin levels were observed at the top dose (both sexes), but the changes were significant in females at week 52 only. Increased spleen extramedullary haematopoiesis was reported in one top dose male.

The clinical signs observed in this study are consistent with those observed in the subchronic studies in dogs. The isolated reports of histopathological effects do not present a cause for concern.

Summary of findings in dogs

In dogs, clinical signs of generalised toxicity were consistently observed at dose levels relevant for classification. In addition, there is some limited evidence of blood toxicity. Degenerative myelinopathy or myelin digestion chambers were reported in all of the dog studies.

Re-evaluation of the histopathological findings in dogs

In light of the findings in dogs, the neurotoxic potential of pyridate was reviewed. The slides of the sciatic nerves from all three dog studies were peer reviewed in 1997. The CLH report does not provide specific details of the terms of reference for this review. Originally, the

findings in the first dog study had been described as 'degenerative myelinopathy'. The reviewing pathologists agreed with the grading of this effect but renamed it to 'myelin digestion chambers' and noted the finding in additional dogs. The incidences and severity of myelin digestion chambers in the dog studies after a re-evaluation of the histopathology of the sciatic nerves are tabulated below.

90 day dog study (Tompkins, 1987)

	Males				**Females**			
Dose (mg/kg bw/d)	0	20	60	200	0	20	60	200
Myelin digestion chambers (grade)	0/4	0/3	0/4	3/4 (1, 1, 2)	0/4	0/4	0/4	3/4 (1, 1, 1)

90 day dog study (Vandaele, 1990)

	Males				**Females**			
Dose (mg/kg bw/d)	0	40	80	120	0	40	80	120
Myelin digestion chambers (grade)	1/5 (1)	0/5	0/5	2/5 (1)	0/5	2/5 (1)	0/5	3/5 (1)

One year dog study (Bailey, 1989)

	Males				**Females**			
Dose (mg/kg bw/d)	0	5/10/30	20/60/ 80/100	60/100/120/ 140/150	0	5/10/30	20/60/ 80/100	60/100/120/ 140/150
Myelin digestion chambers (grade)	0/5	1/5 (1)	2/5 (1)	1/5 (2)	0/5	0/5	0/5	1/5 (1)

The reviewing pathologists commented that these changes are commonly observed in sections of sciatic nerves from a number of different species/strains of laboratory animals and tend to be more common and severe in older animals, particularly in mice and rats. They did not consider that these changes represented toxic effects of the test substance.

In response to a comment submitted during the public consultation, the DS considered that while Wallerian degeneration is a hallmark of neurodegenerative diseases, it is not observed in isolation. Since no other histopathological observations were made in any of the long-term studies in any species tested, RAC agrees with the DS that the findings in dogs described above do not support classification for STOT RE.

<u>Effects observed below the guidance values for classification after repeated oral dosing of pyridate</u>

Study	**Guidance values (mg/kg bw/d)**	**Effects for Cat. 1**	**Effects at or below guidance value for classification in Cat. 2**
Rats, Wistar, 4 weeks	*Cat. 1 ≤ 30* *30 < Cat. 2 ≤ 300*	N/A	None
Rats, Wistar and SD, 4 weeks	*Cat. 1 ≤ 30* *30 < Cat. 2 ≤ 300*	N/A	N/A

CD rats, Albino, 90 days with 28 day recovery	*Cat. 1 ≤ 10* *10 < Cat. 2 ≤ 100*		<u>62.5 mg/kg bw/d</u> ↓ creatinine (males, 0.6 vs 0.7 mg/dl) ↑ alkaline phosphatase (females, 44.6 vs 33.9 U/l in controls) ↑ SGPT (males, 31.4 vs 29.9 U/l in controls) ↑ pigment in the spleen (females) ↑ RBC cholinesterase – females (1.245 v 0.97 mM/l/min in controls)
Rats, CD, 90 days	*Cat. 1 10 ≤* *10 < Cat. 2 ≤ 100*	N/A	None
Rats, Wistar, 121 weeks	*Cat. 1 ≤ 1.25* *1.25 < Cat. 2 ≤ 12.5*	N/A	<u>3.6 mg/kg bw/d</u> ↓ haemoglobin and red blood cells (females, no dose response) ↑ LDH levels (males)
Mice, Swiss, 4 weeks	*Cat. 1 ≤ 30* *30 < Cat. 2 ≤ 300*	N/A	<u>188 & 224 mg/kg bw/d in males and females, respectively</u> ↑ spleen and liver weight (females, non-significant)
Mice, B6C3F1, 18 months	*Cat. 1 ≤ 1.67* *1.67 < Cat. 2 ≤ 16.7*	N/A	N/A
Dogs, beagle, 90 days (4/sex/ group)	*Cat. 1 ≤ 10* *10 < Cat. 2 ≤ 100*	N/A	<u>20 mg/kg bw/d</u> Emesis (1 male, 2 females) ↑ relative liver weight (significant at this dose only) ↓ SGPT (males) ↑ Erythrocyte cholinesterase levels (males) (Broncho)pneumonia (1 female) <u>60 mg/kg bw/d</u> Emesis (all animals), Salivation (2 males, 2 females), Ataxia (1 male, 2 females), Mydriasis (3 males, 1 female), Nystagmus (1 female) ↓ SGPT (both sexes, significant in males) ↑ Erythrocyte cholinesterase levels (males)
Dogs, beagle, 90 days, 5/sex/ group	*Cat. 1 ≤ 10* *10 < Cat. 2 ≤ 100*	N/A	<u>40 mg/kg bw/d</u> Head shaking (1 male) Myelin digestion chambers (grade 1) (2 females) <u>80 mg/kg bw/d</u> Head shaking (1 female), salivation (2 males, 2 females), ataxia (1 male, 4 females), vacant expression (1 male), underactivity (2 males, 2 females), hunched posture (3 females), emesis (1 female), pupils dilated (2 females) Heinz bodies (4 males, 1 female), ↓ haemoglobin and red blood cells (both sexes), ↑ platelets (both sexes, significant) ↓ ALT (both sexes) ↑ incidence of pigmentation of Kupffer cells Lung bronchopneumonia (1 female)

Dogs, beagle, 1 year	*Cat. 1 ≤ 2.5* *2.5 < Cat. 2 ≤ 25*	N/A	Ascending dose of 5/10/30 mg/kg bw/d Emesis (1/5 males at 5 mg/kg bw/d in week one of dosing) Myelin digestion chambers (grade 1) in 1 male

N/A = not applicable

The dog appears to be more sensitive than rats and mice to the effects of oral administration of pyridate. However, there does not appear to be a specific target organ effect following repeated exposure to dose levels relevant for classification.

Dermal

SD rats (5/sex/dose) were exposed to 0 or 1000mg/kg bw/d. Pyridate (91.5% purity) was applied to an area of approximately 10% of the total body surface area on 21 consecutive days.

There was no treatment-related effect on bodyweight. ALT values increased in treated animals (significantly in males only). After adjustment for differences in final bodyweight, liver weight was significantly higher in treated animals (both sexes) compared to controls.

Skin encrustation was observed in 3/5 treated males and dark areas on the skin were reported in 1/5 treated males and 1/5 treated females. Two treated males had scab(s) on the treatment site and epidermal hyperplasia was observed on the treatment site in 4/5 males and 4/5 females. There were no signs of adverse systemic toxicity.

Inhalation

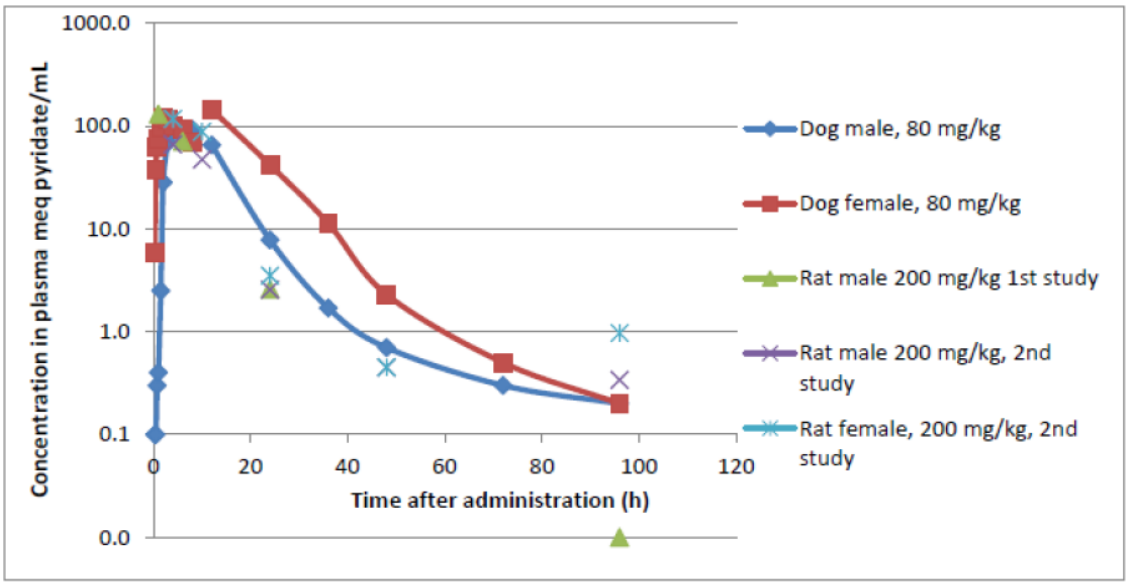
No data were presented in the CLH report.

RAC Conclusion on STOT RE

RAC considers that no specific toxicity developed after repeated exposure to pyridate. The observed effects were of a general nature and were most likely due to incomplete recovery from acute effects as daily doses were administered. Therefore, RAC concludes that **no classification for STOT RE is appropriate**.

Supplemental information - In depth analyses by RAC

The applicant submitted position papers regarding neurotoxicity and classification and labelling. Kinetic data of the dog (80 mg/kg bw/d) and rat (200 mg/kg bw/d) were compared, as shown in the graph below.



This graph shows that 24 hours after exposure to pyridate, the test substance was still present in the blood of both rats and dogs. This is considered to support the assertion that there is no specific repeated dose effect of pyridate. Rather, the observed effects are the result of multiple acute doses, which were given before the substance has been cleared from the blood following the previous exposure(s).

3.9 Germ cell mutagenicity (Mutagenicity)

3.9.1 Non-human information

Table 51: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
***In vitro* studies**			
Reverse mutation assay (S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100)	1, 10, 100, 500, 1000, 2500, 5000, 10000 µg/plate **Negative** (+/- S-9 mix)	-	Hoorn, 1986a
Reverse mutation assay (E. coli WP2uvrA$^-$)	01, 10, 100, 500, 1000, 2500, 5000, 10000 µg/plate **Negative** (+/- S-9 mix)	-	Hoorn, 1986b
Reverse mutation assay (*Bacillus subtilis* H17 and M4))	1, 10, 100, 500, 1000, 2500, 5000, 10000 µg/plate **Negative** (+/- S-9 mix)	-	Hoorn, 1986c
Reverse mutation assay (S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100)	1, 10, 100, 500, 1000, 2500, 5000, 10000 µg/plate **Negative** (+/- S-9 mix)	-	Hoorn, 1986a
Reverse mutation assay (S. typhimurium TA 98, TA 100, TA102, TA 1535 and TA 1537)	10, 31.6, 100, 316, 1000 and 3160 µg/plate suspended in DMSO **Negative** (+/- S-9 mix)	-	Flügge, 2012
Chromosome aberration test in CHO cells	0, 20, 50, 100 and 200 µg/ml (- S-9 mix) 0, 20, 50, 100 and 167 µg/ml (+ S-9 mix) dissolved in HEPES-buffered cell culture medium **Negative** (+/- S-9 mix)	-	Taalam, 1987
Unscheduled DNA synethesis test in rat hepatocytes	3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500 nl/ml **Negative**	-	Myhr & Brusick, 1981
***In vivo* studies**			
Micronucleus test in mice (Swiss Random)	400, 1300, 4000 mg/kg bw **Negative**	-	Taalman, 1986
Somatic cell mutation (Mouse spot test)	0.073, 0.242, 0.725 g/kg On day 9, 10 and 11 of pregnancy p.o. **Negative**	-	Nguyen & Brusick, 1980
Method of Mirsalis 1982, FIFRA § 84-2 (Rats (Fisher 344), rat hepatocytes)	40, 160, 800 ml/kg p.o. **Negative**	-	Curren, 1988

3.9.2 Human information

Not available.

3.9.3 Other relevant information

Not available.

3.9.4 Summary and discussion of mutagenicity

All studies evaluated in support of the Annex I listing of pyridate showed no genotoxic potential. All tests were performed under GLP and are still considered to be valid. Furthermore a new bacterial point mutation assay (Ames test) was conducted (Flügge 2012). The tests did not indicate any genotoxic potential.

3.9.5 Comparison with criteria

Pyridate was tested negative for genotoxicity in a battery of *in vitro* and one *in vivo* test.

The substance therefore does not meet the criteria for classification for germ cell mutagenicity.

3.9.6 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: No classification proposed

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The CLH repprt includes several old mutagenicity studies performed under GLP which are considered to be valid. Furthermore a new bacterial point mutation assay (Ames test) was conducted (Flügge 2012). The tests did not indicate any genotoxic potential.

Since a battery of *in vitro* and one *in vivo* test on pyridate were negative , the DS considered that the substance does not meet the criteria for classification for germ cell mutagenicity.

Comments received during public consultation

No comments relating to mutagenicity were submitted during the public consultation, although one MSCA made a general comment supporting the classification proposal.

Assessment and comparison with the classification criteria

The results of the available studies are tabulated below.

Method	Results	Reference
***In vitro* studies**		
Reverse mutation assay (S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100)	1 - 10000 µg/plate **Negative** (+/- S-9 mix)	Hoorn, 1986
Reverse mutation assay (E. coli WP2uvrA-)	1, - 10000 µg/plate **Negative** (+/- S-9 mix)	Hoorn, 1986
Reverse mutation assay (*Bacillus subtilis* H17 and M4))	1 - 10000 µg/plate **Negative** (+/- S-9 mix)	Hoorn, 1986
Reverse mutation assay (S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100)	1 - 10000 µg/plate **Negative** (+/- S-9 mix)	Hoorn, 1986
Reverse mutation assay (S. typhimurium TA 98, TA 100, TA102, TA 1535 and TA 1537)	10 - 3160 µg/plate suspended in DMSO **Negative** (+/- S-9 mix)	Flügge, 2012
Chromosome aberration test in CHO cells	0 - 200 µg/ml (- S-9 mix) 0 - 167 µg/ml (+ S-9 mix) dissolved in HEPES-buffered cell culture medium **Negative** (+/- S-9 mix)	Taalam, 1987
Unscheduled DNA synthesis test in rat hepatocytes	3.91 - 500 nl/ml **Negative**	Myhr & Brusick, 1981
***In vivo* studies**		
Micronucleus test in mice (Swiss Random)	400, 1300, 4000 mg/kg bw **Negative**	Taalman, 1986
Somatic cell mutation (Mouse spot test)	0.073, 0.242, 0.725 g/kg On day 9, 10 and 11 of pregnancy p.o. **Negative**	Nguyen & Brusick, 1980
Unscheduled DNA synthesis test Method of Mirsalis 1982, FIFRA § 84-2 (Rats (Fisher 344), rat hepatocytes)	40, 160, 800 ml/kg p.o. **Negative**	Curren, 1988

Negative results were obtained in all *in vitro* (reverse mutation, chromosome aberration and UDS) and *in vivo* (micronucleus, somatic cell mutation, method of Mirsalis) studies, therefore there is no evidence of mutagenicity. RAC concludes that no classification is appropriate for this endpoint.

3.10 Carcinogenicity

Table 52: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Chronic toxicity / Oncogenicity study in Wistar rats	0, 3.6, 18 and 115 mg/kg bw/d (diet) NOAEL = 18 mg/kg bw/d LOAEL = 115 mg/kg bw/d based on: ↓ food consumption, bw and bw gain, changes in haematology (F) no carcinogenic effects	-	Til, 1982 (revised 1990)
Oncogenicity study in B6C3F1 mice	0, 400, 800, 1200/1400/1600 (ascending dose) and 7000 ppm/ diet (equivalent to 0, 48, 98, 170 and 853 mg/kg bw/d for males; 0, 55, 115, 204 and 1045 mg/kg bw/d for females) NOAEL = 800 ppm LOAEL = 1200/1400/1600 ppm based on: ↓ bw at 1600 ppm and ↑mortality at 7000 ppm; Non significantly increased tumour incidence in the liver in top dose males above MTD, within the historical control range	-	Lindamood, 1991 (historical control data from Kobel, 2013)

3.10.1 Non-human information

3.10.1.1 Carcinogenicity: oral

Rat

The following study was evaluated in support of Annex I listing of pyridate and is no longer granted data protection:

IIA 5.5.1/01 Til H.P et al. (1982, revised 1990) Lifespan oral carcinogenicity study of Pyridate in rats. TNO Project No.: B 80-0023

Pyridate (batch No. 1001410, CL 11344, purity: 90.3%) was applied at 0, 3.6, 18 and 115 mg/kg bw/d to groups of 75 male and female SPF Wistar rats via diet for 121 weeks. Ten animals per sex and dose were sacrificed after one year. Fifteen animals per sex and dose were sacrificed after two years. The terminal sacrifice was in week 121.

Table 53: Body weight and food consumption in the oral long term and carcinogenicity study in rats from the DAR (Til et al., 1982, revised 1990)

Dose (mg/kg bw/day)	0		3.6		18		115	
	male	female	male	female	male	female	male	female
Body weight in g (day 0)	81.2	72.6	81.5	72.8	81.1	72.2	81.2	72.7
Body weight in g (day 364)	480.7	274.8	485.8	275.5	481.5	271.4	465.1	252.2*
Body weight in g (day 686)	485.5	283.9	494.4	307.4	470.3	311.8*	446*	250.1*
Body weight in g (day 845)	455.3	282.4	419.8	295.5	420.2	291.1	479.2	281.1
Mean food intake in g (day 7)	109.1	92.2	105.8	89.7	108.6	93.3	95*	85.5*

* statistically significant

Body weight and food intake was transiently decreased > 10% in top dose animals. No treatment related mortality or clinical signs could be observed in the study.

Table 54: Haematology in the oral long term and carcinogenicity study in rats from the DAR (Til et al., 1982, revised 1990)

Dose (mg/kg bw/day)	0		3.6		18		115	
	male	female	male	female	male	female	male	female
Week 79								
HB (mmol/l)	8.7	10.1	9.3	9.2*	9.3	10	9.3	9*
PCV (l/l)	0.458	0.528	0.489*	0.482*	0.488	0.529	0.484	0.485*
RBC (10E12/l)	7.2	7.6	7.5	6.9*	7.4	7.5	7.3	6.8*
WBC (10E9/l)	17.1	15.9	19.9	12.7	18	13.7	16.7	12*
Week 120								
HB (mmol/l)	9.2	9.6	9.5	8.6*	8.9	9.2	9.3	9.1
PCV (l/l)	0.478	0.505	0.491	0.441*	0.468	0.463*	0.466	0.468*
RBC (10E12/l)	7.2	7.1	7.5	6.2*	7.2	6.7	7.5	6.3*
WBC (10E9/l)	17.8	16.8	18.7	12.2*	19.9	16.3	19.6	13.4*

* statistically significant

Reduction in erythrocyte parameters and white blood cells (> 10%) were seen in top dose females.

Table 55: Clinical chemistry in the oral long term and carcinogenicity study in rats from the DAR (Til et al., 1982, revised 1990)

Dose (mg/kg bw/day)	0		3.6		18		115	
	male	female	male	female	male	female	male	female
Week 5								
GOT (U/l)	59.2	56.3	59.8	51.7	60.7	51.8	57.8	49.9*
LDH (U/l)	123.6	90.8	142	69*	131.7	78.1	94.3*	90.5
T3 Uptake (%)	50.1	49.2	49.4	49.5	48.6*	47.4	47.5*	47.8
T4 (nmol/l)	72.2	47	68	53.4	70.7	47.2	65.8	43
Week 53								
ALP (U/l)	**82.4**	**66.5**	**76.3**	**67**	**77.8**	**60.8**	**66.4***	**47.9***
GOT (U/l)	54.1	65.8	63.5	57	59.4	64.8	49.4*	48.5*
LDH (U/l)	132.5	128.9	146.1	83.6	125.8	100.8	129.5	72.6*
T3 Uptake (%)	51.9	54.3	50.7	55.4	54.1	56.3	53.1	52.3
T4 (nmol/l)	55.9	40.8	58.5	41.6	57.4	46.3	63.7	47.7
Week 121								
ALP (U/l)	98.2	88.5	104.8	72.1	97	91.6	95.9	91.1
GOT (U/l)	69	99.7	66.7	94.4	69.9	103.9	69.4	88.8
LDH (U/l)	495.6	502.5	550.4	434.2	593.1	488.1	755.8*	495.6
T3 Uptake (%)	40.4	43.3	39.6	44.8	42.4	42.5	41.3	43.6
T4 (nmol/l)	31.2	27.8	23.9	30.1	27.7	28.3	33.4	27.3

* statistically significant

Transient decrease in GOT, ALP and LDH was found in top dose animals. However at week 121 LDH was significantly increased in top dose males. Furthermore T3 uptake was significantly decreased at week 5 in males after application of 18 and 115 mg/kg bw/d pyridate.

Table 56: Organ weights in the oral long term and carcinogenicity study in rats from the DAR (Til et al., 1982, revised 1990)

Dose (mg/kg bw/day)	0		3.6		18		115	
	male	female	male	female	male	female	male	female
Week 52								
Relative kidney weight	5.3	6.4	5.6	6.4	5.8	6.2	6*	6.6
Relative thyroid weight	0.08	0.08	0.063*	0.075	0.053*	0.072	0.054*	0.089
Week 121								
Relative kidney weight	7.51	7.83	8.38	7.33	8.46	7.74	6.84	7.66
Relative thyroid weight	0.079	0.1	0.089	0.1	0.084	0.1	0.077	0.095

After 1 year kidney weight was increased in males of the 18 mg/kg bw/d group. Furthermore thyroid weight was decreased in males in all test groups after one year of treatment.

Absolute and relative organ weights were not affected by treatment at the end of the study. No treatment related histopathological changes could be found.

In the DAR the NO(A)EL was set at 18 mg/kg bw/d at 1, 2 year and terminal sacrifice, based on decreased body weight and body weight gain in top dose animals, which can be confirmed. In addition, haematological parameters were affected in top dose females as well as liver enzymes were transiently decreased in top dose animals.

Mouse

The following study was evaluated in support of Annex I listing of pyridate and is no longer granted data protection:

IIA 5.5.1/02 Lindamood C. et al. (1991) Oncogenicity Study of pyridate, administered by dosed feed to B6C3F1 mice. Project: SRI-Chm-01-603

Pyridate (Batch 2759523, CL 11344/AR-22 #2659427, purity: 91.5%) was applied to groups of 50 male and female B6C3F1 mice via diet for 18 months. Initial dose levels were 0, 400, 800, 1200/1400/1600 ppm (ascending dose). The dose of 1200 ppm was increased to 1400 ppm on day 91 and to 1600 ppm on day 179. An additional control group and a dose group receiving 7000 ppm were used directly after the original study. The dose levels corresponded to 0, 48, 98, 170 and 853 mg/kg bw/d in males and 0, 55, 115, 204 and 1045 mg/kg bw/d in females. Histophathology was evaluated in all animals of the top dose and concurrent control group as well as on early death animals from the low and mid dose groups. In addition gross lesions of lung, liver and kidney taken from mice in the low and mid dose groups were evaluated microscopically. In the Table 57 – Table 60 results are summarised.

Table 57: Mortalities in the carcinogenicity study in the mouse from the DAR (Lindamood et al., 1991)

Dose (ppm)	**0**	**0**	**400**	**800**	**1200/1400/1600**	**7000**
males						
mortality	2/50	2/50	2/50	1/50	0/50	3/50
females						
mortality	3/50	3/50	3/50	4/50	7/50	10/50

* statistically significant

Increased mortality was seen in females from 1200 ppm onwards and in males in the top dose group. No treatment related clinical signs were observerd.

Table 58: Body weight in the carcinogenicity study in the mouse from the DAR (Lindamood et al., 1991)

Dose (ppm)	**0**	**0**	**400**	**800**	**1200/1400/1600**	**7000**
males						
Body weight in g (day 0)	20.7	21.7	21.4	21.1	21.1	21.1
Body weight in g (day 536/534)	40.7	43.2	38.5	37.3	37.3	37.2
% final body weight	100	100	95	92	92	86
females						
Body weight in g (day 0)	17	17.1	17.6	16.4	16.9	17
Body weight in g (day 536/537)	41	45.1	39.1	38.7	35.6	34
% final body weight	100	100	95	94	87	75

* statistically significant

No treatment related effect was seen on food consumption.

Body weight was decreased > 10% in top dose animals and in females of the 1200/1400/1600 ppm group.

No treatment related effects were observed regarding haematological parameters.

Table 59: Organ weights in the carcinogenicity study in the mouse from the DAR (Lindamood et al., 1991)

Dose (ppm)	**0**	**0**	**400**	**800**	**1200/1400/1600**	**7000**
males						
Adrenal glands (absolute) in g	**0.0127**	**0.0076**	**0.0125**	**0.0109**	**0.0092***	**0.0114**
Adrenal glands (relative to bw)	0.3	0.2	0.3	0.3	0.2	0.3
Liver (relative to bw)	45.9	49.2	43.1	49.3	51.1	55.7
females						
Adrenal glands (absolute) in g	0.0118	0.0138	0.0122	0.0134	0.0124	0.0113
Adrenal glands (relative to bw)	0.3	0.3	0.3	0.4*	0.3	0.3
Liver (relative to bw)	45.4	38.6	43.7	47.5	50	55.1*

* statistically significant

Liver weight was increased > 10% from 1200/1400/1600 ppm onwards. Absolute weight of adrenal glands was significantly reduced in 1200/1400/1600 ppm males but not in top dose ones.

Table 60: Histopathology in the carcinogenicity study in the mouse from the DAR (Lindamood et al., 1991)

Dose (ppm)	0	0	400	800	1200/1400/1600	7000
Males (animals with effect/animals evaluated)						
Adrenal hyperplasia capsule	0/50	0/0	0/2	0/1	0/0	1/50
Adrenal hyperplasia focal	0/50	0/0	0/2	0/1	0/0	1/50
Adrenal hypertrophy focal	13/50	0/0	0/2	0/1	0/0	3/50
Liver basophilic focus	3/50	0/0	3/50	1/50	0/0	4/50
Liver eosinophilic focus	1/50	0/0	0/50	0/50	0/0	3/50
Salivary gland atrophy	3/50	0/0	0/2	0/1	0/0	5/50
Adrenal gland cortex adenoma	0/50	0/0	0/2	0/1	0/0	1/50
Adrenal gland-medulla pheochromocytoma malignant	0/50	0/0	0/2	0/1	0/0	1/50
Liver Hepatocellular adenoma	7/50	0/0	12/50	10/50	0/0	5/50
Liver Hepatocellular carcinoma	5/50	0/0	7/50	7/50	0/0	11/50
Lung: malignant lymphoma	0/50	0/0	0/50	0/50	0/0	1/50
Lymph node malignant-mediastinal	0/0	0/0	0/1	0/0	0/0	1/3
Pancreas: Hemangiosarcoma	0/50	0/0	0/2	0/2	0/0	1/50
Females (animals with effect/animals evaluated)						
Adrenal gland degeneration fatty zone	0/50	0/0	0/3	0/3	0/0	1/50
Kidneys cast proteins	11/50	0/0	15/50	9/49	0/0	19/50
Kidneys dilatation renal tubule	0/50	0/0	0/50	1/49	0/0	3/50
Liver eosinophilic focus	0/50	0/0	0/50	0/49	0/0	3/50
Liver hema. cell proliferation	1/50	0/0	2/50	1/49	0/0	6/50
Lung hyperplasia alveolar epithelium	1/50	0/0	2/49	1/49	0/0	4/50
Lung inflammation subacute	0/50	0/0	0/49	0/49	0/0	1/50
Pancreas inflammation suppurative	0/50	0/0	2/4	0/0	0/0	5/50
Pancreas necrosis	0/50	0/0	0/4	0/0	0/0	1/50
Thymus necrosis	0/49	0/0	0/3	0/3	0/3	3/48
Ovary inflammation suppurative	1/49	0/0	3/14	2/11	0/0	10/50
Liver: Hepatocellular adenoma	6/50	0/0	4/50	3/49	0/0	8/50
Liver Hepatocellular carcinoma	7/50	0/0	0/50	1/49	0/0	2/50

* statistically significant

Several histopathological findings were observed in top dose animals. Tumour incidence in the liver is (not statistically significant) increased in top dose males. No historical control data are available within the study report. The top dose exceeds the MTD (maximum tolerated dose), i.e. mortality was observed (3/50 in males, 10/50 in females). The increase in liver tumour incidence in top dose males is considered as effect associated with excessive toxicity, i.e. cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development, rather than as substance related (cf. ECHA: Guidance on the Application of the CLP Criteria, Version 4, November 2013). In order to verify the relevance of the findings for classification, historical control data were requested from the notifier.

In the DAR the NO(A)EL was set at 800 ppm, equivalent to 98 mg/kg bw/d in males and 115 mg/kg bw/d in females, based on decreased body weights and reduced survival rates in females, which can be confirmed.

Reference:	**Pyridate Historical control data for mouse liver tumours**
Author(s), year:	Kobel W., 2013
Report/Doc. number:	Expert statement
Guideline(s):	Not applicable
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Yes

The contract facility having conducted the study was contacted and historical control data relating to hepatocellular adenoma and carcinoma were requested. As the study under discussion had started in November 1989, the period to be covered was set from 1985 to 1992.

Within the set period of 1985 – 1992 (starting date of dosing: 25th August 1986 to 14th January 1991) a total of 12 studies of comparable size and design had been conducted at the facility with B6C3F1 mice.

Their duration was 103 – 105 weeks, the group size for majority of studies was 50 – 60 (49-110) animals per sex and group. The routes of applications were: gavage 6, feed 4, water 1, subcutaneous 1.

