CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

TOLPYRALATE

EC Number: **Not assigned**

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

Index Number: **Not listed**

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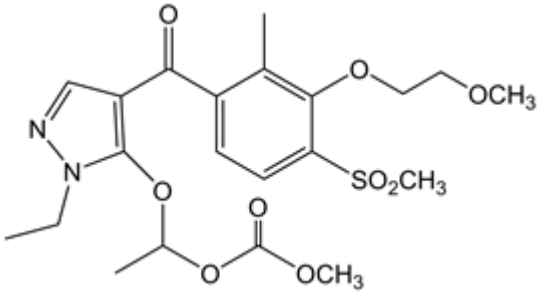
Version number: 2 **Date: March 2018**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(RS)-1-{1-ethyl-4-[4-mesyl-3-(2-methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate
Other names (usual name, trade name, abbreviation)	tolpyralate, SL-573
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	1101132-67-5
Other identity code (if available)	Not available
Molecular formula	$C_{21}H_{28}N_2O_9S$
Structural formula	
SMILES notation (if available)	Not available
Molecular weight or molecular weight range	484.52
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The ratio of enantiomers (R and S isomers) is 50:50 (the active substance is a racemic mixture). The two isomers can be considered equally biologically active with regard to herbicidal activity. In addition *in vivo* mammalian toxicity testing indicates it is stable to chiral conversion. The ratio of stereoisomers (1:1) was unchanged during hydrolysis, indicating that the rate of hydrolysis of the two stereoisomers is the same in the aquatic environment.
Description of the manufacturing process and identity of the source (for UVCB substances only)	n/a – tolpyralate is not a UVCB substance
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum 95%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Tolpyralate, CAS 1101132-67-5	Minimum 95%, 950 g/kg	Not listed	-

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant				

There are a number of process impurities in the substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier.

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	Substance not listed on Annex VI										
Dossier submitters proposal	n/a	(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 (eyes, kidney) Aquatic Acute 1 Aquatic Chronic 1	H351 H373 H400 H410	GHS08 GHS09 Wng	H351 H373 H410	n/a	M = 10 M = 100	n/a
Resulting Annex VI entry if agreed by RAC and COM	n/a	(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 (eyes, kidney) Aquatic Acute 1 Aquatic Chronic 1	H351 H373 H400 H410	GHS08 GHS09 Wng	H351 H373 H410	n/a	M = 10 M = 100	n/a

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

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Tolpyralate is a new active substance in Plant Protection Products (in the meaning of Regulation (EC) No. 1107/2009). Tolpyralate does not have an existing entry in Annex VI of CLP and has not been considered for harmonised classification and labelling previously in the EU.

At the time of submitting this CLH report, tolpyralate was not registered under REACH.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Tolpyralate is a pesticide active substance and CLH is required in accordance with Article 36(2) of CLP.

5 IDENTIFIED USES

Tolpyralate is a new pesticide active substance with a proposed use as a broad spectrum herbicide, effective against broad leaf weeds in maize crops.

6 DATA SOURCES

This classification and labelling proposal has been prepared based on data in the Draft Assessment Report (DAR).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Off-white powder. No discernible odour.	Turner B (2013a) DAR: B.2.3/01	
Melting/freezing point	Melting range of 127 – 129°C	Turner B (2013b) DAR : B.2.1/01	EEC Method A1, OECD Method 102 – metal block method
Boiling point	Not determined	Turner B (2013b) DAR: B.2.1/02	EEC Method A2, OECD Method 103 – Siwolodoff method The boiling temperature was not determinable as the test substance started to decompose after melting. The pale yellow liquid darkened in colour as the temperature rose, becoming a dark yellow liquid at approximately 225°C and a brown liquid at approximately 255°C, until the sample formed a thick black solid at approximately 305°C.
Relative density	1.32 at 20°C	Turner (2013b) DAR: B.2.14	
Vapour pressure	5.9 x 10^{-4} Pa at 25°C	Turner B (2013c) DAR: B.2.2/01	EEC Method A4, OECD Method 104
Surface tension	68.0 mN/m (90% saturated solution at 20°C	Turner B (2013b) DAR: B.2.12/01	EEC Method A5, OECD Method 115 Harkins-Jordan corrected value = 67.0 mN/m The substance is not considered surface

Property	Value	Reference	Comment (e.g. measured or estimated)
			active.
Water solubility	26.5 mg/L at 20°C Moderately soluble	Comb (2012) DAR: B.2.5/01	EEC Method A6, OECD Method 106 – flask method and content determined by HPLC
Partition coefficient n-octanol/water	Log P_{ow} = 1.9 at 25°C (eluent: methanol/ultra pure water pH 6.4)	Furatani (2014) DAR: B.2.7/01	OECD Method 117
Flash point	Not relevant since the melting point is >40°C	DAR: B.2.10/01	
Flammability	Not highly flammable	Turner B (2013a) DAR: B.2.9/01	EEC Method A10
Explosive properties	Not explosive	Turner B (2013a) DAR: B.2.11/01	EEC Method A14
Self-ignition temperature	The sample did not self - ignite up to 400°C.	Turner B (2013a) DAR: B.2.9/02	EEC Method A16
Oxidising properties	Not oxidising	Turner B (2013a) DAR: B.2.13/01	EEC Method A17
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	Tolpyralate does not have a dissociation constant in the environmental pH range (pH 4-10)	Turner B (2013d) DAR: B.2.8/01	OECD Method 112 – Spectrophotometric method
Viscosity	Not relevant since the substance is a solid with a melting point >40 °C		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC Method A14	Not explosive	In the mechanical sensitivity test there were no signs of ignition or explosion observed in any of the six tests but decomposition, indicated by a dark mark on the porcelain plate, was observed.	Turner B (2013a) DAR: B.2.11/01

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

In a standard explosivity study (EEC, A14) there was no evidence of shock, friction or thermal sensitivity.

8.1.2 Comparison with the CLP criteria

A substance is considered for classification as explosive where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation. There was no evidence of shock, friction or thermal sensitivity when tolpyralate was tested in a standard explosivity study.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – data conclusive but not sufficient for classification

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (tolpyralate is not a gas)

8.3 Oxidising gases

Hazard class not applicable (tolpyralate is not a gas)

8.4 Gases under pressure

Hazard class not applicable (tolpyralate is not a gas)

8.5 Flammable liquids

Hazard class not applicable (tolpyralate is not a liquid)

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC Method A10	Not highly flammable		Turner B (2013a) DAR: B.2.9/01

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

In a standard flammability study, tolpyralate was found to be not highly flammable.

8.6.2 Comparison with the CLP criteria

The criteria for classification as a flammable solid (Table 2.7.1 of Annex 1 of CLP) are not met.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified – data conclusive but not sufficient for classification.

8.7 Self-reactive substances

No studies are available for this end point.

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

No studies are available. However, the structure of tolpyralate does not contain any functional groups known to confer self-reactive properties (as indicated in Tables A6.1 and A6.2 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria).

8.7.2 Comparison with the CLP criteria

According to Section 2.8.4.2 of Annex 1 of CLP, if a substance does not contain any chemical groups associated with explosive or self-reactive properties, then the classification procedure need not be applied.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Hazard class not assessed in this dossier; hazard class not applicable.

8.8 Pyrophoric liquids

Hazard class not applicable (tolpyralate is not a liquid).

8.9 Pyrophoric solids

No studies are available for this end point.

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

No studies are available. However, tolpyralate has been handled in air in the available studies and no incidences of self-ignition have been reported.

8.9.2 Comparison with the CLP criteria

According to Section 2.10.4.1 of Annex 1 of CLP, the classification procedure for pyrophoric solids need not be applied when experience in manufacture and handling shows that the substance does not spontaneously ignite upon coming into contact with air at normal temperatures. There are no reports in the available studies of tolpyralate spontaneously igniting when in contact with air.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Hazard class not assessed in this dossier; hazard class not applicable.

8.10 Self-heating substances

Table 10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC Method A16	The sample did not self-ignite up to 400°C		Turner B (2013a) DAR: B.2.9/02

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

A study conducted in accordance with EEC A16 is available. In this study, tolpyralate did not self-ignite up to a temperature of 400°C.

