Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

(RS)-1-{1-ethyl-4-[4-mesyl-3-(2-methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate

EC Number: -
CAS Number: 1101132-67-5

CLH-O-0000001412-86-268/F

Adopted

15 March 2019

15 March 2019

CLH-O-0000001412-86-268/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: **(RS)-1-{1-ethyl-4-[4-mesyl-3-(2-methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate**

EC Number: **-**

CAS Number: **1101132-67-5**

The proposal was submitted by the **United Kingdom** and received by RAC on **10 April 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom 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 **3 May 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 July 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

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

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 **15 March 2019** 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 statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	(RS)-1-{1-ethyl-4-[4-mesyl-3-(2-methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate	n/a	1101132-67-5	Carc. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H373 (eyes, kidney) H400 H410	GHS08 GHS09 Wng	H351 H373 (eyes, kidney) H410		M=10 M=100	
RAC opinion	TBD	(RS)-1-{1-ethyl-4-[4-mesyl-3-(2-methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate	n/a	1101132-67-5	Carc. 2 STOT RE 2 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H373 (eye) H361fd H400 H410	GHS08 GHS09 Wng	H351 H373 (eye) H361fd H410		M=10 M=100	
Resulting Annex VI entry if agreed by COM	TBD	(RS)-1-{1-ethyl-4-[4-mesyl-3-(2-methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate	n/a	1101132-67-5	Carc. 2 STOT RE 2 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H373 (eye) H361fd H400 H410	GHS08 GHS09 Wng	H351 H373 (eye) H361fd H410		M=10 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Tolpyralate is a new pesticide-active substance (4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor) proposed for use as a broad spectrum herbicide, effective against broad leaf weeds in maize crops. There is no existing entry in Annex VI to CLP.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Five physical hazards have been assessed by the dossier submitter (DS) and these were open for public consultation. For self-heating substances, oxidising solids and corrosive to metals the DS proposed no classification due to lack of data. For explosives and flammable solids the DS proposed no classification based the following data:

- Explosives: A negative test conducted according to A.14 (Turner, 2013a)
- Flammable solids: A test conducted according to A.10 (Turner, 2013a) giving a result "not highly flammable"

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

For self-heating substances, oxidising solids and corrosivity to metals, RAC agrees with the DS on **no classification due to lack of data** (or lack of data generated in line with CLP).

RAC also agrees on **no classification on the basis of conclusive data for flammable solids**.

RAC agrees with **no classification for explosive properties** based on the negative A.14 test but highlights the lack of the time/pressure test prescribed by the CLP regulation and not included in the A.14 testing method.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for acute toxicity based on the results of the following GLP compliant studies:

- Acute oral toxicity study in the rat conducted according to OECD TG 423 (Anonymous, 2012a): No mortality and no clinical signs of toxicity at 2000 mg/kg bw/d.
- Acute dermal toxicity study in the rat conducted according to OECD TG 402 (Anonymous, 2012b): No mortality or overt clinical signs of toxicity at 2000 mg/kg bw/d.

- Acute inhalation toxicity study in the rat conducted according to OECD TG 436, nose-only (Anonymous, 2012c): No mortality or overt clinical signs of toxicity at 2.0 mg/L (aerosol) with a MMAD of 4.0 µm (GSD 2.6). The DS explained that it was not possible to achieve a test atmosphere with appropriate characteristics at the higher concentration of 5.0 mg/L.

Comments received during public consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

As no mortalities occurred in the oral and dermal acute toxicity studies at the limit dose of 2000 mg/kg bw and no mortalities occurred in the acute inhalation toxicity study at the highest achievable concentration to produce an acceptable test atmosphere (2.0 mg/L), RAC agrees that **no classification** for acute toxicity is appropriate.

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

Summary of the Dossier Submitter's proposal

Information relating to specific target organ toxicity following single exposure is available from the acute toxicity studies via the oral, dermal and inhalation routes and from the acute neurotoxicity study (Anonymous, 2013f) in the rat. According to the DS, the clinical and behavioural observations in these studies did not show any effects indicative of specific target toxicity following single exposure. Therefore, the DS proposed no classification for STOT SE.

Comments received during public consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

In the acute neurotoxicity study conducted according to OECD TG 424 (GLP compliant; Anonymous, 2013f), male and female rats were administered a single dose of up to 2000 mg/kg bw tolpyralate and observed for 14 days. Top dose males showed significantly reduced activity 2 hours after administration of the test item. There was no comparable effect on day 8 or day 15. RAC agrees with the DS that this finding can indicate general toxicity and is not sufficient for classification.

No clinical signs of toxicity were observed in the acute toxicity studies (Anonymous, 2012a,b,c) or within the first few days of the repeated dose studies.

No human information related to STOT SE is available.

RAC agrees with the DS that the available studies do not show effects requiring classification and therefore **no classification is appropriate for STOT-SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation based on a negative *in vivo* study in the rabbit conducted according to OECD TG 404 (GLP compliant; Anonymous, 2012d).

Comments received during public consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

Tolpyralate did not elicit any signs of dermal irritation in the available *in vivo* dermal irritation study (Anonymous, 2015d). As the criteria for classification are not met, RAC agrees with the DS that **no classification for skin irritation/corrosion** is appropriate.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for serious eye damage/irritation based on a negative *in vivo* study in the rabbit according to OECD TG 405 (GLP compliant; Anonymous, 2012e).

Comments received during public consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

In the available *in vivo* eye irritation study (Anonymous, 2012e), signs of slight eye irritation were observed in the first hour following administration. All reactions had reversed by 24 hours and all scores at 24 h, 48 h and 72 h were zero. As the criteria for classification are not met, RAC agrees with the DS that **no classification for serious eye damage/irritation** is appropriate.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

There were no specific data available relating to the respiratory sensitisation potential of tolpyralate. The DS proposed no classification because of lack of data.

Comments received during public consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

In the absence of information on respiratory sensitisation, RAC agrees with the DS's proposal of **no classification due to lack of data**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of tolpyralate was investigated in a local lymph node assay (LLNA) (Anonymous, 2013b) and a guinea pig maximisation test (GPMT) (Anonymous, 2012f). Both studies were negative. The DS proposed no classification based on conclusive data.

Comments received during public consultation

One Member State Competent Authority (MSCA) commented on the choice of the highest concentration, which was 50% in both the LLNA and GPMT. As the concentration of 50% did not cause irritation, the MSCA asked why 100% had not been tested. The DS explained that it was not possible to test at a higher concentration as the test material was a solid that required moistening before application.

Assessment and comparison with the classification criteria

The LLNA and GPMT are summarised in the following table.

<table>
<tr><th colspan="3">Skin sensitisation studies</th></tr>
<tr><th>Type of study; Reference</th><th>Method</th><th>Observations</th></tr>
<tr><td>LLNA
Anonymous, 2013b</td><td>OECD TG 429
GLP
0, 10, 25, 50% w/v tolpyralate in N,N-dimethylformamide
5 females/group
Positive control: 25% α-hexylcinnamaldehyde</td><td>Negative
Stimulation indices:<table><tr><td>Treatment</td><td>SI</td></tr><tr><td>10% tolpyralate</td><td>0.5</td></tr><tr><td>25% tolpyralate</td><td>1.1</td></tr><tr><td>50% tolpyralate</td><td>1.0</td></tr><tr><td>Positive control</td><td>9.2</td></tr></table></td></tr>
<tr><td>GPMT
Anonymous, 2012f</td><td>OECD TG 406
GLP
Intradermal induction: 10% in liquid paraffin
Topical induction: 50% in acetone (suspension); dermal irritation induced by sodium lauryl sulphate (SLS) pre-treatment
Challenge: 50% in acetone
10 females/test group
5 females/control group
No concurrent positive control; the laboratory reliability check (1-chloro-2,4-dinitrobenzene) from the respective period (within 2 months of the present study) was stated to have shown an acceptable response</td><td>Negative
No skin reactions in the test group or the control group</td></tr>
</table>

RAC accepts the DS' justification for the choice of the highest concentration, *i.e.*, that the tested material, being a solid, had to be suspended in a suitable vehicle before application. The highest concentration of 50% in both studies is considered to fulfil the requirements of the respective OECD testing guidelines. RAC also notes that in the GPMT, SLS pre-treatment was applied as prescribed by the guideline for non-irritant substances.

As both studies are negative and neither of them shows any major methodological deficiency, RAC agrees with the DS that **no classification on skin sensitization** based on conclusive data is justified.

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

Summary of the Dossier Submitter's proposal

The DS proposed classification with STOT RE 2 (eyes, kidneys) based on increased incidence of various kidney effects and ocular opacity associated with keratitis in the rat studies.

The DS also discussed findings in the liver, pancreas, cerebellum, sciatic nerve and gall bladder but did not consider them sufficient for classification.

Comments received during public consultation

3 MSCAs commented on this hazard class.

2 MSCAs supported the proposed classification in Category 2 for effects on the eye and the kidney. The third MSCA, while supporting classification for eye and kidney effects, requested Category 1 because ocular effects occurred below the guidance value (GV) for STOT RE 1 in the 28-day and 2-generation rat studies. The DS replied that due to the low incidence of ocular effects below the GV for Category 1, Category 2 is more appropriate.

All three MSCAs pointed out the very large dose spacing (a 100-fold interval) in several rat studies. As a result, effects on several other organs below the GVs for classification cannot be excluded. The DS agreed that the dose spacing was very large and that effects on some organs below the GVs for classification cannot be excluded. However, they decided not to classify in the absence of evidence of an adverse effect below the GVs.

One MSCA proposed to additionally classify for effects on the thyroid based on increased incidence of follicular cell hypertrophy in the rat below the GVs for classification. The DS explained that whilst the finding is treatment-related, it does not represent a significant change affecting function or morphology and in the absence of other thyroid histopathological findings is not considered sufficient for classification.

Assessment and comparison with the classification criteria

Repeated dose toxicity of tolpyralate was investigated in rats, mice and dogs. Information from reproductive toxicity studies and from mechanistic studies is also available. The following target organs have been identified:

- Eye
- Kidney
- Liver and gall bladder
- Thyroid

- Pancreas
- Nervous system

RAC notes the 100-fold gap between dose levels in the rat studies (90-day, 1-year, 2-year), which creates considerable uncertainties in relation to STOT RE classification in cases where an effect is present at the higher dose but not at the lower dose. Interpolation, the generally recommended practice according to the Guidance on the application of the CLP criteria, would be difficult and rather speculative here and RAC would normally use it only in cases where the LOAEL is above but still reasonably close to the GV. RAC acknowledges that such large dose spacing is not in line with the OECD test guidelines, which advise against intervals greater that 10-fold.