Table 61: Summary Historical control data from chronic B6C3F1 mouse studies started at SRI 1986 - 1991

	# with lesion [route via feed and water only]	**# examined [route via feed and water only]**	**% (range) [route via feed and water only]**
Males			
Liver, Hepatocellular Adenoma	179	718	25 (17–34)
Liver, Hepatocellular Adenoma, Multiple	74	658	11
Liver, Hepatocellular Adenoma, Multiple, Two	2	119	2
Liver, Hepatocellular Adenoma, includes all Hepatocellular Adenoma above	**255 [121]**	**718 [298]**	**36 (18-68) [18-68]**
Liver, Hepatocellular Carcinoma	141	718	20 (8-34)
Liver, Hepatoicellular Carcinoma, Multiple	45	599	8
Liver, Hepatocellular Carcinoma, Multiple Two	1	60	2
Liver, Hepatocellular Carcinoma, Multiple Three	3	60	5
Liver, Hepatocellular Carcinoma, includes all Hepatocellular Carcinoma above	**190 [64]**	**718 [298]**	**26 (13-42) [13-42]**
Females			
Liver, Hepatocellular Adenoma	129	607	21 (10-34)
Liver, Hepatocellular Adenoma, Multiple	49	547	9
Liver, Hepatocellular Adenoma, Multiple Two	1	60	2
Liver, Hepatocellular Adenoma, includes all Hepatocellular Adenoma above	**179 [72]**	**607 [298]**	**29 (12-49) [12-49]**
Liver, Hepatocellular Carcinoma	91	607	15 (7-27)
Liver, Hepatoicellular Carcinoma, Multiple	29	427	7
Liver, Hepatocellular Carcinoma, Multiple Two	1	60	2
Liver, Hepatocellular Carcinoma, Multiple Five	1	60	2
Liver, Hepatocellular Carcinoma, includes all Hepatocellular Carcinoma above	**122 [53]**	**607 [298]**	**20 (8-42) [8-42]**

The incidence of hepatocellular neoplastic findings observed in the study under discussion are summarised in the table below.

Table 62: Comparison of hepatocellular neoplastic findings of mouse pyridate study with historical control data in B6C3F1 mice at SRI

		Incidence in study		Historical controls [route via feed and water only]
males	Hepatocellular ademona	5/50	10%	18-68% [18-68%]
	Hepatocellular carcinoma	11/50	22%	13-42% [13-42%]

Hepatocellular adenomas and carcinomas of male mice are within the historical control ranges of all application routes and when applied via feed and water.

3.10.1.2 Carcinogenicity: inhalation

No data available.

3.10.1.3 Carcinogenicity: dermal

No data available.

3.10.2 Human information

Not available.

3.10.3 Other relevant information

Not available.

3.10.4 Summary and discussion of carcinogenicity

All tests evaluated in support of the Annex I listing of pyridate were performed under GLP and are still considered to be valid.

Pyridate showed no cancerogenic potential in rats and mice. The lowest relevant NOAEL was 18 mg/kg bw/day (long-term toxicity in rats).

3.10.5 Comparison with criteria

No increase in tumour incidence was found in the chronic rat study conducted with pyridate. The chronic mouse study showed an increase in liver tumour incidence in top dose male mice, which were within the historical control range. In the Guidance on the Application of the CLP Criteria (ECHA, Version 4, November 2013) liver tumours in B6C3F1 mice are listed as an example of animal tissue with a high spontaneous tumour incidence. According to the respective guidance *where the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories*. For pyridate tumours were observed only in one sex in the liver of a sensitive strain of mice, without a plausible explanation from ADME studies why only male animals are concerned. Furthermore, the MTD in the top dose group was exceeded, i.e. mortality was observed (3/50 in males, 10/50 in females) and the tumours were within the historical control range. Therefore in conclusion no classification and labelling regarding carcinogenicity is required. The experts in the Pesticide Peer Review Meeting 109 (January 2014) supported this conclusion.

3.10.6 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: No classification proposed

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Following treatment with pyridate, no increase in tumour incidence was found in a 2-year study conducted in rats.
In a 18 month mouse study, there was an increase in tumour incidence in the livers at the top dose male mice, which were within the laboratory historical control range. In the Guidance on the Application of the CLP Criteria (ECHA, Version 4, November 2013) liver tumours in B6C3F1 mice are listed as an example of animal tissue with a high spontaneous tumour incidence. According to the respective guidance "*where the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories"*. For pyridate, tumours were observed in just one sex, in the liver of a sensitive strain of mice and without a plausible explanation from ADME studies why only male animals are concerned. Furthermore, the MTD in the top dose group was exceeded, i.e. mortality was observed (3/50 in males, 10/50 in females) and the tumours were within the historical control range. Therefore, the DS proposed no classification for carcinogenicity.

Comments received during public consultation

Carcinogenicity was not open for commenting during the public consultation. One MSCA made a general comment, supporting the classification proposal.

Assessment and comparison with the classification criteria

Two carcinogenicity studies are available: one in rats and one in mice.

Rats

Wistar rats (75/sex/dose) were exposed to pyridate (90.3% purity) in the diet at doses of 0, 3.6, 18 or 115 mg/kg bw/d for 121 weeks. No increase in tumour incidence was reported. Non-neoplastic effects are described in the repeated dose section.

Mice

B6C3F1 mice were exposed to 0, 400, 800 and 1200/1400/1600 (ascending dose) pyridate (91.5% purity) in the diet (equivalent to 0, 48, 98 and 170 mg/kg bw/d for males; 0, 55, 115 and 204 mg/kg bw/d for females) for 18 months. The dose of 1200 ppm was increased to 1400 ppm on day 91 and to 1600 ppm on day 179. After the original study, there was also an additional control group and a group exposed to 7000ppm pyridate (853 and 1045 mg/kg bw/d in males and females, respectively).

Incidences of mortality increased in females only at the top 2 doses (3/50, 3/50, 3/50, 4/50, 7/50 and 10/50 at 0, 0, 400, 800, 1200/1400/1600 and 7000ppm, respectively. There were no reports of treatment-related clinical signs. Non-neoplastic findings are covered in the repeated dose section.

Tumours were observed in the adrenal gland, liver and pancreas, as shown in the table below.

Dose (ppm)	0	0	400	800	1200/1400/1600	7000
Males (animals with effect/animals evaluated)						
Adrenal gland cortex adenoma	0/50	0/0	0/2	0/1	0/0	1/50
Adrenal gland-medulla pheochromocytoma malignant	0/50	0/0	0/2	0/1	0/0	1/50
Liver: Hepatocellular adenoma	7/50	0/0	12/50	10/50	0/0	5/50
Liver: Hepatocellular carcinoma	5/50	0/0	7/50	7/50	0/0	11/50
Lung: malignant lymphoma	0/50	0/0	0/50	0/50	0/0	1/50
Lymph node malignant-mediastinal	0/0	0/0	0/1	0/0	0/0	1/3
Pancreas: Hemangiosarcoma	0/50	0/0	0/2	0/2	0/0	1/50
Females (animals with effect/animals evaluated)						
Liver: Hepatocellular adenoma	6/50	0/0	4/50	3/49	0/0	8/50
Liver: Hepatocellular carcinoma	7/50	0/0	0/50	1/49	0/0	2/50

In top dose males, there were single incidences of adrenal gland cortex adenoma, adrenal gland-medulla pheochromocytoma (malignant), malignant lymphoma in the lung, lymph node malignant-mediastinal and hemangiosarcoma in the pancreas. Although these tumours were not observed at lower dose levels or in controls, these isolated incidences did not reach statistical significance and are not considered to be clear evidence of a carcinogenic effect.

Hepatocellular adenoma and carcinoma were observed in both sexes. However, a dose related increase in tumour incidence was observed in males only (hepatocellular carcinoma).

The historical control data (HCD) covered 12 studies of comparable size and design in B6C3F1 mice in the same laboratory over the period from 1985-1992 (start of study: November 1989). According to the HCD, hepatocellular carcinoma were observed in 26% (mean value) (range: 13-42%) males. In the available study on pyridate, hepatocellular carcinoma was observed in 22% males at the top dose and was therefore within the historical control data range. Furthermore, RAC notes that liver tumours in B6C3F1 mice have a high spontaneous tumour incidence (CLP guidance). Therefore, RAC considers the increased incidence of hepatocellular carcinoma in male mice following exposure to pyridate no reliable evidence of treatment-related carcinogenicity.

Since there is no evidence of carcinogenicity in rats and no reliable evidence of carcinogenicity in mice, RAC considers that **classification of pyridate for carcinogenicity is not warranted**.

3.11 Toxicity for reproduction

Table 63: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Three generation study in Wistar rats	0, 80, 400 and 2500 ppm (equivalent to 0, 3.6, 18.8 and 110 mg/kg bw/d) Reproduction NOAEL: 2500 ppm Parental NOAEL: 80 ppm Parental LOAEL: 400 mg/kg bw/d based on: ↑ relative kidney weights in both sexes (F1 and F2 generation), ↓ thyroid weight in males of F2 Offspring NOAEL: 400 ppm Offspring LOAEL: 2500 mg/kg bw/d based on: ↓ pup weight (1st mating)	-	Til, 1982
Developmental toxicity study in Sprague Dawley CD rats	0, 55, 165, 400 and 495 mg/kg bw/d from day 6 through day 15 of pregnancy oral gavage Maternal NOAEL: 165 mg/kg bw/d Maternal LOAEL: 400 mg/kg bw/d based on: Mortality, ↓ bw gain and food consumption Fetal NOAEL: 165 mg/kg bw/d Fetal LOAEL: 400 mg/kg bw/d based on: ↓ bw and delayed ossification No teratogenic effects	-	Becker, 1986
Developmental toxicity study in NZW rabbits	0, 150, 300 and 600 mg/kg bw/d from day 7 through day 19 of pregnancy oral gavage Maternal NOAEL: 150 mg/kg bw/d Maternal LOAEL: 300 mg/kg bw/d based on: ↓ bw gain and abnormal faeces Fetal NOAEL: 300 mg/kg bw/d Fetal LOAEL: 600 mg/kg bw/d based on: Abortions and minimal ↓in foetal bw No teratogenic effects above historical control range	-	Hoberman, 1987

3.11.1 Effects on fertility

3.11.1.1 Non-human information

The following study was evaluated in support of the Annex I listing of pyridate and is no longer granted data protection:

IIA 5.6.1/01 Til H.P. et al. (1982) Multigeneration study with Pyridate in rats. TNO Project No.: B 80-0696

The relevance of the findings in this study has been re-assessed by the SCP in the year 2000 (Opinion of the Scientific Committee on Plants regarding the evaluation of Pyridate in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market (Opinion expressed by the Scientific Committee on Plants on 6 June 2000) (SCP/PYRID/002-Final)) and found to be pertinent as basis for the derivation of the AOEL and implicitly also for the ADI.

Pyridate (Batch: 1001410, CL 11344, purity: 90.3%) was applied to groups of 20 male and female Wistar (Cpb:Wu) rats via diet at dose levels of 0, 80, 400 and 2500 ppm (equivalent to 0, 3.6, 18.8 and 110 mg/kg bw/d) throughout mating period, gestation and rearing in a reproduction study over 3 generations. 2 successive litters were reared in each generation from each female. In addition a 4-week feeding study was conducted with weanling rats of the F_3^b generation. Blood samples were collected from 10 rats/sex/group of the F1 generation in week 61. The blood was investigated for the following parameters: (Differential) WBC. Furthermore blood samples were taken from 10 rats/sex/group of the F1 and F2 generation for Cl and K measurements (F1) and LDH (F1 and F2) determination. Histopathology was only performed in control and top dose parents ot the F3 generation and in the pituitary of all animals of the F1 generation (parents and pups). The results are summarised in the following table:

Table 64: Body weight and food consumption in partents in the 3-generation study in rats from the DAR (Til et al., 1982)

Dose (mg/kg bw/day)	0		3.6		18.8		110	
	male	female	male	female	male	female	male	female
F0								
Body weight in g (day 0)	81.4	65.5	81.2	66.8	81.9	67	81.9	68.7
Body weight in g (day 84)	337.2	199.2	332.6	198.7	334.2	199	319.5	190.3
Body weight in g (day 170)	-	306.7	-	300.2	-	296.2	-	268.7*
Mean food intake g/rat/week (day 7)	106.9	83.8	105	84.2	101.8	88.5	94.4	81.2
Mean food intake g/rat/week (day 84)	120.1	83.6	113	85.6	114.6*	85.4	110.1*	82.4
F1								
Body weight in g (day 0)	83	76.5	81.9	73.9	75.2	69.9	69	63.5
Body weight in g (day 84)	374.8	220.3	374	212.9	349.5	211.2	339.5	203.4
Body weight in g (day 170)	-	334.9	-	318.7	-	324	-	301.1
Mean food intake g/rat/week (day 7)	116.9	97.6	110.1	88	105.9	93.6	93.6*	86.6
Mean food intake g/rat/week (day 84)	132.5	95.6	131.7	89.5	125.5	90.4	121.1	87.9
F2								
Body weight in g (day 0)	43.3	40.9	45.1	42	44.7	42	43.4	40.4
Body weight in g (day 84)	322	198.9	311.5	196.2	318.5	191.2	319.2	200.9
Body weight in g (day 170)	-	300	-	291.6	-	286.5	-	292.2
Mean food intake g/rat/week (day 7)	77.5	63.6	60.1*	52.4*	72.4	58.1	65.2*	57.3
Mean food intake g/rat/week (day 84)	118.4	89.3	107	86.7	117.3	81.7	116	91.5
F3								

Dose (mg/kg bw/day)	0		3.6		18.8		110	
	male	female	male	female	male	female	male	female
Body weight in g (day 0)	41.4	41.2	39.2	40.7	39	37.3	38.2	37.7
Body weight in g (day 28)	180.7	132.4	177.6	128.5	174.3	127.6	168.1	127.4
Mean food intake g/rat/week (day 7)	55.6	47.5	50.1*	51.8*	51.9*	51.7*	50.1*	50.1*
Mean food intake g/rat/week (day 28)	139.1	102.6	139.4	99.8	135.5	99.2	139.8	101.9

* statistically significant

There were no treatment related mortalities nor clinical signs.

Body weight was reduced > 10% in top dose females of the F0 and F1 generation.

No treatment related effect could be seen for WBC parameters of F1 animals.

Table 65: Clinical chemistry in the 3-generation study in parental rats from the DAR (Til et al., 1982)

Dose (mg/kg bw/day)	0		3.6		18.8		110	
	male	female	male	female	male	female	male	female
F1								
LDH (U/l)	344.3	116.4	225.9*	111.4	223.5*	116.7	175.3*	92.7
F2								
LDH (U/l)	179.6	132.4	137.7	145.1	192.8	152.8	135.8	127.8

* statistically significant

LDH was significantly decreased in males of the F1 generation in all dose groups. No treatment related effect could be seen for Cl and K measurements.

Table 66: Organ weights in the 3-generation study in parental rats from the DAR (Til et al., 1982)

Dose (mg/kg bw/day)	0		3.6		18.8		110	
	male	female	male	female	male	female	male	female
F1								
Thyroid (relative in g/kg bw)	0.058	0.085	0.056	0.087	0.049	0.087	0.052	0.09
Kidney (relative in g/kg bw)	5.49	6.35	5.61	6.43	5.59	6.57	5.98*	6.93*
Liver (relative in g/kg bw)	29.7	30.1	30	30.8	28.9	30.9	30.5	31.6
F2								
Thyroid (relative in g/kg bw)	0.068	0.084	0.065	0.091	0.056*	0.074	0.053*	0.091
Kidney (relative in g/kg bw)	5.53	6.54	5.78	6.74	5.76	7.11*	6.31*	7.29*
Liver (relative in g/kg bw)	30.1	31.9	30	32.5	29.4	32.6	31.3	35.6*
F3								
Thyroid (relative in g/kg bw)	0.081	0.123	0.085	0.131	0.099	0.122	0.088	0.12
Kidney (relative in g/kg bw)	8.27	8.96	8.67	9.18	8.88*	9.76*	9.07*	9.84*
Liver (relative in g/kg bw)	49.1	45.7	50	46.3	49.2	47.5	52.8*	49.3

* statistically significant

According to the opinion expressed by the Scientific Committee on Plants on 6 June 2000 (SCP/PYRID/002-Final) the reproductive study indicates that pyridate induces dose-dependent effects in the weights of thyroid and kidney. A slight increase in kidney weights was observed in the top-dose group of both sexes in the F1 and F2 generation, in females of the mid-dose group of the F2 generation, and in both sexes of the mid-dose group of the F3 generation. A dose-dependent decrease of the thyroid weights was observed in males of the mid- and top-dose groups of the F2 generation. Liver weight was increased in F2 females and F3 males of the top-dose group.

Table 67: Histopathology of F3 parents in the 3-generation study in parental rats from the DAR (Til et al., 1982)

Dose (mg/kg bw/day)	**0**		**3.6**		**18.8**		**110**	
No of animals with effect/ all animals investigated	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Liver: Single hepatocellular necrosis, minimal to slight	3/10	6/10	0/0	0/0	0/0	0/0	5/10	6/10
Liver: Cell necrosis around central vein, slight	0/10	0/10	0/0	0/0	0/0	0/0	1/10	0/10
Spleen: Extramedullary haematopoiesis, minimal to slight	7/10	4/10	0/0	0/0	0/0	0/0	8/10	5/10
Spleen: Extramedullary haematopoiesis, moderate	0/10	0/10	0/0	0/0	0/0	0/0	1/10	0/10

* statistically significant

No treatment related effects were observed for gross pathological changes for parental animals of all generations and histopathology of the pituitary of F1-parents and pups. Slight increases in single cell necrosis in the liver as well as slight increased extramedullary haematopoisesis could be found in top dose F3 parental animals.

No treatment related changes were observed for gross pathology of pups of all generations.

Table 68: Reproductive performance in the 3-generation study in parental rats from the DAR (Til et al., 1982)

Dose (mg/kg bw/day)	**0**	**3.6**	**18.8**	**110**
F0, first breeding				
Lactation index (%)	92	98*	98*	95
Pup weight in g (day 14)	27.8	27.6	27.4	24.6*
Pup weight in g (day 21)	40.9	40.9	41.2	36.2*
F0, second breeding				
Lactation index (%)	81	94*	92*	91*
Pup weight in g (day 14)	28.5	29.2	27.4	27.6
Pup weight in g (day 21)	43.8	42.9	40.7	40.1
F1, first breeding				
Lactation index (%)	91	97*	97*	91
Pup weight in g (day 14)	27.7	25.9	26.4	24.2*

Dose (mg/kg bw/day)	0	3.6	18.8	110
Pup weight in g (day 21)	40.2	36.5*	38.6	34.5*
F1, second breeding				
Lactation index (%)	95	97	100*	99*
Pup weight in g (day 14)	24.6	25.9	26.9*	25.7
Pup weight in g (day 21)	38.3	38.8	39.7	38.1
F2, first breeding				
Lactation index (%)	90	85	71*	76*
Pup weight in g (day 14)	20.4	18.6	19.1	17.1*
Pup weight in g (day 21)	30.9	27.7*	28.4	27.1*
F2, second breeding				
Lactation index (%)	81	76	85	90*
Pup weight in g (day 14)	25.5	24.2	24.5	24.7
Pup weight in g (day 21)	36	36.5	34.4	34.6

* statistically significant

No treatment related effect was observed for the duration of the gestation period, fertility- and gestation index and sex ratio. Doses up to 110 mg/kg bw/d did not induce malformations. In the F2 generation the lactation index was lower in the mid and top dose animals of the first breeding but not in the second. The mean pup weight was significantly decreased in the top dose group in all generations in the first mating.

In the DAR the parental NO(A)EL was set at 3.6 mg/kg bw/d based on increased relative weight in kidneys in all generations and decreased thyroid weight in males of F2, which can be confirmed.

According to the Opinion expressed by the Scientific Committee on Plants on 6 June 2000) (SCP/PYRID/002-Final)) the notifier argued that, since the kidney effect observed in the reproductive study is not associated with deviations in clinical chemistry data or histopathological findings, they should not be considered as adverse but more as “adaptive” effects, “due to an overload of the organism as the result of a higher feed intake, especially in young animals”. The notifier considered also that the findings on thyroid were not related to the treatment. It is opinion of the SCP, however, that the observation of similar results in the short-term study, and in the long-term study after one year may rather indicate a higher sensitivity of younger and middle age animals. In conclusion, the NOAEL of 3.6 mg/kg bw was confirmed by the SCP.

No adverse effects were seen for reproductive performance, therefore the NOAEL for reproduction is 110 mg/kg bw, the highest dose tested.

For offspring the NOAEL can be set at 18.8 mg/kg bw/d, based on decreased pup weight in top dose animals in the first mating.

3.11.1.2 Human information

Not available.

3.11.2 Developmental toxicity

3.11.2.1 Non-human information

Developmental toxicity studies according to GLP and international guidelines were conducted in rats and rabbits.

Rats:

The following study was evaluated in support of the Annex I listing of pyridate and is no longer granted data protection:

IIA 5.6.10/01 Becker H. et al. (1986) Embryotoxicity (including teratogenicity) study with Pyridate technical in the rat. RCC Project 055934

Pyridate (Batch: 2420966, purity: 92%) was applied to groups of 35 (control) – 25 (dose groups) female Wistar/HAN rats via gavage (vehicle: distilled water with 4% carboxy methylcellulose sodium salt) at dose levels of 0, 55, 165, 400 and 495 mg/kg bw/d from day 6 through day 15 of pregnancy. The dosage is based upon the results of the dose-finding embryotoxicity study (RCC project 055923, 1985) which is not available and not considered necessary. In the Table 69 - Table 70 results are summarised.

Table 69: Mortality, body weight gain and food consumption in maternal rats the developmental study in rat from the DAR (Becker et al., 1986)

Dose (mg/kg)	**0**	**55**	**165**	**400**	**495**
Mortality	0	0	0	5 (after 1 application)	16 (13 after 1 application, 1 each after 5,6 and 10 applications)
Non pregnant animals (besides dead ones)	0	1	2	1	0
Body weight gain in %[1] (% of control)	9.7±3.7 (100%)	9.3±3.9 (95.88%)	8.2±3.9 (84.54%)	8.6±3.4 (88.66%)	4±3.7 (41.23%)
Body weight gain in g[2] (% of control)	22.23±8.24 (100%)	21.00±8.28 (94.47%)	18.61±8.21 (83.72%)	19.16±7.7 (86.19%)	9.33±8.49 (41.99%)
Food consumption (day 6-11)	21.3	22.2	20.9	16.1	15.9
Food consumption (day 11-16)	23.6	23.4	23.5	21.9	19.3

[1] corrected weight gain in % of weight on day 6 (weight on day 21- weight on day 6 – uterus weight)
[2] (weight on day 21)-(weight on day 6) – (uterus weight)
* statistically significant

Clinical signs (i.e. ventral body position, dyspnea, sedation, somnolence, lack of response to external stimuli, tonic and clonic muscle spasms, ruffled fur) were noted in animals treated with 400 mg/kg bw/d pyridate upwards. These symptoms were most pronounced on the first day of dosing, and decreased in incidence as the study progressed, except in the animals that died. No symptoms were recorded after the 6th day of application.

Increased mortality of the dams was noted from 400 mg/kg bw/d upwards. Body weight gain was reduced > 10% from 165 mg/kg bw/d upwards. However the decrease in bw gain does not follow linear dose reponse kinetics as there is no decrease in bw gain from 165 mg/kg bw/d to 400 mg/kg bw/d, even if the dose was more than doubled. Food consumption was reduced > 10% from 400 mg/kg bw/d upward.

No treatment related effect was observed for mean number of implantations, corpora lutea/dam, pre- and postimplantation loss.

Table 70: Offspring parameter in the developmental study in rat from the DAR (Becker et al., 1986)

Dose (mg/kg)	**0**	**55**	**165**	**400**	**495**
Weight of live fetuses	**4.8**	**4.9**	**4.8**	**4.6**	**4.4**
Skeletal abnormalities (ossification delay) in %	2	0.7	1.4	4.9	13.5

* statistically significant

Mean body weights of the pups were slightly (< 10%) decreased from 400 mg/kg bw/d upward. Incomplete ossification was increased in the top two doses. Skeletal abnormalities in the two top doses included: absent thoracic vertebral body 1, absent sternebra 5 and/or 6, wavy ribs, bipartite cervica vertebra 1, bipartite sternebra 1 and irregularly ossified sternebrae 3 and 4.

No evidence of treatment related malformations was found.

In the DAR the maternal NO(A)EL was set at 165 mg/kg bw/d based on mortalities and reduction in body weight gain and food consumption > 10% from 400 mg/kg bw/d upward, which can be confirmed. The offspring NO(A)EL in the DAR was set at 165 mg/kg bw/d based on delayed ossification from 400 mg/kg bw/d upward, which can be confirmed.

Rabbits:

The following study was evaluated in support of the Annex I listing of pyridate and is no longer granted data protection:

IIA 5.6.11/01 Hoberman A.M. (1987) Developmental toxicity (embryo/fetal toxicity and teratogenic potential) study of Pyridate technical administered as the neat test substance orally via stomach tube to New Zealand white rabbits. Argus Research Laboratories Project ID: 512-001.

Pyridate (Batch: 2659427, purity: 93.1%) as the neat test substance was applied to groups of 20 female New Zealand White rabbits via gavage at dose levels of 0 (Control R.O. water), 150, 300 and 600 mg/kg bw/d from day 7 through day 19 of pregnancy. The dosage is based upon the results of a pilot dose-finding study (512-001PA, 1987) which is summarized in the study report. In the Table 71 - Table 72 results are summarised.

Table 71: Abortion, premature delivery, clinical signs, body weight gain and food consumption in maternal rabbits the developmental study in rabbits from the DAR (Hoberman, 1987)

Dose (mg/kg)	**0**	**150**	**300**	**600**
Abortion (day)	0	0	1 (22)	4* (19, 14 or 26)
Premature delivery (day)	0	0	0	1 (28)

Dried faeces (days observed/ rabbits observed)	0/0	0/0	7/1	39*/9*
No faeces (days observed/ rabbits observed)	0/0	0/0	0/0	12*/3*
Body weight gain days 0-7 in kg	+ 0.17 ± 0.07	+ 0.15 ± 0.08	+0.18 ± 0.09	+0.17 ± 0.08
Body weight gain days 7-20 in kg	+0.23 ± 0.13	+0.21 ± 0.12	+0.15 ± 0.12	-0.17 ± 0.3*
Body weight gain days 20-29 in kg	+0.02 ± 0.14	+0.08 ± 0.13	+0.11 ± 0.16	+0.02 ± 0.26
Food consumption (day 7-20)	165.1	161.4	157.5	92.2*
Food consumption (day 20-29)	110	125	120.1	80

* statistically significant

There was no treatment related mortality. Abortion was significantly increased in the top dose, one additional animal aborted at 300 mg/kg bw/d. Threre was an increased incidence of dried or absent faeces in the mid and high dose groups. Body weight gain was reduced > 10% in the mid and high dose group, with high dose animals loosing weight during dosing. Food consumtion was significantly reduced in top dose animals.

Table 72: Offspring parameter in the developmental study in rabbits from the DAR (Hoberman, 1987)

Dose (mg/kg)	**0**	**150**	**300**	**600**	**Historical control data (570 litters from 1983-1986) N (%) (range in %)**
Live fetal body weights g/litter	42.71	44.25	45.59	39.26	
Head domed (% litter incidence)	0	0	0	1 (8.3) [3]	4 (0.7) (0-8.3)
Menigocele lumbar-sacral (% litter incidence)	0	0	1 (6.7) [1]	0	2 (0.35) (0-9.1)
Menigocele cervical (% litter incidence)	0	0	1 (6.7)	0	2 (0.35) (0-9.1)
Right paw, rotated inward (% litter incidence)	0	0	1 (6.7) [1]	0	1 (0.18) (0-5.9)
Hydrocephalus (% litter incidence)	0	0	0	1 (8.3) [3]	5 (0.97) (0-8.3)
Hydronephrosis, dilatation of the renal pelvis (% litter incidence)	0	0	0	1 (8.3)	No data
Kidneys fused (% litter incidence)	0	0	1 (6.7) [2]	0	No data
Diaphragmic hernia (% litter incidence)	0	0	1 (6.7)	0	No data
Adrenals absent (% litter incidence)	0	0	1 (6.7) [2]	0	No data
Fontanelle, anterior enlarged (% litter incidence)	0	0	0	1 (8.3)	9 (1.74) (0-8.3)
Vertebrae thoracic : hemivertebra, centrum bifid, centra assymetric (% litter incidence)	0	0	0	1 (8.3)	9 (1.74) (0-20), 2 (0.39) (0-33.3), 1 (0.19) (0-33.3)
Sternebra 1st incomplete ossified (% litter incidence)	0	0	0	1 (8.3)	1 (0.19) (0-6.25)
Pelvis pubes incomplete ossified (% litter incidence)	1 (5.6)	0	0	1 (8.3)	Not ossified: 5 (0-13.3) No data for incomplete ossified

[1,2,3] same individual

* statistically significant

No treatment related effect was noted on reproduction parameter (numer of corpora lutea, implantations, litter size, live foetuses, resorptions, sex ratio, viablility/litter). Pub weight was slightly decreased < 10% in the top dose group. No developmental effects or malformations in the top dose group were above the historical control range. At 300 mg/kg bw/d one inward rotated right paw was detected which was slightly above the historical control range, however as this finding was not repeated or increased in the high dose group it is considered incidentially.

In the DAR the maternal NO(A)EL was set at 150 mg/kg bw/d based on clinical signs (dried feces) and reduction in bw gain > 10% from 300 mg/kg bw/d upward, which can be confirmed. The offspring NO(A)EL in the DAR was set at 300 mg/kg bw/d based on abortions and slightly decreased body weight of live fetuses, which can be confirmed.

3.11.2.2 Human information

Not available.

3.11.3 Other relevant information

Not available.

3.11.4 Summary and discussion of reproductive toxicity

In a three generation study in rats no influence was seen on fertility, appearance and behaviour of the parental animals, and no effect on survival, malformation or influence on the sex ratio or any other effect on reproduction could be seen in the offspring. In developmental studies in rats and rabbits no treatment-related differences were noted on the mean number of implantations, foetuses and embryonic deaths. Increased number of abortions in the rabbit study was associated with maternal toxicity. The lowest relevant (parental) NOAEL was 80 ppm, equivalent to 3.6 mg/kg bw/day (3 generation reproduction toxicity), based on increased relative kidney weights in the F2 and F3 generation and decreased relative thyroid weights in males of the F2 generation at the next higher dose. No teratogenic potential was found in a rat and in a rabbit developmental toxicity study. Developmental toxicity (decreased body weight and ossification effects) was associated with maternal toxicity in both species.

3.11.5 Comparison with criteria

In a three generation study in rats no influence was seen on fertility, appearance and behaviour of the parental animals, and no effect on survival, malformation or influence on the sex ratio or any other effect on reproduction could be seen in the offspring. Furthermore in developmental studies in rats and rabbits no treatment-related differences were noted on the mean number of implantations, foetuses and embryonic deaths. Increased number of abortions in the rabbit study was associated with maternal toxicity.

No teratogenic potential was found in a rat and in a rabbit developmental toxicity study. Developmental toxicity (decreased body weight and ossification effects) was associated with maternal toxicity in both species.

The substance therefore does not meet the criteria for classification for reproductive toxicity.