8.10.2 Comparison with the CLP criteria

Studies conducted according to EEC Method A16 are not sufficient for classification under CLP.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not classified - data lacking

8.11 Substances which in contact with water emit flammable gases

No studies are available.

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

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

8.11.2 Comparison with the CLP criteria

According to Section 2.12.4.1 of Annex 1 of CLP, the classification procedure for this hazard class need not be applied if experience in production or handling shows that the substance does not react with water. Therefore, classification for this class is not applicable for tolpyralate.

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

Hazard class not assessed in this dossier; hazard class not applicable.

8.12 Oxidising liquids

Hazard class not applicable (tolpyralate is not a liquid).

8.13 Oxidising solids

Table 11: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC Method A17	The test substance was not found to possess oxidising properties.		Turner B (2013a) DAR: B.2.13/01

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

A study conducted according to EEC Method A17 indicated that the substance is not oxidising. However, results generated using this study method are not suitable for classification using the CLP criteria.

The waiving criteria specified in Section 2.13.4.1 of Annex 1 of CLP are not met, since the substance contains an oxygen chemically bonded to something other than carbon or hydrogen (i.e., sulphur).

8.13.2 Comparison with the CLP criteria

Data lacking.

8.13.3 Conclusion on classification and labelling for oxidising solids

Not classified - data lacking

8.14 Organic peroxides

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

8.15 Corrosive to metals

No data are available.

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

No data are available.

8.15.2 Comparison with the CLP criteria

No data are available.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – data lacking

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The toxicokinetics of tolpyralate has been investigated in a range of studies conducted in the rat, as described in Table 12. Note that "SL-573" is the development code for tolpyralate.

Table 12: Summary table of toxicokinetic studies

Study	Reference
[^{14}C]SL-573 – Absorption, distribution, metabolism and excretion in the rat	Anonymous (2013a) DAR: B.6.1
[^{14}C]SL-573 - Absorption, distribution, metabolism and excretion in the rat following repeated oral administration	Anonymous (2014a) DAR: B.6.1
[^{14}C]SL-573 - Investigation of the chiral conversion of tolpyralate in the rat	Anonymous (2014e) DAR: B.6.1
[^{14}C]SL-573: Mouse, rat and human liver microsomal metabolism	Foster, J.R. (2015) DAR: B.6.1
14C]SL-573 – Investigation of metabolite MMTA following oral administration of [Phenyl-14C]-tolpyralate to the rat and laying hen	Anonymous (2014b) DAR: B.6.1

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

The absorption, distribution, excretion and metabolism of tolpyralate have been investigated in a range of studies conducted using two different radiolabelled forms of the technical material, with radiolabels located at either the phenyl or pyrazole moieties.

Data informing on the plasma kinetics of tolpyralate indicate rapid absorption following oral administration. Oral absorption is estimated to be in the region of 60%. Tolpyralate was found to be widely distributed with the highest levels of radioactivity being associated with the liver and kidney. There was some indication of saturation of absorption at high dose levels. There was no evidence of a potential for tolpyralate to accumulate in fat.

Data from a single dose ADME study in the rat indicates that at the early time point the blood and the bone marrow are well exposed following oral exposure in the rodent. From the available data, the t_{max} of tolpyralate can be estimated to be in the range of 0.5 – 2 hours.

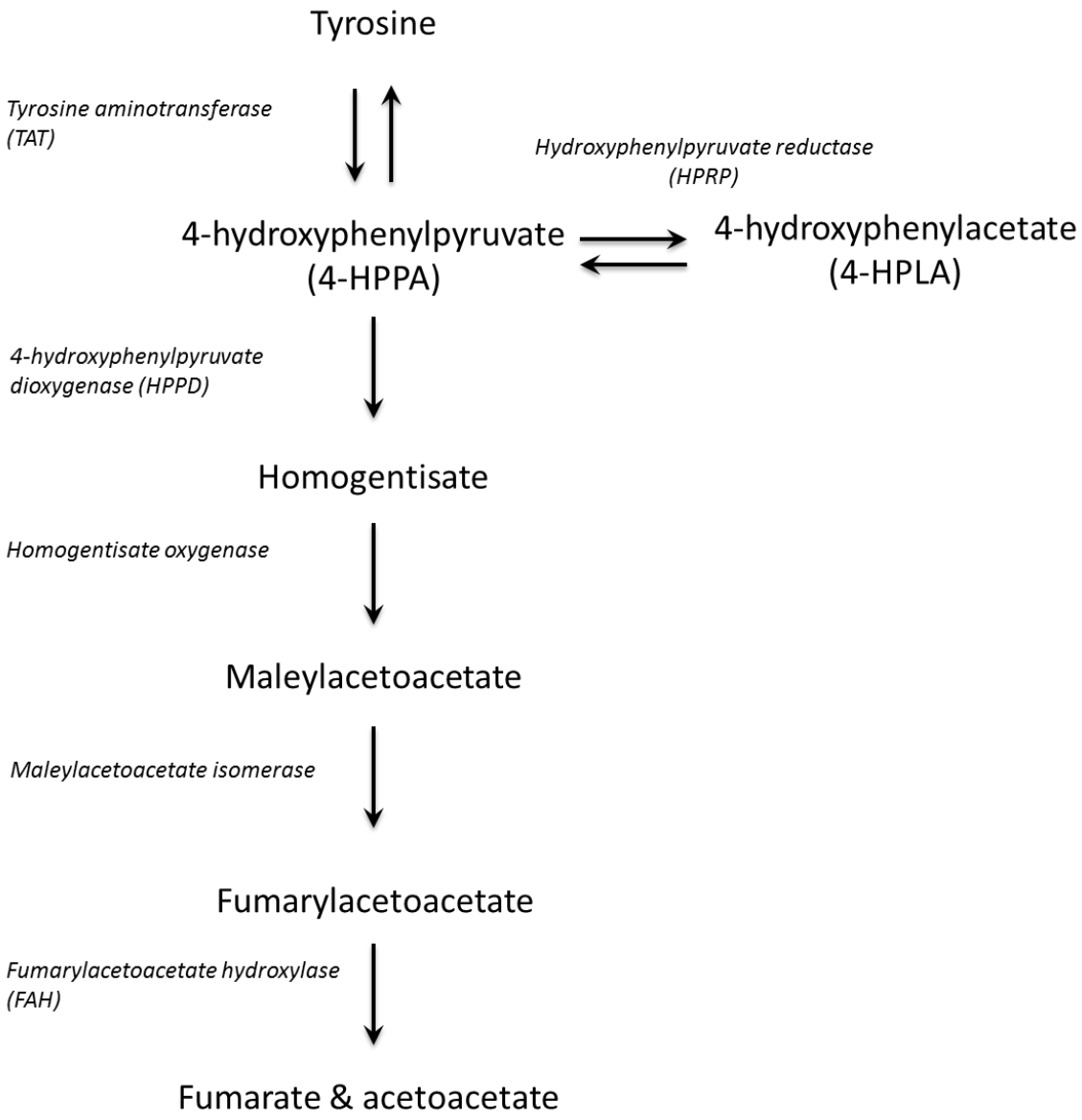
Tolpyralate was found to be extensively metabolised, the primary route of metabolism appears to be by dealkylation and subsequent conjugation with glucuronic acid. Comparative metabolism data from an *in vitro* study did not indicate any significant differences in metabolism between rats, mice and humans. Tolpyralate is eliminated in both the urine and faeces in approximately equal proportions; some radioactivity was also associated with the bile, indicated by studies in bile duct cannulated animals.

Dermal penetration of pure tolpyralate technical material has not been specifically investigated. An *in vitro* study investigated the dermal penetration of tolpyralate from a formulated pesticide product in both its concentrated and in use forms in human skin. Dermal penetration values of 0.1% and 9% were calculated, respectively.

