Committee for Risk Assessment

RAC

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

Adopted

5 December 2017

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CLH-O-0000001412-86-186/F

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

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

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

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

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

The proposal was submitted by **Austria** and received by RAC on **10 November 2016.**

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

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **10 January 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24 February 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **5 December 2017** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	607-232-00-7	pyridate (ISO); 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	-	-	-
Dossier submitters proposal	607-232-00-7	pyridate (ISO); O-(6-chloro-3-phenylpyridazin- 4-yl) S-octyl thiocarbonate	259-686-7	55512-33-9	**Retain :** Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 **Modify :** Skin Sens. 1B **Add :** STOT SE 1	**Retain :** H315 H400 H410 H317 **Add :** H370	GHS07 GHS08 GHS09 Dgr	**Retain:** H315 H410 H317 **Add :** H370	-	**Add:** M = 1 M = 10	-
RAC opinion	607-232-00-7	pyridate (ISO); O-(6-chloro-3-phenylpyridazin-4-yl) S-octyl thiocarbonate	259-686-7	55512-33-9	**Retain:** Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 **Add:** Acute Tox. 4	**Retain:** H315 H317 H400 H410 **Add :** H302	**Retain:** GSH07 GSH08 GHS09 Wng	**Retain:** H315 H317 H410 **Add:** H302	**-**	**Add:** M = 1 M = 10 ATE = 500	**-**
Resulting Annex VI entry if agreed by COM	607-232-00-7	pyridate (ISO); O-(6-chloro-3-phenylpyridazin-4-yl) S-octyl thiocarbonate	259-686-7	55512-33-9	Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H315 H317 H400 H410	GSH07 GSH08 GHS09 Wng	H302 H315 H317 H410	-	M = 1 M = 10 ATE = 500	-

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

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 in background document), 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.

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).

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 (see background document).

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

Study	Clinical signs	Mortality
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.

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**.

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.

Effects observed below the guidance values for classification after repeated oral dosing of pyridate

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*		62.5 mg/kg bw/d ↓ 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*	N/A	3.6 mg/kg bw/d ↓ haemoglobin and red blood cells (females, no dose response) ↑ LDH levels (males)

	1.25 < Cat. 2 ≤ 12.5		
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	<u>Ascending dose of 5/10/30 mg/kg bw/d</u> 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**.

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

Method	Results	Reference
***In vitro* studies**		
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.

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**.

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**.

ENVIRONMENTAL HAZARD EVALUATION

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**.

ANNEXES:

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

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