3.11.6 Conclusions on classification and labelling

Regulation (EC) No. 1272/2008: No classification proposed

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

In a three-generation study in rats no influence was seen on fertility, appearance and behaviour of the parental animals, and no effect on survival, malformation or influence on the sex ratio or any other effect on reproduction could be seen in the offspring. Furthermore, in developmental studies in rats and rabbits, no treatment-related differences were noted on the mean number of implantations, foetuses and embryonic deaths. An increased number of abortions in the rabbit study was associated with maternal toxicity.

No teratogenic potential was found in a rat or in a rabbit developmental toxicity study. Developmental toxicity (reduced body weight and ossification effects) was associated with maternal toxicity in both species.

Therefore, the DS did not propose classification for reproductive toxicity.

Comments received during public consultation

Although there were no specific comments, the proposal for no classification for this endpoint was supported by all 3 of the MSCAs that responded.

Assessment and comparison with the classification criteria

Pyridate has been tested in a multi-generation study in rats and two developmental toxicity studies, one in rats and one in rabbits (all performed in the 1980s).

Fertility and sexual function

Groups of 20 males and female Wistar rats received pyridate in the diet at concentrations of 0, 80, 400 and 2500 ppm (equivalent to 0, 3.6, 18.8 and 100 mg/kg bw/d) throughout the mating, gestation and lactation periods for three generations. Two successive litters were reared in each generation from each female.

There were no treatment-related mortalities or clinical signs observed. Body weight was reduced in top dose females of the F_0 generation (12 % lower than controls). Relative kidney weight was statistically significantly increased in F_1 males and females of the top dose group, F_2 males and females of the top dose group and F_2 females only in the mid dose group and in F_3 males and females of the mid and top dose groups. The increase in kidney weight only rose above 10 % in top dose males and females of the F_2 generation. Thyroid weight was decreased in mid and top dose F_2 males (18 and 22 % lower than controls, respectively). Liver weight was statistically significantly increased in top dose F_2 females and top dose F_3 males. The increase was only above 10% in F_2 females (12% higher than controls). There were no histopathological correlates to the changes in organ weights.

No treatment-related effects were observed to the following reproductive indices; gestation period, fertility index, gestation index or sex ratio. In the F_2 generation, the lactation index was lower in the mid and high dose groups (71 % and 76 % respectively, compared to 90 % in controls). However, there was no clear dose-response and it was not observed in the second breeding nor in any other generations.

Mean pup weight was reduced in all top dose groups in the first mating (11 – 16 % lower than controls, noted at day 14 and day 21). However, only the in first breeding from the F_0 generation did this reduction in weight show any dose-response. Pups in this group had a mean body weight 11.5 % lower than controls. As the dams also suffered reduced body weight in this group, RAC considers the weight loss in pups to be due to undernourishment/generalised toxicity of the mothers rather than a specific toxic effect. There were no effects to pup weights in the second breeding nor at any other dose levels.

Developmental toxicity

Rats

Pregnant female Wistar rats received an oral dose of pyridate of either 0 (n = 35), 55, 165, 400 or 495 mg/kg bw/d (25/dose) by gavage on days 6 – 15 of pregnancy.

The following clinical signs were noted from a dose of 400 mg/kg bw/d: ventral body position, dyspnea, sedation, somnolence, lack of response to external stimuli, tonic and clonic muscle spasms and ruffled fur. These symptoms were more pronounced on the first day of dosing and decreased in incidence as the study progressed, except in animals that died. Mortality occurred in the top two dosing groups. At 400 mg/kg bw/d, 1/25 females died after the first application and a further 4 animals died during the course of the study. At 495 mg/kg bw/d, 13/25 animals died after one application and a further 3 animals died after 5, 6 and 10 applications. Body weight gain in dams was reduced from a dose of 165 mg/kg bw/d (16 %, 14 % and 60 % reduction compared to controls at 165, 400 and 495 mg/kg bw/d).

There were no treatment-related effects to the mean number of implantations, corpora lutea/dam or pre-and post-implantion loss. In pups, delayed ossification occurred in litters of the top two doses (4.9 % and 13.5 % at 400 and 495 mg/kg bw/d compared to 2 % in controls). There was no evidence of any treatment-related malformations.

Rabbits

Pyridate was administered by gavage to female New Zealand White rabbits (20/dose) at doses of 0, 150, 300 and 600 mg/kg bw/d from day 7 – 19 of pregnancy.

There was no treatment-related mortality in this study. At the top dose of 600 mg/kg bw/d animals were observed to have lost weight (170 g lost during the dosing period compared to a gain of 230 g in control animals) and food consumption was significantly reduced. At this dose, there was an increase in abortion (4 animals aborted compared to 0 in controls). Mid-dose animals were also observed to have reduced body weight gain (34 % reduction compared to controls) and one mid-dose animal aborted prematurely. In both mid- and top-dose groups there was an increased incidence of absent or dried faeces.

There was no treatment-related effect on reproductive parameters including, number of corpora lutea, implantations, litter size, live foetuses, resorptions, sex ratio or viability/litter.

In pups, there were a number of isolated findings of malformations and variations at the mid and top dose that were above the concurrent control. These are shown in more detail in the table below.

Table showing the incidence of malformations (shaded) and variations in rabbits

	Dose (mg/kg bw/d)				
Finding (% litter incidence)	**0**	**150**	**300**	**600**	**Historical control data (570 litters from 1983 - 1986) N (%) (range in %)**
Head domed	0	0	0	1 (8.3)[3]	4 (0.7) (0-8.3)
Meningocele lumbar-sacral	0	0	1 (6.7)[2]	0	2 (0.35) (0-9.1)
Meningocele cervical	0	0	1 (6.7)	0	2 (0.35) (0-9.1)
Right paw, rotated inwards	0	0	1 (6.7)[2]	0	1 (0.18) (0-5.9)
Hydrocephalus	0	0	0	1 (8.3)[3]	5 (0.97) (0-8.3)
Hydronephrosis, dilatation of the renal pelvis	0	0	0	1 (8.3)	No data
Kidneys fused	0	0	1 (6.7)[1]	0	No data
Diaphragmatic hernia	0	0	1 (6.7)	0	No data
Adrenals absent	0	0	1 (6.7)[1]	0	No data
Fontanelle, anterior enlarged	0	0	0	1 (8.3)	9 (1.74) (0-8.3)
Vertebrae thoracic: hemivertebra, centrum bifid, centra asymmetric	0	0	0	1 (8.3)	9 (1.74) (0-20), 2 (0.39) (0-33.3), 1 (0.19) (0-33.3)
Sternebra 1st incomplete ossified	0	0	0	1 (8.3)	1 (0.19) (0-6.25)
Pelvis pubes incomplete ossified	1 (5.6)	0	0	1 (8.3)	Not ossified: 5 (0-13.3). No data for incomplete ossification

[1,2,3]same individual animal

At 300 mg/kg bw/d, one rabbit was found to have a meningocele in the lumbar-sacral region and also a malrotated paw. Another rabbit was found to have a meningocele in the cervical region and one rabbit was found to have a diaphragmatic hernia. A fourth rabbit had both fused kidneys and absent adrenals. All of these findings are considered to be malformations of high concern. With the exception of the fused kidneys, diaphragmatic hernia and absent adrenals, for which there was no data, they were all within HCD. None of these findings

occurred at the next dose level and are therefore considered incidental and un-related to treatment.

At 600 mg/kg bw/d, one rabbit was found to have both a domed head shape and also hydrocephalus (versus 0 in controls). Both findings were within the HCD provided. A second rabbit had hydronephrosis (versus 0 in controls). There was no HCD provided for this finding. A third rabbit was found to have vertebrae thoracic: hemivertebra, centrum bifid, centra asymmetry (versus 0 in controls). This finding was well within the HCD provided.

The only increase in variations observed was at the top dose, where one animal was found to have incomplete ossification of the sternebra and one animal had an enlarged fontanelle. Both of these findings were within the HCD provided.

For the malformations occurring at the top dose only one cannot rule out an effect of treatment based on a lack of dose-response. However, the increase in each finding was only in 1 animal and in most cases they occurred within the historical control data range provided, and are therefore considered incidental and un-related to treatment. In the case of one finding of hydronephrosis, dilatation of the renal pelvis, there was no historical control data available. This finding is considered a serious malformation, however given that the finding occurred in only one animal from one study, at one dose level and in the presence of maternal toxicity (body weight loss) it is not deemed sufficient evidence of a treatment-related effect worthy of classification with developmental toxicity.

RAC conclusion

In a multigenerational reproductive toxicity study in rats, there were no treatment-related effects to fertility or development observed.

In a developmental toxicity study in rabbits there were single incidences of malformations to the head [domed head, hydrocephalus (occurring in the same animal)], kidneys (hydronephrosis) and vertebra (thoracic – centrum bifid, centra asymmetric). These incidences occurred at the top dose only and in most cases, with the exception of the finding of hydronephrosis for which there was no data, occurred within HCD ranges. There were no treatment-related increases in malformations in a similar study, carried out in rats. RAC concludes that there is no pattern to the findings that could link them to development toxicity, therefore they are likely to be incidental and not related to treatment.

RAC is in agreement with the DS that **pyridate should not be classified for reproductive toxicity**.

3.12 Other effects

3.12.1 Non-human information

Safety pharmacology studies with pyridate

The following study was evaluated in support of Annex I listing of pyridate and is no longer granted data protection:

IIA5.8.2/01 Janiak T. and Braunhofer P. (1989) General pharmacology of Pyridate technical. RCC Project 212837

Pyridate (batch No.: 2759523, 91.5%) was applied at single oral dosages by gavage of 0, 1000, 3000, 5000 and 8000 mg/kg bw to male KFM NMRI mice. Decreased activity and dyspnea (from ≥ 1000 mg/kg bw/d onwards) as well as hunched posture (from ≥ 3000 mg/kg bw/d onwards) were observed but no symptoms of clear pharmacological significance.

Pyridate (batch No.: 2759523, 91.5%) was applied at single oral dosages by gavage of 0, 250, 500, 1000 and 2300 mg/kg bw to male KFM Han Wistar rats. One animal died overnight at 2300 mg/kg bw. No other symptoms occurred.

A more detailed assessment is copied from the DAR for the first Annex I listing of pyridate:

A series of tests were performed to investigate pharmacological properties of Pyridate (91.5 %) in a study conducted under GLP and according to Japanese MAFF toxicity guidelines. The studies are acceptable for evaluating some effects of the test article on central nervous systems and respiratory/circulatory systems and can be seen as additional informations.

General behaviour

Single oral dosage of up to 8000 mg/kg bw to male mice (KFM NMRI) caused decreased activity and dyspnea, but no symptoms of clear pharmacological significance. Hunched posture was also noted at 3000 mg/kg bw. Oral application of up to 2300 mg/kg bw caused no observable symptoms in male rats (KFM Han Wistar), although at this dose level one animal died overnight. These dose levels were slightly lower than those causing severe toxicity in the appropriate acute toxicity studies.

Prolongation of hexabarbitone sleeping time

Oral dosage of up to 8000 mg/kg bw to mice (KFM NMRI) showed no effect on the length of hexabarbitone-induced sleeping time, thus suggesting no interaction with hexabarbitone or influence on hepatic detoxification enzymes. Reference substance diazepam showed a statistically significant increase by 161 % in the sleeping time.

Pentetrazole-, Strychnine-, or Electroshock-induced convulsions

Oral dosage of up to 8000 mg/kg bw to mice (KFM NMRI) had no influence on the time between dosing pentetrazole and appearance of pentetrazole-induced convulsions, or strychnine application and strychnine-induced convulsions; nor on the appearance or duration of electroshock-induced effects.

Effects on locomotory activity

Activity-monitor quantitation confirmed earlier findings that locomotor activity was reduced (i.e. hypoactivity) in mice (KFM NMRI) dosed orally with 8000 mg Pyridate/kg bw. Locomotor activity was decreased to 50 and 30 % from 45 - 60 and 165 - 180 minutes after treatment.

Body temperature

Oral administration of up to 2300 mg Pyridate/kg bw to rats (Wistar) caused no effect on body temperature during the ensuing 6 hours.

Interaction with tremourine

Intraperitoneal injection of 8000 mg Pyridate/kg bw to mice (KFM NMRI) caused no detectable influence on the cholinergic symptoms induced by tremourine.

Effect on the cardiovascular system

Intravenous dose levels of up to 200 mg/kg bw into the catheterized jugular vein of anaesthetized rabbits (New Zealand White) caused no obvious effects on blood pressure or heart rate. One of the two rabbits used for this study died during the test, probably due to solid test material causing obstruction of pulmonary capillaries.

(Janiak, 1989)

Reference:	**Final literature review on Pyridate and metabolites**
Author(s), year:	Criollo R., 2013
Reference:	Dr. Knoell Consult GmbH, Mannheim Germany, Report No.: 467901-1
Guideline(s):	Not applicable
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Yes

A search of the scientific peer-reviewed open literature relevant to the scope of the application for renewal, dealing with side-effects on health, and published within the last 10 years before the date of submission of the dossier, was conducted.

No new information relevant for the classification of pyridate was retrieved in the literature search.

3.12.1.1 Neurotoxicity

No specific study addressing neurotoxic effects of pyridate was submitted. However neurotoxic effects (clinical signs) were observed in a range of acute and repeated dose studies in rats and dogs. Furthermore, expert statements were submitted from the notifier regarding neurotoxic effects and their impact on classification and labelling for renewal of approval of pyridate.

These data are presented in sections 3.2 (Acute Toxicity), 3.3 (STOT SE) and 3.8 (STOT RE).

Two position papers from the manufacturing company regarding neurotoxicity findings in dogs are presented in detail in Annex.

4 ENVIRONMENTAL HAZARD ASSESSMENT

4.1 Degradation

Table 73: Summary of relevant information on degradation of pyridate

Method		Results			Re-marks	Reference
Guideline	**Type of study**	**Matrix**	**Temp.**	**Result/Half-life**		
US-EPA, N, 161-1; OECD A 80/13	Aqueous hydrolysis	pH 5, 7 and 9, sterile buffer solutions	22 and 25 °C	Study 1 (22 °C): pH 5: DT50 = 2.8 d pH 7: DT50 = 0.7 d pH 9: DT50 = 0.1 d Study 2 (25 °C): pH 5: DT50 = 3.7 d pH 7: DT50 = 2.4 d pH 9: DT50 = 0.4 d		Study 1: Zohner et al., 1981, Study 2: Lutringer, 1997
US-EPA, N, 161-2	Aqueous photolysis	pH 5, 7 and 9, sterile buffer solutions	25 °C	No photo-degradation (no significant adsorption > 290 nm)		Van Dijk and Betschart, 1992
OECD 301F	Biological degradation (ready)	Activated sludge obtained from a communal wastewater treatment plant	22 ± 2 °C	Not readily biodegradable		Dietschy, 2001
OECD 308	Water/ sediment study	Water pH: 7.1 – 8.2 Sediment pH: 5.4 – 7.3 OC: 1.0 – 5.0 %	20 °C	DegT50 (whole system) = 0.4 – 0.6 days (n = 2) DisT50 (water) = 0.3 days (n = 2)		Simmonds, 2012
BBA, IV, 4-1	Laboratory studies on aerobic degradation in soil	Soil pH: 6.0 – 7.7 Clay: 4.0 – 11.3 % OC: 1.3 – 2.6 %	20 °C	DegT50 (normalized) = 0.3 – 3.3 days (n = 5)		Schanné and Morgenroth, 1995
US-EPA, N, 161-3	Soil photolysis		20 °C	No photo-degradation (no significant adsorption > 290 nm)		Van Dijk and Baranowski, 1992
US-EPA, N, 164-1; SETAC 1995	Field studies on degradation in soil	Soil pH(H_2O): 5.0 – 7.7 OC: 0.5 – 2.6 %	Ambient conditions	DegT50 (ambient cond.) = 0.4 – 2.2 days (n = 7) DegT50 (normalized) = 1.0 – 2.1 days (n = 3)		Dykeman 1992a, 1992b, 1992c, Krennhuber and Pfarl, 1996, Chambers et al., 2011a, Chambers et al., 2011b

Table 74: Summary of relevant information on degradation of major metabolite **pyridafol (CL 9673)**

Method		**Results**			**Re-marks**	**Reference**
Guideline	**Type of study**	**Matrix**	**Temp.**	**Result/Half-life**		
US-EPA, N, 161-1	Aqueous hydrolysis	pH 4, 5, 7 and 9, sterile buffer solutions	25 and 50 °C	Stable		Goodyear, 1997
OECD 316	Aqueous photolysis	pH 4, 7 and 9, sterile buffer solutions	25 °C	pH 5: DT50 = 0.04 d pH 7: DT50 = 1.1 d pH 9: DT50 = 1.8 d		Mills and Dobson, 2012
OECD 308	Water/ sediment study	Water pH: 7.1 – 8.6 Sediment pH: 5.4 – 7.6 OC: 0.5 – 5.0 %	20 °C	DegT50 (whole system) = 150 – 491 days (n = 4) DisT50 (water) = 54.5 – 278 days (n = 4)		Krüger, 1997; Simmonds, 2012
US-EPA, N, 162-1; BBA, IV, 4-1	Laboratory studies on aerobic degradation in soil	Soil pH: 5.4 – 7.7 Clay: 1.3 – 27.4 % OC: 1.0 – 2.6 %	20 °C	DegT50 (normalized) = 16.5 – 42.8 days (n = 9)		Zohner, 1985; Schanné and Morgenroth, 1995
US-EPA, N, 161-3	Soil photolysis	Soil pH: 5.8 Clay: 5.0 % OC: 1.8 %	20 °C	DT50 = 19.4 days (n = 1)		Van Dijk and Baranowski (1992)
US-EPA, N, 164-1; SETAC 1995	Field studies on degradation in soil	Soil pH: 5.0 – 8.0 OC: 0.5 – 2.6 %	Ambient conditions	DegT50 (ambient cond.) = 5.4 – 97 days (n = 14) DegT50 (normalized) = 5.1 – 44.7 days (n = 9)		Ellgehausen, 1985; Heegemann et al., 1983; Dykeman 1992a, 1992b, 1992c; Krennhuber and Pfarl, 1996, Gasser A., 2001a, 2001b, 2001c; Chambers et al., 2011a, 2011b

4.1.1 Stability

Aqueous hydrolysis

For first Annex I inclusion hydrolysis of pyridate in sterile buffer solutions with varying pH was investigated in two studies:

- Determination of hydrolysis of Pyridate (CL 11.344) in water as a function of pH (Zohner et al., 1981)
- Pyridate: Hydrolysis as Function of pH (Lutringer, 1997)

Pyridate is rapidly hydrolysed in water to form the biologically active metabolite pyridafol (CL-9673), which is considered to be stable under conditions of sterile hydrolysis. Re-calculated DT_{50} values for pyridate at 22 ± 0.1 °C were 2.8, 0.7 and 0.1 days at pH 5, 7 and 9, respectively (RMS assessment based on Zohner et al., 1981). Results from the second study (Lutringer, 1997) were somewhat higher with DT_{50} values of 3.7, 2.4 and 0.4 days at pH 5, 7 and 9 at 25 °C.

Aqueous hydrolysis of the metabolite pyridafol (CL-9673) was not investigated in detail for first Annex I inclusion. For renewal a study conducted on pyridafol (CL-9673) was submitted:

- (^{14}C)-SAN 1367 H: Hydrolytic stability (Goodyear, 1997)

The study submitted for renewal is summarized below:

Reference:	**(^{14}C)-SAN 1367 H: Hydrolytic stability**
Author(s), year:	Goodyear, A., 1997
Report/Doc. number:	Covance Laboratories, 252/227-1015
Guideline(s):	EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-1 (1982)
GLP:	Yes
Deviations:	None
Validity:	Yes

MATERIAL AND METHODS:

Test substance:	[4,5-^{14}C]-pyridafol (CL-9673), batch (^{14}C)-CL-9673-M9230-C, radiochemical purity > 99.0 % by TLC, specific activity 2.79 MBq/mg
Reference substance:	Pyridafol (CL-9673, unlabelled)
Test system:	Sterile aqueous buffer solutions at pH 4 (0.1 M potassium hydrogen phthalate buffer), pH 5 (0.01 M sodium citrate buffer), pH 7 (0.01 M TRIS maleic acid buffer) and pH 9 (0.01 M borate boric acid buffer), sterility confirmed by microbial analysis
Temperature:	50 and 25 °C
Test duration:	5 days (50 °C), 32 days (25 °C)
Test concentration:	25 µg/mL
Analytical techniques:	TLC, HPLC-RAD

Findings:

No degradation of pyridafol (CL 9673) was observed under conditions of sterile aqueous hydrolysis at pH 4, 5, 7 and 9 at 25 and 50 °C. Pyridafol (C-9673) was shown to be stable under these conditions for 32 days (25 °C) and 5 days (50 °C), respectively.

Comments (RMS):

None

Aqueous photolysis

Two aqueous photolysis studies on pyridate and pyridafol (CL-9673) had been assessed for first Annex I inclusion. In addition one study was conducted to derive the quantum yield for pyridate.

- Photodegradation study of ^{14}C-pyridate in water at pH 5, 7 and 9 (Van Dijk and Betschart, 1992)
- Aqueous photolysis study on ^{14}C-CL 9673, the hydrolysis product of ^{14}C-Pyridate (Zohner, 1988) (non-GLP)
- Direct phototransformation study. CL 9673. Determination of quantum yield in water at pH 7 (Werle, 1993)

Pyridate itself does not significantly absorb light at wavelengths > 290 nm but is rapidly hydrolysed in water to pyridafol (CL-9673) which absorbs at wavelengths > 290 nm accompanied by a significant photo-degradation to several metabolites.

The main findings of these studies are summarized below.

In the GLP study from Van Dijk and Betschart (1992) ^{14}C-pyridate was exposed to artificial light (xenon burner, cut-off filter < 290 nm, 12 hrs of light per day) at 25 °C in sterile buffer solutions at pH 5, 7.3 and 9.2, respectively, up to 30 days. Formation of $^{14}CO_2$ in irradiated samples was significant (27 %, 30 % and 25 % AR at pH 5.0, 7.3 and 9.2, respectively). In contrast, negligible amounts of CO_2 were formed in dark samples. Under conditions of irradiation pyridate rapidly hydrolysed to pyridafol (CL-9673) (showing a maximum occurrence of 12.7 %, 40.6 % and 59.7 % AR at pH 5.0, 7.3 and 9.2, respectively). Pyridafol (CL-9673) was photo-degraded further releasing numerous unidentified photolysis products. Based on multiple TLC-analyses it was concluded that, except for one metabolite fraction at pH 5.0 (11.3 % AR by 16 DAT), at all three pH-values no photolytic degradation product accounted for more than 10 % AR. However, no efforts were undertaken to identify the nature of the metabolite fractions.

Table 75: Residues (% AR) of pyridate and pyridafol (CL-9673) in sterile aqueous buffer solutions exposed to artificial light (25 °C) (Van Dijk and Betschart, 1992) (data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	pH 5.0			pH 7.3			pH 9.2		
	Pyridate	Pyridafol (CL-9673)	Others[a]	Pyridate	Pyridafol (CL-9673)	Others[a]	Pyridate	Pyridafol (CL-9673)	Others[a]
0	91.3	5.8	2.9	91.0	6.2	2.8	53.7	44.3	2.0
1/24	91.4	6.1	2.9	77.5	8.7	11.8	na	na	na
0.25	59.2	7.0	10.2	49.7	24.6	13.4	na	na	na
0.5	39.4	11.0	7.1	53.2	21.9	10.7	na	na	na
2	25.8	**12.7**	8.8	32.5	38.8	10.1	3.3	**59.7**	6.6
4	8.5	6.5	16.8	13.6	**40.6**	11.8	1.0	49.7	6.0
8	0.7	3.9	24.7	3.2	38.6	**14.6**	1.8	40.9	10.1
16	nd	nd	**32.2[b]**	nd	23.2	9.0	nd	22.8	9.5
30	na	na	na	na	na	na	nd	7.7	**10.6**

na denotes not analysed
nd denotes not detected
[a] Max of individual unknown metabolite fraction
[b] Shown to consist of numerous minor, highly unstable compounds

Table 76: Re-calculated DT_{50} of pyridate and pyridafol (CL-9673) in sterile aqueous buffer solutions exposed to artificial light (25 °C) (Van Dijk and Betschart, 1992) – RMS assessment.

Substance	Test system	pH 5.0	pH 7.3	pH 9.2
Pyridate	Irradiated	0.5	1.0	0.4
Pyridafol (CL-9673)	Irradiated	2.0	9.8	9.9

In the non-GLP study from Zohner (1988), conducted according to the US EPA guideline 161-2, ^{14}C-pyridafol (CL-9673) was exposed to natural sunlight at outdoor temperature (13 – 22 °C, latitude 48°14', August to September) in sterile aqueous buffer solutions (non-sensitised and sensitised with acetone) at pH 5, 7 and 9. The major photolysis product was carbon dioxide accounting for 27, 53 and 27 % AR at pH 5, 7 and 9, respectively, in non-sensitised samples, and 55, 82 and 80 % AR at pH 5, 7 and 9, respectively, in sensitised samples after 18.5 sun days (= 35 calendar days). Several photolytic products were formed over time showing a high turnover rate. Up to 17 individual metabolite fractions, mainly belonging to highly polar compounds, were detectable by multidimensional TLC, none of them significantly exceeding 10 % AR. Recalculated DT_{50} values (RMS assessment) for pyridafol (CL-9673) were 0.3, 4.9 and 15.0 sun days at pH 5, 7 and 9,

respectively, in non-sensitised buffer solutions and 0.2, 1.1 and 1.9 sun days at pH 5, 7 and 9, respectively, in sensitized buffer solutions.

Table 77: Residues (% AR) of pyridafol (CL-9673) in sterile non-sensitized and sensitized aqueous buffer solutions exposed to natural light at outdoor temperature (13 – 22 °C, latitude 48° 14'. August to September) (Zohner, 1988).

DAT	Sundays	Non-sensitized			Sensitized (adding acetone)		
		pH5	pH 7	pH 9	pH5	pH 7	pH 9
0	0.0	97.9	97.9	97.9	97.9	97.9	97.9
1	1.0	9.5	87.9	92.7	5.1	65.8	77.2
2	1.6	3.6	78.9	87.1	3.9	54.8	64.8
3	2.5	1.5	76.8	83.1	2.8	29.3	39.6
8	5.7	0.7	43.4	77.7	1.1	0.3	0.4
14	9.3	1.6	26.0	59.3	0.25	0.2	0.7
23	13.1	1.1	15.4	53.9	0.71	0.2	1.0
35	18.5	-	7.2	40.9	-	-	1.8

Table 78: Re-calculated DT_{50} (sun days) of pyridafol (CL-9673) in sterile aqueous buffer solutions exposed to natural sunlight under outdoor conditions (Zohner, 1988) – RMS assessment.

Test system	pH 5	pH 7	pH 9
Non-sensitized	0.3	4.9	15.0
Sensitized (with acetone)	0.2	1.5	1.9
Dark	Stable	Stable	Stable

The quantum yield Φ of pyridafol (CL-9673) in water at pH 7 was estimated to be 0.00017 (Werle, 1993).

In both aqueous photolysis studies, assessed for first Annex I inclusion, the metabolite identification was insufficient and consequently a new study is provided for renewal. A new aqueous photolysis study including quantum yield determination was performed with pyridafol (CL-9673). In a separate study the theoretical half life time of pyridafol (CL-9673) in the top layer of aqueous systems was estimated.

- [^{14}C]-CL-9673: Aqueous photolysis and quantum yield determination in sterile buffer solutions (Mills and Dobson, 2012)
- CL9673 (pyridate metabolite): Estimation of environmental photolytic half-life in water. Model calculation according to Frank and Klöpffer (Buntain, 2012)

The studies submitted for renewal are summarized below:

Reference:	**[^{14}C]-CL-9673: Aqueous photolysis and quantum yield determination in sterile buffer solutions**
Author(s), year:	Mills, E.A.M., Dobson, R.M., 2012
Report/Doc. number:	Battelle UK Ltd., OZ/10/019
Guideline(s):	OECD 316 (2008)
GLP:	Yes
Deviations:	None
Validity:	Yes

MATERIAL AND METHODS:

Test substance:	[4,5-^{14}C]-pyridafol (CL-9673), batch 3885NGP024-3, radiochemical purity 99.0 % by HPLC, specific activity 2.95 MBq/mg
Reference substances:	Pyridafol (CL-9673, unlabelled), HHAC-047, HHAC-048, HHAC-060, HHAC-061, HHAC-062
Test system:	Irradiated sterile aqueous buffer solutions at pH 4 (0.01 M acetate buffer), pH 7 (0.01 M phosphate buffer) and pH 9 (0.01 M borate buffer), sterility confirmed by microbial analysis
Light source:	Xenon arc lamp (Heraeus Suntest CPS+), 71.5 W/m^2 from 300 – 400 nm, cut-off filter < 290 nm, measured intensity in the range from 300 - 400 nm used to determine the days of equivalent summer sunlight at a latitude of 30 °N
Temperature:	25 °C (irradiated), 20 °C (non-irradiated)
Test duration:	6, 8.2 and 10 days at pH 4, 7 and 9, respectively
Test concentration:	0.5 mg/L, max. 0.57 % v/v of solvent (acetonitrile); additional experiment (pH 7 only) at 0.05 mg/L without solvent
Volatile traps:	Closed system in the definitive phase, samples were treated with barium chloride prior to HPLC analysis in order to precipitate $^{14}CO_2$; in the preliminary phase polyurethane foam and soda lime were used, ^{14}C-CO_2 confirmed by barium chloride precipitation
Actinometer:	4-Nitroacetophenone (PNAP)/pyridine, 2 µM PNAP, ca. 0.2 M pyridine
Analytical techniques:	HPLC-RAD, LC-MS, LC-MS/MS LOD = 1 % AR
Kinetic evaluation:	SFO kinetics for pyridafol (CL-9673), KinGUI 1.1

Findings:

The recovery of radioactivity was quantitative, with all recoveries within the acceptable range of 90 – 110 % AR. The overall material balance for individual samples was in the range of 90.7 – 99.8 % AR at pH 4, 92.7 – 100.5 % AR at pH 7 and 96.1 – 104.0 % AR for pH 9 for the irradiated samples. For the non-irradiated samples recoveries ranged from 96.4 – 100.0 % AR at pH 4, from 97.4 – 102.1 % AR at pH 7 and from 98.2 – 108.6 % AR at pH 9.

No significant hydrolysis occurred. In the non-irradiated samples, the parent compound was found to be stable for the time period of this study. Amounts of pyridafol (CL-9673) in dark samples were found to be 100.1 ± 2.5 % AR.

In the irradiated samples at pH 4, pyridafol (CL-9673) decreased from 99.3 % AR at time zero to 6.4 % AR after 0.2 DAT. At pH 7 the corresponding decline was from 99.9 % to 8.9 % AR by 4

DAT. At pH 9 the decline in pyridafol (CL-9673) from 99.5 % to 6.0 % AR took 8 days, showing a trend for slower photo-transformation of the parent at higher pH.