10 EVALUATION OF HEALTH HAZARDS

Tolpyralate is a pyrazole herbicide which is reported to inhibit the enzyme 4- Hydroxyphenylpyruvate dioxygenase (HPPD) (see Section 10.12). In mammals, HPPD is the second enzyme in the catabolic pathway of tyrosine (figure 1):

Figure 1: Catabolic pathway of tyrosine



Inhibition of HPPD leads to a build-up of its substrate 4-hydroxypheylpyruvic acid (4-HPPA), which is excreted in urine or converted back to tyrosine. In common with other important amino acids, tyrosine is not excreted by the kidney (Buist *et al.*, 1974; Goldsmith, 1983) and hence only small amounts of free tyrosine may be readily cleared from the body. The clinical consequence of HPPD inhibition is an increase in

circulating levels of plasma tyrosine and increased urinary excretion of the substrate 4-HPPA and related substances, collectively known as phenolic acids (e.g., 4-hydroxyphenylactate (4-HPLA)).

When HPPD is inhibited, studies have shown that the extent of the elevated tyrosine levels is species-specific with a particularly severe effect induced in male rats (reviewed in Lock *et al*, 2006). The basis for the species difference is the relative efficiencies of urinary excretion of phenolic acids between rats and mice due to differences in their inherent activity of Tyrosine Aminotransferase (TAT) (see Table 13).

Table 13: Comparison of innate hepatic TAT (nmol/ HPPA/min/mg protein) activity – rats, mice and humans

	Rat	Mouse	Humans*
Male	1.7 ± 0.2	7.8 ± 1.5	7.17 ± 1.17
Female	3.3 ± 0.5	10.5 ± 1.9	

From Henderson *et al*., 1981

Innate hepatic TAT activity is higher in the mouse and, in the rat, a sex difference in TAT activity results in the male having particularly low HPPD activity. This difference in TAT activity results in a species and sex difference in tyrosine accumulation. Due to a high innate TAT activity, humans are expected to be less sensitive to the effects of HPPD inhibition than rats.

In the Mode of Action (MoA) proposed by the Applicant, a number of the effects seen with tolpyralate are said to be linked to significantly elevated plasma tyrosine levels, rather than a direct result of dosing with tolpyralate. This is discussed further in the relevant sections.

Acute toxicity

The acute toxicity of tolpyralate has been investigated in specific studies conducted *via* the oral, dermal and inhalation routes.

10.1 Acute toxicity - oral route

The acute oral toxicity of tolpyralate was investigated in a GLP compliant study in the rat, conducted in accordance with OECD TG 423.

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test Item	Result	Remarks
Acute toxic class OECD 423 GLP Anonymous (2012a) DAR: B.6.2.1	Rat, SD (Sprague Dawley) 6 females	2000 mg/kg bw Vehicle: 1% methylcellulose Batch: 20111222-1 Purity: 97.27%	LD50 > 2000 mg/kg bw	No overt clinical signs of toxicity. No unscheduled deaths.

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

In a study performed in accordance with the acute toxic class method (OECD 423), a group of 6 female rats was administered tolpyralate by gavage, formulated in 1% methylcellulose, at a single dose level of 2000 mg/kg bw. Animals were observed for mortality and clinical signs of toxicity for a period of 14 days

following dose administration. No unscheduled deaths were recorded. There were no overt clinical signs of toxicity. The acute oral LD_{50} of tolpyralate can be concluded to be > 2000 mg/kg bw in the rat.

10.1.2 Comparison with the CLP criteria

In accordance with the CLP criteria, substances should be classified for acute oral toxicity when the LD_{50} has been reliably determined to be ≤ 2000 mg/kg bw. The acute oral LD_{50} of tolpyralate was found to be >2000 mg/kg bw, therefore the criteria for classification are not met.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification (conclusive but not sufficient for classification).

10.2 Acute toxicity - dermal route

The acute toxicity of tolpyralate *via* the dermal route was investigated in a study in the rat conducted in accordance with OECD TG 402 and in compliance with GLP.

Table 15: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test Item	Result	Remarks
Acute dermal toxicity OECD 402 GLP Anonymous (2012b) DAR: B.6.2.2	Rat, SD (Sprague-Dawley) 5 males/5 females	2000 mg/kg bw Vehicle: 1% methylcellulose Batch: 20111222-1 Purity: 97.27%	LD50 > 2000 mg/kg bw	No overt clinical signs of toxicity. No unscheduled deaths.

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

The acute dermal toxicity of tolpyralate was investigated in a study conducted in accordance with OECD TG 402. Groups of 5 male and 5 female Sprague-Dawley rats of each sex were exposed to tolpyralate, formulated in 1% methylcellulose, applied topically at a single dose level of 2000 mg/kg bw, for a period of 24 hours. Animals were monitored for mortality and clinical signs of toxicity for a period of 14 days following administration of the test item. There were no recorded mortalities or overt clinical signs of toxicity. The acute dermal LD_{50} of tolpyralate was found to be > 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

In accordance with the CLP criteria, substances should be classified for acute dermal toxicity where the LD_{50} has been reliably determined to be ≤ 2000 mg/kg bw. The acute dermal LD_{50} of tolpyralate was shown to be > 2000 mg/kg bw, therefore the criteria for classification are not met.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification (conclusive but not sufficient for classification).

10.3 Acute toxicity - inhalation route

The acute toxicity of tolpyralate *via* the inhalation route was investigated in a study in the rat conducted in accordance with OECD test guideline 436 and in compliance with GLP.

Table 16: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test conditions	Result	Remarks
Acute inhalation toxicity Nose only OECD 436 GLP Anonymous (2012c) DAR: B.6.2.3	Rat, SD 3 males/3 females	4-hour exposure Batch: 20111222-1 Purity: 97.27% Single concentration of 2.01 mg/L (aerosol) MMAD 4.0 µm GSD 2.60	LC50 > 2.01 mg/L	2.01 mg/L was the maximum achieved concentration that was found to produce an acceptable test atmosphere.

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

The acute toxicity of tolpyralate *via* the inhalation route was investigated in a study conducted in accordance with OECD test guideline 436. Three male and three female Sprague-Dawley rats were exposed to tolpyralate, generated as an atmosphere (aerosol) with a concentration of 2.01 mg/L. Animals were exposed to the test atmosphere (nose only) for a period of 4 hours. There were no overt signs of clinical toxicity or unscheduled deaths. The LC_{50} of tolpyralate was concluded to be >2.01 mg/L.

10.3.2 Comparison with the CLP criteria

In accordance with the criteria on CLP, classification for acute inhalation toxicity is appropriate where the 4-hour LC_{50} is ≤ 5 mg/L (dust/mist). In the case of tolpyralate, the LC_{50} was determined to be > 2.01 mg/l, which was the maximum achieved concentration. No mortalities were observed at the maximum achieved concentration, consequently classification is not appropriate.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification (conclusive but not sufficient for classification).

10.4 Skin corrosion/irritation

The skin irritation potential of tolpyralate was investigated in a study conducted in the rabbit in accordance with OECD TG 404 and in compliance with GLP.

Table 17: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test conditions	Result	Remarks
Dermal irritation OECD 404	Rabbit New Zealand	0.5g tolpyralate 4-hour exposure	All scores (24,48,72h) = 0	There were no recorded signs of dermal

Method, guideline, deviations if any	Species, strain, sex, no/group	Test conditions	Result	Remarks
GLP Anonymous (2012d) DAR: B.6.2.4	White 3 females	Batch: 20111222-1 Purity: 97.27%		irritation.

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

The skin irritation potential of tolpyralate was investigated in a study conducted in the rabbit in accordance with OECD TG 404 and in compliance with GLP. 0.5g of tolpyralate was applied to a prepared area of skin on the dorso-lateral area of three New Zealand white rabbits for a period of 4 hours. The animals were observed for clinical signs of toxicity and signs of dermal irritation for 3 days following exposure. There were no mortalities, signs of clinical toxicity or signs of dermal irritation in any of the animals.

10.4.2 Comparison with the CLP criteria

In the study conducted with tolpyralate there were no signs of dermal irritation at any point. Consequently none of the criteria for classification were met.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification (conclusive but not sufficient for classification).

10.5 Serious eye damage/eye irritation

The eye irritation potential of tolpyralate was investigated in a study conducted in the rabbit in accordance with OECD TG 405 and in compliance with GLP.

Table 18: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test conditions	Result	Remarks
Eye irritation OECD 405; GLP Anonymous (2012e) DAR: B.6.2.5	Rabbit, New Zealand White 3 females	0.1g tolpyralate Batch: 20111222-1 Purity: 97.27%	All scores (24,48,72h) = 0	Signs of slight eye irritation were observed in the first hour following administration. All reactions had reversed by 24 hours.