Eye

Ocular opacity and keratitis were observed in the rat at doses below the GVs for classification; the incidences of keratitis in the oral studies are listed in the table below (no ocular effects were observed in the dermal study). No effects on the eye were observed in the mouse studies up to high dose levels (≈ 1100 mg/kg bw/d in a 90-day study, ≈ 700 mg/kg bw/d in an 18-month study). In the dog, there was only one incidence of keratitis and ocular opacity at 670 mg/kg bw/d in a 90-day study, which is above the GV for classification.

Incidence of keratitis in the rat oral repeated dose studies				
Type of study; Reference	**Dose (mg/kg bw/d) m/f**	**Incidence of keratitis**		**GV for Cat. 2 / Cat. 1 (mg/kg bw/d)**
		males	**females**	
28-day, dietary Anonymous, 2013g	46/47	4/6	6/6	300 / 30
	4.5/5.0	2/6	0/6	
	controls	0/6	0/6	
90-day, dietary Anonymous, 2013h	133/159	7/10	10/10	100 / 10
	1.3/1.6	0/10	0/10	
	controls	0/10	0/10	
90-day, dietary ISK, study no. AN-2850	37/43	3/5	3/5	100 / 10
	3.6/4.2	0/5	0/5	
	controls	0/5	0/5	
2-year, dietary Anonymous, 2015f	84/108	51/51	51/51	12.5 / 1.25
	0.77/1.0	4/51	1/51	
	controls	4/51	0/51	
Two-generation, dietary Anonymous, 2015e	55/59: P 55/59: F1	21/24 24/24	23/24 24/24	≈ 70 / 7
	2.7/3.0: P 2.7/3.0: F1	1/24 1/24	0/24 0/24	
	controls: P controls: F1	0/24 0/24	0/24 0/24	

A high incidence of keratitis below or around the GVs for classification in Category 2 was observed in several studies; two of them (the 28-day study and the 2-generation study) reported ocular

effects even below the GVs for Category 1. However, the incidence below the GVs for Category 1 was relatively low and rats are generally more sensitive than humans to the ocular effects from exposure to HPPD inhibitors (see the carcinogenicity section). Therefore, RAC agrees with the DS that classification in Category 2 for effects on the eyes is appropriate.

Kidney

Increased deposition of hyaline droplets in proximal tubular cells was observed in male rats below the GVs for classification in 28-day and 90-day studies. In addition, a range of kidney abnormalities were reported in the 2-generation study, especially in the animals exposed to the test substance *in utero* and as juveniles. Kidney effects in the oral rat studies are summarised in the table below (no kidney effects were seen in the dermal study). Kidney effects were reported also in the dog and mouse studies, but occurred only at doses above the GVs for classification in these species (e.g., glomerulonephritis in female mice at 732 mg/kg bw/d in an 18-month study).

Kidney effects in the rat oral repeated dose studies		
Type of study; Reference	**Effects on the kidneys**	**GV for Cat. 2 / Cat. 1 (mg/kg bw/d)**
28-day, dietary Anonymous, 2013g	46/47 mg/kg bw/d m/f: increased deposition of hyaline droplets in proximal tubular cells (m 3/6 vs 0/6; f not examined) 4.5/5.0 mg/kg bw/d m/f: hyaline droplet deposition (m 1/6 vs 0/6; f not examined)	300 / 30
90-day, dietary Anonymous, 2013h	133/159 mg/kg bw/d m/f: ↑ kidney weight (m relative by 10%), hyaline droplet deposition (m 2/10 vs 0/10) 1.3/1.6 mg/kg bw/d m/f: hyaline droplet deposition (m 2/10 vs 0/10) 0.32/0.38 mg/kg bw/d m/f: no kidney effects	100 / 10
90-day, dietary ISK, study no. AN-2850	37/43 mg/kg bw/d: hyaline droplet deposition (incidence not reported) and increased positive response to α2u-globulin antibody in males 3.6/4.2 mg/kg bw/d: hyaline droplet deposition (incidence not reported) and increased positive response to α2u-globulin antibody in males	100 / 10
1-year, dietary Anonymous, 2015i	97/126 mg/kg bw/d m/f: ↑ kidney weight (m relative by 17%), basophilic change of the renal tubule (m 8/21 vs 2/21) 0.92/1.2 mg/kg bw/d m/f: no kidney effects	25 / 2.5
2-year, dietary Anonymous, 2015f	84/108 mg/kg bw/d m/f: ↑ kidney weight (m relative by 23%) 0.77/1.0 mg/kg bw/d m/f: no kidney effects	12.5 / 1.25
Two-generation, dietary Anonymous, 2015e	55/59 mg/kg bw/d m/f – P generation: ↑ kidney weight (m relative by 13%) 55/59 mg/kg bw/d m/f – F1 generation: ↑ kidney weight (m/f relative by 17%/7%), basophilic change of renal tubule (m 10/24 vs 3/24; f 4/24 vs 0/24), calcification (m 7/24 vs 0/24; f 8/24 vs 3/24), cortical cysts (m 6/24 vs 0/24), hyaline droplet deposition (m 5/24 vs 2/24), nephropathy (m 11/24 vs 0/24; f 4/24 vs 0/24), dilatation of renal pelvis (f 5/24 vs 0/24) ≤ 2.7/3.0 mg/kg bw/d m/f: no kidney effects (very slight, statistically non-significant increase in the incidence of dilated renal pelvis in F1)	≈ 70 / 7

Association of hyaline droplets with α2u-globulin has been demonstrated using immunohistochemical staining in one of the 90-day studies (ISK, study no. AN-2850). Alpha2u-

globulin nephropathy is a mechanism specific to male rats. Therefore, RAC does not consider the increased incidence of hyaline droplet deposition in male rats to support classification.

However, the relationship to α2u-globulin nephropathy of the kidney effects observed in the F1 and F2 generations of the 2-generation study is unclear and consequently, human relevance cannot be excluded. These findings are discussed in the reproductive toxicity section as they occur due to or are aggravated by *in utero* and early postnatal exposure. They are not considered for a STOT RE classification and are used to support classification for developmental toxicity instead.

Liver and gall bladder

Increased liver weight without associated histopathological findings was observed below or around the GVs for Category 2 in two rat studies (28-day oral, 90-day). This effect is not considered sufficiently adverse to warrant classification. No liver effects were observed below the GVs for classification in the mouse and dog studies.

A significantly increased incidence of gall bladder calculi was observed in the 18-month mouse study from ≈ 7 mg/kg bw/d (70 ppm), the lowest dose tested, without any other findings in the gall bladder or the liver at the low dose. Liver hypertrophy was observed from 700 ppm. Although the increased incidence of gall bladder calculi is likely to be a treatment-related effect, it is not considered sufficiently adverse to warrant classification.

Incidence of gall bladder calculi in the 18-month mouse study				
Dose (ppm)	**0**	**70**	**700**	**7000**
Dose (mg/kg bw/d) (m/f)	0	7.4/7.3	79/73	793/732
No. of animals examined (m/f)	52/52	52/52	51/52	52/52
Incidence of gall bladder calculi – males	6	14*	18**	16*
Incidence of gall bladder calculi – females	4	11*	17**	20**

* $p \leq 0.05$; ** $p \leq 0.01$ (Fisher's exact test)

Thyroid

Thyroid effects in the rat oral repeated dose studies with tolpyralate are summarised in the table below. A relatively high incidence of follicular cell hypertrophy was observed especially in males in the 28-day and 90-day studies; the effects were far less prominent in the long-term studies. No effects on the thyroid were observed at doses below or around the GVs in the mouse and dog studies or in the rat dermal study.

Effects on the thyroid in the rat oral repeated dose studies		
Type of study; Reference	**Effects on the thyroid**	**GV for Cat. 2 / Cat. 1 (mg/kg bw/d)**
28-day, dietary Anonymous, 2013g	46/47 mg/kg bw/d m/f: follicular cell hypertrophy (m 6/6 vs 0/6) 4.5/5.0 mg/kg bw/d m/f: follicular cell hypertrophy (m 2/6 vs 0/6)	300 / 30
90-day, dietary Anonymous, 2013h	133/159 mg/kg bw/d m/f: follicular cell hypertrophy (m 9/10 vs 0/10; f 4/10 vs 0/10) 1.3/1.6 mg/kg bw/d m/f: no thyroid effects	100 / 10

1-year, dietary Anonymous, 2015i	97/126 mg/kg bw/d m/f: follicular cell hypertrophy (m 3/21 vs 0/21; f 2/21 vs 0/21) 0.92/1.2 mg/kg bw/d m/f: no thyroid effects	25 / 2.5
2-year, dietary Anonymous, 2015f	84/108 mg/kg bw/d m/f: colloid degeneration (f 16/51 vs 0/51) 0.77/1.0 mg/kg bw/d m/f: no thyroid effects	12.5 / 1.25
Two-generation, dietary Anonymous, 2015e	55/59 mg/kg bw/d m/f: no thyroid effects	≈ 70 / 7

Additionally, the DAR summarised a mechanistic study investigating the effects on the thyroid in the rat. Tolpyralate was administered in the diet at 0 or 20000 ppm (1930 mg/kg bw/d) to 6 male rats per group for 14 days. Serum TSH was significantly increased by 16% relative to control animals, with an associated but non-significant decrease in circulating T4 (by 18%). Total liver cytochrome P450 content was significantly increased, as was UDPGT activity towards both 4-nitrophenol and 4-hydroxybiphenyl (by ≈ 60%). This indicates that the thyroid effects might be due to UDPGT induction. However, alternative modes of actions have not been investigated.

The follicular cell hypertrophy is considered a treatment-related effect and although it is an effect of an adaptive rather than adverse nature, it may be associated with reduced T4 levels, which is an adverse effect. However, the T4 reduction was less than 20% at a dose as high as 1930 mg/kg bw/d in a 2-week study, and the long-term studies show that the hypertrophy did not follow Haber's rule. In fact, hypertrophy diminished with increasing exposure duration and the lack of clear increase in thyroid hyperplasia or neoplasia in the 2-year study indicates that it was indeed weak and transient. Therefore, RAC agrees with the DS that classification for effects on the thyroid is not warranted.

Pancreas

Effects on the pancreas in the rat repeated dose studies with tolpyralate are summarised in the table below. No effects on the pancreas were observed in the mouse and dog studies.