Mineralisation to carbon dioxide occurred at all pH values. It was the major final degradate at pH 7 and pH 9, but was only a minor degradate at pH 4. At pH 7 $^{14}CO_2$ rose to 37.3 % AR after 8.2 days of irradiation. At pH 9 carbon dioxide rose to a plateau value at the last two test points; a maximum of 44.0 % and 42.0 % AR by 8 and 10 DAT, respectively.

Across all pH values there were two other major degradates (> 10 % AR). They were identified with supplied reference standards. HHAC-062 reached a maximum of 63.1 % AR by 3 day of irradiation at pH 4, declining a little thereafter. HHAC-060 reached a maximum of 23.6 % AR after 1 hour irradiation at pH 4, thereafter declining to zero by 1 day of irradiation. A third degradate was identified as HHAC-047, which reached a maximum of 8.7 % AR after 1 days of irradiation at pH 9 and then declined.

A partially characterised degradate (rrt 1.30) reached a maximum of 9.4 % AR at the end of irradiation at pH 7. It was shown by LC-MS to have a molecular mass greater than the parent test item, which implied a bi-molecular reaction which could be an artefact caused by the artificially high concentration in the study. A repeat experiment at a lower concentration showed that significantly less of this degradate, maximum 3.7 % AR, was formed under the environmentally more relevant concentrations.

The phototransformation of pyridafol (CL-9673) resulted in a complex pattern of degradation, including regions where a large number of minor degradates with similar HPLC retention behaviour formed groups of poorly-resolved peaks. The only important group (rrt 0.25) was collected and found to separate into several minor degradates (none > 10 % AR) by re-analysis using a different HPLC system.

Table 79: Transformation of pyridafol (CL-9673) in pH 4 buffer solution, irradiated samples (% AR, mean of two replicates, data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	rrt 0.25[a]	rrt 0.31[b]	rrt 0.47	rrt = 0.63 (HHAC-062)	rrt 0.90 (HHAC-047)	Pyridafol (CL-9673)	rrt 1.29 (HHAC-060)	Sum of minor peaks	^{14}C-CO_2[c]
0.0	nd	nd	nd	nd	nd	99.3	nd	nd	0.0
0.02	3.2	1.1	1.0	4.3	nd	73.1	16.3	0.8	0.0
0.04	6.3	1.8	1.0	14.9	1.2	41.5	**23.6**	6.8	0.7
0.1	8.6	2.5	1.3	23.5	1.2	29.5	21.2	8.3	1.0
0.2	10.9	3.0	nd	54.8	**3.6**	6.4	11.7	6.1	0.0
1.0	15.7	**4.7**	0.2	62.7	3.4	nd	nd	5.0	0.3
3.0	17.8	3.8	1.1	**63.1**	2.8	0.2	nd	7.4	**1.6**
6.0	**32.6**	2.0	**3.5**	49.6	1.3	nd	nd	0.4	**1.6**

nd denotes not detected
[a] Several overlapping peaks none exceeding 9.1 % AR for any interval
[b] Three overlapping peaks none exceeding 2.8 % AR for any interval
[c] Dissolved in aqueous phase

Table 80: Transformation of pyridafol (CL-9673) in pH 7 buffer solution, irradiated samples (% AR, mean of two replicates, data in grey indicate exceedance of 10 % of AR for an individual

compound, bold data indicate maximum values).

DAT	rrt 0.25[a]	rrt 0.31[b]	rrt 0.36[c]	rrt 0.47	rrt 0.65 incl. HHAC-062[d]	rrt 0.90 (HHAC -047)	Pyridafol (CL-9673)	rrt 1.27 (HHAC -060)	rrt 1.30	Sum of minor peaks	^{14}C-CO_2[e]
0.0	nd	nd	nd	nd	nd	nd	99.9	nd	nd	0.1	0.0
0.2	1.4	nd	0.5	0.3	1.1	2.5	91.9	1.0	nd	nd	0.4
0.3	3.8	0.2	0.6	0.8	4.1	4.3	82.2	**1.2**	0.3	0.3	1.3
1.0	9.9	**3.2**	1.2	1.4	10.4	**6.6**	59.0	0.9	1.1	0.7	2.3
2.0	17.9	1.7	1.1	2.1	16.8[f]	5.4	26.1	nd	2.4	1.9	22.1
4.0	21.1	2.0	0.8	3.6	**21.9**[f]	4.4	8.9	nd	6.2	**3.0**	22.4
6.1	24.1	3.0	3.1	2.9	15.8[f]	0.6	1.4	nd	8.0	1.1	31.5
8.2	**26.6**	2.7	**3.9**	**3.8**	10.7	0.1	0.4	nd	**9.4**	0.8	**37.3**

nd denotes not detected
[a] Seven overlapping peaks none exceeding 7.8 % AR for any interval
[b] Three overlapping peaks none exceeding 2.5 % AR for any interval
[c] Two overlapping peaks none exceeding 1.7 % AR for any interval
[d] Region includes HHAC-062 and two other peaks not exceeding 4.7 % AR for any interval as well as a number of minor peaks whose sum never exceeded 2.2 % AR
[e] Dissolved in aqueous phase
[f] RMS: Residues of HHAC-062 are 8.6 %, 11.5 % and 11.2 % AR at 2, 4 and 6.1 DAT, respectively (based on additional HPLC analysis)

Table 81: Transformation of pyridafol (CL-9673) in pH 7 buffer solution, irradiated samples, additional experiment at reduced application rate and without solvent (% AR, mean of two replicates, data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	Pyridafol (CL-9673)	rrt 1.27 (HHAC-060)	rrt 1.30	Sum of other peaks	^{14}C-CO_2[b]
0.0	99.7	nd	nd	0.2	0.0
1.0	65.8	**1.2**	nd	24.1	13.1
3.0	14.2	0.2	1.9	51.9	26.0
6.0	2.4	nd	2.3	**56.2**	35.8
7.0	0.6	nd	1.0	49.9	49.4
7.9	0.6	nd	**3.7**	50.8	43.0
10.1	0.2	nd	2.7	47.9[a]	43.0
11.0	nd	nd	1.5	45.7	**49.6**

nd denotes not detected
[a] RMS: rrt 0.25: 24.7 % AR, rrt 0.31: 1.8 % AR, rrt 0.36: 1.8 % AR, rrt 0.47: 1.3 % AR, rrt 0.65 (including HHAC-062): 16.6 % AR
[b] Dissolved in aqueous phase

Table 82: Transformation of pyridafol (CL-9673) in pH 9 buffer solution, irradiated samples (% AR, mean of two replicates, data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	rrt 0.25[a]	rrt 0.31[b]	rrt 0.65 (HHAC-062)	rrt 0.73	rrt 0.90 (HHAC-047)	Pyridafol (CL-9673)	Sum of minor peaks	^{14}C-CO_2[c]
0.0	nd	nd	nd	nd	0.1	99.5	0.5	0.0

0.3	3.2	0.3	0.1	nd	4.3	91.3	0.2	2.0
1.0	10.0	1.8	0.2	0.3	**8.7**	69.8	1.8	8.2
2.0	17.6	3.5	1.0	1.5	8.4	49.3	3.8	13.5
4.0	28.1	4.0	3.2	**3.7**	3.7	17.7	9.1	28.4
6.0	28.8	4.6	3.2	3.0	1.9	10.1	8.1	38.5
8.0	29.7	4.7	**4.0**	2.5	0.2	6.0	**9.6**	44.0
10.0	**34.4**	**5.6**	3.9	1.3	nd	2.2	7.0	42.0

nd denotes not detected
[a] Seven overlapping peaks none exceeding 10.0 % AR for any interval
[b] Three overlapping peaks none exceeding 2.8 % AR for any interval
[c] Dissolved in aqueous phase

$DegT_{50}$ and $DegT_{90}$ for the decline of pyridafol (CL-9673) were calculated assuming SFO kinetics. The DT_{50} of pyridafol (CL-9673) was 0.04 days, 1.12 days and 1.81 days at pH 4, 7 and 9, respectively, equivalent to 0.15, 3.5 and 5.3 natural sunlight days.

Table 83: Photo-degradation half-life of pyridafol (CL-9673).

Test System	**pH**	**Experimental**		**Natural sunlight[a]**		**Chi2 error (%)**	**Kinetics**
		$DegT_{50}$ (days)	**$DegT_{90}$ (days)**	**$DegT_{50}$ (days)**	**$DegT_{90}$ (days)**		
Pyridafol (CL-9673)	4	0.04	0.14	0.15	0.49	7.0	SFO
	7	1.12	3.74	3.50	11.6	3.5	SFO
	9	1.81	6.00	5.31	17.7	3.4	SFO
Actinometer	4	0.17	0.56	-	-		SFO
	9	1.63	5.41	-	-		SFO

[a] One experimental day corresponds to 3.40, 3.12 and 2.94 days of natural sunlight at pH 4, 7 and 9, respectively (summer, 30 °N)

A 4-Nitroacetophenone (PNAP)/pyridine actinometer was irradiated simultaneously with the pyridafol (CL-9673) samples. The quantum yield for pyridafol (CL-9673) was determined from a comparison of actinometer and pyridafol (CL-9673) photolytic decay kinetics. The quantum yield for pyridafol (CL-9673) was found to be 0.0094 at pH 4 and 0.00019 at pH 9, respectively.

Conclusion:

Following exposure to artificial light, pyridafol (CL-9673) was degraded by photolysis to a significant extent. $DegT_{50}$ of pyridafol (CL-9673) was 0.04, 1.12 and 1.81 days at pH 4, 7 and 9, respectively, equivalent to 0.15, 3.50 and 5.31 natural sunlight days (30 °N). At pH 7 and 9 mineralisation to CO_2 was the most significant degradation pathway. The photolytic degradation route was complex and pH dependent, with two degradation products exceeding 10 % AR during the course of the study. None of the major degradates was persistent under the conditions of the test. HHAC-062 reached a maximum of 63.1 % AR after 3 days of irradiation at pH 4, declining to < 50 % AR after 6 days. HHAC-060 reached a maximum of 23.6 % AR after 1 hour irradiation at pH 4, thereafter declining to < LOQ by 1 DAT. A third degradate was HHAC-047, which reached a maximum of 8.7 % AR after 1 day at pH 9 and then declined.

The quantum yield for pyridafol (CL-9673) was found to be 0.00944 at pH 4 and 0.000188 at pH 9.

Comments (RMS):

None

Reference:	**CL9673 (pyridate metabolite): Estimation of environmental photolytic half-life in water. Model calculation according to Frank and Klöpffer**
Author(s), year:	Buntain, I.G., 2012
Report/Doc. number:	Battelle UK, report no. OZ/12/002
Guideline(s):	ECETOC Technical report No. 12; German UBA Test Guideline
GLP:	Yes
Deviations:	No
Validity:	Yes

The estimation method described in ECETOC Technical report No. 12 (1984) and the solar light intensity determined by Frank and Klöpffer (1988) were used in this study.

The UV/visible spectra and quantum yields used for the calculation were those determined in the study by Mills and Dobson (2012).

With a pK_a of 6.7, pyridafol (CL-9673) would be expected to exist largely in an ionised (deprotonated) form at pH 9 but to remain largely protonated at pH 4. The calculations in this report were therefore performed using experimental data obtained at both pH 4 and pH 9.

In the study by Mills and Dobson (2012), absorbance data for pyridafol (CL-9673) at both pH 4 and pH 9 were determined at 5 nm intervals in the range 290 to 340 nm and at 10 nm intervals at wavelengths above 340 nm (until absorbance was too weak to measure). Since the calculation of environmental photolytic half-life in water following the method of Frank and Klöpffer requires the molar extinction coefficient at these and intermediate, wavelengths, it was necessary to calculate these. The molar extinction coefficients at each wavelength were calculated using the Beer-Lambert law.

Table 84: Photolytic half-life of pyridafol (CL-9673) (pH 4 and pH 9) when dissolved in the top millimetres of natural aquatic systems (Central Europe latitude, 52 °N).

Month	pH 4	pH 9	Month	pH 4	pH 9	Month	pH 4	pH 9	Month	pH 4	pH 9
Jan	1.2	55.8	Apr	0.2	8.0	Jul	0.2	6.4	Oct	0.4	18.3
Feb	0.6	26.7	May	0.2	6.5	Aug	0.1	6.2	Nov	1.0	44.3
Mar	0.3	13.3	Jun	0.1	5.9	Sep	0.2	10.0	Dec	1.8	89.1

Conclusion:

The photolytic half-life of pyridafol (CL-9673) in the top millimetres of natural aquatic systems was estimated to range from ca. 0.1 days in June to 1.8 days in December in acidic waters (pH 4) and from 5.9 days in June to 89 days in December in high pH waters (pH 9). Thus it can be concluded that pyridafol (CL-9673) in the top few millimetres of aquatic systems may undergo rapid degradation by direct photolytic processes under the conditions prevailing in Central Europe, dependent upon the season and the pH of the water body.

Comments (RMS):

None

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

Reference:	**Ready biodegradability of SAN 319 tech. (pyridate tech.), (Manometric respirometry test)**
Author(s), year:	Dietschy, A., 2001
Report/Doc. number:	Solvias AG, report no. LO1 - 006745
Guideline(s):	OECD 301 F (1993), 92/69/EEC, C.4-D (1992)
GLP:	Yes
Deviations:	No
Validity:	Yes

MATERIAL AND METHODS:

Test substance:	Pyridate (tech.), unlabelled, purity 91.8 %
Reference substance:	Na-benzoate
Inoculum:	Mixture of polyvalent bacteria (activated sludge obtained from a communal wastewater treatment plant)
Treatments:	• Blank control • Reference substance: Na-benzoate • Test substance: Pyridate • Toxicity control: Pyridate and Na-benzoate • Abiotic sterile control: Pyridate, Na-benzoate and sterilizing agent ($HgCl_2$)
Analysis:	Biochemical oxygen demand (BOD)
Incubation conditions:	22 ± 2 °C, 28 days

Findings:

Table 85: Biodegradation of pyridate and reference compound (% of theoretically possible degradation).

DAT	Reference substance	Test substance	Toxicity control	Abiotic control
14	85	0	40	0
28	90	0	48	0

No biodegradation was observed within the test period of 28 days. As a conclusion, the test item pyridate was not readily biodegradable after 28 days of incubation.

Conclusion:

Pyridate is considered to be not readily biodegradable.

4.1.2.2 Screening tests

No data

4.1.2.3 Simulation tests

Water/sediment studies

Water sediment studies (Zohner, 1985 and Zohner, 1988) assessed for first Annex I inclusion are not guideline compliant since conducted with rice soil in a water-soil slurry:

- Aerobic metabolism study of ^{14}C-pyridate in water/sediment (Zohner, 1985)
- Partition of ^{14}C-pyridate in water sediment (Zohner, 1988)

Although not guideline compliant the RMS notes that the outcome of these two studies are in line with the new studies submitted for renewal: Pyridate was rapidly hydrolysed to pyridafol (CL-9673), which was the only metabolite detectable in the dark water/sediment systems beside significantly lower amounts of pyridafol-O-methyl (CL-9869).

For renewal new studies with pyridate and pyridafol (CL-9673) are provided. In addition the degradation kinetics in both studies was recalculated following the FOCUS recommended procedure for determining modelling endpoints.

- [^{14}C]-Pyridate: Degradation and retention in two water-sediment systems (Simmonds, 2012)
- SAN 1367 H AI: Route and rate of degradation of ^{14}C-labelled SAN 1367 H AI in water/sediment systems (Krüger, 1997)
- Kinetic modelling analysis of pyridate and CL9673 from water sediment studies to derive modelling endpoints (Hardy, 2012a)

The studies submitted for renewal are summarized below:

Reference:	**[^{14}C]-Pyridate: Degradation and retention in two water-sediment systems**
Author(s), year:	Simmonds, M., 2012
Report/Doc. number:	Battelle UK Ltd., OZ/10/017
Guideline(s):	OECD 308 (2002)
GLP:	Yes
Deviations:	None
Validity:	Yes

MATERIAL AND METHODS:

Test substance:	[4,5-^{14}C]-pyridate, batch 3885NGP025-6, radiochemical purity 99.3 % by HPLC, specific activity 1.59 MBq/mg
Reference substances:	Pyridate (unlabelled), pyridafol (CL-9673), pyridafol-O-methyl (CL-9869)
Test system:	Two natural aquatic sediment systems in the dark under aerobic conditions (aerated with moistured air)
Temperature:	20 ± 2 °C
Test duration:	101 days
Test concentration:	0.3 mg/L (corresponding to approx. 900 g ai/ha and 12 cm water phase)
Water/sediment ratio:	Approx. 4 : 1 (v/v)

Volatile traps:	Ethylene glycol, 2 x 2M KOH
Monitoring:	O_2, pH, redox potential
Analytical techniques:	LSC, HPLC-RAD, LC-MS, LC-MS/MS LOQ = 0.14 % AR

Table 86: Physicochemical parameters of the water sediment systems

System	Swiss Lake (Chatsworth, Derbyshire)		Calwich Abbey Lake (Calwich, Ashbourne, Derbyshire)	
	Start	End	Start	End
Water phase				
Conductivity (µS/cm) (at collection)	82		440	
pH (at collection)	6.36		7.94	
pH	7.1		8.2	
Calcium (mg/kg)	5.7		68	
Magnesium (mg/kg)	2.0		6.6	
Hardness (mg equiv. $CaCO_3$/L)	23		198	
Total Organic Carbon (mg/kg)	8.0		3.9	
Dissolved Organic Carbon (mg/kg)	8.0		3.7	
Total Nitrogen (mg/kg)	0.7		1.9	
Total Phosphorus (mg/kg)	1.4		1.6	
Sediment				
Textural classification (USDA) Sand (50 – 2000 µm) Silt (2 – 50 µm) Clay (< 2 µm)	Sand 89 7 4		Silt Loam 33 59 8	
pH Deionised Water 1 M KCl 0.01 M $CaCl_2$	6.0 5.3 5.4		7.4 7.2 7.3	
Organic Carbon (%)	0.95		5.0	
Cation Exchange Capacity (meq/kg)	33		101	
Total Nitrogen (%)	0.08		0.39	
Total Phosphorus (mg/kg)	98		713	
Moisture Content (%)	41.8		168.9	
Microbial Biomass (mg C_{mic}/kg)	175	319	1067	958

Findings:

The pH of the water remained at around pH 7 for the Swiss Lake system (average pH 7.2) and pH 8 for the Calwich Abbey Lake system (average pH 8.0) during the incubation period. The redox potentials showed that the water phases remained aerobic throughout the study. Both sediments were anaerobic and remained in this state for the duration of the study. The oxygen content of both water phases were in the range of ca. 6 – 7 mg/L at the start of the study dropping slightly to ca. 5 – 6 mg/L during the latter stages of the study.

The overall recovery of radioactivity was good, with all individual flasks within 90 – 110 % AR and mean recoveries of 96.2 % (range 92.1 - 98.6 %) and 96.7 % (range 92.6 - 98.6 %) for the Swiss Lake and Calwich Abbey systems, respectively.

Only trace amounts of radioactivity were released as volatile products during the study with ≤ 1.5 % AR collected in the traps after 101 days incubation in both systems. All radioactivity was recovered from the potassium hydroxide traps, with none detected in the ethylene glycol (no more than 0.01 %), and thus was assumed to be carbon dioxide.

In the Swiss Lake (sand sediment) system the radioactivity was found to gradually transfer from the water phase to the sediment phase with time. Thus the radioactivity recovered from the water phase declined from 86.8 % at time zero to 70.3 % AR after 101 days. The radioactivity associated with the sediment increased from 5.4 % to 31.5 % after 1 day, decreased to 10.6 % after 3 days and then increased steadily to 25.0 % AR at the end of the study. Of this 25.0 % associated activity, a maximum of 9.3 % was determined as unextractable.

In the Calwich Abbey (silt loam sediment) system the radioactivity was also found to gradually transfer from the water phase to the sediment phase with time. Thus the radioactivity recovered from the water phase declined from 89.5 % at time zero to 64.4 % AR after 101 days. The radioactivity associated with the sediment increased from 3.8 % to 21.3 % after 1 day, decreased to 15.8 % after 7 days, increased steadily to a maximum of 31.6 % after 60 days before declining slightly to 30.8 % AR at the end of the study. Of this 30.8 % associated activity, a maximum of 7.9 % was determined as unextractable.

The degree of mineralisation was low with only around 1–1.5% of the applied radioactivity converted to carbon dioxide in both sediments by the end of the study.

In the total system, pyridate rapidly degraded in both systems, declining to undetectable levels after just 7 days in both systems. The only significant metabolite, pyridafol (CL-9673) achieved maximum mean levels of 96.2 % AR after 7 days in the Swiss Lake system, declining to 81.6 % AR at the end of the study. Similarly in the Calwich Abbey system, pyridafol (CL-9673) reached maximum mean levels of 96.6 % AR after 3 days incubation and declined to 83.7 % AR at the end of the study.

Table 87: Distribution of radioactivity in two aerobic water/sediment systems after application of ^{14}C-labelled pyridate (% AR).

Water/ sediment system	DAT	Water	Sediment				Total Volatiles[a]	Total
			ACN / water shake	ACN / water soxhlet	Total extractable	NER		
Swiss lake	**0**	86.8	5.3	0.1	5.4	< 0.1	na	92.2
	1	64.7	30.6	0.8	**31.4**	0.1	< 0.1	96.2
	3	87.2	9.9	0.5	10.4	0.3	< 0.1	97.9
	7	84.8	11.4	0.9	12.3	0.7	0.1	97.8
	13	82.6	11.1	1.2	12.3	2.1	0.1	97.1
	31	76.6	13.9	1.4	15.3	4.2	0.2	96.2
	60	73.9	14.7	1.4	16.1	5.8	0.4	96.2
	101	70.3	14.1	1.6	15.7	**9.3**	**1.0**	96.3
Calwich Abbey	**0**	89.5	3.7	0.1	3.8	< 0.1	na	93.3
	1	76.5	20.2	0.7	20.9	0.4	< 0.1	97.8
	3	80.4	16.5	0.6	17.1	0.4	< 0.1	97.9
	7	82.7	13.9	1.0	14.9	0.9	0.1	98.5
	13	75.1	18.9	0.9	19.8	2.7	0.1	97.7
	31	68.7	22.3	1.8	24.1	3.2	0.4	96.2
	60	63.2	22.2	2.1	**24.3**	7.4	**1.5**	96.3
	101	64.4	21.9	1.0	22.9	**7.9**	0.8	96.0

na denotes not analysed
[a] Volatiles exclusively in the KOH traps

Table 88: Characterization of extractable radioactivity (% AR) after application of ^{14}C-labelled pyridate to 'Swiss lake' the water/sediment system (data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	Water		Sediment		Total system		
	Pyridate	Pyridafol (CL-9673)	Pyridate	Pyridafol (CL-9673)	Pyridate	Pyridafol (CL-9673)	Sum of unknowns
0	76.5	10.9	1.3	3.3	77.8	14.2	0.0
0	71.5	14.7	3.1	3.0	74.6	17.7	0.0
Mean	74.0	12.8	2.2	3.1	76.2	15.9	0.0
1	8.5	58.0	13.5	16.1	21.9	74.0	0.0
1	12.8	50.2	19.7	11.9	32.4	62.1	0.0
Mean	10.6	54.1	**16.6**	**14.0**	27.2	68.1	0.0
3	1.0	84.6	2.6	8.5	3.6	93.0	0.0
3	0.0	88.9	2.4	6.2	2.4	95.2	0.0
Mean	0.5	**86.7**	2.5	7.4	3.0	94.1	0.0
7	0.0	86.4	0.0	10.9	0.0	97.3	0.0
7	0.0	83.1	0.0	11.9	0.0	95.1	0.0
Mean	0.0	84.8	0.0	11.4	0.0	**96.2**	0.0
13	0.0	84.6	0.0	9.8	0.0	94.4	0.2
13	0.0	80.6	0.0	12.2	0.0	92.8	0.0
Mean	0.0	82.6	0.0	11.0	0.0	93.6	0.1
31	0.0	71.7	0.0	14.1	0.0	85.8	0.9
31	0.0	78.2	0.0	13.2	0.0	91.4	2.9
Mean	0.0	74.9	0.0	13.7	0.0	88.6	1.9
60	0.0	73.2	0.0	14.7	0.0	87.9	3.1
60	0.0	72.1	0.0	12.4	0.0	84.5	1.7
Mean	0.0	72.7	0.0	13.6	0.0	86.2	2.4
101	0.0	67.1	0.0	16.0	0.0	83.1	3.8
101	0.0	69.2	0.0	11.0	0.0	80.2	1.8

Mean	0.0	68.2	0.0	13.5	0.0	81.6	**2.8**

Table 89: Characterization of extractable radioactivity (% AR) after application of ^{14}C-labelled pyridate to the 'Calwich Abbey' water/sediment system (data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	Water		Sediment		Total system		
	Pyridate	Pyridafol (CL-9673)	Pyridate	Pyridafol (CL-9673)	Pyridate	Pyridafol (CL-9673)	Sum of unknowns
0	71.4	17.9	0.0	4.5	71.4	22.4	0.0
0	67.2	22.4	0.0	2.9	67.2	25.4	0.0
Mean	69.3	20.2	0.0	3.7	69.3	23.9	0.0
1	11.1	66.3	0.3	19.1	11.4	85.4	0.0
1	11.2	64.3	2.8	18.2	14.0	82.5	0.0
Mean	11.2	65.3	**1.5**	18.6	12.7	83.9	0.0
3	0.1	85.6	0.4	11.1	0.5	96.7	0.0
3	0.0	75.0	0.0	21.5	0.0	96.5	0.0
Mean	0.1	80.3	0.2	16.3	0.3	**96.6**	0.0
7	0.0	83.5	0.0	13.1	0.0	96.6	0.0
7	0.0	81.8	0.0	14.7	0.0	96.6	0.0
Mean	0.0	**82.7**	0.0	13.9	0.0	**96.6**	0.0
13	0.0	76.2	0.0	18.0	0.0	94.2	0.2
13	0.0	74.0	0.0	19.5	0.0	93.5	0.2
Mean	0.0	75.1	0.0	18.7	0.0	93.9	0.2
31	0.0	69.4	0.6	22.6	0.6	92.0	0.2
31	0.0	67.1	0.0	20.6	0.0	87.7	1.3
Mean	0.0	68.2	0.3	**21.6**	0.3	89.8	0.7
60	0.0	59.3	0.0	20.7	0.0	80.0	3.1
60	0.0	64.7	0.0	21.4	0.0	86.0	1.6
Mean	0.0	62.0	0.0	21.1	0.0	83.0	2.3
101	0.0	60.5	0.0	22.5	0.0	82.9	0.9
101	0.0	63.9	0.0	20.6	0.0	84.5	4.3
Mean	0.0	62.2	0.0	21.5	0.0	83.7	**2.6**

Conclusion:

In water/sediment systems, pyridate was found to rapidly degrade to the hydroxyl-metabolite pyridafol (CL-9673) in both the water and sediment phases. The applied radioactivity dissipated gradually from the water phase to the sediment to form bound residues and minor amounts of carbon dioxide.

Comments (RMS):

- Dissipation/degradation rates of pyridate and pyridafol (CL-9673) in the water phase and in the entire system were re-calculated by Hardy (2012a) following pertinent FOCUS guidance.

Reference:	**SAN 1367 H AI: Route and rate of degradation of ^{14}C-labelled SAN 1367 H AI in water/sediment systems**
Author(s), year:	Krüger, B., 1997
Report/Doc. number:	Agrolinz Melamin, Study No. M 95-18, Report No. 1291
Guideline(s):	OECD 308 (2002)
GLP:	Yes
Deviations:	None
Validity:	Yes

MATERIAL AND METHODS:

Test substance:	[4,5-^{14}C]-pyridafol (CL-9673), batch ^{14}C-CL-9673-M9230-C, radiochemical purity > 98.8 % by HPLC, specific activity 2.79 MBq/mg
Reference substances:	Pyridafol (CL-9673) (unlabelled), pyridafol-O-methyl (CL-9869)
Test system:	Two natural aquatic sediment systems in the dark under aerobic conditions (aerated with moistured air), two samples sterilized by twofold autoclavation at 120 °C
Temperature:	20 ± 2 °C
Test duration:	120 days (Irrsee), 175 days (Rodl)
Test concentration:	0.173 mg/L (corresponding to approx. 520 g ai/ha and 30 cm water depth)
Water/sediment ratio:	Approx. 3 : 1 (v/v)
Volatile traps:	Ethylene glycol, 2 x 2 N NaOH (100 mL each), identity of $^{14}CO_2$ proven by $Ba(OH)_2$ precipitation
Monitoring:	O_2, pH, redox potential
Sediment extraction:	Acetonitrile (three times), acetonitrile/0.2 M HCl (1:1, v/v) (two times), harsh: acetonitrile/water (4/1, v/v) at 80 °C, acetonitrile/1 N HCl (9:1; v/v) at 80 °C
Analytical techniques:	LSC, HPLC-RAD, TLC LOQ = 0.1 % AR

Table 90: Physicochemical parameters in the two water sediment systems.

System	Irrsee (lake), Upper Austria		Rodl (river), Upper Austria	
	Start	End	Start	End
Water phase				
pH	8.32		8.59	
Hardness (°dH)	8.4		2.6	
P_{tot} (mg PO_4/L)	< 0.010	0.060	0.137	0.380
T_{OC} (mg/L)	2.3		3.7	
N_{tot} (mg NO_3/L)	124	39.3	74.1	72.7
Sediment				
Clay (< 2 µm) (%)	5.2		1.0	
Silt (2 - 63 µm) (%)	26.8		4.6	
Sand (> 63 µm) (%)	68.0		94.4	
MAFF classification	Sandy loam		Sand	
Biomass (mg C_{mic}/kg)	1335	854	385	164
C_{org} (%)	3.03		0.53	
N_{tot} (%)	0.2		0.03	
P_{tot} (%)	0.392		0.699	

pH ($CaCl_2$)	7.6		6.7	
CEC (meq/kg)	100		30	

Findings:

The pH value in the 'Irrsee' test system ranged between 8.1 and 8.9. The oxygen saturation of the water phase decreased slightly after application of the test substance, but increased afterwards again and reached a plateau of 70 – 95 % after 3 DAT. The redox potential of the water phase was nearly constant during the course of the study, the redox potential of the sediment decreased from about 160 mV to 100 mV. In the 'Rodl' test system the pH values ranged between 8.2 and 8.9. The oxygen saturation of the water phase was between 71 – 92 %. The redox potential of the water phase was nearly constant during the course of the study. The redox potential of the sediment varied between 125 and 198 mV.