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

The eye irritation potential of tolpyralate was investigated in a study conducted in the rabbit in accordance with OECD TG 405 and in compliance with GLP. 0.1g of the test item was instilled into a single eye of three New Zealand White rabbits. Ocular reactions to the test item were recorded at 1, 24, 48 and 72 hours following administration. Conjunctival redness (grade 1), chemosis (grade 1 or 2) and discharge (grade 2 or 3) were observed in 2 or more animals at the 1 hour observation. However, all ocular reactions to the test item were resolved by the 24 hour observation and no further signs of eye irritation were recorded for the

remainder of the study. Mean scores for all measured parameters, calculated per animal and as the mean of scores at 24, 48 and 72 hours were 0 in all instances.

10.5.2 Comparison with the CLP criteria

The mean scores for all parameters were 0 for all animals at 24, 48 and 72 hours. Therefore tolpyralate does not meet the criteria for classification for eye damage/ irritation.

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

No classification (conclusive but not sufficient for classification).

10.6 Respiratory sensitisation

There are no specific data available relating to the respiratory sensitisation potential of tolpyralate.

10.6.1 Conclusion on classification and labelling for respiratory sensitisation

Because of the lack of data, a definitive conclusion on respiratory sensitisation cannot be made.

10.7 Skin sensitisation

The skin sensitisation potential of tolpyralate was investigated in both a local lymph node assay (LLNA) and a Guinea pig maximisation test (GPMT), both conducted to appropriate test guidelines and in compliance with GLP.

Table 19: Summary table of animal studies on skin sensitisation

Method, guideline	Species, strain, sex, no/group	Test conditions	Results
Local lymph node assay (LLNA) OECD 429 GLP Anonymous (2013b) DAR: B.2.6.2	Mouse, CBA/J, 5 females/group	0, 10, 25, 50% w/v tolpyralate in *N*,*N*-dimethylformamide Batch: 20111222-1 Purity: 97.27% Positive control : 25% α-hexylcinnamaldehyde (HCA)	There was no evidence of a potential for tolpyralate to initiate skin sensitisation. Stimulation indices; Vehicle control = 0 10% tolpyralate = 0.5 25% tolpyralate = 1.1 50% tolpyralate = 1.0 25% HCA = 9.2
Guinea pig maximisation assay OECD 406 GLP Anonymous (2012) DAR: B.2.6.2	Guinea pig, Hartley 5 females/ control group & 10 females/test group	Intra-dermal induction: 10% in liquid paraffin Topical induction: 50% in acetone Challenge: 50% in acetone Batch: 20111222-1 Purity: 97.27%	No evidence of skin sensitisation potential

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

The skin sensitisation potential of tolpyralate has been investigated in both a local lymph node assay (LLNA) and a Guinea pig maximisation test, both were conducted to appropriate test guidelines and in compliance with GLP.

Local Lymph Node Assay (LLNA)

In the LLNA, female CBA/J Mice (five per group) were each given three consecutive daily topical applications to the dorsal surface of both ears of 0, 10, 25 or 50% w/v tolpyralate in *N,N*-dimethylformamide. The proliferative response of the lymph node cells from the draining auricular lymph nodes was assessed five days after the first application by measuring the incorporation of ^{3}H-methyl thymidine in suspensions of the lymph node cells. The ratio of ^{3}H-methyl thymidine incorporation into the nodes from treated mice to that from controls was calculated (the Stimulation Index).

There were no significant differences in mean lymph node weights between the tolpyralate treatment groups and the vehicle control group. In the positive control group, there was a significant increase in mean lymph node weight compared to the vehicle control group.

Stimulation indices (SIs) were 0.5, 1.1 and 1.0 in the 10, 25 and 50% w/v tolpyralate treatment groups, respectively. There were no significant differences in mean cellular proliferation activities between the tolpyralate treatment groups and the vehicle control group. There was a significant increase in mean cellular proliferation activity in the positive control group with an SI value of 9.2.

Guinea Pig Maximisation Test (GPMT)

In the GPMT, groups of 10 test and 5 control animals were used. Intradermal induction was performed at a concentration of 10% w/v in paraffin, topical induction and challenge were performed with 50% w/v in acetone. As the test item was found to be not-irritating at 50%, dermal irritation was induced with application of 10% sodium lauryl sulphate (SLS). Observation for skin reactions was performed at 24 and 48 hours after challenge. No skin reactions were observed on the site challenged with tolpyralate in either the test substance sensitisation group or the control group. A concurrent positive control group was not included for this study, but laboratory control data for the positive control article 1-chloro-,2,4-dinitrobenzene (DNCB) from the same the test facility (period of 11 September 2012 to 30 November 2012) was showed an acceptable response.

10.7.2 Comparison with the CLP criteria

There was no evidence of a potential for tolpyralate to induce skin sensitisation in either of the available studies. Consequently tolpyralate does not meet any of the criteria for classification.

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification (conclusive but not sufficient for classification).

10.8 Germ cell mutagenicity

The genotoxic potential of tolpyralate has been investigated in a range of studies, conducted both *in vitro* and *in vivo*. *In vitro* studies are a bacterial reverse mutation assay, a mammalian chromosomal aberration assay and mammalian gene mutation assay. *In vivo* studies are a mouse micronucleus assay and two comet assays in the rat.

Table 20: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test system (Organism, strain)	Test substance	Conc. tested (range)	Results		Remarks (information on cytotoxicity)
				–S9	+S9	
Gene mutation assay in bacteria (Ames) Pre-incubation OECD 471, GLP Wada, K. (2012) DAR: B.6.4.1	*S.typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; *E.coli* strain WP2 uvrA	tolpyralate Batch : 20111222-1 Purity: 97.27%	Up to 5000 µg/plate	Neg	Neg	Study consisted of a range finding assay and two main assays. No evidence of cytotoxicty.
Chromosome aberration test OECD 473, GLP Matsumoto, K. (2012a) DAR: B.6.4.1	Chinese hamster lung cells CHL/IU	tolpyralate Batch : 20111222-1 Purity: 97.27%	6 hour 200-1200 µg/mL	Neg	Neg	
			24 hour 75-300 µg/mL	Neg	Neg	
			48 hour 50-200 µg/mL	Neg	Neg	
Gene mutation test OECD 476, GLP Matsumoto, K. (2012b) DAR: B.6.4.1	Mouse lymphoma cells L5178Y TK$^{+/-}$	tolpyralate Batch : 20111222-1 Purity: 97.27%	3 hour 78 – 1250 µg/mL	Pos	Pos	Precipitation at 625 and 1250 µg/mL without metabolic activation and at 1250 µg/mL with metabolic activation.
			24 hour 39 – 1250 µg/mL	Pos	N/A	Precipitation at 625 and 1250 µg/mL.

The genotoxic potential of tolpyralate has been investigated in 3 *in vitro* studies, all conducted in accordance with appropriate OECD test guidelines and in compliance with GLP. In a reverse mutation assay (Ames test) and in an *in vitro* chromosome aberration assay there was no evidence of genotoxicity under any of the tested conditions.

In a mammalian cell gene mutation test, there was evidence of genotoxicity in both the three hour test (in the presence and absence of metabolic activation), and in the 24 hour test. As shown below, in both the 3 hour assay and in the 24 hour assay, there were clear and statistically significant increases in mutant frequency, and a clear dose-response relationship. The findings of this study are summarised in Table 21. Clearly tolpyralate induced gene mutations in this assay. There was a slight increase in the ratio of small colonies with increasing exposure to tolpyralate, which, in some instances might indicate the potential of the test substance to induce structural damage to chromosomes. However, given the negative result seen in a chromosome aberration test, this seems unlikely for tolpyralate.