Effects on the pancreas in rat repeated dose studies		
Type of study; Reference	**Effects on the pancreas**	**GV for Cat. 2 / Cat. 1 (mg/kg bw/d)**
28-day, dietary Anonymous, 2013g	447/496 mg/kg bw/d m/f: single acinar cell necrosis (m 3/6 vs 0/6) 46/47 mg/kg bw/d m/f: no pancreatic effects	300 / 30
90-day, dietary Anonymous, 2013h	133/159 mg/kg bw/d m/f: single acinar cell necrosis (m 3/10 vs 1/10; f 1/10 vs 0/10) 1.3/1.6 mg/kg bw/d m/f: no pancreatic effects	100 / 10
1-year, dietary Anonymous, 2015i	97/126 mg/kg bw/d m/f: acinar cell fibrosis (m 11/21 vs 2/21), acinar cell necrosis (m 7/21 vs 1/21) 0.92/1.2 mg/kg bw/d m/f: acinar cell fibrosis (m 7/21 vs 2/21)	25 / 2.5

2-year, dietary Anonymous, 2015f	84/108 mg/kg bw/d m/f: acinar cell fibrosis (m 46/51 vs 14/51; f 23/51 vs 10/51), fat infiltration (m 26/51 vs 3/51) 0.77/1.0 mg/kg bw/d m/f: no pancreatic effects	12.5 / 1.25
Two-generation, dietary Anonymous, 2015e	55/59 mg/kg bw/d m/f: no pancreatic effects	≈ 70 / 7
28-day, dermal Anonymous, 2013l	1000 mg/kg bw/d: acinar cell apoptosis and inflammatory cell infiltration (m 3/10 vs 1/10; f 1/6 vs 0/10)	600 / 60

An increased incidence of single cell acinar cell necrosis and of acinar cell fibrosis was reported in several studies. Although this is considered a treatment-related adverse effect, the incidence was increased mainly at doses above the GVs for classification and where the increase occurred marginally above the GVs, the incidence was relatively low. The only dose level with pancreatic effects below the GVs is 20 ppm (approx. 1 mg/kg bw/d) in the 1-year rat study; however, this increase was not statistically significant and was not replicated at the same dose level in the 2-year study. Taking a weight of evidence approach, classification for effects on the pancreas is not justified.

Nervous system

Increased incidence of vacuolation of the cerebellum was observed at 2000 ppm (approx. 100 mg/kg bw/d) in the 1-year and 2-year studies in the rat. Additionally, increased incidence of sciatic nerve degeneration was observed in female rats of the 2-year study at this dose. No such effect was observed at 2000 ppm in a 90-day rat study. As the effects appeared at doses above the GV for classification, classification for effects on the nervous system is not warranted.

Conclusion

RAC agrees with the DS on classification in Category 2 based on the eye effects (keratitis, opacity) in the rat.

As to the kidney effects, the hyaline droplet deposition in male rats treated with tolpyralate has been shown to be associated with accumulation of α2u-globulin and is not considered relevant to humans. The kidney findings in F1 and F2 generations of the 2-generation study are used to support classification for developmental toxicity.

Thus, RAC concludes that tolpyralate **should be classified as STOT RE 2; H373 (eye)**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The genotoxic potential of tolpyralate has been investigated both *in vitro* and *in vivo*.

In *in vitro* studies, an Ames test and an *in vitro* chromosome aberration assay were negative while a mouse lymphoma assay was positive.

The *in vivo* dataset consists of a mouse micronucleus assay and two comet assays in the rat. All three *in vivo* studies were negative.

According to the DS, the negative findings from three *in vivo* studies are sufficient to dismiss concerns relating to genotoxic potential arising from the positive *in vitro* gene mutation assay. Therefore, the DS proposed no classification based on conclusive data.

Comments received during public consultation

One MSCA questioned whether the *in vivo* tests conducted (one micronucleus and two comet assays) were an appropriate follow-up to the positive mouse lymphoma assay.

Assessment and comparison with the classification criteria

The available genotoxicity studies are summarised in the following table.

Genotoxicity studies		
Type of study; Reference	**Method**	**Observations**
In vitro		
Ames test Wada, 2012	OECD TG 471 GLP *S. typhimurium* TA98, TA100, TA1535, TA 1537; *E. coli* WP2 uvrA Pre-incubation method Up to 5000 µg/plate	Negative ±S9 No cytotoxicity up to the limit concentration
Chromosomal aberration test *in vitro* Matsumoto, 2012a	OECD TG 473 GLP Chinese hamster lung cells (CHL/IU) 6-hour treatment ±S9 up to 1200 µg/mL 24-hour treatment ±S9 up to 300 µg/mL 48-hour treatment ±S9 up to 200 µg/mL	Negative ±S9 Selection of top concentrations based on cytotoxicity (growth inhibition of at least 50%); in the short-term test +S9 a reduction of 50% not achieved but precipitation observed
Mouse lymphoma assay Matsumoto, 2012b	OECD TG 476 GLP Mouse lymphoma cells L5178Y $TK^{+/-}$ 3-hour treatment ±S9 up to 1250 µg/mL 24-hour treatment –S9 up to 1250 µg/mL	Positive ±S9 A slight shift towards small colonies with increasing concentrations
In vivo		
Micronucleus test in the bone marrow Anonymous, 2012g	OECD TG 474 GLP Mouse 5 males/group Sampling at 24 h: single oral (gavage) dose of 0, 500, 1000 or 2000 mg/kg bw Sampling at 48 h: single oral (gavage) dose of 2000 mg/kg bw	Negative Positive control worked properly No increase in PCE to NCE ratio No clinical signs of toxicity
Comet assay Anonymous, 2014c	OECD TG 489 GLP Rat 6 males/group Tissues: liver and stomach	Negative Positive control responded appropriately

	Two oral (gavage) doses, at 0 h and at 21 h; sacrifice at 24 h Doses: 0, 500, 1000, 2000 mg/kg bw	
Comet assay Anonymous, 2015a	OECD TG 489 GLP Rat 5 males/group Tissues: liver, stomach and thyroid Two oral (gavage) doses, at 0 h and at 21 h; sacrifice at 24 h Doses: 0, 500, 1000, 2000 mg/kg bw	Negative Positive control responded appropriately

A set of one *in vivo* micronucleus assay and two *in vivo* comet assays (from two different laboratories) is in principle considered as an appropriate follow-up to a positive mouse lymphoma assay.

No evidence of bone marrow exposure and no evidence of systemic toxicity were observed in the mouse micronucleus test itself. In an ADME study in rats (Anonymous, 2013a) the radioactivity in the bone marrow at a T_{max} of 2 h after a single dose of 200 mg/kg bw tolpyralate was approx. 2–3 µg equiv/g compared to 14–18 µg equiv/g in the plasma and 25–32 µg equiv/g in the liver. This is considered as evidence of bone marrow exposure in the rat. Although bone marrow exposure in the mouse is likely, direct evidence is not available.

The first comet assay in the rat (Anonymous, 2014c) utilised the stomach, representing the site of first contact, and the liver, a site with high systemic perfusion and high exposure to the test substance as confirmed by ADME studies (Anonymous, 2013a; Anonymous, 2014a). The second assay (Anonymous, 2015a) also examined the stomach and the liver and in addition examined the thyroid in order to investigate genotoxic potential specific to this tissue. The tissues were sampled at an appropriate time (3 h after the last exposure; T_{max} is 0.5–2 h) and the positive controls responded appropriately. Both studies were negative in all examined tissues.

RAC agrees with the DS that the three available *in vivo* studies collectively remove the concern raised by the positive mouse lymphoma assay and that **no classification for germ cell mutagenicity** is therefore warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of tolpyralate was investigated in two carcinogenicity studies, one in the rat and one in the mouse. The only treatment-related neoplastic finding was malignant squamous cell carcinoma in the eye of male rats.

The applicant proposed a mode of action for these tumours involving the following key stages:

1. Inhibition of HPPD; reduced catabolism of tyrosine
2. Induction and maintenance of high plasma and eye levels of tyrosine
3. Sustained damage to the corneal epithelium
4. Regenerative hyperplasia, increased DNA synthesis and cell replication, leading to tumour formation

The DS agreed that tolpyralate produces a significant increase in plasma tyrosine in rats (as demonstrated by the studies Anonymous, 2013o; Anonymous, 2016a; Anonymous, 2016b) and

considered it plausible that the eye effects are related to the increase in plasma tyrosine concentrations.

Regarding the hypothesis that the ocular tumours may have been caused by persistent keratitis, the DS noted that eye tumours had been reported in rat studies with some other HPPD inhibitors (e.g., tembotrione) but not with others (e.g., sulcotrione and mesotrione) despite a high level of keratitis with the latter ones. In addition, the tumours were observed only in males although there were comparable levels of keratitis in females. Although the DS considered the proposed mode of action (MoA) plausible, they did not deem the available information sufficient to conclude that the corneal tumours were definitively induced as a result of tissue damage and regenerative hyperplasia from elevated tyrosine levels.

As to human relevance, the DS acknowledged the particularly high sensitivity of the rat to the effects of HPPD inhibition but, on the other hand, pointed out that NTBC is capable of producing significant increases of plasma tyrosine in healthy human subjects (up to 1100 µmol/L) and that ocular effects were observed patients with chronically increased tyrosine concentrations due to NTBC intake.

Overall, the DS considered that there is limited evidence for the carcinogenicity of tolpyralate in rats, thus proposing classification in Category 2.

Comments received during public consultation

Three MSCAs and 1 industry association provided comments during the public consultation.

One MSCA supported the proposed classification with Carc. 2.

The other two MSCAs requested discussion of Category 1B. The following arguments were raised in support of a more stringent classification:

- The tumours are clearly treatment-related.
- According to the definition of "sufficient" evidence (CLP, Annex I, 3.6.2.2.3), a single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset. One of the MSCAs considered the tumours to occur to an unusual degree with regard to incidence (rare tumours), unusual site and unusual tumour type (not a spontaneous tumour type).
- The tumours are relevant to humans (relevant MoA; the type of tumour also occurs in humans).
- Genotoxicity may be an additional MoA. A positive mouse lymphoma assay was followed up by a comet assay which however did not investigate the eye or bone marrow. The DS replied that the available comet assay was sufficiently comprehensive as it included tissues from the site of first contact (stomach), point of metabolism (liver) and tissue which is known to be a target for the test item (thyroid).

One of the MSCAs also recommended discussing the limitations of the data presented on hepatic TAT enzyme activity, in particular with regard to representativeness and allometric scaling.

The industry association proposed no classification based on the following arguments:

- The differences between the severity of ocular lesions between male and female rats as well as between rats and mice can be explained by interspecies differences in the activity of TAT (quantified as the maximum rate, V_{max}). Human TAT activity is similar to that of mice, a species in which ocular effects from HPPD inhibitors have not been seen, and considerably higher than the TAT activity in rats.

- In addition, mice and humans, unlike rats, are able to use an alternative pathway for tyrosine catabolism via formation of 4-HPLA if HPPD is inhibited.
- Humans experiencing ocular problems would seek medical help before keratitis can develop into ocular tumours.

In response to the comments, the DS maintained that Category 2 is more appropriate than 1B due to the lack of genotoxicity and due to carcinogenic responses having been observed only in a single sex and a single species. At the same time, they did not consider no classification appropriate for reasons explained in the CLH report.