The recoveries of radiocarbon were between 89.8 % and 105.5 % AR (individual replicates) with an average of 98.2 % for 'Irrsee' and 101.5 % for 'Rodl'. During the course of the study pyridafol (CL-9673) was slowly mineralized to carbon dioxide. The amount of $^{14}CO_2$ increased steadily and amounted to 5.7 % and 10.7 % AR at study termination in test system 'Irrsee 'and 'Rodl', respectively. Only traces of organic volatiles were observed (< 0.1% AR).

The amounts of radiocarbon in the water phase of test system 'Irrsee' decreased from 98.3 % AR (0 DAT) to 25.7 % AR (120 DAT). The radiocarbon content of the sediment reached a plateau value of about 50 % AR from 65 DAT onwards. Sediment extractable radioactivity increased to 37.1 % by 30 DAT and decreased thereafter to 27.3 % at 120 DAT. The amounts of radiocarbon in the water phase of test system 'Rodl' decreased from 98.5 % AR (0 DAT) to 28.8 % AR (175 DAT). The radiocarbon content of the 'Rodl' sediment increased to 62.9 % at 175 DAT. Sediment extractable radioactivity increased to 31.2 % by 51 DAT and remained fairly stable thereafter (30.7 % at 175 DAT). The non-extractable residues in the sediments increased steadily and reached 30.2 % at 120 DAT ('Irrsee') and 32.3 % at 175 DAT ('Rodl') and were characterized as belonging mainly to the fulvic acid fraction. Approximately 12 to 15 % of the fulvic acid fractions were identified as pyridafol (CL-9673).

Pyridafol (CL-9673) decreased in the whole test system 'Irrsee" (water phase and sediment) from 96.4 % (0 DAT) to 49.8 % (120 DAT). For 'Rodl' the respective values were 98.8 % (0 DAT) and 47.1 % (175 DAT).

Four minor metabolites were found in the course of the study with maximum individual amounts of 0.3 % and 1.8 % AR in the entire test systems 'Irrsee' and 'Rodl', respectively. One metabolite was identified by co-chromatography as pyridafol-O-methyl (CL-9869). The maximum amounts found were 0.2 % ('Irrsee') and 0.4 % ('Rodl').

Table 91: Recovery of radioactivity in two water sediment systems after application of ^{14}C-pridafol (CL-9673) (% AR, mean of 2 replicates, one replicate from 105 DAT onwards).

Water/ sediment system	DAT	Water	Sediment						$^{14}CO_2$	Organic volatiles	Unit wash	Recovery
			ACN	HCl/ ACN	Harsh	Sum extrac-table	NER	Total				
Irrsee	0	98.3	1.9	0.2	0.1	2.1	< 0.1	2.2	na	na	0.6	101.1
	1	89.1	9.4	1.2	0.4	11.1	0.3	11.4	< 0.1	< 0.1	1.1	101.7
	3	83.3	12.7	1.9	1.1	15.7	0.6	16.3	0.4	nd	0.9	100.9
	7	72.4	19.3	3.1	2.4	24.8	1.4	26.1	0.2	nd	1.2	99.9
	14	64.5	21.1	4.2	4.6	29.9	1.9	31.8	0.2	nd	1.4	97.9

	30	51.7	24.2	4.5	8.4	**37.1**	6.1	43.2	0.5	< 0.1	1.6	96.9
	51	45.5	20.7	4.7	9.8	35.2	10.5	45.6	1.2	< 0.1	3.1	95.4
	65	41.4	19.8	4.6	10.9	35.3	16.0	51.3	1.7	< 0.1	1.5	95.9
	105	35.8	17.1	3.5	9.9	30.6	23.9	54.4	2.7	< 0.1	1.7	94.7
	120	25.7	12.7	3.4	11.2	27.3	**30.2**	**57.5**	**5.7**	< 0.1	0.9	89.8
Sterile	**126**	67.3	21.2	2.8	2.6	26.6	3.7	30.3	0.2	< 0.1	1.9	99.8
	0	98.5	2.1	0.1	< 0.1	2.3	< 0.1	2.3	na	na	0.5	101.3
	1	93.0	6.8	0.8	0.3	7.8	0.2	8.1	0.1	< 0.1	0.7	101.8
	3	89.1	9.0	1.3	0.4	10.7	0.8	11.5	0.1	nd	0.7	101.4
	7	80.8	14.1	2.7	0.8	17.6	2.6	20.1	0.2	nd	1.1	102.3
	14	71.4	17.6	4.5	2.2	24.4	3.2	27.6	0.6	< 0.1	1.4	100.9
Rodl	**30**	60.3	18.1	6.9	3.8	28.8	4.4	33.1	1.3	< 0.1	1.5	96.3
	51	56.8	17.3	8.3	5.6	**31.2**	11.3	42.6	2.8	< 0.1	0.9	103.0
	65	55.8	15.9	8.0	5.8	29.7	14.1	43.8	3.5	< 0.1	1.1	104.1
	105	49.8	13.7	8.7	7.1	29.5	12.1	41.6	5.0	nd	0.8	97.2
	120	48.6	11.8	9.2	6.7	27.8	20.0	47.8	7.2	nd	0.6	104.3
	175	28.8	9.9	9.8	11.0	30.7	**32.3**	**62.9**	**10.7**	< 0.1	0.4	102.9
Sterile	**126**	70.8	16.9	4.2	2.2	23.3	7.0	30.3	0.4	< 0.1	1.3	102.8

na denotes not analysed
nd denotes not detected

Table 92: Characterization of extractable residues (% AR) after application of ^{14}C-pridafol (CL-9673) to 'Irrsee' water/sediment system (data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	**Water**		**Sediment**		**Total system**		
	Pyridafol (CL-9673)	**Pyridafol-O-methyl (CL-9869)**	**Pyridafol (CL-9673)**	**Pyridafol-O-methyl (CL-9869)**	**Pyridafol (CL-9673)**	**Pyridafol-O-methyl (CL-9869)**	**Max. of unknown**
0	95.4	nd	1.7	nd	97.1	nd	nd
0	93.6	nd	2.0	nd	95.6	nd	nd
Mean	94.5	nd	1.8	nd	96.4	nd	nd
1	88.1	nd	9.3	nd	97.4	nd	nd
1	88.2	nd	9.3	nd	97.5	nd	nd
Mean	88.2	nd	9.3	nd	97.5	nd	nd
3	82.5	nd	12.5	nd	95.0	nd	nd
3	82.9	nd	12.5	nd	95.4	nd	nd
Mean	82.7	nd	12.5	nd	95.2	nd	nd
7	71.7	nd	23.8	nd	95.5	nd	nd
7	72.5	nd	24.0	nd	96.5	nd	nd
Mean	72.1	nd	23.9	nd	96.0	nd	nd
14	64.4	nd	28.8	nd	93.3	nd	0.1
14	63.4	nd	29.0	nd	02.4	nd	0.1
Mean	63.9	nd	28.9	nd	92.8	nd	0.1
30	51.1	nd	36.1	< 0.1	87.2	< 0.1	0.2
30	51.4	nd	36.3	< 0.1	87.7	< 0.1	0.2
Mean	51.3	nd	**36.2**	< 0.1	87.5	< 0.1	0.2
51	44.9	nd	35.8	< 0.1	80.6	< 0.1	0.2
51	45.4	nd	32.8	< 0.1	78.2	< 0.1	0.2
Mean	45.1	nd	34.3	< 0.1	79.4	< 0.1	0.2
65	40.7	nd	35.1	< 0.1	75.7	0.1	0.3
65	41.0	nd	33.3	< 0.1	74.3	0.1	0.2
Mean	40.8	nd	34.2	< 0.1	75.0	0.1	0.2
105	35.3	nd	28.9	**0.2**	64.2	**0.2**	**0.3**

120	23.6	nd	26.2	< 0.1	49.8	0.1	**0.3**
126 (sterile)	66.1	nd	25.6	< 0.1	91.7	< 0.1	< 0.1

Table 93: Characterization of extractable residues (% AR) after application of ^{14}C-pridafol (CL-9673) to 'Rodl' water/sediment system (data in grey indicate exceedance of 10 % of AR for an individual compound, bold data indicate maximum values).

DAT	Water		Sediment		Total system		
	Pyridafol (CL-9673)	**Pyridafol-O-methyl (CL-9869)**	**Pyridafol (CL-9673)**	**Pyridafol-O-methyl (CL-9869)**	**Pyridafol (CL-9673)**	**Pyridafol-O-methyl (CL-9869)**	**Max. of unknown**
0	96.0	nd	2.2	nd	98.2	nd	nd
0	97.5	nd	2.0	nd	99.5	nd	nd
Mean	96.8	nd	2.1	nd	98.9	nd	nd
1	92.7	nd	6.1	nd	98.8	nd	nd
1	91.5	nd	7.3	nd	98.9	nd	nd
Mean	92.1	nd	6.7	nd	98.9	nd	nd
3	88.6	nd	9.1	nd	97.7	nd	< 0.1
3	88.0	nd	8.4	nd	96.4	nd	< 0.1
Mean	88.3	nd	8.7	nd	97.0	nd	< 0.1
7	78.4	nd	16.7	nd	95.1	nd	0.8
7	81.7	nd	12.4	nd	94.1	nd	1.0
Mean	80.1	nd	14.5	nd	94.6	nd	0.9
14	71.1	nd	20.8	nd	91.9	nd	1.2
14	69.7	nd	22.9	nd	92.5	nd	0.7
Mean	70.4	nd	21.8	nd	92.2	nd	0.9
30	60.8	nd	27.0	< 0.1	87.7	< 0.1	1.1
30	56.8	nd	23.6	< 0.1	80.4	< 0.1	1.3
Mean	58.8	nd	25.3	< 0.1	84.1	< 0.1	1.0
51	54.7	nd	27.8	0.1	82.6	0.1	0.7
51	55.1	nd	26.7	0.1	81.8	0.1	1.6
Mean	54.9	nd	**27.3**	0.1	82.2	0.1	1.2
65	57.6	nd	24.4	< 0.1	82.0	< 0.1	0.9
65	49.2	nd	25.7	< 0.1	74.9	< 0.1	1.8
Mean	53.4	nd	25.0	< 0.1	78.5	< 0.1	1.4
105	47.1	nd	24.0	< 0.1	71.1	< 0.1	0.4
120	46.1	nd	21.9	< 0.1	68.1	< 0.1	**1.8**
175	25.3	nd	21.7	**0.4**	47.1	**0.4**	0.3
126 (sterile)	69.7	nd	20.4	0.6	90.1	0.6	0.1

Conclusions:

From the results of the study it can be concluded that pyridafol (CL-9673) was degraded in both test systems, albeit at slow rate. Endpoint of the degradation is the mineralization to carbon dioxide and the formation of bound residues.

Comments (RMS):

- Dissipation/degradation rates of pyridafol (CL-9673) in the water phase and in the entire system were re-calculated by Hardy (2012a) following pertinent FOCUS guidance.

Reference:	**Kinetic modelling analysis of pyridate and CL9673 from water sediment studies to derive modelling endpoints**
Author(s), year:	Hardy, I.A.J., 2012a
Report/Doc. number:	Battelle UK, report no. OZ/11/017
Guideline(s):	FOCUS guidance
GLP:	Not applicable
Deviations:	None
Validity:	Yes

The aim of this evaluation was to conduct a kinetic modelling analysis of the data from water / sediment degradation studies with pyridate and pyridafol (CL-9673) in order to derive DT_{50} values for use as modelling endpoints.

Following the recommended procedure for determining modelling endpoints (FOCUS degradation kinetics), all datasets were initially evaluated using SFO kinetics with free optimisation of M_0 and K_p. If datasets were statistically and or visually unacceptable, further evaluation with FOMC, DFOP and HS kinetics were considered. The kinetic evaluations were performed according to the respective decision flowchart for the determination of level P-I parent endpoints for use in modelling and level M-I metabolite endpoints.

The sampling times and residue data were entered into KinGUI2 and optimisations carried out for Simple First Order (SFO) kinetics. The model fits were evaluated using a chi-square (X^2) error statistic and visual inspection of residual plots.

Findings:

Table 94: Pyridate parameter optimisation results (SFO) all datasets – free optimisation.

Compartment	Water/Sediment system	DT_{50} (days)	DT_{90} (days)	Chi^2 error (%)	t-test (-)	Visual assessment	Kinetics
Water phase	Calwich Abbey	0.33	1.10	0.1	< 1E-5	Excellent	SFO
	Swiss Lake	0.32	1.06	0.6	< 1E-5	Excellent	SFO
Geometric mean		**0.32**	**1.06**				
Total system	Calwich Abbey	0.35	1.16	0.1	< 1E-5	Excellent	SFO
	Swiss Lake	0.57	1.89	0.8	< 1E-5	Excellent	SFO
Geometric mean		**0.45**	**1.49**				

Table 95: Pyridafol (CL-9673) parameter optimisation results (SFO) all datasets – free optimisation.

Compartment	Water/Sediment system	DT_{50} (days)	DT_{90} (days)	Chi^2 error (%)	t-test (-)	Visual assessment	Kinetics
Water phase	Calwich Abbey	228	758	4.4	9.8E-4	Acceptable	SFO → SFO
	Swiss Lake	278	923	2.1	2.2E-5	Good	SFO → SFO
	Irrssee	54.5	181	10.2	< 1E-5	Acceptable	SFO
	Rodl	85.9	285	9.3	< 1E-5	Acceptable	SFO
Geometric mean		**131**					
Total system	Calwich Abbey	491	1631	2.0	< 1E-5	Excellent	SFO → SFO
	Swiss Lake	436	1447	1.1	< 1E-5	Excellent	SFO → SFO
	Irrssee	150	497	2.7	< 1E-5	Excellent	SFO
	Rodl	209	694	2.0	< 1E-5	Excellent	SFO
Geometric mean		**286**					

Conclusion:

For pyridate, the water phase and total system degradation data were well described by SFO kinetics. For $FOCUS_{SW}$ evaluations, the total system geometric mean DT_{50} value of 0.45 days for pyridate can be used for both the water and sediment phase degradation rates due to the rapid degradation and limited transfer to the sediment.

For pyridafol (CL-9673), the water phase and total system degradation data were adequately described by SFO kinetics. For $FOCUS_{SW}$ evaluations, the total system geometric mean DT_{50} value of 286 days for pyridafol (CL-9673) can be used for the water phase degradation rate along with a conservative default DT_{50} value of 1000 days for the sediment.

Comments (RMS):

None

Degradation in soil under aerobic conditions

The rate of degradation of pyridate in **soil laboratory conditions** was investigated for first Annex I inclusion using ^{14}C-pyridate:

- Metabolism and degradation of ^{14}C-labelled pyridate in four soils (Schanné and Morgenroth, 1995)
- Aerobic soil metabolism study on ^{14}C-pyridate at two specified temperatures (26 °C and 7 °C) in a laboratory test (Zohner, 1988)

No further study was submitted for renewal.

The kinetics re-assessment and the normalization to reference conditions at 20 °C and pF2, done by the RMS according to pertinent guidance (FOCUS degradation kinetics report, 2006[1]), is summarized below. Degradation of pyridate in the study from Zohner (1988) is considered not fully reliable owing to serious shortcomings during extraction (no acidification) and was not considered further.

[1] FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp

Table 96: Normalization factors for moisture and temperature of soils used for aerobic incubation studies with ^{14}C-pyridate (Schanné and Morgenroth, 1995) – RMS assessment.

Soil	Classification (USDA)	Incubation moisture content	Moisture content (g/100 g)		Temp. (°C)		f_{Moist}[b]	t_{Temp}[c]	f_{Tot}[d]
			Inc.	pF2[a]	Inc.	Ref.			
Speyer 2.2	Sand	40 % of MWHC	15.8	12.0	20	20	1.00	1.00	1.00
Speyer 2.2 SP 211	Loamy sand	55 % of MWHC	24.4	14.0	20	20	1.00	1.00	1.00
Auboden	Silt loam	40 % of MWHC	19.1	26.0	20	20	0.81	1.00	0.81
Collombey	Sand/loamy sand	40 % of MWHC	16.8	14.0	20	20	1.00	1.00	1.00
Les Evouettes	Silt loam/loam	40 % of MWHC	22.1	25.0	20	20	0.92	1.00	0.92

[a] FOCUS default values (FOCUS groundwater report, 2000[2])
[b] $f_{Moist} = (MC_{Inc} / MC_{pF2})$ ^ 0.7 (maximum $f_{Moist} = 1$)
[c] $f_{Temp} = 2.58$ ^ $((T_{Ref} - T_{Inc}) / 10)$
[d] $f_{Tot} = f_{Moist}$ x f_{Temp}

Table 97: Re-calculated degradation rates of pyridate in aerobic soil incubation studies conducted with ^{14}C-pyridate (Schanné and Morgenroth, 1995) – RMS assessment, KinGUI 2.0.

Soil	$DegT_{50}$ (d)	$DegT_{90}$ (d)	X^2-error (%)	t-test (-)	Visual fit	Kinetics	Norm. $DegT_{50}$ (d)
Speyer 2.2[a]	1.3	4.3	18.5	k: 2e-4	Medium	SFO	nc
	0.9	10.8	7.9	α/β: 5e-5/0.003	Very good	FOMC	3.3[b]
Speyer 2.2 SP 211[a]	0.7	2.4	19.3	k: 7e-5	Medium	SFO	nc
	0.4	4.6	5.2	α/β: 2e-5/0.002	Very good	FOMC	1.4[b]
Auboden	0.4	1.4	22.7	k: 9e-4	Good	SFO	0.3
Collombey	0.4	1.2	18.7	k: 4e-4	Medium	SFO	0.4
	< 0.1	1.5	4.7	α/β: 0.001/0.154	Very good	FOMC	ns
Les Evouettes	0.5	1.7	6.6	k: 4e-7	Very good	SFO	0.5

nc denotes not calculated (not best fit)
ns denotes not significant (no reliable fit)
[a] Including data up to 126 DAT
[b] Based on pseudo SFO-$DegT_{50}$ (i.e. FOMC-$DegT_{90}$ divided by 3.32)

The rate of degradation of pyridafol (CL-9673) in soil was investigated for first Annex I inclusion using ^{14}C-labelled pyridate and pyridafol (CL-9673):

- Metabolism and degradation of ^{14}C-labelled pyridate in four soils (Schanné and Morgenroth, 1995)
- Aerobic soil metabolism study on ^{14}C-pyridate at two specified temperatures (26 °C and 7 °C) in a laboratory test (Zohner, 1988)
- Aerobic soil metabolism study of ^{14}C-CL 9673, the main metabolite of ^{14}C-pyridate in soil (Zohner, 1985, including attachment, 1989)

As mentioned earlier residue data in Zohner (1988) are considered not fully reliable owing to serious shortcomings during extraction (no acidification). The study is not considered further therefore.

[2] FOCUS (2000) "FOCUS groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp

Main findings of the other two studies can be found in the route of degradation section. The kinetic re-assessment and the normalization to reference conditions at 20 °C and pF2, done by the RMS according to pertinent guidance (FOCUS degradation kinetics report, 2006), is summarized below. Normalization factors for the study Schanné and Morgenroth (1995) are provided in Table 113.

Table 98: Normalization factors for moisture and temperature of soils used for aerobic incubation studies with ^{14}C-pyridafol (CL-9673) (Zohner, 1985) – RMS assessment.

Soil	Classification (USDA)	Incubation moisture content	Moisture content (g/100 g)		Temperature (°C)		f_{Moist}[b]	t_{Temp}[c]	f_{Tot}[d]
			Inc.	pF2[a]	Inc.	Ref.			
Auboden	Silty loam	40 % of MWHC	12.8	26.0	18 – 23[e]	20	0.61	1.00	0.61
Pararendzina	Silty loam	40 % of MWHC	12.8	26.0	18 – 23[e]	20	0.61	1.00	0.61
Rendzina	Silty loam	40 % of MWHC	12.8	26.0	18 – 23[e]	20	0.61	1.00	0.61
Ranker	Sandy loam	40 % of MWHC	10.8	19.0	18 – 23[e]	20	0.67	1.00	0.67

[a] FOCUS default values (FOCUS groundwater report, 2000)
[b] $f_{Moist} = (MC_{Inc} / MC_{pF2})$ ^ 0.7 (maximum $f_{Moist} = 1$)
[c] $f_{Temp} = 2.58$ ^ $((T_{Ref} - T_{Inc}) / 10)$
[d] $f_{Tot} = f_{Moist}$ x f_{Temp}
[e] Average temperature considered to be approx. 20 °C

Table 99: Re-calculated degradation rates of pyridafol (CL-9673) in aerobic soil incubation studies conducted with ^{14}C-pyridate (Schanné an Morgenroth, 1995) and ^{14}C-pyridafol (CL-9673) (Zohner, 1985) – RMS assessment.

Soil	$DegT_{50}$ (d)	$DegT_{90}$ (d)	ff (-)	X^2-error (%)	t-test (-)	Visual fit	Kinetics	Norm. $DegT_{50}$ (d)	Ref.
Speyer 2.2	46.6	155	0.64	6.1	2e-6	Very good	SFO → SFO	42.8	Schanné and Morgen-roth, 1995
Speyer 2.2 SP 211	16.8	56.0	0.82	5.8	8e-8	Very good	SFO → SFO	16.8	
Auboden	21.7	72.1	0.94	8.0	5e-5	Medium	SFO → SFO	17.6	
Collombey	16.5	54.9	0.85	3.1	7e-10	Very good	SFO → SFO	16.5	
Les Evouettes	23.8	79.1	0.99	3.6	9e-10	Very good	SFO → SFO	21.9	
Auboden	33.2	110	na	2.6	3e-8	Very good	SFO	20.3	Zohner, 1985
Pararendzina	33.3	111	na	3.8	5e-7	Very good	SFO	20.3	
Rendzina	38.6	128	na	4.7	6e-6	Very good	SFO	23.5	
Ranker	42.0	140	na	2.3	8e-8	Very good	SFO	28.2	

na denotes not applicable

For first Annex I inclusion, **field dissipation studies** conducted with pyridate in CH (n = 1) and in the US (n = 4) and with pyridafol (CL-9673) in AT (n = 1) were submitted:

- Examination of degradation of Pyridate and CL 9673 in soil (Hegemann et al., 1983)
- Dissipation of Pyridate residues from an Iowa loam and an Illinois sandy clay loam corn field treated with Tough 3.75 EC Herbicide (Dykeman, 1992a)
- Dissipation of Pyridate residues from a Wisconsin silt loam cabbage field treated with Tough 3.75 EC Herbicide (Dykeman, 1992b)
- Dissipation of Pyridate residues from a California sandy loam cabbage field treated with Tough 3.75 EC Herbicide (Dykeman, 1992c)
- ^{14}C-Pyridate: Soil metabolism in a model study under outdoor conditions (Elgehausen, 1995)

However, none of these studies could be subjected to a normalization procedure in line with the FOCUS degradation kinetic report (2006).

For renewal of pyridate 10 additional field degradation studies, conducted on representative sites in the EU with either pyridate (n = 3) or pyridafol (CL-9673) (n = 7) applied as parent, were submitted. Field trials were conducted in DE (n = 6), FR (n = 3) and UK (n = 1):

- Residues of pyridate and its main metabolites CL-9673 and CL-9673-O-methyl in soil treated with 2.5 kg Lentagran 45 WP/ha (Krennhuber and Pfarl, 1996)
- Field dissipation and mobility of residues of SAN 1367 H, its O-methyl metabolite and of dicamba and its metabolite dichlorosalicylic acid in cropped soil following application of SAN 1411 H 167 SL 003BS in France (Gasser, 2001a)
- Field dissipation and mobility of residues of SAN 1367 H, its O-methyl metabolite and of dicamba and its metabolite dichlorosalicylic acid in cropped soil following application of SAN 1411 H 167 SL in Germany (Gasser, 2001b)
- Field soil dissipation and mobility of residues of SAN 1367 H and its O-methyl metabolite in bare and cropped soil following application of A-11897 A (SAN 1367 H 490 SC) in Northern Germany (Gasser, 2001c)
- Terrestrial soil dissipation of pyridate and its metabolite CL-9673 after one application of Lentagran 450 g/kg WP on bare soil in Northern France, 2010-2011 (Chambers et al., 2011a)
- Terrestrial soil dissipation of pyridate and its metabolite CL-9673 after one application of Lentagran 450 g/kg WP on bare soil in Germany, 2010-2011 (Chambers et al., 2011b)

Based on best fit kinetics, non-normalized DT_{50} (dissipation) values for pyridate (US field studies and new field dissipation studies as assessed by the RMS) were in a range from 0.4 – 2.2 days (n = 7, all SFO with the exception of the Wisconsin US field trial (FOMC)). Non-normalized dissipation rates for pyridafol (CL-9673) were in a range from 5.4 – 97 days based on reliable best fit kinetics (n = 14, all SFO).

The 10 EU field trials, submitted for renewal, were subjected to thorough kinetic evaluation based on the time step normalization procedure in line with the FOCUS degradation kinetic report (2006) and with EFSA (2010). Normalised to reference conditions at 20 °C and pF 2 applying a Q_{10} factor of 2.58 $DegT_{50}$ values for pyridate were in a range from 1.0 – 2.1 days (n = 3).

Normalized field $DegT_{50}$ values for pyridafol (CL-9673) were in a range from 5.1 – 44.7 (n = 9, no reliable fit could be obtained for one field trial).

Table 100: Summary on $DegT_{50}$ (degradation) values for pyridate obtained in in field trials normalised to reference conditions at 20 °C and pF 2 applying a Q_{10} factor of 2.58 (reliable best fit kinetics).

System	Soil/Field trial	Data used[a]	$DegT_{50}$ (days)	$DegT_{90}$ (days)	Chi^2 error (%)	t-test (-)	Visual assessment	Kinetics
Field	Kings Newton (UK)	10 mm	2.0	6.5	13.9	k: < 0.001	Very good	SFO
	La Chapelle (FR)	All	2.1	7.1	5.3	k: 0.004	Excellent	SFO
	Harthau (DE)	All	1.0	3.1	1.6	k: < 0.001	Excellent	SFO
Geometric mean			**1.0**	**3.4**	-	-	-	-

na denotes not applicable

[a] Field data, All: All data used; 10 mm: Only data following 10 mm of precipitation used (in line with EFSA, 2010)

[b] Based on pseudo-SFO-$DegT_{50}$ (i.e. FOMC-$DegT_{90}$ divided by 3.32)

Table 101: Summary on $DegT_{50}$ (degradation) values for pyridafol (CL-9673) obtained in field trials normalised to reference conditions at 20 °C and pF 2 applying a Q_{10} factor of 2.58 (reliable best fit kinetics).

Field trial	Data used[a]	$DegT_{50}$ (days)	$DegT_{90}$ (days)	Chi^2 error (%)	t-test (-)	ff (-)	Visual assessment	Kinetics
Kings Newton (UK)	10 mm	3.7	12.4	30.3	k: < 0.001	1.00	Good	SFO → SFO
Cuxac (FR)[b]	*All*	*0.63*	*19.7*	*9.9*	*k_1: 0.4, k_2: 0.05*	-	*Excellent*	*DFOP*
Peyrens (FR)	10 mm	9.7	32.4	22.0	k: 0.004	-	Good	SFO
Hilgermissen (DE)	10 mm	43.4	144	13.4	k: < 0.001	-	Very good	SFO
Eicklingen (DE)	10 mm	44.7	149	12.5	k: < 0.001	-	Very good	SFO
Geinsheim (DE)	10 mm	5.3	17.8	2.8	k: < 0.001	-	Excellent	SFO
Stetten (DE)	10 mm	6.6	21.9	29.0	k: < 0.001	-	Acceptable	SFO
Brunne (DE)	10 mm	13.8	45.7	43.7	k: 0.017	-	Acceptable	SFO
Harthau (DE)	All	5.1	16.9	16.6	k: < 0.001	1.00	Excellent	SFO → SFO
La Chapelle (FR)	All	20.6	68.5	22.7	k: 0.003	1.00	Acceptable	SFO → SFO
Geometric mean		**11.5**	**38.3**			-		

[a] All: All data used; 10 mm: Only data following 10 mm of precipitation used (in line with EFSA, 2010)
[b] Fit not considered reliable (k_1 not significant), excluded from averaging

Soil photolysis

The following studies were submitted to assess soil photolysis of pyridate for first Annex I inclusion:

- Photodegradation study of ^{14}C-pyridate on a silty loam soil (Zohner, 1990)
- Photodegradation study of ^{14}C-pyridate on soil (Van Dijk and Baranowski, 1992)

Data residues in Zohner (1990) are considered not fully reliable owing to significant shortcomings during extraction (no acidification). The study is not considered further therefore.

Table 102: Re-calculated degradation rates of pyridate and pyridafol (CL-9673) under conditions of soil photolysis - RMS assessment.

Substance	Conditions	DT_{50} (days)	DT_{90} (days)	X^2-error (%)	t-test (-)	Visual assess-ment	Kinetics	Ref.
Pyridate	Irradiated	1.8	5.9	3.5	1e-9	Very good	SFO	Van Dijk and Baranowski, 1992
	Dark	No reliable fit owing to data scattering						
Pyridafol (CL-9673)	Irradiated	19.4	64.6	6.4	1e-4	Very good	SFO → SFO	
	Dark	No reliable fit owing to data scattering						

4.1.3 Summary and discussion of degradation

Summary: Biotic degradation	Test guideline / design	GLP (y/n)	Reliability
Ready biodegradability: **In a ready biodegradability study pyridate was found to be not readily biodegradable.**	OECD 301	Yes	Yes
Water/sediment systems: In two water/sediment systems pyridate was rapidly hydrolysed to its major metabolite pyridafol (CL-9673) with DT50 values in a range from 0.4 – 0.6 days. Two additional water/sediment systems were incubated with pyridafol (CL-9673) as parent compound. DT50 values for pyridafol (CL-9673) in the entire water/sediment system of all four studies were in the range from 150 – 491 days.	OECD 308	Yes	Yes
Degradation in soil: Under aerobic laboratory soil conditions pyridate is rapidly hydrolysed to its main metabolite pyridafol (CL-9673) with a non-normalized laboratory DT50 in the range of 0.3 – 3.3 days (5 soils). The main soil metabolite pyridafol (CL-9673) is degraded further with non-normalized laboratory DT50 values in the range from 16.5 – 42.8 days (9 soils). Non-normalized DT50 (dissipation) values for pyridate in EU and US field studies were in a range from 0.4 – 2.2 days (7 soils). Non-normalized dissipation rates for pyridafol (CL-9673) were in a range from 5.4 – 97 days (14 soils).	OECD 307, SETAC 1995 and similar	Partly	Yes

Summary: Abiotic degradation	Test guideline / design	GLP (y/n)	Reliability
Hydrolysis: Under conditions of sterile aquatic hydrolysis pyridate is rapidly degraded to pyridafol (CL-9673) which is shown to be stable under these conditions. Hydrolysis of pyridate in sterile buffer solutions at 22 ± 0.1 °C and pH 5, 7 and 9 is depending on pH with DT50 values of 2.8, 0.7 and 0.1 days, respectively, in one study and DT50 values of 3.7, 2.4 and 0.4 days at 25 °C in another study.	US-EPA, N, 161-1	Yes	Yes
Aquatic photolysis: Pyridate does not significantly adsorb light in a range > 290 nm. In contrast, its main metabolite pyridafol (CL-9673), which is rapidly released from pyridate by hydrolysis, undergoes significant photo-degradation under conditions of aquatic photolysis releasing numerous metabolites. DT50 values of pyridafol (CL-9673) under conditions of laboratory aquatic photolysis were 0.04, 1.12 and 1.81 days at pH 4, 7 and 9, respectively.	US-EPA, N, 161-2	Yes	Yes
Soil photolysis: Under conditions of soil photolysis pyridate, which does not absorb light > 290 nm, is rapidly hydrolysed to its main metabolite pyridafol (CL-9673). For pyridafol (CL-9673), which is known to undergo extensive photo degradation based on aquatic photolysis studies, photolysis DT50 under experimental conditions was 19.4 days.	US-EPA, N, 161-3	Yes	Yes

Conclusion: The criteria for rapid degradation are not fulfilled because

- pyridate itself is demonstrated to be ultimately degraded in surface water sediment and soil simulation tests with a half-life of << 16 days (corresponding to a degradation of > 70 % within 28 days), however the degradation products (in particular pyridafol, CL-9673) are demonstrated to show half-lifes > 16 days in soil and water/sediment in several cases;

- pyridate is demonstrated to be primarily degraded biotically or abiotically (in particular via hydrolysis) in the aquatic environment with a half-life << 16 days (corresponding to a degradation of > 70 % within 28 days), however the degradation products (in particular pyridafol, CL-9673) do fulfill the criteria for classification as hazardous to the aquatic environment.