Table 21: Results of the mammalian cell gene mutation assay

Exposure time	S9	Treatment	Concentration (µg/mL)	Relative survival at Day 0	Relative total growth	Mutant frequency (× 10^{-6})	Ratio of small colonies

				(%)	(%)		(%)
3 hours	-	Solvent control (DMSO)	1%	100	100	134.3	33
		tolpyralate	78.1	106	102	141.7	34
			156	108	102	125.1	28
			313	102	101	145.3	30
			625 (a)	103	86	163.9	40
			1250 (b)	70	43	212.3*	47
3 hours	+	Solvent control (DMSO)	1%	100	100	166.9	31
		tolpyralate	78.1	97	112	152.5	23
			156	94	99	155.1	27
			313	87	93	162.3	36
			625	65	52	347.1***	32
			1250 (b)	24	14	457.2***	38
24 hours	-	Solvent control (DMSO)	1%	100	100	71.8	32
		tolpyralate	39.1	83	99	84.7	26
			78.1	74	108	101.1	39
			156	73	95	125.6	40
			313	73	76	179.7*	43
			625 (a)	60	50	420.0***	49
			1250 (b)	15	9	445.1***	50

DMSO Dimethyl sulfoxide

MMS Methyl methanesulfonate

(a) Precipitation of the test substance was observed at the end of the treatment

(b) Precipitation of the test substance was observed at the beginning and end of the treatment

Mutant frequency data were analysed by Dunnett's test

Significantly different from the solvent control: * $p \leq 0.05$; *** $p \leq 0.001$

Table 22: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance	Dose levels and sampling times	Results
Micronucleus test OECD 474, GLP Anonymous (2012g)	Mouse, CD1 5 males/group	tolpyralate Batch : 20111222-1 Purity:	24 hour : 0, 500,1000, 2000 mg/kg bw, single oral (gavage) dose	Negative No increases in mean frequency of micronucleated polychromatic erythrocytes or PCE to NCE ratio under any tested conditions.

Method, guideline, deviations if any	Species, strain, sex, No/group	Test substance	Dose levels and sampling times	Results
DAR B.6.4.2		97.27%	48 hour : 2000 mg/kg bw, single oral (gavage) dose	
Comet Assay Liver and stomach OECD 489, GLP Anonymous (2014c) DAR B.6.4.2	Rat, Wistar 6 males/group	tolpyralate Batch : 20111222-1 Purity: 97.27%	0, 500, 1000, 2000 mg/kg bw, two oral (gavage) doses Positive control – 200 mg/kg bw EMS*	Negative No increase in % DNA tail under any tested conditions.
Comet Assay Liver, thyroid and stomach OECD 489, GLP Anonymous (2015a) DAR B.6.4.2	Rat, Wistar 5 males/group	tolpyralate Batch : 20111222-1 Purity: 97.27%	0, 500, 1000, 2000 mg/kg bw, two oral (gavage) doses Positive control – 200 mg/kg bw EMS*	Negative No increase in % DNA tail under any tested conditions.

*EMS – ethyl methanesulfonate

The genotoxic potential of tolpyralate was investigated *in vivo* in 3 separate studies: a mouse micronucleus assay and two *in vivo* comet assays in the rat.

In the micronucleus assay, groups of 5 male CD1 mice were administered a single dose of tolpyralate by oral gavage, at dose levels of 0, 500, 1000 or 2000 mg/kg bw. Animals were sacrificed at 24 hours following dose administration. Under the conditions of this study there were no increases in either the mean frequency of micronucleated polychromatic erythrocytes or the PCE to NCE ratio in the bone marrow. A separate group of mice were administered a single dose at only 2000 mg/kg bw and were sacrificed at 48 hours. There is appropriate information available from the ADME studies conducted with tolpyralate to indicate that the bone marrow is sufficiently exposed to the test item following oral administration, with levels of radioactivity in the bone marrow being in the same order as multiple other tissues that are not associated with primary digestive / excretive organs, such as the lung, pancreas and thyroid. Overall, it is concluded that the result of this test was negative.

Two comet assays were conducted: one investigating the genotoxicity in the liver and stomach, and a second study in the liver, stomach and thyroid. In both studies the highest dose level was 2000 mg/kg bw, and the test item was administered by oral gavage on two consecutive days. In both studies animals were sacrificed 3 hours after the final dose, based on the available information, this is after the t_{max} (0.5-2hours) and so maximum systemic concentration will have been achieved. There was no significant increase in %DNA tail or any other indication of a genotoxic effect of the test item in either study.

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

The genotoxic potential of tolpyralate was investigated in a range of studies conducted both *in vitro* and *in vivo*. In a bacterial reverse mutation (Ames) test and an *in vitro* chromosomal aberration assay, there was no evidence of genotoxicity under any of the tested conditions. In a mammalian cell gene mutation assay, there

was clear evidence of a positive, reproducible and dose related increase in mutant fraction both with and without metabolic activation.

With respect to *in vivo* studies, there was no evidence of genotoxicity in the micronucleus assay under any of the tested conditions. Additionally, two *in vivo* comet assays are available, investigating genotoxic potential in the stomach and liver and in the stomach, liver and thyroid respectively. There was no evidence of genotoxicity in either study. These 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.

10.8.2 Comparison with the CLP criteria

The available information indicates that tolpyralate is not genotoxic.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification (conclusive but not sufficient for classification).

10.9 Carcinogenicity

The carcinogenic potential of tolpyralate was investigated in two carcinogenicity/chronic toxicity studies; one in the rat and one in the mouse.

Table 23: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method
Guideline, Deviation(s) from the guideline (if any)</th><th>Species
Strain
Sex
no/group</th><th>Dose levels
duration of exposure</th><th>Results</th></tr>
<tr><td>Two-year carcinogenicity study
Oral (dietary)
OECD 451
GLP
Batch: 2011222-1
Purity: 97.27%
Anonymous (2015b)
DAR B.6.5</td><td>Rat, Wistar

51/sex/group</td><td>104 weeks
0, 5, 20, 2000, 10000 ppm
Equivalent intake at 104 weeks:
Males: 0, 0.196, 0.765, 83.8, 426 mg/kg bw/d
Females: 0, 0.255, 1.01, 108, 554 mg/kg bw/d</td><td><u>General toxicity</u>
<table>
<tr><th rowspan="2">Dose level
(mg/kg bw/d)</th><th colspan="2">Survival (%)</th></tr>
<tr><th>Males</th><th>Females</th></tr>
<tr><td>0/0</td><td>75</td><td>69</td></tr>
<tr><td>0.196/0.255</td><td>75</td><td>65</td></tr>
<tr><td>0.765/1.01</td><td>78</td><td>71</td></tr>
<tr><td>83.8/108</td><td>71</td><td>71</td></tr>
<tr><td>426/554</td><td>75</td><td>59</td></tr>
</table>
No overt or serious clinical signs of toxicity. Decreased body weight, relative to controls (<20%) in mid-high and high dose groups.
<u>Neoplastic effects</u>
<table>
<tr><th rowspan="2"></th><th colspan="5">dose level (ppm)</th></tr>
<tr><th>0</th><th>5</th><th>20</th><th>2000</th><th>10000</th></tr>
<tr><td>Malignant squamous cell carcinoma in the eye in males
(Total incidence)</td><td>0/51</td><td>0/51</td><td>0/51</td><td>3/51</td><td>5/51*</td></tr>
</table>
*, $p \leq 0.05$; (by Fisher's exact probability test).
There was no incidence of this effect in females and in any of the historical control data.</td></tr>
</table>

<table>
<tr><th>Method
Guideline,
Deviation(s) from the guideline (if any)</th><th>Species
Strain
Sex
no/group</th><th>Dose levels
duration of exposure</th><th>Results</th></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td>18-month carcinogenicity
Oral (dietary)
OECD 451
GLP
Anonymous (2015c)
DAR B.6.5</td><td>Mouse,
CD1
52/sex/group</td><td>78 weeks
0, 70, 700, 7000 ppm

Equivalent intake at 78 weeks:
Males: 0, 7.37, 78.5, 793 mg/kg bw/d
Females: 0, 7.25, 72.6, 732 mg/kg bw/d</td><td>Survival at 78 weeks
<table>
<tr><th rowspan="2">Dose level
(mg/kg bw/d)</th><th colspan="2">Survival (%)</th></tr>
<tr><th>Males</th><th>Females</th></tr>
<tr><td>0/0</td><td>67</td><td>77</td></tr>
<tr><td>7.37/7.25</td><td>62</td><td>71</td></tr>
<tr><td>78.5/72.6</td><td>65</td><td>62</td></tr>
<tr><td>793/732</td><td>60</td><td>73</td></tr>
</table>
No overt clinical signs of toxicity or increase in cumulative mortality at 78 weeks. Small decreases in body weight, relative to controls were observed in males, but were < 10%.
<u>Neoplastic effects</u>
No relevant increases in tumour incidence in any groups.</td></tr>
</table>

10.9.1 Chronic / carcinogenicity study in rats

The carcinogenic potential of tolpyralate was investigated in a 2-year dietary study performed in Wistar rats. Four groups (51/sex/group) were administered tolpyralate in the diet at concentrations of 5, 20, 2000 or 10000ppm for a period of 104 weeks. A concurrent control group was administered the basal diet under the same conditions. Mean achieved dosages during the 104-week treatment period were 0.196, 0.765, 83.8 and 426 mg/kg/day for males and 0.255, 1.01, 108 and 554 mg/kg/day for females receiving 5, 20, 2000 and 10000 ppm respectively.