Assessment and comparison with the classification criteria

Rat carcinogenicity study (Anonymous, 2015b)

In the rat carcinogenicity study, tolpyralate was administered via diet for 2 years at dose levels up to 10000 ppm, which corresponded to 426 mg/kg bw/d in males and 554 mg/kg bw/d in females. Terminal body weights were reduced by 18% and 7% in top dose males and females, respectively, and ocular opacity was present in all animals at 2000 and 10000 ppm (vs 3/51 in the controls of both sexes). Survival was not affected.

The only treatment-related neoplastic finding was malignant squamous cell carcinoma in the eye of males. The incidences are shown in the table below. This type of tumour was not encountered in the historical control database.

Dose level (ppm)	**Males**					**Females**				
	0	**5**	**20**	**2000**	**10000**	**0**	**5**	**20**	**2000**	**10000**
No. of animals examined	51	51	51	51	51	51	51	51	51	51
Malignant squamous cell carcinoma in the eye	0	0	0	3	5*	0	0	0	0	0

* statistically significant difference from control, $p \leq 0.05$ (Fisher's test)

RAC notes that the occurrence of the tumours in males only is consistent with a higher severity of keratitis in this sex. Keratitis graded as severe was present in 16/51 males and 0/51 females at 10000 ppm and in 6/51 males and 1/51 females at 2000 ppm.

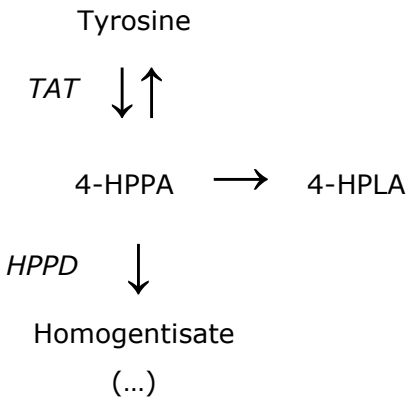
Mouse carcinogenicity study (Anonymous, 2015c)

In the mouse carcinogenicity study, tolpyralate was administered via the diet for 18 months at dose levels up to 7000 ppm, which corresponded to 793 mg/kg bw/d in males and 732 mg/kg bw/d in females. Body weight was transiently reduced in top dose males and increased in females. Survival was not affected and there were no ocular effects. Tolpyralate did not produce a carcinogenic response in this study.

Mode of action and human relevance of the rat ocular tumours

In mammals, HPPD is involved in the catabolism of tyrosine. Inhibition of HPPD leads to a build-up of its substrate 4-hydroxyphenylpyruvate (4-HPPA), which is excreted in urine or converted back to tyrosine. Like other important amino acids, tyrosine is not excreted in the kidney. Thus, HPPD inhibition leads to an increase in plasma tyrosine (tyrosinaemia) and increased urinary

excretion of 4-HPPA and its derivatives (including 4-hydroxyphenyllactate, 4-HPLA), collectively known as phenolic acids. The first two steps of the catabolic pathway of tyrosine are outlined below (TAT = tyrosine aminotransferase).



HPPD inhibitors may cause ocular effects (keratitis) in mammals. The relationship between tyrosinaemia, HPPD inhibition and ocular effects has been studied extensively with NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; also known as nitisinone), a potent HPPD inhibitor used therapeutically in humans with tyrosinaemia type I. Lock *et al.* (1998; 2000; 2006) reported that out of the five animal species tested (rat, mouse, dog, rabbit, rhesus monkey), only rats and dogs were susceptible to ocular effects from NTBC exposure and the corneal lesions in rats administered NTBC were similar to those observed in rats administered tyrosine in a low-protein diet. Interestingly, the interspecies differences in susceptibility to the eye effects only partly correlated with the differences in the extent of tyrosinaemia. Ocular problems have been reported in humans suffering from tyrosinaemia type II and in some patients treated with NTBC.

RAC considers the MoA proposed by the applicant plausible. The absence of ocular tumours in female rats treated with tolpyralate is consistent with a less severe keratitis in this sex.

The applicant ascribed the differences in susceptibility to tyrosinaemia and ocular effects to differences in TAT activity. However, the data obtained with NTBC in several species (Lock *et al.*, 2006) shows that TAT activity alone cannot explain the interspecies differences in susceptibility to tyrosinaemia and ocular effects from exposure to HPPD inhibitors. In addition, the available information on TAT activity does not allow for a robust comparison of the human and animal data (discussed in more detail under 'Supplemental information' in the background document).

An additional argument raised by Industry in support of no classification was the existence of an alternative tyrosine catabolism pathway producing 4-hydroxyphenyl lactic acid (4-HPLA) from 4-HPPA in mice and humans but not in rats, with reference to an *in vitro* study with tembotrione (US EPA, 2007) and to an *in vitro* study with tolpyralate (Yokoyama, 2016). However, neither of the studies indicates that this pathway would not be operative in the rat (for details see 'Supplementary information' in the background document).

On the other hand, tyrosinaemia type III patients, whose HPPD is deficient due to a genetic defect, and tyrosinaemia type I patients treated with NTBC do only show plasma tyrosine levels up to ca 1300 µmol/L compared to ca 2500 µmol/L in rats treated with NTBC (Gissen *et al.*, 2003; Ellaway *et al.*, 2001; Lock *et al.*, 2006). None of the 15 patients with tyrosinaemia type III described in literature so far (Ellaway *et al.*, 2001; Najafi *et al.*, 2018; Szymanska *et al.*, 2015) and less than 10% of the tyrosinaemia type I patients treated with NTBC developed ocular effects. Rhesus monkeys did not develop ocular lesions at doses of NTBC that caused a high incidence of ocular opacity in the rat (Lock *et al.*, 2006). This evidence suggests a lower sensitivity of humans

compared to rats to the effects of HPPD inhibitors. Still, ocular problems do occur in some patients treated with RAC understands Industries claim that the occurrence of ocular tumours from tolpyralate exposure in humans is not very likely in reality, as tumour formation would be preceded by persistent ocular problems that would lead the individual to seek medical help before tumours could develop. Although this reduces the concern, such risk-based considerations are not relevant to hazard classification.

Conclusion on classification

The ocular tumours occurred only in one species (rat) and one sex (males) and the substance is not genotoxic. In addition, tumour formation in the rat is associated with severe keratitis and humans are generally less sensitive to the effects of HPPD inhibitors. Therefore, RAC agrees with the DS that there is limited evidence of carcinogenicity for tolpyralate and classification with **Carc. 2; H351** is appropriate.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for fertility and sexual development based on the results of a 2-generation study in the rat.

No classification was also proposed for developmental toxicity. Although an increased incidence of skeletal variations was observed in both the rat and the rabbit prenatal developmental toxicity (PNDT) studies, these were considered to be of minimal toxicological significance.

The two-generation study showed some effects potentially relevant for classification for adverse effects on or via lactation (reduced pup viability on PND 0, reduced pup weights, ocular and kidney lesions). However, as these could not be unequivocally attributed to intake of the substance via milk, the DS proposed no classification for this endpoint.

Comments received during public consultation

Two MSCAs and 1 Industry association commented on reproductive toxicity.

The Industry association supported the DS proposal of no classification. They emphasised that the toxicological characteristics of tolpyralate are essentially the same as those of NTBC, including ocular opacity, kidney lesions and foetal skeletal variations. Industry argued that all these effects were secondary to tyrosinaemia, which is less likely to occur in humans than in rats. In their position paper, Industry also summarised a negative developmental toxicity study in the rabbit employing a dose level of 1000 mg/kg bw/d. The DS examined the study report and confirmed that this study was negative.

The two MSCAs proposed classification for developmental effects, one in Category 2 and the other in Category 1B. The following findings were mentioned in support of classification:

- Increased incidence of skeletal variations in the rat and rabbit PNDT studies
- Reduced foetal weight in the rat PNDT study
- Low incidence of malformations (left umbilical artery, right subclavian from aortic arch, split cartilage of thoracic centrum) in the treated groups of the rat PNDT study
- More pronounced kidney effects in the F1 generation compared to the P generation, which indicates increased sensitivity of foetuses and juveniles compared to adults
- Increased number of dead pups on lactation day 0 in the 2-generation study

In their response the DS emphasised the following:

- The observed skeletal variations are of minimal toxicological significance (referring to CLP, Annex I, 3.7.2.3.3)
- The small reduction in foetal body weight (by 6.1%) in the rat PNDT study is secondary to maternal toxicity and does not justify classification
- The incidence of the malformations in the rat PNDT study was low and within the historical control data provided by the applicant
- The presence of kidney findings in F1 adults and not in P adults could indicate increased susceptibility during development. However, the effects in the kidney were not considered developmental effects and were covered by the proposed STOT RE classification
- The increased pup death occurred inconsistently between the two generations. In the F1 generation, the dead pups were associated with a small number of deaths (1-3) across 8 litters. In the F2 generation, the increased number of deaths was predominantly associated with the loss of 9 pups from a single litter. Therefore, in the F2 the effect was not considered treatment-related by the DS. As the effect on pup death in the F1 was small and not confirmed in the F2, it was not considered related to treatment overall.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

The two-generation study and the one-generation range-finding study are summarised in the following table.