The substance is demonstrated to be **not readily biodegradable** in a 28-day test for ready biodegradability.

4.2 Environmental distribution

4.2.1 Adsorption/Desorption

Due to the instability of pyridate in soil, soil batch analyses are not feasible for this compound. Based on the HPCL-method (OECD 121) the K_{OC} of pyridate was estimated to be 223,800 L/kg (Simmonds, 2012).

Adsorption/desorption of pyridafol (CL-9673) were measured in equilibrium batch experiments on a large set of representative soils (n = 22) covering a wide spectrum of soil properties (Krüger, 1992; Glänzel, 1996; Burgess and Simmonds, 2012). Freundlich K_f values obtained for pyridafol (CL-9673) were in the range from 0.11 – 10.2 L/kg, respective 1/n values were in range from 0.63 – 0.89. Based on the data available it can be concluded that the adsorption of pyridafol (CL-9673) increases with increasing soil organic matter and decreasing soil pH. Freundlich K_{fOC} values were in a range from 4 – 339 L/kg, significantly depending on soil pH.

4.2.2 Volatilisation

With a vapour pressure of 4.8 x 10^{-7} Pa at 20 °C and a Henry constant of 1.21 x 10-4 Pa m^3 mol^{-1} the active substance pyridate is not considered volatile. The chemical half-life in the troposphere calculated according to Atkinson is ~ 0.7 days for pyridate (Glänzel, 1996; Buntain, 2011).

For pyridafol (CL-9673) new data submitted for renewal also show a very low vapour pressure of 4 x 10^{-7} Pa at 20 °C and Henry constant of ≤ 2.07 x 10-6 Pa m^3 mol^{-1}.

4.2.3 Distribution modelling

No data/information available

4.3 Aquatic Bioaccumulation

Table 103: **Summary of relevant information on aquatic bioaccumulation**

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water OECD A 80/8 equivalent to EEC/A.8 not GLP (study before 1993)	^{14}C Pyridate (purity > 98%, estimated)] Log Pow = 4.01 ± 0.16 at room temperature	Value due to rapid hydrolysis not reliable. Effect of pH (4-10) on the n-octanol/water partition co-efficient not relevant since pyridate does not dissociate.	Zohner, A. et al. 1982
Partition coefficient n-octanol/water EEC method A.8 GLP	14C-CL9673 >99% Log Pow distilled water: 0.86 at pH 4: 2.0 at pH 7: 0.56 at pH 9: -0.52 (all at 20 ° C)	-	Krüger, B. 1997
Bioconcentration in fish OECD 305 E	Bioconcentration factor (BCF): whole fish: 116; edible tissues: 27; non-edible tissues: 180 Clearance time (days) (CT50): whole fish: 0.19; edible tissues: 0.05; non-edible tissues: 0.08 (CT90): Not specified Level of residues in organisms after the 14 day depuration phase: Two weeks after depuration 98.5, 99.5 and 99.0% of radioactivity were eliminated from edibles, non-edibles and whole fish.	-	Ellgehausen and Wüthrich, 1984

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

4.3.1.2 Measured bioaccumulation data

The bioconcentration study in fish by Ellgehausen and Wüthrich (1984) was evaluated and accepted for the first Annex I inclusion of the active ingredient Pyridate. A new detailed study summary was not deemed necessary in course of the Annex I renewal procedure according to Commission Regulation (EU) 1141/2010 as no new data are available.

4.3.2 Summary and discussion of aquatic bioaccumulation

The determined BCF demonstrates that pyridate does not bioconcentrate in fish.

Summary Bioconcentration	
	Active substance Pyridate
$logP_{O/W}$	4.01 ± 0.16
Bioconcentration factor (BCF)	116 (whole fish)
Conclusion	Pyridate is not considered to have **a potential for bioconcentration.**

4.4 Aquatic toxicity

Table 104: **Summary of relevant information on aquatic toxicity for Pyridate**

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
In agreement with EPA 72-1	Rainbow trout	static	96 h	Mortality	Mean measured	0.53	>1.01	-	Bowman, Schuster and Franklin, 1986a/Yes
In agreement with EPA 72-1	Bluegill sunfish	static	96 h	Mortality	Mean measured	1.3	>1.3	-	Bowman, Schuster and Frannklin, 1986b/yes
OECD 202	*Daphnia magna*	static	48h	Immobility	Mean measured	-	>0.78	-	Egeler, Goth and Seck, 2011a
OECD 202	*Daphnia magna*	semistatic	48h	Immobility	Mean measured	-	0.49	-	Egeler, Goth and Seck, 2011b
OECD 211	*Daphnia magna*	semistatic	21d	Immobility reproduction	Mean measured	0.01	0.4 (immobility) 0.49 (reproduction)	-	Wüthrich, 1992
ASTM E 1218-9, FIFRA 122-2 and 123-2	*Anabaena flos-aquae*	static	96h	Growth rate Biomass	Mean measured	0.75	>0.75	-	Grade, 1998

Table 105: **Summary of relevant information on aquatic toxicity for CL9673**

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
OECD 203	Rainbow trout	flow through	96 h	Mortality	Mean measured	16.2	>16.2	-	Wüthrich, 1993
EPA 72-1	Bluegill sunfish	flow through	96 h	Mortality	Mean measured	48	140	-	Sword and Cramer, 1991
OECD 204	Rainbow trout	Flow trough	21d	mortality sublethal effects	nominal	17	>17	-	Wüthrich, 1991a
OECD 210	Rainbow trout	Flow trough	69d	Sublethal effects	nominal	0.1	-	-	Gilberg and Seck, 2011
OECD 202/EPA 72-1	*Daphnia magna*	static	48h	mortality	Mean measured	21.5	30.7	-	Wüthrich, 1991b
OECD 211	*Daphnia magna*	static	21d	Immobility reproduction	nominal	5	>5	-	Wüthrich, 1991c
OECD 201	*Selenastrum capricornutum*	static	96h	Biomass	nominal	1.7	4.93	-	Grade, 1998
OECD 201	*Anabaena flos-aquae*	static	96h	Growth rate	nominal	3.2	>10	-	Hermes and Erk, 2013
OECD 221	*Lemna gibba*	static	7d	Growth rate	nominal	1.8	8.64	-	Grade, 1997

4.4.1 Fish

Short-term toxicity to fish

Several acute toxicity studies with fish were available for the first Annex I inclusion of Pyridate. No new studies were provided in course of the Annex I renewal procedure according to Commission Regulation (EU) 1141/2010. Two studies including the study generating the lowest valid and reliable endpoint accepted for the first Annex I inlcusion were still considered to be valid and were only corrected for the level of measured concentrations. New detailed study summaries were not deemed necessary in course of the Annex I renewal procedure as no new data are available.

Table 106: Summary of relevant information on available acute toxicity studies with fish for Pyridate

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
In agreement with EPA 72-1	Rainbow trout	static	96 h	Mortality	Mean measured	0.53	>1.01	-	Bowman, Schuster and Franklin, 1986a
In agreement with EPA 72-1	Bluegill sunfish	static	96 h	Mortality	Mean measured	1.3	>1.3	-	Bowman, Schuster and Frannklin, 1986b

As for the active ingredient Pyridate several acute toxicity studies with fish were available for the first Annex I inclusion of the main metabolite CL9673 (Pyridafol). No new studies were provided in course of the Annex I renewal procedure according to Commission Regulation (EU) 1141/2010. Two studies including the study generating the lowest valid and reliable endpoint accepted for the first Annex I inlcusion were still considered to be valid and were only corrected for the level of measured concentrations. New detailed study summaries were not deemed necessary in course of the Annex I renewal procedure as no new data are available.

Table 107: Summary of relevant information on available acute toxicity studies with fish for CL9673

Method	Results							Remarks	Reference
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
OECD 203	Rainbow trout	flow through	96 h	Mortality	Mean measured	16.2	>16.2	-	Wüthrich, 1993
EPA 72-1	Bluegill sunfish	flow through	96 h	Mortality	Mean measured	48	140	-	Sword and Cramer, 1991

Long-term toxicity to fish

No long term studies with fish are available for Pyridate.

One long-term toxicity studies with fish with CL9673was available for the first Annex I inclusion of Pyridate. The study was still considered to be valid and was only corrected for the level of measured concentrations. A new detailed study summary was not deemed necessary in course of the Annex I renewal procedure.

Table 108: Summary of relevant information on available long-term toxicity studies with fish for CL9673

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
OECD 204	Rainbow trout	Flow trough	21d	mortality sublethal effects	nominal	17	>17	-	Wüthrich, 1991a

A fish early life stage study was provided for the Annex I renewal procedure according to Commission Regulation (EU) 1141/2010

Reference:	**CL9673: A Study on the Toxicity to Early-Life Stages of Rainbow Trout.**
Author(s), year:	Gilberg D., Seck, C., 2011
Report/Doc. number:	ECT Oekotoxikologie and Battelle, DOC No. 11BK1FV; OZ/11/006
Guideline(s):	OECD Guideline No. 210 "Fish, Early-life Stage Toxicity Test".
GLP:	Yes (certified laboratory)
Deviations:	None of relevance
Validity:	Acceptable
Material and methods:	
Test substance:	CL 9673 (pyridafol), purity: 98.5 %, batch: Cl- 9673- 1103001
Test species:	*Oncorhynchus mykiss* Walbaum 1792 (Rainbow trout)
Number of organisms:	2 replicates of 30 fertilized eggs per concentrations level (60 at start of exposure, reduced to 30 per replicate after 1 to 2 hours of exposure, damaged, injured eggs that appeared abnormal (e.g. opaque, milky-white, under- or oversized) and supernumerous eggs were removed.
Age:	Freshly fertilized eggs
Type of test, duration:	Flow-through test. The test period (exposure of test organisms to the test solutions) was 69 days.
Applied concentrations:	
Nominal:	0 (control), 10, 3.16, 1.0, 0.32 and 0.1
Test conditions:	
Test medium:	Reconstituted water (OECD guidelines No. 203) mixed with deionised water (1:1; v/v), supplemented with 1 % artificial sea water)
Total water hardness:	9.1 – 10.8 °dH; 161 – 193 mg $CaCO_3$/L
Temperature:	Temperature until day 29: 10 ± 2 °C (target); measured: 9.8 – 11.9 °C (online), 10.2 – 11.9 °C (manual) Temperature after hatch (day 33 – 69): 12 ± 2 °C (target); measured: 11.3 – 13.0 °C (online), 11.8 – 12.7 °C (manual)
pH:	7.0 – 7.6 during the total test period

O_2 content:	8.1 – 12.0 mg O_2/L (76 – 109 % saturation)
Light regime:	Before hatch: 24 hours darkness except during inspection of test vessels After hatch: light/dark cycle 16 h/8 h
Feeding	On the day the first larvae per test vessel was recorded to swim up (day 43 of exposure), feeding was started by adding food to the vessels. The test organisms were fed dry food (6 – 8 mg dry food per swim up larva; trout starter food), live *Artemia nauplius* larvae (0.5 – 1 drop *Artemia* nauplius suspension oer swim up larva). The daily ration was fed in 2 – 3 portions. The food ration was adjusted to the number of living fish per test vessel. Food was withheld from the fish for 24 hours prior to the test end.
Test parameters:	Chemical analysis of test concentrations was performed once per week at 0.1, 1.0 mg/L and 10.0 mg/L; the control medium, and the test concentrations 0.32 mg/L and 3.16 mg/L were verified in week one and at end of test. Samples were analysed via LC-MS/MS after dilution with water. Temperature was assessed manually in each test vessel once per week during the test, at the start and the end of the exposure period and in addition in week 2 (2 replicates), 5, 6, 8 and 9 in each test vessel. Dissolved oxygen was measured once per week during the test, at the start and the end of the exposure period and in addition in week 2 (2 replicates). pH was assessed in each test vessel once per week during the test, at the start and the end of the exposure period and in addition in week 2 (2 replicates). Total water hardness was checked in each test vessel at the start and the end of the exposure period. The following parameters were recorded during and/or at the end of the test: cumulative mortality, numbers of healthy fish, time to start of hatching and end of hatching, number of larvae each day, number of deformed larvae, number of organisms exhibiting abnormal behaviour and length and weight of surviving fish.
Statistics:	Hatching success, mortality and numbers of healthy fish were compared to the control by Fisher`s Exact Binomial Test. Dry weight and length of the surviving fish (per treatment means) were tested for statistically significant effects by Williams`Multiple t-test. ECx values were not calculated due to an apparent lack of concentration-response relationship. The normal distribution was checked with Shapiro-Wilk's Test. Variance homogeneity was checked with Bartlett`s Test.
<u>Findings:</u>	
Analytical data:	The measured concentrations in test solutions were stable throughout the exposure period. The calculated recoveries of each measured sample ranged from 79 % to 113 % of nominal concentration (mg ai/L) and the total mean recovery was 99 %.
Biological observation:	Hatching success: In the controls the hatching success was 73.3 %. In the treatments the mean hatching success was in a range of 33.3 – 63.3 %. An influence of the test item on the hatching success was observed at the two highest levels. The effect determined at 0.32 mg/L was due to the low hatching success in one replicate. The hatching success in the next higher level was not statistically significant different from control and it was decided to disregard the statistically significant effect at the level 0.32 mg/L. Post-hatch success and survival of the fish: No influence of the test item concentrations on post-hatch success and total survival could be observed.

Numbers of healthy fish at the end of exposure: The surviving fish appeared healthy at the end of exposure, without behavioural abnormalities, except some fish lying on bottom of the test vessel. At all treated concentration levels some morphological abnormalities were observed (fish were showing curved spine and reduced growth). The morphological abnormalities ranged from 3 – 9 % expressed in % of surviving fish and no correlation was observed between the concentration of the test item and the number of healthy fish at the end of the exposure. No separate statistical evaluation was performed on the number of healthy fish.

Length and weight of fish at test end: No statistically significant differences of weight and length were observed between the treated and control groups at

$p \leq 0.05$. No correlation was observed between the concentration of the test item and the length and weight of fish.

Effects: See tables below.

Table 109: Cumulative number and percentage of hatched fish in the control and treatments

CL9673 [mg ai/L] (nominal)	Introduced eggs	Hatched fish	n Not hatched	% Not hatched
Control	60	44	16	26.7
0.1	60	34	26	43.3
0.32	60	27	33	55.0*
1.0	60	38	22	36.7
3.16	60	31	29	48.3*
10.0	60	20	40	66.7*

* Statistically significant differences on the hatching success were observed between treated and control groups at $p \leq 0.05$.

Table 110: Post-hatch success and survival of the fish

CL9673 [mg ai/L] (nominal)	Survived fish (n)	Post-hatch success [% of hatched eggs]
Control	35	79.4
0.1	33	96.2
0.32	22	80.1
1.0	35	93.2
3.16	28	92.1
10.0	16	87.5

Table 111: Weight of the surviving fish at test end

CL9673 [mg ai/L] (nominal)	Mean	Min	Max	n
Control	114.8	102.63	127.1	2
0.1	80.5	75.27	85.68	2
0.32	75.4	47.01	103.82	2
1.0	106.2	89.04	123.27	2
3.16	113.7	100.2	127.15	2
10.0	73.2	63.65	82.7	2

Values of two pooled replicates per treatment

Table 112: Length of the fish at test end

CL9673 [mg ai/L] (nominal)	Mean	Min	Max	n
Control	30.29	30.01	30.57	2
0.1	28.05	27.53	28.56	2
0.32	27.47	25.83	29.10	2
1.0	29.21	27.99	30.43	2
3.16	30.16	29.25	31.07	2
10.0	29.51	29.39	29.63	2

Values of two pooled replicates per treatment

Conclusion: NOEC = 1.0 mg/L (hatching success)
NOEC = 10.0 mg/L (mortality)
NOEC = 10.0 mg/L (number of healthy fish, no statistic performed)
NOEC = 10.0 mg/L (length of surviving fish)
NOEC = 10.0 mg/L (dry weight of surviving fish)
based on nominal concentrations

Comment RMS: Though included in the study report it is not considered possible to derive a NOEC for the parameter "number of healthy fish" as no statistics were performed and morphological abnormalities were observed at all test concentrations. However, assumed that all of the unhealthy fish die, the NOEC for mortality would probably not change as the numbers are quite low.
In the pesticides peer review meeting 111 the NOEC was set to 0.1 mg/L, as there is no clear indication as to which dose group the outlier was.

4.4.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Reference:	**Pyridate technical : A Study on the Acute Toxicity to Daphnia magna**
Author(s), year:	Egeler P., Goth M., Seck C., 2011a
Report/Doc. number:	ECT Oekotoxikologie and Battelle, DOC No. 11BK4DA; OZ/11/021
Guideline(s):	OECD Guidelines for Testing of Chemicals, Guideline 202 "Daphnia sp., Acute Immobilisation Test"
GLP:	Yes (certified laboratory)
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:	
Test substance:	Pyridate technical, Batch H1012008, purity 91.4%
Test species:	*Daphnia magna* (Straus)
Number of organisms:	5/replicate, 6 replicates/test group
Age:	Less than 24h
Type of test, duration:	Static, 48h

Applied concentrations:	
Nominal:	1.2 mg Pyridate technical/L
Measured (mean):	0.979 mg ai/L at 0h, 0.459 mg ai/L at 48h
Solvent:	0.1 mL acetone/L in the solvent control
Test conditions:	
Water quality:	pH adjusted Elendt medium M4
Temperature:	19.8 – 20.1°C (manual measurement); 19.1 - 20.2°C (online measurement)
pH:	6.1 – 6.8 adjusted
O_2 content:	9.5 -9.8 mg/L
Light regime:	16h light : 8h dark
Test parameters:	Immobility, behavioural effects
Statistics:	Fishers exact test with Bonferroni correction was used to compare immobility at the limit concentration with the immobility's in the solvent control.
Findings:	
Analytical data:	Concentrations were 89% and 42% of nominal at 0h and 48h, respectively
Effects:	Mortality was 36.7% after 48h of exposure

Table 113: Observations concerning the behaviour of the daphnids

Number of animals							
Concentration [mg test item/L] (mean measured)	**Introduced**	**Showing observation a**		**Showing observation c**		**Showing observation g**	
		24h	**48h**	**24h**	**48h**	**24h**	**48h**
0.0 (control)	30	2	2	0	0	0	0
0.0 (Solvent control)	30	1	0	0	0	0	0
0.78	30	28	21	3	8	2	0

Explanations:
0 No adverse observations compared to the controls, no special observation
a Daphnids were trapped in the water surface tension
c Daphnids were showing reduce swimming activities compared to the control
g Two daphnids were sticking to each other and were separated by use of a Pasteur pipette

Table 114: Effects on daphnids (*D. magna*) exposed to technical Pyridate

Concentration [mg test item/L] (mean measured)	**Mean cumulative immobilised organisms [%]**	
	24 hours	**48 hours**
0 (control)	0	0
0 (Solvent control)	3.3	3.3
0.78	3.3	37.7
EC_{50} (48 h) > 0.78mg/L		

Conclusion: 48 h EC_{50} > 0.78 mg/L (corrected for purity of test substance: 0.72 mg ai/L)
based on mean measured concentrations

Reference:	**Pyridate technical: A study on the acute toxicity to Daphnia magna**
Author(s), year:	Egeler P., Goth M., Seck C. (2011b)
Report/Doc. number:	ECT Oekotoxikologie and Battelle, DOC No. 11BK1DA; OZ/11/007
Guideline(s):	OECD Guidelines for Testing of Chemicals, Guideline 202 "Daphnia sp., Acute Immobilisation Test"
GLP:	Yes, certified laboratory
Deviations:	None of relevance
Validity:	Acceptable
Material and methods:	
Test substance:	Pyridate technical, Batch H1012008, purity 91.4%
Test species:	Daphnia magna (Straus)
Number of organisms:	5/replicate, 4 replicates/test group, control and solvent control
Age:	Less than 24h
Type of test, duration:	Semistatic, 48h, Test solutions were renewed after one day of exposure.
Applied concentrations:	
Nominal:	Nominal: 0.075, 0.150, 0.300, 0.600 and 1.2 mg/L technical pyridate
Measured (mean):	The overall mean recovery was 74% of the nominal concentrations.
Solvent:	0.1 mL acetone/L in the solvent control
Test conditions:	
Water quality:	pH adjusted Elendt medium M4
Temperature:	19.1 – 21.0 °C (manual measurement), 18.8 – 19.7 °C (online measurement)
pH:	6.1 – 6.6 adjusted
O_2 content:	8.0 -9.8 mg/L
Light regime:	16h light : 8h dark
Test parameters:	Immobility, behavioural effects
Statistics:	Probit analysis using linear maximum likelihood regression was used to calculate the EC50 value as based on nominal concentrations.
Findings:	
Analytical data:	The measured concentrations ranged from 69% to 108% in the new and from 41% to 77% in the old test media in relation to the nominal values. The overall mean measured concentration was 74%.
Effects:	Mortality was 55% in the highest dose group, no mortality was observed in the lowest dose group. Behavioural symptoms like being trapped in the water surface tension or reduced swimming activities occurred in all dose groups and a tendency for dose dependency was observed.

Table 115: Observations concerning the behaviour of the daphnids

Number of animals							
Concentration [mg test item/L] nominal	**Introduced**	**Showing observation a**		**Showing observation c**		**Showing observation g**	
		24h	**48h**	**24h**	**48h**	**24h**	**48h**
0 (control)	20	1	1	0	0	0	0
0 (Solvent control)	20	1	0	0	0	0	0
0.075	20	0	3	0	0	0	0
0.150	20	4	11	0	4	0	0
0.300	20	6	8	0	0	0	0
0.600	20	19	9	11	6	2	0
1.20	20	20	13	8	7	0	2

Explanations:
0 No adverse observations compared to the controls, no special observation
a Daphnids were trapped in the water surface tension
c Daphnids were showing reduce swimming activities compared to the control
g Two daphnids were sticking to each other and were separated by use of a Pasteur pipette

Table 116: Effects on daphnids (*D. magna*) exposed to technical Pyridate

Concentration [mg test item/L], nominal	**Mean cumulative immobilised organisms [%]**	
	24 hours	**48 hours**
0 (control)	0	0
0 (Solvent control)	0	0
0.075	0	0
0.150	0	30
0.300	0	40
0.600	5	50
1.20	5	55
EC_{50} (48 h) = 0.66 mg/L (95 % C.I. 0.41 – 1.64), based on nominal concentrations EC_{50} (48 h) = 0.49 mg/L (95 % C.I. 0.30 – 1.21), based on mean measured concentrations		

Conclusion: 48 h EC_{50} = 0.49 mg/L
based on mean measured concentrations

Two acute toxicity studies with daphnids with CL9673were available for the first Annex I inclusion of Pyridate. One of the studies was still considered to be valid and was only corrected for the level of measured concentrations. A new detailed study summary was not deemed necessary in course of the Annex I renewal procedure as no new information was provided.

Table 117: Summary of relevant information on available acute toxicity studies with daphnids for CL9673

Method	**Results**							**Remarks**	**Reference / acceptability**
	Test organism	**Test condition**	**Duration**	**Endpoint**	**Test conc**	**NOEC [mg ai/L]**	**EC_{50}/LC_{50} [mg ai/L]**		

OECD 202/EPA 72-1	Daphnia magna	static	48h	mortality	Mean measured	21.5	30.7	-	Wüthrich, 1991b

Long-term toxicity to aquatic invertebrates

The long-term toxicity studies with daphnids with Pyrdiate and CL9673 available for the first Annex I inclusion of Pyridate were still considered to be valid. No new information was provided, but as required in the accordance check report (submission number: EJ013413-58) a summary of the study is included below table 124.

Table 118: Summary of relevant information on available long-term toxicity studies with daphnids for Pyridate

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
OECD 211	*Daphnia magna*	semistatic	21d	Immobility reproduction	Mean measured	0.01	0.4 (immobility) 0.49 (reproduction)	-	Wüthrich, 1992

Reference:	**Influence of Pyridate technical on the reproduction of Daphnia magna.**
Author(s), year:	Wüthrich, V., 1992a
Report/Doc. number:	RCC Umweltchemie AG Report N°211386
Guideline(s):	OECD 202, Part II
GLP:	Yes
Deviations:	See comments below
Validity:	Acceptable

Material and methods:	
Test substance:	Pyridate technical, purity: 91.5%,
Test species:	*Daphnia magna*,
Number of organisms:	10 individuals/test glass beaker, 20 individuals/concentration, 10 daphnids with eggs in the brood pouch were separated and kept individually in beakers containing 50 ml medium, reproduction rates were followed individually,
Age:	<24 h old
Type of test, duration:	Semi-static, 21 days
Applied concentrations:	
Nominal:	Control, solvent control, 1.6, 0.4, 0.1, 0.025 and 0.006 mg ai/L
Measured (mean):	
Solvent:	Acetone at 0.01% at the maximum
Test conditions:	

Water quality:	Mixture of 2/3 reconstituted water and 1/3 filtered pond water, renewed on days 3, 5, 7, 10, 12, 14, 17 and 19
Temperature	21-22.5°C
pH	The initial pH od the systems was on average 8.2 for untreated and acetone treated control, 8.2 and 8.1 for the treated samples. At the end of the exposure period, the respective pH values were 8.2, 8.2, 8.1 and 8.8 respectively.
O_2 content:	The initial oxygen concentration was, on average, 8.5 mg oxygen/L for untreated and acetone treated control, 8.5 and 8.1 mg/L for the treated samples. At the end of the exposure periods, the oxygen concentration of the respective samples ranged, on average, from 8.3 to 8.9 mg oxygen/L.
Light regime:	16h light : 8h dark, 500-2000lux,
Feeding	Mixture of yeast and algae (*Scenedesmus subspicatus*) at days of test water renewal
Test parameters:	pH and oxygen concentrations of the test samples were controlled at all treatment periods at the beginning and at the end. Immobility of adults and number of young released were recorded three times per week at the renewal of test media. Analyses of test concentrations were performed with low (0.025 mg/L), medium (0.4 mg/L) and high (1.6 mg/L) concentrations by HPLC. Measured values were expressed as the sum of measured pyridate plus the amount of measured pyridafol converted to pyridate.
Statistics:	Results for mortality and reproduction were statistically analysed using the Steel-test and the Logit-model. In addition, NOEC and LOEC values were determined.
<u>Findings:</u>	
Analytical data:	The concentration of the 0.025 mg/L treated vessels was below the lowest calibration point. The concentration of 0.4 mg/L and 1.3 mg/L showed actual concentrations of 18.2% to 38.7% of nominal concentrations.
Effects:	No mortality of control samples with or without acetone was observed. From day 0-5, no mortality was observed at the lowest test concentration, i.e. nominal 0.006 mg/L up to nominal 0.1 mg/L (actual 0.028 mg/L). At the second highest concentration of 0.4 mg/L (actual 0.112 mg/L) a mortality rate of 30% was observed within 21 days. At the highest concentration (1.6 mg/l, actual 0.446 mg/l) 100% mortality were observed within 3 days of exposure. Reproduction of young daphnids started from day 10 of the exposure period on. Finally, at the end of the study, a total of 455, 444, 380, 462, 544 and 238 live, neonates were produced by the 10 adult daphnids in the untreated and acetone treated medium and to nominal concentrations of 0.006, 0.025, 0.1 and 0.4 mg/L treated test medium, respectively.

Table 119: Summary of effects of long-term exposure of pyridate on *Daphnia magna*

Pyridate [mg ai/L] (nom)	Pyridate [mg ai/L] (mm*)	Mean parent mortality at day 21 [%]	Offspring of surviving females at day 21
Control	-	0	455
Solvent control	-	0	444
0.006	<0.01	10	380
0.025	<0.01	10	462
0.1	0.028	0	544

Pyridate [mg ai/L] (nom)	Pyridate [mg ai/L] (mm*)	Mean parent mortality at day 21 [%]	Offspring of surviving females at day 21
0.4	0.112	30	238
1.6	0.446	100	0

* Based on sum of measured pyridate and measured pyridafol converted to pyridate

Conclusion: Reproduction: NOEC = 0.1 mg ai./L (based on nominal concentrations)
NOEC = 0.028 mg ai/L (based on measured concentrations)
EC_{50} = 0.4 mg ai./L (based on nominal concentrations)
EC_{50} = 0.112 mg ai./L (based on measured concentrations)
Adult mortality: EC_{50} = 0.49 mg ai./L (based on nominal concentrations)
EC_{50} = 0.137 mg ai./L (based on measured concentrations)

Comment RMS: The study is considered to be acceptable and the validity criteria according to the cited guidelines were met: The mortality in the controls did not exceed 20% at the end of the test. The dissolved oxygen concentration was >60% (5 mg/L) throughout the test. The deviation of the pH from the initial value was <0.3 units (This value was exceeded for the highest test concentration, but this was not relevant for the reproduction assessment as all individuals died). The results are based on measured concentrations. The first young should be born in the controls after a maximum of nine days (actually on day 10, but this is likely due to the sampling regime: 0 young on day 7, 86 young on day 10). The average cumulative number of young per female in the controls was 46.
The number of tested animals and replicates deviated from the cited guideline as 10 daphnids (out of 20) were tested individually instead of 4 replicates with ten daphnids each. As this test regime as followed in the study is in line with current recommendations according to OECD 211, this deviation is not considered to invalidate the test.
It further is noted that the endpoints as presented above are based on the sum of measured pyridate and pleasured pyridafol (CL9673) expressed as pyridate. The NOEC for effects on reproduction and parent mortality was therefore set to 0.01 mg ai/L (the limit of detection) based on the low measured concentrations of pyridate alone. Though this value represents a quite conservative endpoint it is considered to be appropriate for classification purposes.