Non-neoplastic findings

There were no severe or overt clinical signs of toxicity in any of the treated groups. Survival was ≥59% in all treated groups. There were small reductions in body weight when compared to control animals in both sexes at the top two dose levels; these were greater than 10% only in males, and less than 20% in all cases.Non neoplastic findings are summarised in section 10.12

Neoplastic findings

Eye

A small increase in the incidence of malignant squamous cell carcinoma in the eye was observed in males at 83.8 and 426 mg/kg bw/d. This effect was statistically significant at the top dose. As there were no such tumours seen in the control, low and lower mid dose males, and none were evident in the laboratory's historical control data set, these tumours are considered to be related to treatment. This observation is consistent with macroscopic and histopathological findings in the eye.

Thyroid

A slight increase in the incidence of benign follicular cell adenoma in the thyroid was observed in males at the highest tested dose (incidences of 3, 0, 3, 1 and 5 at 0, 5, 20, 2000 and 10000 ppm respectively), occurring without statistical significance. Historical control data from the same laboratory, from 9 studies conducted between 2007 and 2013, shows a mean incidence of 3.92% (range 0/51 -6/51) of this finding in males. Noting the lack of statistical significance and dose-response relationship and the historical control

data, the observation of benign follicular cell adenoma in the thyroid is not considered to be a treatment related finding.

10.9.2 Chronic / carcinogenicity study in mice

The carcinogenic potential of tolpyralate was investigated in a 78 week study conducted in the mouse. Groups of 52 CD1 mice of each sex were administered the test item (tolpyralate, batch 20111222-1, purity 97.27 %) in the diet at concentrations of 70, 700 and 7000 ppm, equivalent to 7.37, 78.5 and 793 mg/kg bw/day in males and 7.25, 72.6 and 732 mg/kg bw/day in females.

Observed clinical signs of toxicity which are attributable to administration of the test item were increased incidence in loss of fur and tactile hair in both sexes and small (less than 10%) reductions in body weight in males. Non-neoplastic findings are summarized in section 10.12. There were no increases in either benign or malignant tumors in any treated groups. Changes in the number of tumours were limited to sporadic decreases which occurred without a dose response relationship. Consequently, it was concluded that tolpyralate did not produce a carcinogenic response in this study.

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

The carcinogenic potential of tolpyralate has been investigated in a rat and a mouse carcinogenicity study (rat, 2 years duration; mouse, 18 months). In both studies the test item was well tolerated, with minimal clinical signs of toxicity other than small decreases in body weights. There were no relevant increases in mortality and survival at study termination was acceptable (>50%) in both studies. As such these studies provide for a valid assessment of the carcinogenicity of tolpyralate.

In the mice, there were no relevant increases in the incidence of tumours in any of the treated groups. Similarly, there were no significant tumour findings in female rats.

In male rats there was an increase in the incidence of squamous cell carcinoma in the cornea affecting 3 and 5 males (6% and 10% of animals) at the mid (83.8 mg/kg bw/d) and high (426 mg/kg bw/d) doses respectively. This type of tumour was not observed in any other rats in the study, and was not evident in historical data from the laboratory that performed the study. Therefore, the increase in corneal squamous cell carcinoma is considered to be related to treatment with tolpyralate.

The Applicant proposed a mode of action for the rat corneal tumours involving the following key stages:

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

The effect of 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibition on tyrosine metabolism is discussed at the beginning of Section 10.

As discussed in Section 10.12.1 (repeated dose toxicity), a single dose (1000 mg/kg bw) of tolpyralate produced a significant increase in plasma tyrosine levels in rats, rabbits and mice (Anonymous 2013o; Anonymous 2013p; Anonymous 2013q). The increase was the highest in rats (25-fold), followed by rabbits (20-fold) and then mice (13-fold). These results indicate that rats might be more sensitive to tolpyralate-induced tyrosinemia compared to mice, although it is noted that plasma tyrosine levels were similar to controls after 72 hours in the rat, but were still elevated in mice at this time point. Repeated daily administration of tolpyralate in the diet to female Wistar rats (144 mg/kg bw) and female CD1 mice (1089 mg/kg bw) produced a significant increase in terminal plasma concentrations of tyrosine (Anonymous, 2016a). Although no male rats were included in the repeated dose study, a single gavage dose of tolpyralate (100 or 1000 mg/kg bw) was sufficient to produce a comparable significant increase in male Wistar rat plasma tyrosine levels (Anonymous, 2016b). This study also demonstrated significant increases in tyrosine concentrations in the aqueous humour of the eye following a single dose of tolpyralate; indeed, tyrosine

concentrations were higher in the aqueous humour than in the plasma at all dose levels (1, 100 and 1000 mg/kg bw). No information was gained on the reversibility of these effects. No direct evidence that tolpyralate inhibits HPPD in rats, mice or humans is available.

Keratitis was observed in male and female rats in the 28-day, 90-day, 1-year (Anonymous 2016a) and 2-year carcinogenicity studies. Keratitis was also observed in the parents (both P and F1) in the 2-generation study, whilst corneal opacity was recorded in the F1 and F2 offspring. There were no adverse effects in the eyes of mice or dogs treated with tolpyralate. The non-neoplastic eye effects are discussed further in Section 10.12.1.

Such effects in the eyes are reported to be common to substances that inhibit the metabolism of tyrosine and consequently cause tyrosinemia (Antonenko *et al.*, 2015). Therefore, from the information available, it is plausible that the effects in the eye are related to the increase in plasma tyrosine concentrations which are, in turn, due to the inhibitory action of tolpyralate on 4-HPPD.

Chronic keratitis is described as the persistent inflammation of the cornea. It is possible that this persistent inflammatory response may be the cause of the tumorigenesis observed in male rats following life-time exposure to tolpyralate. Eye tumours have been reported in the rat in studies conducted with some other HPPD inhibitors (e.g., tembotrione). However, there are a number of other related substances (e.g., sulcotrione and mesotroine) in which such findings were not reported, even though there was also a high level of keratitis. In addition, the tumours were observed in males only despite comparable levels of keratitis in females. Consequently, although the mode of action is considered plausible, there is insufficient information available to conclude that the corneal tumours were definitively induced as a result of tissue damage and regenerative hyperplasia leading on from elevated tyrosine levels. This uncertainty should be reflected in the classification.

It is widely documented that the rat is particularly sensitive to the effects of 4-HPPD inhibition and it is generally accepted that humans are less sensitive owing to species differences in alternative metabolic pathways. However, humans who have chronically elevated plasma tyrosine levels (through 4-HPPD inhibition) are known to exhibit ocular effects. NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) is a pharmaceutical drug used to inhibit HPPD. In healthy adult volunteers, a single dose of 1 mg/kg bw of NTBC has been shown to significantly increase plasma tyrosine levels (from ~100 to 1100 µmol/L) (Hall *et al.*, 2001). Patients treated with this drug (i.e., repeated exposure) have been shown to have greatly elevated plasma tyrosine concentrations which can result in adverse effects in the eyes (Ahmad *et al.*, 2002; Lock *et al*., 2006; Wisse *et al.*, 2012, Schauwvlieghe *et al*, 2013). Given the proposed mode of action of tolpyralate, it is possible that tolpyralate could cause adverse eye effects in humans. There is a lack of information on the relative sensitivities of rats and humans to tolpyralate.