Generational studies		
Type of study; Reference	**Method**	**Observations**
1-generation reproductive range-finding, dietary, rat Anonymous, 2015d	Non-guideline GLP Doses: 0, 5, 20, 200, 2000, 20000 ppm; corresponding to 0, 0.26/0.50, 1.0/1.9, 11/20, 107/193, 1090/1940 mg/kg bw/d (m/f) 8 parental animals/sex/dose	<u>Parental findings</u> 20000 ppm (1090/1940 mg/kg bw/d): • ↓ bw gain (by 20%/23% m/f) • ↓ food consumption in f during lactation (by up to 20%) • ↑ liver weight (relative by 19%/15% m/f) • ↑ kidney weight (relative by 12%/19% m/f) • Ocular opacity (m 2/8, f 8/8) 2000 ppm (107/193 mg/kg bw/d): • ↓ bw gain (m) • ↑ liver and kidney weight • Ocular opacity 200 ppm (11/20 mg/kg bw/d): • ↓ bw gain (m) • ↑ liver weight • Ocular opacity (low incidence) ≤ 20 ppm (1.0/1.9 mg/kg bw/d): no adverse effects <u>Reproductive findings</u> No treatment related effects

		<u>Offspring findings</u> 20000 ppm: • ↑ pup mortality PND 1-4 (80% viable pups vs 99% in the controls) • ↓ pup weight (by up to ≈ 20%) throughout the lactation period • Renal abnormalities on PND 4 and 26 (pelvic dilatation, kidney cysts, kidney atrophy, white material in the urinary bladder) • Ocular opacity on PND 26 (19% vs 0% in the controls) 2000 ppm: • ↑ pup mortality PND 1-4 (90% viable pups vs 99% in the controls) • ↓ pup weight (by ≈ 10%, not stat. sign.) • Renal abnormalities on PND 4 and 26 (pelvic dilatation, kidney cysts, kidney atrophy) 200 ppm: • Renal abnormalities on PND 26 (kidney cysts, pelvic dilatation) ≤ 20 ppm: no adverse effects
2-generation reproductive, dietary, rat Anonymous, 2015e	OECD TG 416 GLP Doses: 0, 5, 50, 1000 ppm; corresponding to 0, 0.27/0.29, 2.7/3.0, 55/59 mg/kg bw/d (m/f) 24 parental animals/sex/dose	<u>Parental findings</u> 1000 ppm (55/59 mg/kg bw/d) – P generation: • ↑ liver and kidney (m) weight • Ocular opacity and keratitis (high incidence) 1000 ppm – F1 generation: • Mortality f 2/24 during lactation • ↓ bw and bw gain (m ↓ terminal bw by 10%) • ↑ liver weight • Macroscopic and histopathological kidney findings (nephropathy, calcification, basophilic change of renal tubules, cortical cysts) • Ocular opacity and keratitis (high incidence) 50 ppm (2.7/3.0 mg/kg bw/d) – P generation: • ↑ liver weight • Keratitis (single animal) 50 ppm – F1 generation: • Keratitis (single animal) 5 ppm (0.27/0.29 mg/kg bw/d): no adverse effects <u>Reproductive findings</u> 1000 ppm: • ↑ duration of gestation in F1 (22.7 days vs 22.2 days in controls, stat. sign.) • ↓ no. of pups per litter (10.9 vs 12.7, not stat. sign.) and no. of implantation sites (11.9 vs 13.6, not stat. sign.) in F2

		≤ 50 ppm: no adverse effects <u>Offspring findings</u> 1000 ppm – F1 generation: • ↓ pup weight (by ≈ 10% PND 14 and 21) • ↓ pup viability index (P 96.3 vs 99.4, stat. sign.; F1 90.6 vs 98.9, not stat. sign.); ↑ no. of pups found dead on PND 0 • Gross kidney findings on PND 4 and in weanlings (cysts, pelvic dilation, white material in the pelvic space) • Eye opacity in weanlings (1.9%) • Delayed preputial separation (45.7 vs 41.7, stat. sign.) and vaginal opening (35.4 vs 31.4, stat. sign.) 1000 ppm – F2 generation: • ↓ pup weight (by ≈ 7% PND 14 and 21) • Gross kidney findings on PND 4 and in weanlings (cysts, pelvic dilation, white material in pelvic space) • Eye opacity in weanlings (5.6%) ≤ 50 ppm: no adverse effects

These two studies showed no effects on fertility and sexual function except for a reduction in the number of implantation sites and delayed puberty onset. Both effects are discussed below.

<u>Reduced number of implantation sites and reduced litter size</u>

A reduction in the mean number of implantation sites (by 1.7) and a corresponding reduction in mean litter size (1.8) were observed at the top dose in the F1/F2 generation, relative to controls. The decreases were not statistically significant. The finding is difficult to interpret in the absence of HCD. RAC notes that no effect on these two parameters was observed in the dose-range finding study at a 2-fold higher dose and only a slight reduction (by 0.9) at a 20-fold higher dose (albeit with a lower number of animals). Overall, RAC does not consider that the reduced number of implantation sites supports classification.

<u>Delayed sexual maturation</u>

A statistically significant delay in preputial separation (PS, by 4 days) and vaginal opening (VO, by 4 days) was observed at the top dose in the 2-generation study. Although the delayed sexual maturation was associated with reduced body weight (bw on PND 21 was reduced by 10/11% in m/f and there was no significant difference in bw on the day of PS/VO), RAC is of the opinion that a delay in puberty onset by 4 days cannot be fully explained by the observed body weight reductions. As other MoAs cannot be excluded, the effect has to be considered for classification.

Sexual maturation in the 2-generation study				
Dose (ppm)	**0**	**5**	**50**	**1000**
Dose (mg/kg bw/d) m/f	0	0.27/0.29	2.7/3.0	55/59
Preputial separation				
Day of preputial separation	41.7	41.4	42.9	45.7**

Bw on the day of prep. separation (g)	225	218	226	223
Bw on PND 21 (g)	64.9	64.8	64.6	58.1**
Vaginal opening				
Day of vaginal opening	31.4	31.9	31.8	35.4**
Bw on the day of v. opening (g)	116	121	118	126
Bw on PND 21 (g)	63.6	63.4	62.3	56.3**

* Significantly different from control, $p \leq 0.05$; ** Significantly different from control, $p \leq 0.01$

Conclusion on classification

Delayed sexual maturation by 4 days in both sexes was observed at the top dose in the 2-generation study in association with some body weight reduction. Although there were no other effects related to fertility (e.g., no effect on sperm parameters or on the reproductive outcome), RAC considers delayed puberty onset as an adverse effect on its own (cf. CLP, Annex I, 3.7.1.3) and proposes classification in **Category 2** for adverse effects on sexual function and fertility.

Adverse effects on development

The developmental toxicity of tolpyralate was investigated in two standard PNDT studies, one in the rat and one in the rabbit. A follow-up study to the rat PNDT study tested the hypothesis that one of the observed malformations (misshapen urethral orifice) was of genetic origin. In addition, one non-standard developmental toxicity study in the rat is available, investigating developmental consequences of hypertyrosinaemia. All studies are summarised in the following table.

Developmental toxicity studies		
Type of study; Reference	**Method**	**Observations**
Rat		
PNDT study, gavage Anonymous, 2013c	OECD TG 414 GLP Doses: 0, 1, 10, 500 mg/kg bw/d Dosing GD 6-19 23-24 females/group	Maternal toxicity 500 mg/kg bw/d: • ↓ food consumption until GD 15 (by 6-20%); no effect on corrected terminal bw ≤ 10 mg/kg bw/d: no adverse effects Developmental toxicity 500 mg/kg bw/d: • ↓ foetal weight (by 6%) • ↑ incidence of skeletal variations (discontinuous rib cartilage, supernumerary rib) • Misshapen urethral orifice (3 foetuses in 1 litter) ≤ 10 mg/kg bw/d: no adverse effects
Follow-up to the PNDT study,	Non-guideline Non-GLP	F1 offspring 26 males, 27 females

testing if the misshapen urethral orifice is of genetic origin Anonymous, 2013e	1 male (suspected of a recessive mutation) was mated with 4 females of a different strain; then the same male was mated with 4 F1 females, producing N2 offspring F1 and N2 offspring examined for external genital anomalies	No external genital abnormalities <u>F2 offspring</u> 58 males, 38 females 7 female foetuses from 5 litters had the same misshapen genital orifice observed in the tolpyralate-treated group in the PNDT study Anonymous (2013c); this agrees with the 1 : 3 hypothesis for autosomal recessive inheritance
Developmental toxicity study, investigations into developmental consequences of hypertyrosinaemia, dietary Anonymous, 2016c	Non-guideline Non-GLP Tolpyralate group: 1000 ppm (corresponding to 89 mg/kg bw/d) NTBC group: 10 ppm (corresponding to 0.84 mg/kg bw/d) Control group Administration GD 0 – PND 21, terminated on PND 21 12 females/group Comprehensive developmental analysis (malformations, variations) not conducted	<u>Maternal findings</u> 1000 ppm tolpyralate (89 mg/kg bw/d): • Ocular opacity (10/11, starting from LD 7) • Plasma tyrosine concentration 2020 µmol/L (vs 66.5 µmol/L in control) 10 ppm NTBC: • Ocular opacity (11/11, starting from LD 4) • Plasma tyrosine concentration 2160 µmol/L <u>Offspring findings</u> 1000 ppm tolpyralate: • Plasma tyrosine concentration 3200 µmol/L (vs 191 µmol/L in control) • Ocular opacity (1.1% of pups vs 0 in control) • Abnormalities of the kidney: small kidney (2/64 vs 0 in control), rough surface (4/64 vs 0 in control) 10 ppm NTBC: • Plasma tyrosine concentration 3650 µmol/L • Increased pup mortality at PND 4 (22% vs 9.0% in control) • Ocular opacity (8.0% of pups) • Abnormalities of the kidney: small kidney (6/52), rough surface (2/52)
Rabbit		
PNDT study, gavage Anonymous, 2013d	OECD TG 414 GLP Doses: 0, 0.5, 5, 500 mg/kg bw/d Dosing GD 6-27 25 females/group	<u>Maternal toxicity</u> No adverse effects <u>Developmental toxicity</u> 500 mg/kg bw/d: • ↑ incidence of skeletal variations (supernumerary rib, 27 presacral vertebrae) ≤ 5 mg/kg bw/d: no adverse effects

Rat PNDT study

The rat PNDT study showed a small reduction in foetal weight and an increased incidence of skeletal variations (discontinuous rib cartilage, supernumerary rib) in the absence of maternal toxicity at the top dose of 500 mg/kg bw/d. Although these effects are not sufficiently severe to warrant classification, RAC notes that probably a higher dose, possibly the limit dose of 1000 mg/kg bw/d, would have been tolerated by the dams.

As to the litter containing 3 foetuses with the misshapen urethral orifice, RAC agrees with the DS conclusion that this finding can be attributed to a recessive mutant allele present in both the male and female which sired the affected litter as shown in the study Anonymous (2013e).

Regarding the incidences of several anomalies mentioned in the public consultation (left umbilical artery, right subclavian from aortic arch, split cartilage of the thoracic centrum), RAC agrees with the DS that these are within the ranges of relevant HCD provided by the applicant after the public consultation.

Rabbit PNDT study

The only developmental effect at the top dose of 500 mg/kg bw/d was the increased incidence of skeletal variations (supernumerary rib, 27 presacral vertebrae) in the absence of maternal toxicity. An additional study testing 1000 mg/kg bw/d with a limited number of animals (8 females per group) confirmed this finding. The effect is not considered sufficiently severe to warrant classification.

Rat study investigating developmental consequences of tyrosinaemia

In this mechanistic study, pregnant females were exposed to tolpyralate via diet from the beginning of gestation until PND 21. The study confirmed the eye and kidney findings seen in the 2-generation study and showed that comparable effects were caused by NTBC at a dose level causing a similar degree of tyrosinaemia. RAC considers the proposed causal relationship between tyrosinaemia and the eye and kidney effects plausible.

NTBC additionally caused an increased incidence of pup mortality on PND 4.

Rat 1-generation and 2-generation studies

Reduced pup viability on the day of birth was observed in the P/F1 generation of the main 2-generation study. The data on pup viability in this study are presented below.