Table 120: Summary of relevant information on available long-term toxicity studies with daphnids for CL9673

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
OECD 211	*Daphnia magna*	static	21d	Immobility reproduction	nominal	5	>5	-	Wüthrich, 1991c

4.4.3 Algae and aquatic plants

Two toxicity studies with algae with Pyridate were available for the first Annex I inclusion of Pyridate. One of the studies was still considered to be valid and was only corrected for the level of

measured concentrations. A new detailed study summary was not deemed necessary in course of the Annex I renewal procedure as no new information was provided.

Table 121: Summary of relevant information on available acute toxicity studies with algae for Pyridate

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
ASTM E 1218-9, FIFRA 122-2 and 123-2	*Anabaena flos-aquae*	static	96h	Growth rate Biomass	Mean measured	0.75	>0.75	-	Grade, 1998

The toxicity study with algae with CL9673 available for the first Annex I inclusion of Pyridate was still considered to be valid. A study with a second species was additionally provided in course of the Annex I renewal procedure according to Commission Regulation (EU) 1141/2010 and is summarised below.

Table 122: Summary of relevant information on available acute toxicity studies with algae for CL9673

Method	Results							Remarks	Reference / acceptability
	Test organism	Test condition	Duration	Endpoint	Test conc	NOEC [mg ai/L]	EC_{50}/LC_{50} [mg ai/L]		
OECD 201	*Selenastrum capricornutum*	static	96h	Biomass	nominal	1.7	4.93	-	Grade, 1998

Reference:	**Toxicity of CL9673 to Anabaena flos-aquae in an Algal Growth Inhibition Test**
Author(s), year:	Hermes, H. and Erk, T., 2013
Report/Doc. number:	Ibacon project no. 81931210
Guideline(s):	OECD Guideline No. 201; Commission Regulation (EC) No 761/2009, Annex, Part C, C.3
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:	
Test substance:	CL9673; Batch No.: Cl-9673-1103001; content of a.i.: 99.4 % (analytical)
Test species:	*Anabaena flos-aquae*, UTEX B1444
Number of organisms:	Initial cell density: 15000 cells/mL; 3 replicates per treatment group and 6 replicates per control (medium)

Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	10, 3.2, 1, 0.32 and 0.1 mg test item/L, control
Measured:	Endpoints were reported as nominal values as the measured concentration were in the range of 80 – 120 % of nominal.
Solvent:	None
Test conditions:	
Water quality:	Reconstituted Water: 20 x AAP Medium; The pH was adjusted with 2 M HCl to pH 7.4. The culture medium was prepared 4 days before test start to allow pH to stabilise.
Temperature:	22 °C
pH:	7.6 to 8.0 at test start and 9.0 to 9.4 at test end
Light regime:	Continuous illumination, Mean light intensity: 3445 lux (range: 3040 to 3780 lux)
Test parameters:	The temperature was measured daily in an Erlenmeyer flask filled with water and incubated under the same conditions as the test flasks. The pH was measured in all test item concentrations and the control at the start and the end of the test. The cell densities in the samples were determined by spectrophotometrical after 24, 48 and 72 hours of exposuremeasurement. For analytical confirmation of the test concentrations duplicate samples from the freshly prepared test media (containing algae) of all test concentrations and from the controls were taken at the start of the test. For the determination of the stability of the test item under the test conditions and of the maintenance of the test item concentrations during the test period, duplicate samples from the test media of all test concentrations and the controls (containing algae) were taken at the end of the test (after the 72 hours test period).
Statistics:	Based on the calculated cell densities, the 72-hour E_rC_{50} and the 72-hour E_yC_{50}, the corresponding EC_{20} and EC_{10} values and where possible their 95 % - confidence limits were calculated by Probit analysis. For the determination of the 72-hour LOEC and the 72-hour NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by Williams t-test. The software used to perform the statistical analysis was ToxRat Professional.
Findings:	
Analytical data:	Mean recovery in the test samples: freshly prepared: 120 %, aged test media: 119 %
Biological results	The microscopic examination of the shape of the algal cells after 72 hours of test duration did not show any difference between the algae that had been growing at a nominal test concentration of 10 mg test item/L and the algal cells in the control.

Table 123: Effects of CL9673 on mean cell density of *Anabaena flos-aquae* exposed for 72

hours in a static test

CL9673 [mg/L] (nominal)	Mean density of algal cells [10^4/mL] * (S.D.)		
	24 h	48 h	72 h
Control	8.842 (0.943)	21.107 (2.051)	76.020 (4.907)
0.1	9.064 (0.884)	21.368 (0.547)	79.047 (2.860)
0.32	8.960 (1.701)	21.614 (1.646)	74.894 (11.268)
1	6.857 (0.472)	22.614 (1.959)	76.838 (3.221)
3.2	6.750 (0.586)	21.311 (1.720)	75.666 (2.741)
10	5.036 (0.329)	10.980 (0.692)	37.345 (2.416)

* initial value: 15000 cells/mL

Table 124: Effects of CL9673 on growth rate and yield of *Anabaena flos-aquae* exposed for 72 hours in a static test

CL9673 [mg/L] (nominal)	Percent inhibition relative to the control [%]	
	Yield	Growth rate
Control	-	-
0.1	-4.1	-1.0
0.32	1.5	0.5
1	-1.1	-0.3
3.2	0.5	0.1
10	51.9 *	18.1 *
72 h EyC50 = 9.76 mg/L (95 % C.I. 8.11 – 11.1) 72 h ErC50 >10 mg/L (95 % C.I. n-d-) Based on nominal concentrations		

* significantly different from control

Conclusion: 72 h E_yC_{50} = 9.76 mg/L
72 h E_rC_{50} >10 mg/L
72 h NOE_yC = 3.2 mg/L
72 h NOE_rC = 3.2 mg/L
based on nominal concentrations

Reference:	**Acute toxicity test of SAN 1367 H tech. to the duckweed *Lemna gibba* G3 under static conditions**
Author(s), year:	Grade R., 1997
Report/Doc. number:	Novartis Crop Protection, Doc N°: 971559
Guideline(s):	ASTM Guideline E1415-91, OPPTS 850.4400, 712-C-96-156, April 1996; OECD guideline Draft proposal dated July 1996 Study compliant with OECD 221
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Material and methods:

Test substance:	SAN 1367 H, purity: 95.4 %, batch: CH.4101
Test species:	Duckweed (*Lemna gibba)*
Number of organisms:	3 replicates per treatment group, 3 replicates per control, initial frond number: 18

Type of test, duration:	Static test, 7 days
Applied concentrations:	
Nominal:	0 (medium control), 1.8, 3.2, 5.8, 10, 18 and 32 mg ai/L
Measured (mean):	At test start: 0 (medium control), 1.7, 2.9, 5.1, 8.9, 16.4 and 28.7 mg/L At test end: 0 (medium control), 1.6, 2.8, 5.3, 9.1, 16.8 and 29.5 mg/L
Test conditions:	
Water quality:	Reconstituted bi-distilled nutrient containing water (according to guideline), total hardness: 298 mg/L as $CaCO_3$
Temperature:	24 ± 1 °C
pH:	7.6 (0 d), 8.6 – 9.0 (7 d)
Light regime:	Intensity: 68 µE/m^2 sec (approx. 7000 lux)
Test parameters:	For the analytical determination of test concentrations 2 samples were taken at start of exposure. 3 samples were taken at the end of exposure. Analyses were performed via HPLC-UV. Fronds were counted at 0, 3, 5 and 7 days of exposure. Temperature was continuously measured. The pH of the test solutions was measured at the start and at the end of exposure.
Statistics:	The EC50 values were calculated according to the maximum likelihood method, logit model. In addition, EC-values were graphically determined on gaussi-logarithmic probability paper. For determination of the NOEC/LOEC values a Dunnett`s Multiple Comparison Test was used.
Findings:	
Analytical data:	Mean measured concentrations were in the range of 88 – 94 and 88 - 93 % of nominal concentrations at test start and test end respectively.
Morphological effects:	After 5 and 7 days of exposure, plants were smaller at test concentrations 10, 18 and 32 mg/L in comparison to control. At test concentration 32 mg/L, many roots were swimming in the test water, separated from the plants. At test day 7, the roots in the test water of the concentrations 18 and 32 mg/L were bleached out.
Effects on biomass:	See table below

Table 125: Effects of SAN 1367 H on mean frond number of *Lemna gibba*

SAN 1367 H [mg/L] (nominal)	Frond number (mean of 3 replicates)			
	0 d	3 d	5d	7 d
control	18	40	94	221
1.8	18	37	83	198
3.2	18	44	89	180
5.8	18	41	74	129
10.0	18	34	56	84
18.0	18	27	37	40
32.0	18	27	28	29

Table 126: Dry weight and inhibition of growth rate and area under the growth curve

SAN 1367 H [mg/L] (nominal)	Growth rate (µ)		Area under the growth curve (A)		Dry weight
	mean	Inhibition	mean	Inhibition	mean [mg]
control	358	0.0	41	0.0	29.7
1.8	340	4.8	36	12.6	25.8
3.2	329	8.0	37	10.0	21.3 ***

SAN 1367 H [mg/L] (nominal)	Growth rate (μ)		Area under the growth curve (A)		Dry weight
	mean	Inhibition	mean	Inhibition	mean [mg]
5.8	281	21.3 **	28	31.3 **	14.0 ***
10.0	220	38.5 **	18	55.5 **	7.8 ***
18.0	115	67.8 **	8	79.8 **	3.9 ***
32.0	66	81.4 **	5	87.1 **	1.9 ***
7d EbC50 = 9.2 mg/L (95 % C.I. 7.0 – 11.3), based on frond number 7d ErC50 = 12.6 mg/L (95 % C.I. 1.4 – 13.9), based on frond number					

* Significant on 5 % level
** Significant on 1 % level
**** Significant on 0.1 % level

Conclusion:

7d E_bC_{50} = 9.2 mg/L (frond number)
7d E_rC_{50} = 12.6 mg/L (frond number)
7d NOE_bC = 3.2 mg/L (frond number)
7d NOE_rC = 1.8 mg/L (frond number)
7d NOEC =1.8 mg/L (dry weight)
based on nominal concentrations

Comment RMS: The notifier additionally presented endpoints related to growth rate and yield based on biomass using probit analyses calculated with the program “Toxrat” (Anonynmous, 2013d).

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7d E_yC_{50} = 4.84 mg/L (95 % C.I. 4.41 – 5.3) (dry weight)
7d E_rC_{50} = 8.64 mg/L (95 % C.I. 7.61 – 9.81) (dry weight)
based on nominal concentrations

4.4.4 Other aquatic organisms (including sediment)

-

4.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Endpoint	Classification Criteria - CLP (2nd ATP) (criteria in bold)	Evidence for Pyridate
Degradation Pyridate	***Hydrolytic degradation:*** **Pyridate:** pH 5: DT50 = 2.8 days at 22 °C / 3.7 days at 25 °C pH 7: DT50 = 0.7 days at 22 °C / 2.4 days at 25 °C pH 9: DT50 = 0.1 days at 22 °C / 0.4 days at 25 °C **Pyridafol (CL-9673):** Stable under conditions of sterile hydrolysis ***Photodegradation:*** **Pyridate:** Pyridate does not significantly absorb light at wavelengths > 290 nm **Pyridafol (CL-9673):** pH 5: DT50 = 0.04 days pH 7: DT50 = 1.1 days pH 9: DT50 = 1.8 days ***Degradation in aquatic water sediment systems:*** Pyridate is considered to be not ready biodegradable according to a ready biodegradability study. In water sediment systems pyridate is rapidly hydrolysed with entire system DT50 values < 1 day releasing the main metabolite pyridafol (CL-9673). Pyridafol (CL-9673) is considered persistent in water sediment systems (entire system DT50 > 150 days). ***Degradation in soil:*** Under aerobic laboratory soil conditions pyridate is rapidly hydrolysed with laboratory DT50 < 1 day releasing the main metabolite pyridafol (CL-9673). Laboratory DT50 values for Pyridafol (CL-9673) are in a range from 16.5 – 42.8 days.	The active substance is **not considered as ready biodegradable/rapid degradable**.
Bioaccumulation Pyridate	**Log K_{ow} is < 4** Pyridate Log K_{ow} = 4.01 ± 0.16 at room temperature	The measured log P_{OW} is 4.01 (at room temperature) and is slightly above the classification criteria of 4. The bioconcentration factor for the whole fish was 116

Endpoint	Classification Criteria - CLP (2nd ATP) (criteria in bold)	Evidence for Pyridate
	BCF >500 Pyridate BCF = 116 (whole fish)	and therefore below the criteria of 500. Pyridate is **not** considered **to have a potential for bioconcentration**.
Acute aquatic toxicity Pyridate	**LC_{50} > 1 mg/L (freshwater invertebrates)** LC_{50} = 0.49 mg/L	The classification is based on data with Pyridate. Pyridate is of moderate toxicity to fish (LC50 = >1.01 mg/L) and algae (E_rC_{50} >0.75 mg/L) and of high toxicity to fish and aquatic invertebrates (LC_{50} < 1 mg/L) and fulfills the criteria for the proposed classification as **H400** according to Regulation EC 1272/2008.
Chronic aquatic toxicity Pyridate	**For not rapidly degradable substances: NOEC ≤ 0.1 mg/L** NOEC = 0.01 mg/L (daphnids)	Pyridate is of high chronic toxicity to aquatic invertebrates with a NOEC = 0.01 mg/L. Therefore Pyridate fulfills the criteria for the proposed classification as **H410** according to Regulation EC 1272/2008. Pyridate is not indicated to be rapidly biodegradable. Though in the water sediment study a DT50 of 0.45 days (geomean) was determined for the whole system, the persistent main metabolite CL9673 fulfils the classification criteria (fish: NOEC = 0.1 mg/L,
SUMMARY	**H400 / H410**	**PROPOSED CLASSIFICATION**

4.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Conclusion of environmental classification according to Regulation EC 1272/2008

Pictogram: GHS 09

Signal word: Warning!

Aquatic Acute 1 – H400 'Very toxic to aquatic life'

Aquatic Chronic 1 - H410 'Very toxic to aquatic life with long lasting effects'

M factor = 1 (acute) and 10 (chronic)

Justification for the proposal

H400 follows from the toxicity of the active substance pyridate to aquatic invertebrates (*Daphnia magna*, LC50 = 0.49 mg/L, Egeler, Goth and Seck, 2011).

H410 follows from the long-term toxicity of the active substance pyridate to aquatic invertebrates (Daphnia magna, NOEC = 0.01 mg/L, Wüthrich, 1992a) and the fact that the active substance is not readily biodegradable (Dietschy, 2001). Though in the water sediment study a DT50 of 0.45 days (geomean) was determined for the whole system, the persistent metabolite CL9673 fulfils the classification criteria (NOEC = 0.1 mg/L; Gilberg and Seck, 2011). Further, the log Pow of pyridate is 4.01 (Zohner et al., 1982). Pyridate is therefore not indicated to be rapidly degradable.

Based on the fish bioconcentration study (Lentz, N.R., 2010) with *L. macrochirus* a steady-state BCF (whole fish) of 116 was determined (Ellgehausen and Wüthrich, 1984). The substance Mandestrobin does not meet the CLP criteria (BCF ≥ 500) based on the measured fish BCF.

Pyridate fulfils the criteria for classification as aquatic environmental hazard based on the CLP Regulation and should be classified.

The statements **P273**, **P391** and **P501** follow a general precautionary approach for dangerous substances.

Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Under conditions of sterile hydrolysis pyridate undergoes rapid degradation (DT50 < 4 days) releasing the main metabolite pyridafol (CL-9673) which is considered hydrolytically stable.

Pyridate is not readily biodegradable according to a ready biodegradability study. In water sediment systems pyridate undergoes rapid hydrolysis (DT50 < 1 day) to release the main metabolite pyridafol (CL-9673). Pyridafol (CL-9673) is considered persistent in water sediment systems (DT50 ≥ 150 days). Mineralisation of the active substance is below 10 % of applied radioactivity after 120 days after application.

Pyridate has a lowpotential of bioaccumulation in aquatic system because of a measured fish BCF of 116 (Ellgehausen and Wüthrich, 1984).

Pyridate is acute toxic to freshwater invertebrates (*Daphnia magna*, LC_{50} = 0.49 mg/L, Egeler, Goth and Seck, 2011). Pyridate is chronic toxic to aquatic invertebrates (*Daphnia magna*, NOEC = 0.01 mg/L, Wüthrich, 1992).

Hazard pictogram		Environment
Hazard class and category:	Hazardous to the aquatic environment, Acute Hazard Category 1 Hazardous to the aquatic environment, Chronic Hazard Category 1	
Signal word	Warning!	
Hazard statement:	H400 H410	Very toxic to aquatic life Very toxic to aquatic life with long lasting effects
Precautionary statements - Prevention	P273	Avoid release to the environment
Precautionary statements - Response	P391	Collect spillage
Precautionary Statement Disposal	P501	Proper disposal of contents/container

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Pyridate is a herbicide used in agriculture and horticulture. The existing harmonised environmental classification in Annex VI to the CLP Regulation is Aquatic Acute 1, H400 and Aquatic Chronic 1, H410. The DS proposed to add an acute M-factor of 1 and a chronic M-factor of 10. The proposed revised aquatic acute classification was based on an LC_{50} of 0.49 mg/L for *Daphnia magna* and the revised chronic classification was based on a NOEC of 0.01 mg/L for *Daphnia magna*. The substance is not rapidly degradable and it not considered to be bioaccumulative.

Degradation

In a GLP study performed according to OECD TG 111 with 97.9% pyridate, the hydrolysis DT_{50} values were: 3.7 days (pH 5), 2.4 days (pH 7) and 0.4 days (pH 9) at 25°C. In another hydrolysis study, the DT_{50} values were 2.8, 0.7 and 0.1 days at pH 5, 7 and 9, respectively, at 22°C (recalculated from original Zohner *et al*. (1981) by the RMS). The biologically active degradant pyridafol (CL-9673) is considered to be stable since no degradation was observed under conditions of sterile hydrolysis at pH 4, 5, 7 and 9 at 25°C. Pyridafol was shown to be stable under these conditions for 32 days at 25°C.

There are two aqueous photolysis studies available on pyridate and pyridafol. Pyridate does not significantly absorb light at wavelengths > 290 nm. Pyridafol, on the other hand, absorbs light at wavelengths > 290 nm accompanied by a significant photodegradation to several degradation products. None of the degradation products significantly exceeded

10% of the applied radioactivity (AR). A new aqueous photolysis study, including quantum yield determination, was performed with pyridafol where, following exposure to artificial light, pyridafol was degraded by photolysis to a significant extent. The photodegradation DT_{50} of pyridafol was 0.04, 1.12 and 1.81 days at pH 4, 7 and 9, respectively, equivalent to 0.15, 3.50 and 5.31 natural sunlight days (30°). At pH 7 and 9, mineralisation to CO_2 was the most significant pathway. The photolytic degradation route was complex and pH dependent, with two degradation products exceeding 10% AR during the course of the study. None of the major degradants were persistent under the conditions of the test. In a separate study, the theoretical half-life ($t_{0.5}$) of pyridafol in the surface layer of aqueous systems was estimated to range from ca. 0.1 days to 89 days, depending on the season and the pH of the water body.

There is one ready biodegradability test available on pyridate performed according to GLP and OECD TG 301F. No biodegradation was observed within the test period of 28 days. Consequently, pyridate is considered not readily biodegradable.

In a GLP OECD TG 308 study, degradation and retention of pyridate were tested in two natural aquatic sediment systems in the dark under aerobic conditions. The pH for the Swiss Lake system remained at around 7 and for the Calwich Abbey Lake system remained at around 8. Mean recoveries of radioactivity were 96.2% and 96.7% for the Swiss Lake and Calwich Abbey systems, respectively. Only trace amounts of radioactivity were released as volatile products during the duration of the study (101 days). In both systems, radioactivity was found to gradually transfer from the water phase to the sediment phase with time. The degree of mineralisation was low with only around 1-1.5% of the AR converted to CO_2 in both sediments at the end of the study. Non-extractable residues were determined in the sediment phase to account for a maximum of 9.3% of the 25% of AR in sediment and for a maximum of 7.9% of the 30.8 % of AR in sediment at the end of the study in the Swiss Lake and Calwich Abbey, respectively. In the total system, pyridate rapidly degraded in both systems, declining to undetectable levels after just 7 days in both systems. The only significant degradation product, pyridafol, achieved maximum mean levels of 96.2% AR after 7 days in the Swiss lake system, declining to 81.6% AR at the end of the study. Similarly, in the Calwich Abbey system, pyridafol reached maximum levels of 96.6 AR after 3 days incubation and declined to 83.7% AR at the end of the study.

In another GLP OECD TG 308 study, radiolabelled pyridafol (4,5-^{14}C-pyridafol) was tested for 120 days (Irrsee) and for 175 days (Rodl). The pH ranged from 8.1 to 8.9 in the 'Irrsee' test system and from 8.2 to 8.9 in the 'Rodl' test system, respectively. The mean recoveries of radiocarbon were 98.2% for 'Irrsee' and 101.5% for 'Rodl'. During the course of the study, pyridafol was slowly mineralised to CO_2. The amount of $^{14}CO_2$ increased steadily and amounted to 5.7 % and 10.7 % AR at study termination in test system 'Irrsee' and 'Rodl', respectively. Only traces of organic volatiles were observed (< 0.1% AR).

Using the water/sediment test data presented above, DT_{50} values were estimated with FOCUS. For pyridate in the total system, a geometric mean DT_{50} value of 0.45 days can be used for both the water and sediment phase degradation rates due to the rapid degradation and limited transfer to the sediment (Simmonds, 2012). The total system geometric mean DT_{50} value of 286 days for pyridafol (from Krüger 1997 and Simmonds

2012) can be used for the water phase degradation rate along with a conservative default DT_{50} value of 1000 days for the sediment (Hardy, 2012a).

The CLH report includes data for soil degradation but as these are not directly relevant for the decision on rapid degradability they are not summarised here.

The DS concluded that the criteria for rapid degradation were not fulfilled.

Bioaccumulation

The measured partition coefficient log Kow of 4.1 for ^{14}C-pydirate was not reliable due to rapid hydrolysis. In a newer study following GLP and EEC method A.8, the log Kow for pyridafol was 2.0 (pH 4), 0.56 (pH 7) and -0.52 (pH 9) at 20°C. In an OECD TG 305E study, the bioconcentration factor (BCF) for the whole fish was 116 L/kg. Further details on the BCF study were provided by the DS as a response to comments following Public Consultation (PC): 'In a flow-through test bluegill sunfish were exposed to a nominal concentration of 0.05 mg/L of radiolabelled test substance for 28 days followed by a 14 days depuration period. The level of radioactivity in the treated tanks was 0.05 mg pyridate equivalent per litre, 24.8 % of the total radioactivity in the test medium was determined as pyridate, 55.3 % as pyridafol. Pyridate and its main degradation product pyridafol were accumulated to a limited extent. The plateau level was achieved after 3 days of exposure and represented on average accumulation of 116, 27 and 180 L/kg, respectively for the fish, edible and non-edible tissues. Depuration was very rapid with a calculated elimination half-life of 1.2 to 4.5 hours, which was in line with the instability of pyridate and the polar nature of the degradation products. The log Kow of pyridafol is 0.5 at pH 7 (Ellgehausen, Wüthrich 1984). The DS concluded that pyridate is considered to have no potential for bioconcentration.

Aquatic toxicity

Table. Relevant acute aquatic toxicity studies on pyridate

Test substance	Test organism	Test method	Test conditions	EC/LC$_{50}$ mg ai/L
Pyridate	*Rainbow trout*	In agreement with EPA 72-1	Static, 96 h, mortality	> 1.01 mm
Pyridate	*Bluegill sunfish*	In agreement with EPA 72-1	Static, 96 h, mortality	> 1.3 mm
Pyridate technical, purity 91.4%	*Daphnia magna*	OECD TG 202, GLP	Static, 48 h, immobility, limit test	> 0.78 mm 0h:89%, 48h: 42% of nominal
Pyridate technical, purity 91.4%	*Daphnia magna*	OECD TG 202, GLP	Semistatic, 48 h, immobility	0.49 mm
Pyridate	*Anabaena flos-aquae*	ASTM E 1218-9, FIFRA 122-2 and 123-2	static, 96 h, growth rate, biomass	>0.75 mm
mm= mean measured concentrations				

The lowest acute toxicity test result is a 48 h EC_{50} of 0.49 mg/L (mean measured) from a semistatic *Daphnia magna* test. Test solutions were renewed after one day of exposure.

The measured concentrations ranged from 69% to 108% in the new and from 41% to 77% in the old test media in relation to the nominal values.

Table. Relevant chronic aquatic toxicity studies on pyridate

Test substance	Test organism	Test method	Test conditions	NOEC mg ai/L
Pyridate technical, purity 91.5%	*Daphnia magna*	OECD TG 202 Part II, GLP	semistatic, 21 d, immobility, reproduction	0.028 * LoD reproduction 0.01 mm**
Pyridate	*Anabaena flos-aquae*	ASTM E 1218-9, FIFRA 122-2 and 123-2	static, 96 h, growth rate, biomass	0.75 mm

* sum of measured pyridate plus the amount of measured pyridafol converted to pyridate
** value set by the DS, limit of detection (LoD - based on the low measured concentrations of pyridate alone)

There are no long-term fish studies for pyridate as chronic data from OECD TG 204 is not admissible under CLP. The 21 days NOEC of 0.028 mg/L is based on the sum of measured pyridate and measured pyridafol converted to pyridate. The NOEC was set to 0.01 mg ai/L (limit of detection) based on the low measured concentrations of pyridate alone. The DS provided further information on the study following the PC. The DS clarified that the validity criteria in the test were met and that the mortality in the controls did not exceed 20% at the end of the test. The dissolved oxygen concentration was > 60% (5 mg/L) throughout the test. The deviation of the pH from the initial value was < 0.3 units. The first young were born in the controls after nine days. The average cumulative number of young per female in the water control was 85 and in the solvent control 83. The number of tested animals and replicates deviated from the cited guideline as 10 daphnids (out of 25) were tested individually instead of 4 replicated with 10 daphnids each. As this test regime is in line with current recommendations according to OECD TG 211, this deviation is not considered to invalidate the test. The study result of 0.028 mg/L (21 days NOEC) is based on nominal values. In addition to mortality and effects on reproduction, effects on the body length of all surviving daphnids were investigated. No significant difference was noted.

As it can be seen in the tables below, the acute EC/LC_{50} values for the hydrolysis product pyridafol range from 8.64 mg ai/L to 140 mg ai/L. The chronic NOEC values are from 0.1 mg ai/L to 17 mg ai/L. Consequently, pyridafol is less toxic than pyridate. Comparison cannot be made for chronic fish toxicity because data on pyridate is lacking.

Table. Relevant acute toxicity studies on the hydrolysis product pyridafol (CL-9673)

Test substance	**Test organism**	**Test method**	**Test conditions**	**EC/LC_{50} mg ai/L**
PYRIDAFOL	*Rainbow trout*	OECD TG 203	flow through, 96 h, mortality	>16.2 mm
PYRIDAFOL	*Bluegill sunfish*	EPA 72-1	flow through, 96 h, mortality	140 mm
PYRIDAFOL	*Daphnia magna*	OECD TG 202/EPA 72-1	static, 48 h, mortality	30.7 mm
PYRIDAFOL	*Selenastrum capricornutum*	OECD TG 201	static, 96 h, biomass	4.93 nominal

PYRIDAFOL purity 99.4%	*Anabaena flos-aquae*	OECD TG 201, GLP	static, 72 h, growth rate	>10 nominal (measured conc. 120 of the nominal)
SAN 1367 H purity 95.4%	*Lemna gibba*	OECD TG 221, GLP	static, 7 d, growth rate	8.64 nominal (mm at start 88-94 %, at end 88-93%)
mm=based on mean measured concentrations				

Table. Relevant chronic toxicity studies on the hydrolysis product pyridafol (CL-9673)

Test substance	Test organism	Test method	Test conditions	NOEC mg ai/L
Pyridafol	Rainbow trout	OECD TG 204	Flow through, 21 d, mortality, sublethal effects	17 nominal (mm 99% of the nominal)
CL 9673 purity 98.5%	*Oncorhynchus mykiss*	OECD TG 210, GLP	Flow through, 69 d, sublethal effects	0.1 value set in the pesticides peer review meeting (uncertainty of the outlier dose group)
Pyridafol	*Daphnia magna*	OECD TG 211	static, 21 d, immobility, reproduction	5 nominal
Pyridafol	*Selenastrum capricornutum*	OECD TG 201	static, 96 h, biomass	1.7 nominal
Pyridafol purity 99.4%	*Anabaena flos-aquae*	OECD TG 201, GLP	static, 72 h, growth rate	3.2 nominal (measured conc. 80-120 of the nominal)
SAN 1367 H purity 95.4%	*Lemna gibba*	OECD TG 221, GLP	static, 7 d, growth rate	1.8 nominal (mm at start 88-94 %, at end 88-93%)
mm=based on mean measured concentrations				

Comments received during public consultation

Three MSCAs agreed with the environmental classification proposed by the DS. One industry organisation had no comments on the proposal. One MSCA had questions related to details of the BCF study. The DS gave a more detailed description of the test as a response. The same MSCA also questioned the use of the 21-days NOEC for *Daphnia magna*. Due to the hydrolysis, it is unclear if the observed response is induced by the parent, the degradant, or a combination effect. The DS replied that the endpoints were based on the sum of measured pyridate and measured pyridafol expressed as pyridate. Setting the NOEC to 0.01 mg ai/L (the limit of detection) is based on the low measured concentrations of pyridate alone, which is considered to be a conservative approach. The DS agreed that effects are likely to be caused by a mixture of the parent and the

degradation product. The proportion of the parent can be increased by flow-through or semistatic tests. The DS regarded this as a worst case to assign the observed effects to the test compound tested, namely pyridate. The MSCA also asked for further details on the chronic toxicity study on *Daphnia magna* for pyridafol. The DS gave the details in the response to PC. Another MSCA pointed out that the pyridate study with *Anabaena flos-aquae* does not fulfil the validy criteria for OECD TG 201 because the coefficient of variation of sectional specific growth rate does not meet the validity criteria (≤ 35%). Consequently, there are no reliable information on the toxicity of pyridate to algae. The DS informed that the study had been considered valid for the first Annex I inclusion and also for the renewal procedure. The coefficient of variation of sectional specific growth rate was not a validity criterion at the time the study was conducted and it would be questionable to use it now.