Overall, although a plausible mode of action involving inhibition of tyrosine catabolism has been proposed, uncertainties remain in understanding the basis for the ocular tumours seen in male rats treated with tolpyralate. Further, even if this mode of action is applicable, further uncertainties remain about its relevance to humans. There is limited evidence for the carcinogenicity of tolpyralate in rats.

10.9.4 Comparison with the CLP criteria

As there is no information on the carcinogenicity of tolpyralate in humans, a classification in Category 1A would not be appropriate.

There was an increased incidence of squamous cell carcinoma in the cornea of male rats treated with tolpyralate in the diet. No tumours were seen in female rats or in mice. Whilst it is plausible that the tumours may have been a consequence of chronic keratitis (which likely resulted from HPPD inhibition and sustained elevation of plasma tyrosine), it is notable that they were only observed in males and not females despite a comparable level of keratitis in both sexes. Further, whilst humans might be expected to be less sensitive to the effects of HPPD inhibition, there is no definitive information regarding the relative potency of tolpyralate compared to other known HPPD inhibitors (e.g., NTBC) and no definitive information on the relative sensitivity of humans to this carcinogenic effect.

In accordance with the CLP criteria and associated guidance, the following factors are considered in proposing how to classify tolpyralate for carcinogenicity.

Table 24: Factors considered when assessing tolpyralate for carcinogenicity.

Factor	Evidence	Classification
Tumour type and background incidence	Ocular tumours, very rare	1B or 2
Multi-site response	No, only eyes	2
Progression of lesions to malignancy	Yes	1B or 2
Reduced tumour latency	No data	1B or 2
Whether responses are in single or both sexes	Males only	2
Whether responses are in a single species or several species	Rat only (mouse negative)	2
Structural similarity to a substance for which there is good evidence of carcinogenicity	No	No impact
Routes of exposure	Tumours after dietary treatment; no significant information on applicability of other routes	No impact
Comparison of absorption, distribution, metabolism and excretion between test animals and humans	Limited data; no differences expected.	No impact
The possibility of a confounding effect of excessive toxicity at test doses	Tumours were evident at doses observed to cause ocular opacity and keratitis. No evidence that tumours can occur at doses that don't produce these lesions.	2 or 0
Mode of action and its relevance to humans	Non-mutagenic. Possible mode of action involving production of excessive levels of tyrosine, tissue damage, regenerative hyperplasia and associated increased DNA replication. Significant uncertainty about this mode of action for tolpyralate and about its relevance to humans	2

Taking into account all of these factors, the observation of ocular tumours in male rats is considered to present limited evidence of carcinogenicity and the criteria for classification in Category 2 appear to be met.

10.9.5 Conclusion on classification and labelling for carcinogenicity

Classification in Category 2 - Suspected human carcinogen

10.10 Reproductive toxicity

The potential for tolpyralate to adversely affect reproductive performance and the survival, growth and development of offspring has been investigated in a two generation dietary reproductive toxicity study performed in the rat. The potential for tolpyralate to cause developmental toxicity has been investigated in specific developmental toxicity studies conducted in both the rat and the rabbit. In addition a generational range finding study in the rat is available.

10.10.1 Adverse effects on sexual function and fertility

Table 25: Summary table of animal studies on adverse effects on sexual function and fertility

Study details	Dose levels	Critical effects
Rat, SD 8 / sex / group GLP tolpyralate Batch: 20111222-1 Purity 97.27% (range finding study) Anonymous (2015d) DAR B.6.6.1	0, 5, 20, 200, 2000, 20000 ppm Equivalent to; *Males:* 0, 0.257, 1.04, 10.7, 107, 1093 mg/kg bw/d Females 0, 0.503, 1.94, 19.6, 193, 1943 mg/kg bw/d Test item administered from the beginning of rearing until the weaning of F1 pups (Approx. 11 weeks) NOAELs were not determined from this range finding study	**Parental toxicity** <u>5ppm & 20 ppm</u> - No adverse effects <u>200ppm</u> Ocular opacity in a single animal in each sex (clinical observation only, not noted at necropsy) Reduced body weight gain in males (13%) Increased relative liver weight in both sexes (male 16%, female 12%) <u>2000ppm</u> Ocular opacity in both sexes Reduced body weight gain in males (14%) Increased relative liver weight in both sexes (male 17%, female 7%) Increased relative kidney weight in both sexes (male 12%, female 9%) <u>20000ppm</u> Ocular opacity in both sexes Reduced body weight gain in males (20%) and females (23%) Increased relative liver weight in males (19%) and females (15%) Increased relative kidney weight in males (12%) and females (19%) **Offspring toxicity** <u>*Lactation day 4*</u> <u>5, 20 and 200 ppm</u> No adverse effects <u>2000ppm</u> Reduced pup viability at day 4 (9% lower than controls) Pelvic dilatation (18.7% incidence) Pale-coloured kidney (9.8% incidence) <u>20000ppm</u> Reduced pup viability at day 4 (19% lower than controls) Reduced pup weight (19% males, 18% females) Pelvic dilatation (14.3% incidence) Pale-coloured kidney (14.3% incidence) White material in urinary bladder lumen (4.8% incidence) <u>*Day 26*</u> <u>5ppm and 20 ppm</u> - No adverse effects

Study details	Dose levels	Critical effects
		200ppm Kidney cysts (15.6% incidence) and pelvic dilatation (6.3% incidence) 2000ppm Kidney atrophy (16.1% incidence), kidney spots (8.9%) kidney cysts (21.4%) and pelvic dilatation (5.4%) 20000ppm Ocular opacity (18.8% incidence), kidney atrophy (4.2%), kidney spots (2.1%), kidney cysts (8.3%), and pelvic dilatation (15.2%). Kidney absent (2.1% incidence)
Rat, SD 24 / sex / group OECD 416 GLP tolpyralate Batch: 20111222-1 Purity 97.27% (main study) Anonymous (2015e) DAR B.6.6.1	0, 5, 50, 1000ppm Equivalent to; *Males:* 0, 0.270, 2.70, 54.9 mg/kg bw/d Females 0, 0.287, 2.97, 59.3 mg/kg bw/d	**Parental toxicity** P generation 5ppm No adverse effects 50ppm Increased relative liver weight (8% in both sexes) Ocular keratitis (1/24 males) 1000ppm Increased incidence of gross eye findings; Ocular opacity (18/24 males, 11/24 females) Increased incidence of microscopic eye findings; Keratitis (21/24 males, 23/24 females) Increased relative organ weights; Liver (16% males, 13% females). Kidney (13% males) Testis (10%) Decreased relative organ weights; Brain (6% females) F_1 generation 5ppm Increased incidence of microscopic findings; Dilatation, renal pelvis (3/24 females) 50ppm Increased incidence of gross kidney findings; Pelvic dilatation (3/24 males) Increased incidence of microscopic eye and kidney findings; Ocular keratitis (1/24 males) Dilatation, renal pelvis (3/24 males, 1/24 females) 1000ppm Decreased terminal body weight in males (10%). Increased incidence of gross eye findings; Ocular opacity (24/24 males, 12/24 females) Increased incidence of gross kidney findings; Coarse surface (2/24 males, 1/24 females) Cyst(s) (5/24 males, 1/24 females) Depressed area (4/24 males, 5/24 females) Pale in colour (1/24 males) Pelvic dilatation (3/24 males, 3/24 females) Decreased relative organ weights; Prostate (21%) Increased relative organ weights; Liver (22% males, 13% females)