Pup mortality in the 2-generation study (Anonymous, 2015e)				
Dose (ppm)	**0**	**5**	**50**	**1000**
Dose (mg/kg bw/d) m/f	0	0.27/0.29	2.7/3.0	55/59
F1: PND 0				
Number of live pups	287	303	320	288
Number of dead pups	2	1	8	11
Dead pups per litter (no. of litters affected)	1,1 (2)	1 (1)	1,6,1 (3)	2,1,1,1,3, 1,1,1 (8)
% found dead	0.6	0.4	2.4	3.7*
Viability index (%)	99.4	99.6	97.6	96.3*
F1: PND 1-4				
Number of dead pups	1	1	0	0

Dead pups per litter (no. of litters affected)	1 (1)	1 (1)	0 (0)	0 (0)
% found dead	0.4	0.4	0.0	0.0
% lost	2.4	0.3	1.2	5.4
Viability index on LD 4 (%)	97.2	99.3	98.8	94.6
F2: PND 0				
Number of live pups	301	307	297	228
Number of dead pups	3	4	6	11
Dead pups per litter (no. of litters affected)	2,1 (2)	1,1,1,1 (4)	1,1,1,2,1 (5)	9,1,1 (3)
% found dead	1.1	1.2	1.9	9.4
Viability index (%)	98.9	98.8	98.1	90.6
F2: PND 1-4				
% lost	0.6	2.5	6.6	2.1
Viability index on LD 4 (%)	99.4	97.6	93.4	97.9

* Significantly different from control, $p \leq 0.05$

The reduction in pup viability on PND 0 was statistically significant in the first generation but not in the second generation and no such finding was reported at a 20-fold higher dose in the dose-range finding study.

On the other hand, the dose range-finding study reported increased pup mortality on PND 1-4 from 2000 ppm (107/193 mg/kg bw/d). The data on pup mortality in the dose-range finding study are presented in the following table. Although the reduction in pup viability at 2000 ppm was not statistically significant, 5 out of 7 litters were affected at this dose and the mortality was even more pronounced at the top dose, which indicates a treatment-related effect. In addition, increased pup mortality on PND 1-4 was seen with other HPPD inhibitors (NTBC: Anonymous, 2016c; sulcotrione: RAC, 2011; mesotrione: RAC, 2018) and may be secondary to tyrosinaemia.

Pup mortality in the 1-generation dose-range finding study (Anonymous, 2015d)						
Dose (ppm)	**0**	**5**	**20**	**200**	**2000#**	**20000#**
Dose (mg/kg bw/d) m/f	0	0.26/0.50	1.0/1.9	11/20	107/193	1090/1940
PND 0						
Number of live pups	113	104	116	111	101	91
Number of dead pups	1	1	1	2	1	3
Dead pups per litter (no. of litters affected)	1 (1)	1 (1)	1 (1)	2 (1)	1 (1)	1,1,1 (3)
PND 1-4						
Number of dead pups	1	0	8	1	10	18
Dead pups per litter (no. of litters affected)	1 (1)	0 (0)	6,2 (2)	1 (1)	2,1,4,1,2 (5)	6,2,1,4,1, 1,3 (7)

Viability index on PND 4 (%)	99.0	100	93.8	99.1	90.0	79.7**

[#] 7 litters examined as 1 female was not pregnant

Significantly different from control: *, p ≤ 0.05; **, p ≤ 0.01

RAC is of the view that the increased pup mortality observed on PND 0 in the main study and on PND 1-4 in the range-finding study is an adverse effect on development warranting classification.

Classification is further supported by the occurrence of various renal abnormalities in pups in the generational studies (pelvic dilation, kidney cysts, white material in pelvic space, kidney atrophy). The presence of renal findings (nephropathy, calcification, basophilic change of renal tubules, cortical cysts) in F1 but not in P adults indicates persistence of the effects and increased sensitivity of foetuses and juveniles to the renal toxicity of tolpyralate.

Conclusion on classification for development

RAC proposes classification in **Category 2** for adverse effects on development based on increased pup mortality and an increased incidence of renal findings in the offspring in the rat generational studies. Classification in Category 1B is not considered appropriate due to the low incidence and shallow dose-response relationship for pup mortality and in view of the relatively low severity and incidence of the kidney findings.

Adverse effects on or via lactation

Statistically significant reductions in pup body weight on PND 14 and 21 were observed at the top dose of 1000 ppm (55/59 mg/kg bw/d m/f) in the 2-generation study (data provided in the table below). As the animals started to feed on the diet before PND 14, this reduction in body weight gain cannot be unequivocally attributed to an effect on or via lactation.

Pup body weights (g) in the 2-generation study (Anonymous, 2015e)

		Males				Females			
	Dose (ppm)	**PND 0**	**PND 7**	**PND 14**	**PND 21**	**PND 0**	**PND 7**	**PND 14**	**PND 21**
F1	**0**	7.0	18.1	39.2	64.9	6.6	18.0	39.4	63.6
	5	6.9	18.0	39.7	64.8	6.5	18.0	39.4	63.4
	50	7.0	18.5	39.6	64.6	6.5	17.8	38.7	62.3
	1000	6.9	16.2	35.9*	58.1**	6.5	16.4	36.0	56.3**
F2	**0**	7.3	20.0	41.9	64.4	6.8	19.0	40.5	62.0
	5	6.9	19.5	40.9	63.8	6.4	18.3	40.3	62.4
	50	7.0	19.6	42.4	65.7	6.6	18.7	41.1	62.8
	1000	7.3	19.2	39.0*	60.9	6.8	18.4	37.4**	57.9**

Significantly different from control: *, p ≤ 0.05; **, p ≤ 0.01

Statistically significant reductions in pup body weights were also observed in the one-generation dose-range finding study at 20000 ppm (1090/1940 mg/kg bw/d). The effect in male pups was a significant effect already on PND 7 (reduction by 17% compared to controls; maternal body weight in this period was lower by ca. 10% than in controls). However, the relatively low magnitude of effect at such a high dose level is not considered sufficient to trigger classification.

Pup body weights (g) in the 1-generation range-finding study (Anonymous, 2015d)									
		Males				Females			
	Dose (ppm)	PND 0	PND 7	PND 14	PND 21	PND 0	PND 7	PND 14	PND 21
F1	0	6.7	18.4	38.4	63.0	6.3	17.7	37.4	61.0
	5	6.9	19.3	39.9	65.5	6.6	18.5	38.9	63.3
	20	6.5	15.9	36.2	60.8	6.0	15.5	36.1	60.1
	200	6.6	17.5	36.7	60.3	6.3	16.9	36.2	58.0
	2000	6.5	16.4	35.5	56.7	6.4	16.5	35.7	56.2
	20000	6.4	15.3**	32.3**	51.3**	5.9	15.4	32.5**	50.1**

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$

The reduced pup viability between PND 1 and 4 in the one-generation dose-range finding study and the kidney lesions are considered to be manifestations of developmental toxicity.

In conclusion, RAC agrees with the DS that **classification** for adverse effects on or via lactation **is not warranted**.

Overall conclusion of reproductive toxicity

RAC recommends that tolpyralate be classified as **Repr. 2; H361fd (suspected of damaging fertility or the unborn child)**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The major aquatic metabolite of tolpyralate MT-2153 is persistent and toxic to aquatic organisms and is formed very fast and in significant amounts under normal environmental conditions. Although the DS considered that tolpyralate does not meet the criteria for 'rapidly degradable', the degradant MT-2153 together with the active substance was considered by the DS as relevant for hazard classification of tolpyralate. The DS proposed classification as:

Aquatic Acute 1, with an M factor of 10 based on the lowest 7 d E_rC_{50} value for the major degradant MT-2153 for aquatic plant (*Lemna gibba*) of 0.0315 mg/L.

Aquatic Chronic 1, with M factor of 100 based on the lowest 7 d NOE_rC value for the major degradant MT-2153 for aquatic plant *(Lemna gibba)* of 0.0009 mg/L.

Degradation

The aquatic hydrolysis study indicates that there is no ultimate degradation i.e. >70% within 28 d, as at 10 °C DT50 was 231 d at pH 7 for tolpyralate in purified water (respectively at pH 4 – 1050 d and at pH 9 - 2.31 d). However, tolpyralate hydrolyses more rapidly under conditions of higher pH and under increasing temperature. One hydrolysis product MT-2153 reached > 5 % of

the applied radioactivity. At 25 ˚C degradant MT-2153 reached maximum concentrations of 6.6 % AR (pH 4), 47.6 % AR (pH 7), and 97 % AR (pH 9). At 10 ˚C MT-2153 reached maximum concentrations of 1.1, 7.8 and 86.3 % AR respectively at pH 4, 7, and 9 (Unsworth, 2013). As MT-2153 was stable at all pH values at 50 ˚C and no degradation of MT-2153 was seen, no further testing was conducted as the DT_{50} value would be greater than one year at all environmentally relevant pH and temperature (Hori, K. 2014b).

In a ready biodegradation study (OECD TG 301F), biodegradation of tolpyralate observed after 25 days was 9% (Hammesfahr, 2013).

An aquatic photolysis study (OECD TG 316) indicated that tolpyralate degrades rapidly via photolysis with DT_{50} values of 4.41 days in natural water and 2.86 days in purified water (6 – 7 pH, 25 ˚C) (Unsworth, 2014). The degradant MT-2153 was formed but at equal or lower amounts than in dark controls. That suggests that it was formed by hydrolysis, not photolysis. However, the DS considered that photolysis is of uncertain relevance and is not useful for classification purposes as the route of degradation is dependent on local conditions and the information is insufficient for classification purposes.

Aerobic mineralization in the surface water study (OECD TG 309) indicated that in microbially active surface water at 20 ºC, tolpyralate degraded rapidly to less than 2% of applied activity after 14 days incubation (Van den Bosch, M.M.H. 2014). The DT_{50} values for tolpyralate were 2.06 days at an initial concentration of 10 µg/mL and 2.02 days at an initial concentration of 100 µg/mL. The degradant MT-2153 reached >90 % AR at 7 days at both initial concentrations but there was no clear evidence of decline at the end of study after 62 days. The maximum amount of CO_2 formed was 3.8% AR.

An aerobic water/sediment simulation study (OECD TG 308) was conducted in two natural aquatic systems, Calwich Abbey Lake and Swiss Lake, UK. Whole system DT_{50} values of tolpyralate were 1.39 and 1.88 days (geometric mean 1.62) for the two systems (Kane, T. 2014). Based on the results of the study, the DS considered that tolpyralate declined rapidly in both systems. Degradant MT-2153 in the whole system reached a maximum 82.0% and 79.9% AR. In the sediment, the amounts of MT-2153 reach a maximum 19.8 % and 32.7 % AR. DT_{50} values of MT-2153 in the whole system was 206 and 176 days (geometric mean 190).