Assessment and comparison with the classification criteria

Pyridate is not readily biodegrable. It hydrolyses rapidly and DT_{50}s range from 0.1 to 0.4 days at pH9, from 0.7 to 2.4 days at pH 7 and from 2.8 to 3.7 days at pH 5. The degradation product pyridafol is hydrolytically stable. Pyridate does not degrade through photolysis. Pyridafol, on the other hand, photodegrades to several degradation products. DT_{50}s ranged from 0.04, 1.12 and 1.81 days at pH 4, 5, and 9, respectively. None of the major degradates were persistent. The degradation product pyridafol fulfils the criteria for classification as hazardous to the aquatic environment.

In water/sediment tests, radioactivity was gradually transferred from the water phase to the sediment phase. Only trace amounts of radioactivity were released as volatile products. The degree of mineralisation was low in sediments. Non-extractable residues accounted for a maximum of 25-30.8% of AR in sediment. In the total system, pyridate rapidly degraded and declined to undetectable levels after 7 days. The only significant degradation product, pyridafol, accounted for 81.6-83.7% of the AR at the end of the study. For pyridate, the total system geometric mean DT_{50} value of 0.45 days for both water and sediment phases was estimated with FOCUS. For pyridafol, the total system geometric mean DT_{50} value of 286 days for the water phase and DT_{50} value of 1000 days for the sediment was estimated. In conclusion, RAC agrees with the DS that pyridate does not fulfil the criteria of rapid degradation for the purposes of classification.

The log Kow for pyridate was 2.0 (pH 4), 0.56 (pH 7) and -0.52 (pH 9). The BCF value for the whole fish was 116 L/kg. RAC agrees with the conclusion of the DS that pyridate is not bioaccumulative.

The lowest acute aquatic toxicity value for pyridate is a 48 h EC_{50} of 0.49 mg/L for *Daphnia magna* based on mean measured concentrations of pyridate in a semistatic test. The lowest chronic toxicity value is a 21 d NOEC of 0.01 mg/L (limit of detection) for *Daphnia magna* based on low measured concentrations on pyridate alone in a semistatic test. The NOEC was 0.028 mg/L based on the sum of measured pyridate and measured pyridafol expressed as pyridate. Although there is no chronic toxicity data available for fish, the available values for acute toxicity are above the water solubility limit in each study's test system, so it is not possible to use the surrogate approach. Also, there is no toxicity data for macrophytes (*e.g*. *Myriophyllum* sp. or *Lemna* sp.) available. These species might be sensitive for the herbicide pyridate which could effect the M-factors if tests become available.

The hydrolysis product pyridafol is not rapidly degradable and the lowest acute toxicity value is a 96 h E_bC_{50} of 4.93 mg/L for the algae *Selenastrum capricornutum* based on nominal pyridate concentration in a static test. The lowest chronic toxicity value is a 69 d NOEC of 0.1 mg/L for hatching success in the fish *Oncorhynchus mykiss* based on nominal concentrations in a flow-through test.

Conclusion on use of pyridafol for classification of Pyridate

Although an $L(E)C_{50}$ may be calculated based on the geometric mean of the degradation product concentration, back calculated to the parent substance (ECHA Guidance on the Application of the CLP Criteria (I.4.1 (c))), there is no analytical data available for the parent and degradant substances. It is very difficult or almost impossible to test pyridate alone as it rapidly degrades to pyridafol. The proportion of the parent is increased by flow-through or semistatic tests but both compounds will still be present. RAC agrees with the DS's view that basing the classification on Pyridate represents a worst-case scenario. On this basis the classification is based on data from Pyridate rather than pyridafol.

Conclusion

Overall, RAC agrees with the DS's proposal. The lowest aquatic acute toxicity value for pyridate is 0.49 mg/L which leads to a classification as **Aquatic Acute 1, H400** with an **acute M-factor of 1**. Pyridate is not rapidly degrable and the lowest chronic aquatic toxicity value is 0.01 mg/L justifying a classification as **Aquatic Chronic 1, H410** with a **chronic M-factor of 10**.

1 OTHER INFORMATION

Human health hazard assessment of this CLH report is based on the Evaluation report for plant protection product active substances.

Environmental fate properties and environmental hazard assessments of this CLH report are based on studies and summaries of the Draft Assessment Report.

2 REFERENCES

2.1 Toxicological properties

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Til, H.P. et al.	1982	Multigeneration study with Pyridate in rats. TNO Project No.: B 80-0696; GLP; Unpublished	N	BCP
Til, H.P. et al.	1979b	Subacute (4-week) oral toxicity study with pyridate in mice. TNO report No.: R 6396; GLP; Unpublished	N	BCP
Til, H.P. et al.	1980	Sub-acute (4-week) oral toxicity study with pyridate in Wistar and Sprague Dawley rats. TNO Report No.: R 6367; GLP; Unpublished	N	BCP
Til, H.P. et al.	1982, revise d 1990	Lifespan oral carcinogenicity study of Pyridate in rats. TNO Project No.: B 80-0023; GLP; Unpublished	N	BCP
Tomkins, E.C.	1987	90 day dog oral subchronic toxicity study. TRL Study No.: 043-002	N	BCP
Ullmann, L., Kups, A.	1998	Determination of skin irritation and capacity of allergenic sensitization by the open epicutaneous test on guinea pigs (OET) with pyridate tech. RCC project 098144, GLP; Unpublished.	N	BCP
Ullmann, L. and Sacher, R.	1984	Acute oral (LD_{50}) study with Pyridate technical in rats. RCC Report No.: 036990 ; GLP; Unpublished	N	BCP
Ullmann, L. et al	1984	Acute dermal toxicity (LD_{50}) study with Pyridate techn. in rabbits. RCC Project 037001; GLP; Unpublished	N	BCP
Ullmann, L. et al	1983	4-hour aerosol inhalation toxicity study (LC_{50}) with pyridate techn. in rats RCC Project 016255; GLP; Unpublished	N	BCP
Ullmann, L. et al.	1988	Acute oral toxicity study with Pyridate technical in rats. RCC Report No.: 202623; GLP; Unpublished	N	BCP
Ullmann, L. et al.	1987	Acute oral toxicity study with Pyridate technical in mice. RCC Report No.: 088323; GLP; Unpublished	N	BCP
Vandaele, E.A.N.	1990	Pyridate technical: Toxicity study by oral (capsule) administration to Beagle dogs for 13 weeks. LSR Report No.: 89/AGL001/0806; GLP; Unpublished	N	BCP

2.2 Environmental effects

2.2.1 Environmental fate and behaviour

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Creeger, S.M., Cohen, S.P.	1988	Environmental fate of the S-octyl thiocarbonate moiety of pyridate. Center of Hazardous Materials Research, University of Pittsburgh; report no. 4807A; No GLP; Unpublished	N	BCP
Schanné, C., Morgenroth, U.	1995	Metabolism and degradation of ^{14}C-labelled pyridate in four soils; RCC Umweltchemie AG report no 287550 GLP; Unpublished	N	BCP
Zohner, A.	1985, add-endum 1989	Aerobic soil metabolism study of ^{14}C-CL 9673, the main metabolite of ^{14}C-pyridate in soil; Chemie Linz AG report no 832	N	BCP
Ellgehausen, H.	1985	^{14}C-Pyridate: Soil metabolism in a model study under outdoor conditions; RCC Umweltchemie AG report no 049511	N	BCP
Zohner, A.	1992	^{14}C-Pyridate: Degradation and metabolism in one soil incubated under anaerobic conditions; Agrolinz report no. 1116	N	BCP
Van Dijk, A. Baranowski, D.	1992	Photodegradation study of ^{14}C-pyridate on soil; RCC Umweltchemie AG report no. 287550	N	BCP
Heegemann, W., Bayzer, H., Mayr, H.	1983	Examination of degradation of Pyridate and CL 9673 in soil, site Biolabor Haag, 1983 - Part of a long term test of Lentagran WP in maize;Generated by: Chemie Linz AG, Company file No.: 766, BS 6114	N	BCP
Dykeman, R.G.	1992a	Dissipation of Pyridate residues from an Iowa loam and an Illinois sandy clay loam corn field treated with Tough 3.75 EC Herbicide; EN-CAS resp. ACRES; Company file No.: AGRO-9003, BS 6151	N	BCP
Dykeman, R.G.	1992b	Dykeman, R.G., 1992 Dissipation of Pyridate residues from a Wisconsin silt loam cabbage field treated with Tough 3.75 EC Herbicide;Generated by: EN-CAS resp. GLARS; Company file No.: AGRO-9005, BS 6145	N	BCP
Dykeman, R.G.	1992c	Dissipation of Pyridate residues from a California sandy loam cabbage field treated with Tough 3.75 EC Herbicide; Generated by: EN-CAS resp. Hulst Research; Company file No.: AGRO-9006, BS 6141	N	BCP
Krennhuber, K. Pfarl, Ch.	1996	Residues of pyridate and its main metabolites CL-9673 and CL-9673-O-methyl in soil treated with 2.5 kg Lentagran 45 WP/ha. Agrolinz Melamin report no 1255; GLP; Unpublished	Y	BCP
Gasser, A.	2001a	Field dissipation and mobility of residues of SAN 1367 H, its O-methyl metabolite and of dicamba and its metabolite dichlorosalicylic acid in cropped soil following application of SAN 1411 H 167 SL 003BS in France, 1996. Syngenta AG report 3069/98; GLP; Unpublished	Y	BCP

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Gasser, A.	2001b	Field dissipation and mobility of residues of SAN 1367 H, its O-methyl metabolite and of dicamba and its metabolite dichlorosalicylic acid in cropped soil following application of SAN 1411 H 167 SL in Germany, 1996. Syngenta AG Report no. 3070/98; GLP; unpublished	Y	BCP
Gasser, A.	2001c	Field soil dissipation and mobility of residues of SAN 1367 H and its O-methyl metabolite in bare and cropped soil following application of A-11897 A (SAN 1367 H 490 SC) in Northern Germany. Syngenta Crop Protection AG, report no 304/98; GLP; Unpublished	Y	BCP
Chambers, J.G. et al.	2011a	Terrestrial soil dissipation of pyridate and its metabolite CL-9673 after one application of Lentagran 450 g/kg WP on bare soil in Northern France, 2010-2011. Battelle UK, report no OZ/10/013; GLP; Unpublished	Y	BCP
Chambers, J.G. et al.	2011b	Terrestrial soil dissipation of pyridate and its metabolite CL-9673 after one application of Lentagran 450 g/kg WP on bare soil in Germany, 2010-2011. Battelle UK, report no OZ/10/014; GLP; Unpublished	Y	BCP
Hardy, IAJ.	2011	Pyridate - Kinetic Modelling Evaluation of Data from Field Soil Residue Studies Normalised to 20 ºC (Q10 2.58) and pF2. Battelle UK, report no OZ/11/015A; GLP; Unpublished	Y	BCP
Hardy, IAJ.	2013	Pyridate - Kinetic Modelling Evaluation of US Field Soil Dissipation Studies. Battelle Study Number: OZ/11/015B; no GLP; Unpublished	Y	BCP
Simmonds, R.	2012	Pyridate: Estimation of Adsorption Coefficient (Koc) on Soil by HPLC (OECD 121). Battelle UK, report no OZ/10/022; GLP; Unpublished	Y	BCP
Krüger, B.	1992	^{14}C-CL9673: Adsorption / desorption of four American soils; Agrolinz Agrarchemikalien GmbH; Company file No.: 1126, BS 5754	N	BCP
Glänzel, A.	1996	Adsorption and desorption (non-aged) of CL 9673 (SAN 1367 H AI), the active moiety of pyridate, on soils determined by the batch equilibrium method. Sandoz Agro TDS BS 7426 (study no. E95-11); GLP; Unpublished	N	BCP
Burgess, M., Simmonds, M.	2012	[^{14}C]-CL-9673: Adsorption to and Desorption from Five Soils. Battelle UK, report no OZ/11/024; GLP; Unpublished	Y	BCP
Zohner, A., Bayzer, H., Mayr, H.	1981	Determination of hydrolysis of Pyridate (CL 11.344) in water as a function of pH; Chemie Linz AG; Company file No.: 735, BS 5929	N	BCP
Lutringer, C.	1997	Pyridate: Hydrolysis as Function of pH; Report N° TDS BS 12456; study n° E96-23	N	BCP
Goodyear, A.	1997	(^{14}C)-SAN 1367 H: Hydrolytic stability – Amended Final Report. Covance Laboratories, report no 252/227-1015; GLP; unpublished	Y	BCP

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Van Dijk, A., Betschart, B.	1992	Photodegradation study of ^{14}C-Pyridate in water at pH 5, 7 and 9; RCC Umweltchemie AG; Company file No.: 287548, BS 5933	N	BCP
Zohner, A.	1988	Aqueous photolysis study on ^{14}C-CL 9673, the hydrolysis product of ^{14}C-Pyridate; Agrolinz Agrarchemikalien GmbH; Company file No.: 941, BS 5934	N	BCP
Werle, H.	1993	Direct Phototransformation Study, CL 9673, Determination of Quantum Yield in water at pH 7; BioChem GmBH, Report 92 50 40 035 B	N	BCP
Mills, E.A.M. and Dobson, R.M.	2012	[^{14}C]- CL-9673: Aqueous Photolysis and Quantum Yield Determination in Sterile Buffer Solutions. Battelle UK, report no OZ/10/019; GLP; Unpublished	Y	BCP
Buntain, I.G.	2012	CL9673 (pyridate metabolite): estimation of environmental photolytic half-life in water. Model calculation according to Frank and Klöpffer. Battelle UK, report no OZ/12/002; GLP; Unpublished	Y	BCP
Dietschy, A.	2001	Ready biodegradability of SAN 319 tech. (pyridate tech.), (Manometric respirometry test). Solvias AG, report no LO1 – 006745; GLP; Unpublished.	Y	BCP
Krüger, B.	1997	Route and rate of degradation of ^{14}C-labelled SAN 1367 H AI in water/sediment systems. Agrolinz Melamin GmbH, report no 1291; GLP; Unpublished.	Y	BCP
Simmonds, M.	2012	[^{14}C]- Pyridate: Degradation and Retention in Two Water-Sediment System. Battelle UK, report no OZ/10/017; GLP; Unpublished.	Y	BCP
Hardy, I.A.J.	2012	Kinetic Modelling Analysis of Pyridate and CL9673 from Water Sediment Studies to Derive Modelling Endpoints. Battelle UK, report no OZ/11/017; GLP; Unpublished.	Y	BCP
Ewald, G.	1993	Photochemical-oxidative degradation of Pyridate - Theoretical calculation according to Atkinson; ECON Forschungs GmbH; Company file No.: BS 5941	N	BCP
Glänzel, A.	1996	Estimation of the photochemical oxidative degradation rate of pyridate in the atmosphere. Sandoz Agro, report no TDS BS 7379; GLP; Unpublished.	Y	BCP
Buntain, I.G.	2011	Pyridate and CL9673 (Metabolite): Estimation of chemical lifetime in troposphere. Model calculation according to Atkinson. Battelle UK, report no OZ/10/026; GLP; Unpublished	Y	BCP

2.2.2 Ecotoxicology

Author(s)	**Year**	**Title** **Source** (where different from company) **Company name,** **Report No.,** **GLP status** (where relevant) **published or not**	**Data protect. claimed**	**Owner**
Bowman J, Schuster L., Franklin Bunch B.	1986a	Acute toxicity to rainbow trout. Analytical Bio Chemistry Lab. Inc. Report N° 34819 Unpublished	No	BCP
Bowman J, Schuster L., Franklin Bunch B.	1986a	Acute toxicity to bluegill sunfish. Analytical Bio Chemistry Lab. Inc. Report N° 34303 Unpublished	No	BCP
Egeler P., Goth M., Seck C.	2011a	Pyridate technical : A Study on the Acute Toxicity to *Daphnia magna*. ECT Oekotoxikologie and Battelle, DOC No. 11BK4DA; OZ/11/021 GLP; unpublished	Yes	BCP
Egeler P., Goth M., Seck C.	2011b	Pyridate technical : A Study on the Acute Toxicity to *Daphnia magna*. ECT Oekotoxikologie and Battelle, DOC No. 11BK1DA; OZ/11/007; GLP; unpublished	Yes	BCP
Ellgehausen, H., Wüthrich, V.	1984	Accumulation and elimination of 14C-Pyridate by bluegill sunfish in a dynamic flow-through system. RCC Research & Consulting Company AG Report No.: 020766	No	BCP
Gilberg D., Seck, C.	2011	CL9673: A Study on the Toxicity to Early-Life Stages of Rainbow Trout. ECT Oekotoxikologie and Battelle, DOC No. 11BK1FV; OZ/11/006; GLP; Unpublished	Yes	BCP
Grade R.	1997	Acute toxicity test of SAN 1367 H tech. to the duckweed Lemna gibba G3 under static conditions. Novartis Crop Protection, Doc N°: 971559; GLP; Unpublished	No	BCP
Grade R.	1998	Growth inhibition test of Pyridate (techn.) on Blue Algae Anabaena flos-aquae under static conditions. Project N°971650 Unpublished	No	BCP
Grade R.	1998	Growth inhibition test of Pyridate (techn.) on Blue Algae Anabaena flos-aquae under static conditions. Project N°971650	No	BCP
Hermes H. Erk T.	2013	Toxicity of CL9673 to Anabaena flos-aquae in an Algal Growth Inhibition Test Ibacon GmbH, report number 81931210; GLP, unpublished	Yes	BCP
Krüger, B.	1997	Pyridate: Partition coefficient (n-octanol/water) of the main metabolite CL-9673 using the shake-flask method. Covance, report no. 252/222-1014; GLP; Unpublished	Yes	BCP

Author(s)	**Year**	**Title** **Source** (where different from company) **Company name,** **Report No.,** **GLP status** (where relevant) **published or not**	**Data protect. claimed**	**Owner**
Sword and Cramer,	1991	Acute flow-through toxicity study of CL9673 to bluegill (Lepomis macrochirus) ABC Laboratories Inc Report No.: 39351 Unpublished	No	BCP
Wüthrich, V.	1991a	CL9673 technical: 21-day prolonged toxicity study in rainbow trout under flow through conditions. RCC Umweltchemie AG Report N°274454 Unpublished	No	BCP
Wüthrich, V.	1991b	48-hour acute toxicity of CL9673 technical to Daphnia magna (OECD immobilization test). RRC Umweltchemie AG Report N° 274386	No	BCP
Wüthrich, V.	1991c	Influence of CL9673 technical on the reproduction of Daphnia magna. RCC Umwelchemie AG Report N°274397	No	BCP
Wüthrich, V.	1992	Influence of Pyridate technical on the reproduction of Daphnia magna RCC Umweltchemie AG Report N°211386 Unpublished	No	BCP
Wüthrich, V.	1993	CL9673 technical: 96hour acute toxicity study (LC50) in the rainbow trout under flow through conditions. RCC Umweltchemie AG Report N°: 345328 Unpublished	No	BCP
Zohner, A. et al.	1982	Evaluation of the partition coefficient of pyridate in the system n-octanol/water. Report n°. 748; No GLP, Unpublished	No	BCP

3 ANNEXES

Position papers from notifier regarding neurotoxicity and classification and labelling

Reference:	**Pyridate and neurotoxicity in dogs**
Author(s), year:	Kobel W., 2010
Report/Doc. number:	Expert statement
Guideline(s):	Not applicable
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Yes

The EU documents (DARs) and study reports were studied to analyse the basis of the neurotoxicity assessment. Pyridate toxicology studies were reviewed for neurotoxic effects, separated as functional changes and structural changes.

As additional element kinetics became relevant, kinetic data, primarily plasma and brain concentrations were considered and assessed.

Functional changes:

- Mice: General pharmacology (acute): Some slightly and transiently decreased locomotor activity at levels of 1000 – 8000 mg/kg, observed only on day of administration
- Rat: A 90 day gavage study with daily doses of 0, 62, 5, 177 and 500 mg/kg plus an additional group with 500/600 mg/kg showed hypoactivity & salivation at 177 and 500 mg/kg for several hours after dosing, reversible within 24 hours. Hypoactivity peaked in weeks 3-5 and decreased thereafter. Salivation peaked in weeks 4-5 and diminished only slightly. Additional signs were ataxia and lateral roll. No symptoms were observed during recovery.
- Dog: Clinical signs were observed in both subchronic studies and the 1 year study.
    - In the first subchronic study, emesis, ataxia, opisthotonus, hypoactivity, salivation, mydriasis, nystagmus, head swing, muscle fasciculations, rarely also head tilt were noted at 200, less at 60 mg/kg. Onset was 1 – 3 h after dosing, returning to normal within 24 hours up to 19 days. Recovery thereafter was not always complete. Marked toxicity was observed in the top dose with 7/8 dogs died and an average survival of 36 days.
    - In the second subchronic study, ataxia, emesis, salivation, tremor, opisthotonus, hypoactivity, mainly at 120 mg/kg, but also at 80 mg/kg were observed. Onset occurred within 1.5 hours, and was mostly gone after 6 hours.
    - In the chronic 1-year study, salivation, ataxia, mydriasis, tremors and inability to stand were the major signs. Occurrence in high dose was noted at 120 mg/kg and higher, in mid dose at 100 mg/kg and higher. The report mentions a threshold for “dramatic non-lethal clinical signs” above 80 mg/kg, which seems more dose than duration dependent.

Structural changes:

- Dog: The original reports of the first 90 day capsule study (Tompkins, 1987) reports in both sexes 2/4 incidences of degenerative myelopathy of the sciatic nerve (males 1 slight, 1 minimal, females both slight) at the high dose of 200 mg/kg. The same finding is reported for a high dose (60 – 150 mg/kg) male in 1 year (Bailey, 1989).
- In the second subchronic study (Vandaele, 1990, also capsule administration) there were occasional findings of “Myelin digestion chambers” with no dose effect relationship.

The slides of the sciatic nerves of all 3 dog studies were submitted for a peer review in 1997 (see section 3.7.1.1).

The reviewing pathologists disagreed with the diagnostic terminology “Degenerative Myelinopathy” for the changes seen in the first 90 day study and the 1-year study and changed it

into “Myelin Digestion Chambers”. There was essential agreement with the grading, although the finding was noted in a few more/other dogs.

More important, the reviewing pathologists concluded:

“The changes noted in the three studies are not considered to represent toxic effects of a test article. These changes are commonly observed in sections of sciatic nerves from a number of different species/strains of laboratory animals (e.g. rats, dogs, mice etc.) These changes tend to be more common and severe in older animals, particularly in mice and rats. These histological changes are not sufficient in number and severity to be associated with clinical signs, and the minimal changes may not be recorded by most pathologists concerned about conveying an exaggerated picture. “

Kinetic Aspects:

Rats:

Pyridate is rapidly absorbed from the intestinal tract. The highest plasma levels in animals dosed with 20 or 200 mg/kg were found already one hour after administration. Excretion is equally fast, plasma level were very low after 24 hours. With the dose of 600 mg/kg, resorption was equally rapid, but the high levels persisted over more than 24 hours and were low only after 96 hours.

The brain concentrations were proportional to the plasma levels in the order of 1 (brain): 10 – 15 (plasma), thus no indication of accumulation.

Females tended to have somewhat higher levels in plasma and brain than males.

Dogs:

In dogs the kinetics were found to be similar: Rapid uptake from the intestinal tract with peak plasma levels 3 – 6 hours after administration and also relatively rapid elimination, though slightly slower than in rats (48h vs. 24h in rats).

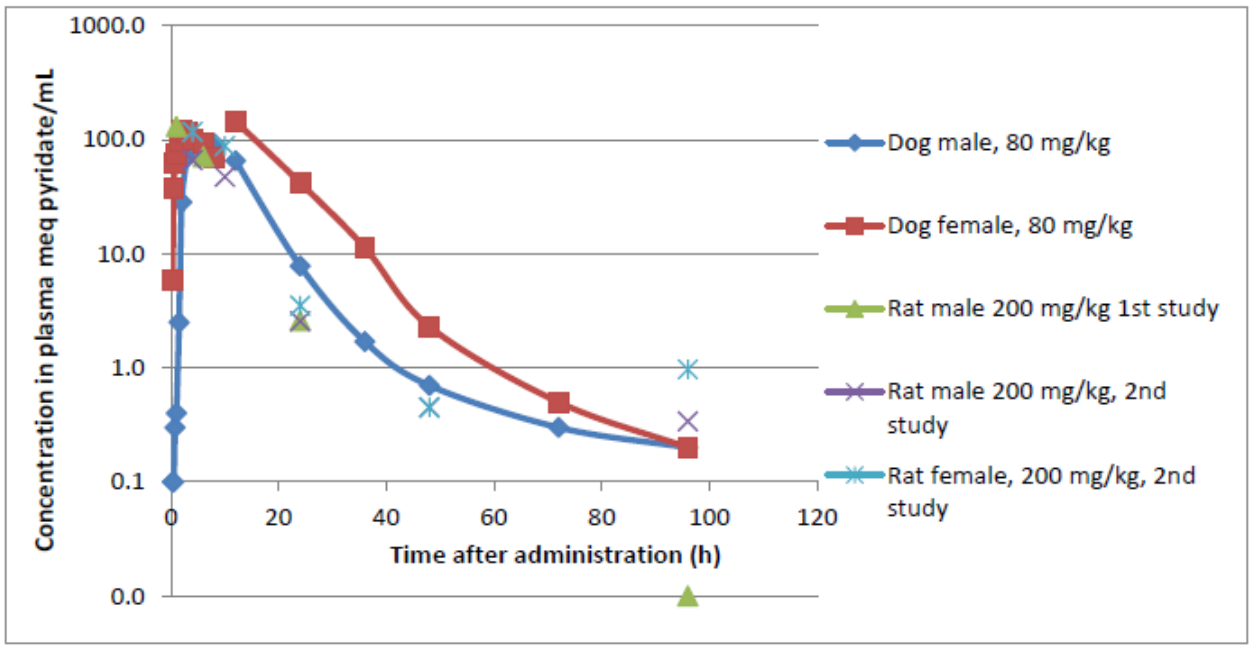
Allometric comparisons:

In order to compare kinetic data an allometric scaling is indicated. The factors used were 0.75 for the exponent and a rat weight of 0.25 kg and dog weights of 10 kg. The “FDA Allometric Scaling” gave the following conversions:

Table 1276: Allometric scaling rat-dog

Dose in Rat, 0.25 kg	**Equivalent dose in Dog, 10 kg**
20 mg/kg	7.95 m/kg
200 mg/kg	79.5 mg/kg
600 mg/kg	239 mg/kg

Figure 1: Allometric scaling rat-dog: Comparison of kinetic data dog 80 mg/kg, rat 200 mg/kg



Pyridate, at high doses and regimes leading to peak effects close to saturation induces neurological clinical signs, such as salivation, hypoactivity and others in rats and dogs.

Their occurrence appears to be mainly C_{max} dependent and fairly duration independent. They are essentially reversible within 24 hours, no accumulation of pyridate has been observed.

For these neurological clinical signs there is a threshold dose of at least 60 mg/kg in the rat (with corn oil as vehicle) and around 80 mg/kg (with capsule administration) in the dog.

Pyridate does not induce structural changes in the nervous system despite doses leading to substantial mortality in dogs. “Myelin digestion chambers” are not considered to be treatment related or associated with clinical signs.

The signs point more to an involvement of the vegetative and central nervous system than to the motoric nervous system.

In view of the nature of the neurological findings and the conditions required to induce them a classification is not deemed appropriate.

Reference:	**Pyridate and neurotoxicity: Considerations regarding potential classification under Regulation (EU) 1272/2008**
Author(s), year:	Kobel W., 2012
Report/Doc. number:	Expert statement
Guideline(s):	Not applicable
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Yes

The neurological findings in rat and dog studies were assessed under the criteria laid out in Regulation (EC) No 1272/2008.

Considering that potentially relevant observations were

- seen mostly at doses which were at least 1000 mg/kg or higher and frequently lethal, rendering the observation unspecific
- reversible in all surviving animals within hours to a few days
- not seen in the initial phases of the repeat dose gavage studies in rats and dogs

a classification as STOT SE is not justified.

For dogs, the guidance values for STOT-RE should be adapted for the higher body weight (ratio body weight/body surface) of this species.

In order to compare data an allometric scaling is indicated. The factors used were 0.75 for the exponent and a rat weight of 0.25 kg and dog weights of 10 kg. The "FDA Allometric Scaling" gives the following conversions:

Table 1287: Allometric scaling rat-dog

Dose in Rat, 0.25 kg	**Equivalent dose in Dog, 10 kg**
10 mg/kg	4 m/kg
100 mg/kg	40 mg/kg

Repeated dose effects

- were seen in rats at 177 mg/kg and above (next lower dose and study NOAEL: 62.5 mg/kg)
- have a threshold dose of about 80 mg/kg in dogs (with a NOAEL of 40 mg/kg) in the repeat dose gavage studies
- were reversible within hours after each application
- were not seen in any of the feeding studies of all durations

are all above the guidance values of 100 mg/kg for rats or the 40 mg/kg for dogs (allometric correction). Therefore a classification as STOT RE is not justified .

RMS (Austria) opinion:

In the CLP guidance there is no advice as to the use of rat-specific guidance values for studies with other experimental species (such as dogs). According to the RAC opinion on cymoxanil (ECHA/RAC/CLH-0-0000002970-73-01/F adopted 14.09.2012) "earlier RAC has considered using the same guidance values for rat and dog studies. In 2006, The Netherlands presented a corresponding thought starter (ECBI/64/06) with considerations on how to translate guidance values for the rat to guidance values to dogs based on allometric scaling and different life spans of species. However, these preliminary discussions on the use of allometric scaling and different life spans of species for RDT classification have not yet been finalized and the corresponding concepts have not yet been integrated into the CLP guidance. Thus for now RAC prefers to generally start with the guidance values for the 90-day oral rat study, to adapt these 90-day rat guidance values for different durations of exposure to rats according to Haber's rule and then to use the original or duration-adjusted rat guidance values without further changes for test results with other animal species."

A table with the guidance values for oral repeated dose studies, adjusted for the duration of exposure is copied from the RAC opinion on cymoxanil (ECHA/RAC/CLH-0-0000002970-73-01/F adopted 14.09.2012).

Table 129: Guidance values for STOT-RE

Study type	**CLP**
28 day rat/dog	**STOT RE 1: C ≤ 30** **STOT RE 2: 30 < C ≤ 300**
90 day rat/dog	**STOT RE 1: C ≤ 10** **STOT RE 2: 10 < C ≤ 100**
1 year rat/dog	**STOT RE 1: C ≤ 2.5** **STOT RE 2: 2.5< C ≤ 25**
2 year rat/dog	**STOT RE 1: C ≤ 1.25** **STOT RE 2: 1.25 < C ≤ 12.5**

For detailed evaluation and discussion please refer to sections 3.3, 3.7.1.6 and 3.8.