Based on an anaerobic water/sediment study (OECD TG 308), the rate of degradation of tolpyralate was similar to the aerobic study, while that for MT-2153 was generally longer (Crowe, 2015). Whole system DT_{50} values of tolpyralate were 1.47 and 2.72 days (geometric mean 2), respectively, at pH 7.3 and 5. MT-2153 reached a max of 89.3 and 93 % in the whole system and 23.6 and 18.8 % in the sediment, respectively. DT_{50} values of MT-2153 were 388 and 394 days (geometric mean 365) for the two systems, respectively, at pH 7.4 and 5.7.

Overall, the DS concluded that although there is rapid primary degradation of the active substance, tolpyralate does not meet the CLH criteria as rapidly degradable because ultimate biodegradation is not achieved to a level greater than or equal to 70% after 28 days and it cannot be demonstrated that the degradation products do not fulfil the criteria as hazardous to the aquatic environment. As MT-2153 indicated high acute and chronic toxicity and formed very fast and in significant amounts, MT-2153 was considered in the classification of tolpyralate by the DS.

Aquatic Bioaccumulation

Using the HPLC method, the determined log K_{ow} of tolpyralate was 1.9 at 25 ˚C and pH 6.4 (Furutami, 2014). No experimental aquatic study to determine the bioconcentration potential (BCF) of tolpyralate was available.

Overall, the DS considered that tolpyralate does not meet the CLP criteria as a bioaccumulative substance as the log K_{ow} value of 1.9 is below the log K_{ow} trigger value of ≥4 and no experimental data (BCF) is available. It is therefore considered to have a low potential for bioaccumulation.

Aquatic Toxicity

The ecotoxicological test results from available acute and chronic studies for all trophic levels of tolpyralate are summarised in the following table and sections. Whilst degradant MT-2153 demonstrates high aquatic toxicity to the aquatic plants and should be considered for classification of tolpyralate, the relevant tests results are provided as well. Accordingly, the new information (generated during Public Consultation) is included and discussed in further detail below.

Only the valid acute and chronic studies on tolpyralate and degradant MT-2153 which are relevant for hazard classification purposes are included in the following table and relevant endpoints from these studies are discussed in further detail below.

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Test material/ remarks	Reference
Fish				
Oncorhynchus mykiss / OECD TG 203, GLP	96 h LC_{50} > 21 mg/L (mm)		Tolpyralate	Anonymous, 2013m
Cyprinus carpio / OECD TG 203, GLP	96 h LC_{50} > 19 mg/L (mm)		Tolpyralate	Anonymous, 2013n
Pimephales promelas / OECD TG 203, GLP	96 h LC_{50} > 19.7 mg/L (mm)		Tolpyralate	Anonymous, 2014f
Cyprinodon variegatus / OECD TG 203, GLP	96 h LC_{50} > 11.6 mg/L (mm)		Tolpyralate	Anonymous, 2015h
Pimephales promelas / OECD TG 210, GLP		28 d NOEC = 0.3 mg/L (mm)	Tolpyralate	Anonymous, 2014g
Oncorhynchus mykiss / OECD TG 203, GLP	96 h LC_{50} > 100 mg/L (n)	96 h NOEC = 100 mg/L (n)	MT-2153	Anonymous, 2014f
Aquatic invertebrates				
Daphnia magna / OECD TG 202, GLP	48 h EC_{50} > 19 mg/L (mm)		Tolpyralate	Yoshikawa, 2013
Americamysis bahia / OPPTS 850.1035, GLP	96 h EC_{50} = 0.66 mg/L (mm)		Tolpyralate	Brougher *et al.*, 2015b
Crassostrea virginica / OPPTS 850.1025, GLP	96 h EC_{50} = 6.8 mg/L (mm)		Tolpyralate	Brougher *et al.*, 2015c
Daphnia magna / OECD TG 211, GLP		21 d NOEC ≥ 8.94 mg/L (mm)	Tolpyralate	Yoshikawa, 2014b
Daphnia magna / OECD TG 202, GLP	96 h LC_{50} > 100 mg/L (n)	96 h NOEC = 100 mg/L (n)	MT-2153	Handlos and Erk, 2014b
Algae / aquatic plants				
Pseudokirchneriella subcapitata / OECD TG 201, GLP	96 h E_rC_{50} = 12.3 mg/L (mm)	96 h NOE_rC = 1.4 mg/L (mm) 96 h E_rC_{10} = 4.44 mg/L (mm)	Tolpyralate	Yoshikawa, 2013b
Pseudokirchneriella subcapitata / OECD TG 201, GLP	72 h E_rC_{50} = 8.88 mg/L (n)	72 h E_rC_{10} = 2.94 mg/L 72 h NOE_rC = 1.0 mg/L	MT-2153	Handlos and Erk, 2014c
Anabaena flos-aquae / OECD TG 201, GLP	96 h E_rC_{50} > 16.7 mg/L (mm)	96 h E_rC_{10} > 16.7 mg/L (mm)	Tolpyralate	Arnie *et al.*, 2013a

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Test material/ remarks	Reference
Navicula pelliculosa / OECD TG 201, GLP	96 h E_rC_{50} > 20.9 mg/L (mm)	96 h E_rC_{10} = 14.8 mg/L (mm)	Tolpyralate	Arnie *et al.*, 2013b
Skeletonema costatum / OECD TG 201, GLP	96 h E_rC_{50} = 1.8 mg/L (mm)	96 h E_rC_{10} = 0.15 mg/L (mm)	Tolpyralate	Arnie *et al.*, 2013c
Lemna gibba / OECD TG 221, GLP	7 d E_rC_{50} = 0.0353 mg/L (n)	7 d E_rC_{10} = 0.00142 mg/L (n) 7 d NOE_rC = 0.00102 mg/L (n)	Tolpyralate	Kuhl and Wydra, 2013
Lemna gibba / OECD TG 221, GLP	7 d E_rC_{50} = **0.0315** mg/L (mm)	7 d E_rC_{10} < 0.0009 mg/L (mm) 7 d NOE_rC = **0.0009** mg/L (mm)	MT-2153	Hermes and Frank, 2015a
Myriophyllum aquaticum / OECD TG 239, GLP	7 d E_rC_{50} > 0.244 mg/L (mm)	7 d NOE_rC < 0.000304 mg/L (mm)	Tolpyralate **	Seeland-Fremer and Wydra, 2014
Myriophyllum spicatum / OECD TG 239, GLP	14 d E_rC_{50} = 0.0611 mg/L (mm)	14 d NOE_rC < 0.000606 mg/L (mm)	MT-2153 **	Hermes and Frank, 2015b

mm – mean measured
n – nominal
bold – lowest aquatic acute and chronic endpoints of tolpyralate and MT-2153 indicated by the DS
** – DS indicated uncertainty in test concentration and/or pH

Acute and chronic aquatic toxicity data on tolpyralate and the degradant MT-2153 are available for fish, invertebrates, algae and aquatic plants. The DS considered aquatic plants as the most sensitive trophic group for both acute and chronic timescales. Therefore, the DS based the classification proposal on the toxicity to aquatic plants.

The DS considered that the lowest overall acute endpoint is a 7-day mean measured E_rC_{50} of 0.0315 mg/L of MT-2153 for *Lemna gibba* (dry weight). Furthermore, the lowest acute endpoint of tolpyralate (7 d E_rC_{50} of 0.0353 mg/L for *Lemna gibba*) was also in the same classification range. In the same classification range was also the acute endpoint of MT-2153 for *Myriophyllum spicatum* (14-day E_rC_{50} of 0.0611 mg/L). Since the effects endpoint is above 0.01 but ≤ 0.1 mg/L, the DS proposed to classify tolpyralate as Aquatic Acute category 1 with an acute M-factor of 10 based on the data for the major degradant MT-2153.

In the absence of reliable *Myriophyllum* chronic toxicity endpoints (for either the active substance or degradant MT-2153), the DS proposed to use the chronic toxicity data derived from the studies with *Lemna gibba*. Since the degradant MT-2153 is not 'rapidly degradable' and slightly more toxic than the parent substance, the chronic classification was based on a 7-day NOEC of 0.0009 mg/L (dry weight) for MT-2153. This endpoint is between 0.0001 and 0.001 mg/L and, since tolpyralate is not 'rapidly degradable', tolpyralate should be classified as Chronic 1 with a chronic M-factor of 100, based on the data for the major degradant MT-2153.

Furthermore, the DS acknowledged that there is an uncertainty in the chronic M-factor due to the absence of a reliable NOE_rC / EC_{10} endpoint for *Myriophyllum*. The DS pointed out that if further reliable chronic data become available on *Myriophyllum* or other sensitive aquatic plant species, then the environmental classification should be reconsidered.

Comments received during public consultation

These studies have been provided during and after the initial CLH public consultation (i.e. as part of the EFSA-led pesticide peer review programme). An additional targeted public consultation was launched on these new studies. The main results of the new studies are provided below.

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)	Test material/ remarks	Reference
Aquatic invertebrates				
Americamysis bahia / OPPTS 850.1035, GLP		21 d NOEC = 0.022 mg/L (mm)	Tolpyralate	Amanda *et al.*, 2017
Crassostrea virginica / OPPTS 850.1025, GLP	96 h EC_{50} = 8.7 mg/L (mm)	96 h NOEC = 3.1 mg/L (mm)	Tolpyralate	Brougher *et al.*, 2016
Algae / aquatic plants				
Myriophyllum spicatum / OECD TG 239, GLP	14 d E_rC_{50} = **0.0102** mg/L (mm)	14_d E_rC_{10} = **0.000129** mg/L (mm) 14 d NOE_rC = **0.000603** mg/L (mm)	Tolpyralate **	Hermes and Emnet, 2018

mm – mean measured
n – nominal
bold – lowest aquatic acute and chronic endpoints indicated after PC
** – DS indicated uncertainty in test concentration and/or pH

At the initial Public consultation, three MSCAs and one company submitted comments on the environmental part of the DS's proposal. One MSCA and Industry referred to a new *Myriophyllum spicatum* study (described above) with a duration of 14 days, performed according to OECD TG 239 with tolpyralate. Nevertheless, the MSCA noted that effect endpoints from this new study (E_rC_{50} 0.0102 mg/L and NOEC 0.00063 mg/L) wouldn't change the proposed classification and M-factors. Additionally, the same MSCA noted that a new chronic toxicity study with *Mysid* (*Americamysis bahia*) is available (also described above), which eliminates the data gap related to the chronic toxicity of invertebrates. In response, the DS noted that the new chronic *Mysid* study has been evaluated and a NOEC could be derived. However, as aquatic plants are clearly more sensitive than invertebrates, this study does not have any impact on the proposed classification.

Regarding the new *Myriophyllum spicatum* study, the DS indicated uncertainty in the lower test concentrations (i.e. due to recovery at the relevant test concentrations being < LOQ or < LOD in the aged media) and, therefore, indicated that the endpoint based on geometric mean measured concentrations may underestimate the toxicity of the test substance. Additionally, the DS pointed out the large difference in pH between the different test solutions. The DS noted that the pH in this study varied from well below to well above the recommended level by OECD over the renewal periods. Therefore, the DS does not deem the study as a reliable one to derive a NOEC although the study report states that all validity criteria were met. The DS noted that it is not possible to currently check two of the criteria, as the raw data do not appear to have been included within the study report. Overall, the DS concluded that as no reliable NOEC for *Myriophyllum* can be derived from this study, the chronic M-factor should be based on the data for *Lemna gibba* for the degradant MT-2153.

A second MSCA requested a better overview of the acute and chronic toxicity studies of the relevant degradant MT-2153 and remarked that, for long-term aquatic hazard classification and labelling purposes, it is recommended to use data for the substance itself (intrinsic toxicity). Hence, the MSCA referred to the chronic data of tolpyralate to aquatic plant *Myriophyllum*

aquaticum (NOErC ≤ 0.000304 mg/L) as the lowest long term value. Furthermore, the MSCA noted that a further study with *Myriophyllum* with a duration of 14 days would be useful and preferable. In their response, the DS noted that further information can be provided regarding the aquatic studies with the degradant MT-2153, however the DS did not provide any detailed explanation on acute and chronic toxicity studies of the MT-2153.

Regarding the long-term aquatic hazard, the DS does not consider the new *Myriophyllum spicatum* study as reliable, hence the chronic M-factor will remain based on the data for *Lemna gibba* for the degradant MT-2153.

A third MSCA specified that they agree with the proposed classification and M-factors as the proposals are based on degradant data and not on the data of the parent substance itself. However, the MSCA indicated that if the classification would be based on toxicity data of the parent substance, the proposal would need to be revised. This MSCA also agreed that the chronic M-factor should be reconsidered if relevant further data become available. In their response, the DS noted that originally the chronic classification and M-factor were based on the parent substance (tolpyralate) data for *Lemna gibba*. However, the proposal was revised and was based on the degradant (MT-2153) aquatic toxicity data. In addition, the DS mentioned that new *Myriophyllum* studies with tolpyralate were provided, although it was not possible to obtain a reliable NOEC from this study.

At the targeted public consultation, three MSCAs submitted comments. All of them agreed with the classification as Aquatic Acute 1 (M = 10) and Aquatic Chronic 1 (M = 100). Although one MSCA noted that the 14-day study with *Myriophyllum spicatum* includes the complicating factor of sediment and analytics at low concentrations and different pH was variable. However, they considered the study reliable and relevant for classification. Additionally, the MSCA suggested to use an E_rC_{10} value of 0.000129 mg/L for classification purposes, which is in same range as the NOE_rC value of 0.000603 mg/L. The other MSCA noted that the 14-day study with *Myriophyllum spicatum* fulfils the CLP requirements and that the endpoints can be considered as reliable for classification purposes.

Assessment and comparison with the classification criteria

A ready biodegradation study with tolpyralate indicated 9% degradation after 25 days, indicating that tolpyralate is not readily biodegradable.

The hydrolysis study indicated that there is no ultimate degradation, with more rapid hydrolysis under conditions of higher pH and increasing temperature. The major aquatic metabolite MT-2153 (is persistent and toxic to aquatic organisms.. Additionally, tolpyralate degrades rapidly via photolysis (< 5 d in purified and natural water) and the major degradant MT-2153 formed in equal or lower amounts than in dark controls, suggesting that it was formed by hydrolysis not photolysis. Moreover, photolysis is of uncertain relevance as a route of degradation.

The aerobic mineralisation study and water/sediment studies (aerobic and anaerobic) indicate that tolpyralate underwent rapid primary degradation in natural waters with DT_{50} values at <3 days. In all studies, the major degradation product was MT-2153, which in the aerobic water/sediment study has in the whole system a geometric mean DT_{50} value of 190 and in anaerobic water/sediment study – 365 days. The rate of degradation of tolpyralate was similar in both studies with DT_{50} of 1.6 – 2 days (geomean). In the aerobic/water sediment study, MT-2153 in the whole system reached a maximum 82.0% and 79.9% AR. In the anaerobic water sediment study MT-2153 reached a max. of 89.3 and 93 %.

RAC agrees that tolpyralate does not meet the CLP criteria as rapidly degradable because ultimate biodegradation of the substance (i.e. full mineralisation) is not achieved and it cannot be demonstrated that the degradation products do not fulfil the criteria as hazardous to the aquatic environment. RAC also agrees that the major degradant MT-2153 should be considered for classification purposes of tolpyralate since MT-2153 is very toxic to aquatic environment and forms very fast in significant amounts under normal environmental conditions, as reported in the different biotic and abiotic degradation studies.

Aquatic Bioaccumulation

The determined log K_{ow} of tolpyralate is 1.9 at 25 °C and pH 6.4 which is less than the CLP trigger of ≥ 4. Therefore, RAC agrees with the DS's conclusion that the substance is not bioaccumulative.

Aquatic Toxicity

There are reliable acute and chronic aquatic toxicity data for all trophic groups with tolpyralate, as well as with the degradant MT-2153. For both, the most acutely and chronically sensitive trophic group were aquatic plants *Lemna gibba* and *Myriophyllum*. The acute toxicity results of tolpyralate and degradant MT-2153 from two studies with *Lemna gibba* and two studies with *Myriophyllum spicatum* were slightly different, but still in the same range for aquatic acute classification purposes and M-factor derivation.

Regarding chronic toxicity, tolpyralate seems be more toxic to *Myriophyllum* than to *Lemna gibba*. However, the chronic toxicity of degradant MT-2153 to *Myriophyllum* and *Lemna gibba* was slightly different, albeit still in the same range for aquatic chronic classification purposes and M-factor derivation. The same was the case (slightly different but still in the same classification range as for the degradant MT-2153) for the tolpyralate results obtained with *Myriophyllum*.

Due to the degradant MT-2153 being formed in levels up to 90% in less than 16 days, RAC considers that MT-2153 should be considered for classification purposes of tolpyralate as it seems slightly more toxic at least to *Lemna gibba* than the parent substance. RAC notes that some key studies have uncertainties related to the concentration and/or pH, however, RAC is of opinion that these uncertainties are unlikely to compromise the reliability of all studies overall. Nevertheless, if additional/more reliable chronic data of tolpyralate or the degradant MT-2153 become available in the future for the most acutely sensitive species, the chronic M-factor(s) may need to be revised.

Therefore, RAC agrees with the DS proposed classification as Aquatic Acute 1 (M-factor 10) and Aquatic Chronic 1 (M-factor 100), but base the classification on the toxicity results of *Myriophyllum spicatum*, from the new 14-day study, performed with tolpyralate. The lowest acute endpoint for aquatic acute classification purposes is 14 d E_rC_{50} for *Myriophyllum spicatum* of 0.0102 mg/L, based on mean measured concentration of tolpyralate. It should be noted that one more study with tolpyralate derived a 7 d E_rC_{50} for *Lemna gibba* of 0.0353 mg/L and two studies with the degradant MT-2153 derived a 7 d E_rC_{50} for *Lemna gibba* of 0.0315 mg/L and 14 d E_rC_{50} for *Myriophyllum spicatum* of 0.0611 mg/L that are all in the same classification range.

For aquatic chronic classification, despite the uncertainties RAC concluded to use the 14 d E_rC_{10} for *Myriophyllum spicatum* of 0.000129 mg/L, based on mean measured concentration of tolpyralate. However, the NOE_rC value from same study with tolpyralate derived a 14 d NOE_rC for *Myriophyllum spicatum* of 0.000603 mg/L, which is in the same classification range.

It should also be noted that as supplemental information, one more study with tolpyralate derived a 7 d NOE_rC for *Myriophyllum aquaticum* of 0.000304 mg/L and two studies with MT-2153 derived a 7 d NOE_rC for *Lemna gibba* of 0.0009 mg/L and a 14 d NOE_rC for *Myriophyllum spicatum* of 0.000606 mg/L, all of which are in the same classification range.

Conclusion on classification

Tolpyralate is considered as not rapidly degradable and does not fulfil the CLP criteria for bioaccumulation. Based on the available and reliable information, RAC is of the opinion that tolpyralate warrants classification as:

Aquatic Acute 1 based on E_rC_{50} = 0.0102 mg/L for *Myriophyllum spicatum*. As this acute toxicity value falls within the 0.01 < $L(E)C_{50}$ ≤ 0.1 mg/L range, the **acute M-factor is 10**.

Aquatic Chronic 1 based on E_rC_{10} = 0.000129 mg/L for *Myriophyllum spicatum*. As this chronic toxicity value falls within the 0.0001 < NOEC ≤ 0.001 mg/L range, the **chronic M-factor is 100.**

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The DS noted that based on molecular structure of tolpyralate it wouldn't qualify as a controlled substance according to the Montreal Protocol. Additionally, it is not expected to enter into contact with stratospheric ozone molecules given its physico-chemical parameters.

Comments received during public consultation

No comments.

Assessment and comparison with the classification criteria

A substance shall be classified as hazardous to the ozone layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer. RAC assumes that it is unlikely that tolpyralate would be available in the stratosphere based on its chemical structure and other available information on its physico-chemical properties and considers that tolpyralate may not present a danger to the structure and/or the functioning of the stratospheric ozone layer.

Consequently, RAC agrees that tolpyralate is not expected to be hazardous to stratospheric ozone and does not require a classification according to the CLP regulation.

Additional references

Ellaway, *et al*. (2001) Outcome of tyrosinaemia type III. Journal of Inherited Metabolic Disease 24:824-832

Gissen, *et al*. (2003) Ophthalmic follow-up of patients with tyrosinaemia type I on NTBC. Journal of Inherited Metabolic Disease 26:13-16

Najafi, *et al.* (2018) A case of tyrosinemia type III with status epilepticus and mental retardation. Advanced Biomedical Research 7:7

Szymanska, *et al*. (2015) Tyrosinemia type III in an asymptomatic girl. Molecular Genetics and Metabolism Reports 5:48-50

Hermes and Emnet, PhD, 2018 Toxicity to the Aquatic Plant Myriophyllum spicatum in a Semi-Static Growth Inhibition Test with a Prior Rooting Phase.

Milligan *et al.* (2017) A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid *(Americamysis bahia)*

Brougher *et al.* (2016) A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)

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 documents).

Annex 3: Records of the targeted public consultation following the submission of additional experimental information on aquatic species