Study details	Dose levels	Critical effects
		Kidney (16% males, 7% females) Testis (12%) Epididymides (13%) Seminal vesicle (19%) Increased incidence of microscopic kidney findings; Basophilic change to renal tubule (10/24 males, 4/24 females) Calcification (7/24 males, 8/24 females) Cortical cysts (6/24 males) Hyaline droplets (5/24 males) Nephropathy (11/24 males, 4/24 females) Dilatation, renal pelvis (2/24 males, 5/24 females) Increased incidence of microscopic eye findings; Keratitis (24/24 males, 24/24 females)
		Fertility and reproductive function
		5ppm and 50 ppm No adverse effects 1000ppm A small but statistically significant increase in duration of gestation was observed in F1 parents (22.7 days, compared to 22.2 days in controls). Slight reduction in total number of pups delivered and mean number of pups per litter in the F2 generation.
		Offspring toxicity
		F_1 generation 5ppm and 50 ppm No adverse effects 1000ppm Reduced pup weight (10%) (Lactation Days 14 and 21). *Day 4 necropsy* Kidney: Pale colour (3.3%) Kidney: Pelvic dilation (6.1%) Kidney: White material in pelvic space/papilla (3.2%) *Day 26 necropsy* Eye opacity (1.9%) Kidney: small in size (4.5%) Kidney: spots (2.4%) Kidney Cysts (12.8%) Kidney: Pelvic dilation (6.1%)
		F_2 generation 5ppm and 50 ppm No adverse effects 1000ppm Reduced pup weight (<10%) (Lactation Days 14 and 21). *Day 4 necropsy* Kidney: Pale colour (1.7%) Kidney: Pelvic dilation (3.6%) Kidney: White material in pelvic space/papilla (3.9%) *Day 26 necropsy* Eye opacity (5.6%) Kidney: small in size (1.9%) Kidney Cysts (11.3%)

*The values for the NOAELs are provided for information only. All NOAEL values in this CLH reports have been taken from the dAR, produced in accordance with Regulation EC 1107/2009.

Range finding investigation for reproductive toxicity study - Anonymous 2015d

A range-finding study was performed in the rat in order to determine appropriate dose levels for a subsequent two generation reproductive toxicity study. Groups of 8 Crl:CD(SD) rats /sex / dose were administered dietary concentrations of 0, 5, 20, 200, 2000 or 20000 ppm tolpyralate.

The mean test substance intakes (mg/kg/day) in each treated group throughout the growth and breeding periods were 0.257 and 0.503 in the 5 ppm group, 1.04 and 1.94 in the 20 ppm group, 10.7 and 19.6 in the 200 ppm group, 107 and 193 in the 2000 ppm group, and 1093 and 1943 in the 20000 ppm group for males and females, respectively.

Parental effects

No deaths occurred in any animals in the parental generation. Ocular opacity was noted in clinical observations among male rats at 200 ppm or greater from treatment week 5 (during the breeding period). The incidence of observed ocular opacity in males was 1, 3 and 2 out of 8 rats in the 200, 2000 and 20000 ppm groups, respectively, although no statistically significant differences were seen between the control and any of these groups. No other clinical signs of toxicity were found in males in any treated groups at any time point.

There were no clinical signs of toxicity in females in any treated groups during the pre-mating growth through mating and gestation periods. During the lactation period, Ocular opacity was noted at clinical observation in 1, 5 and 8 out of 8 rats in the 200, 2000 and 20000 ppm groups, respectively. Ocular opacity was not observed in the control, 5 or 20 ppm group during this period. The difference in incidence between the control and treated groups reached statistical significance for the two higher dose groups. There were no other clinical signs of toxicity during the lactation period.

Reductions in body weight (not statistically significant) were observed in top dose males from weeks 1-10. In the 200, 2000 and 20000 ppm groups, the body weight gains of males were statistically significantly lower than those in the control group during treatment weeks 0-1 through 0-5, in general. There were no statistically significant changes in body weight gains of males in the 5 or 20 ppm group at any time point.

Female body weights were generally comparable among all groups during the pre-mating growth and gestation periods. However, during the lactation period, females in the 20000 ppm group weighed less than those in the control group, with the differences being statistically significant on lactation days 4 and 14. There was also a trend toward lower body weights in the 2000 ppm group on lactation day 4. No other changes in body weights were found in lactating females in any treated groups. There were no changes in body weight gains during the pre-mating growth and gestation periods. However, at 20000 ppm, female body weight gain was statistically significantly lower than in controls during lactation days 0-4 through 0-14. A similar decrease in body weight gain was seen at 2000 ppm group, although statistically significant differences were limited to the change during lactation days 0-4. The body weight gains of females in the other treated groups were unchanged at any time point.

The food consumption of males was generally comparable among all groups throughout the study. The food consumption of females was also similar among all groups during the pre-mating growth period and gestation period. However, a decrease in food consumption was observed in females in the 2000 and 20000 ppm groups during the lactation period, consistent with the decreased body weight gains. These changes were statistically significant for the 20000 ppm group during lactation days 0-7 and 7-14. There were no apparent changes in food consumption of females in the other treated groups.

At necropsy, ocular opacity was recorded in the gross observations in both sexes at 2000 and 20000ppm, occurring in 2 and 2 males, and 2 and 8 females at 2000 and 20000ppm, respectively.

In the 200, 2000 and 20000 ppm groups, the absolute and relative weights of liver and kidneys were generally increased in both sexes when compared to controls. These differences reached statistical significance in the relative liver weights for males at 200, 2000 and 20000 ppm, and the relative kidney

weights for females in the 20000 ppm group. No other statistically significant treatment-related changes in organ weights were noted at 200, 2000 or 20000 ppm. There were no treatment-related changes in organ weights in either sex in the 5 or 20 ppm group.

There were no treatment related effects on any reproductive parameters, in any treated groups, when compared to the control group.

Offspring effects

There were no treatment-related effects on number of pups delivered or sex ratio. There was no notable difference in pup death at lactation day (LD) 0 in any treated group when compared to controls. As summarised in the following table, on lactation days 1-4 clear signs of toxicity were observed in offspring at dose levels of 2000 ppm or more. Observed effects included increased incidence of pup loss, decreased viability index, decreased body weights, and an increased incidence of renal abnormalities. Additionally an increased incidence of opacity in the eye was observed at the highest dose level.

Table 26: Litter data (range finding study)

*7 Litters examined as 1 female was not pregnant

Lactation day(s)	Findings	Dietary level (ppm)					
		0	5	**20**	**200**	**2000***	**20000***
0	Total number of pups delivered	114	105	117	113	102	94
	Number of live pups	113	104	116	111	101	91
	Total number of dead pups	1	1	1	2	1	3
	Percent viable pups	99.1	99.0	99.1	98.2	99.0	96.8
	Number of litters with dead pups	1	1	1	2	1	3
	Dead pups per litter (no. litters affected)	1(1)	1(1)	1(1)	2(1)	1(1)	1(3)
1-4	Number of live pups	112	104	108	110	91	73
	Pups dead/lost	1	0	8	1	10	18
	Percent viable pups (relative to day 0)	99.1	100	93.1	99.0	90.1	80.2
	Dead pups per litter (no. 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)

At 20000 ppm, male and female pups weighed consistently less than controls throughout the lactation period. These differences achieved statistical significance on lactation days 7-21 for males, and on lactation days 14 and 21 for females. Likewise, pups in the 200 and 2000 ppm groups also gained less weight than controls, although the differences did not reach statistical significance. There were no differences in body weights in the 5 or 20 ppm groups.

Necropsy of pups culled on lactation day 4 revealed renal and/or urinary tract abnormalities in the 2000 and 20000 ppm groups; these included pale-coloured kidney, pelvic dilatation, and white material in pelvic space/urinary bladder lumen. One pup in the 20 ppm group exhibited pelvic dilatation in the kidney. The major findings at necropsy of weanlings at day 26 (including those found dead) were opacity of the eye and several renal abnormalities. For the ocular lesion, 18.8% of weanlings exhibited opacity of the eye in the 20000 ppm group, compared to 0% in the control and other treated groups. Several weanlings in the 2000 and 20000 ppm groups showed renal atrophy and/or spots on the kidney surface, with the incidence ranging from 2.1% to 16.1%. No similar findings were seen in the control or other treated groups. There also was a slight increase in the litter incidences of other renal findings, such as cysts and pelvic dilatation, in the 200,

2000 and 20000 ppm groups (8.3-21.4% and 5.4-15.2% for cysts and pelvic dilatation, respectively). 3.1% and 1.6% of control weanlings exhibited cysts and pelvic dilatation, respectively.

In the 2000 and 20000 ppm groups, there was a trend towards lower absolute weights of brain, spleen and thymus relative to the control group, These changes were considered to be secondary to lower body weights of weanlings in these groups. No other changes in organ weights were found in 2000 or 20000 ppm group, or lower dose groups.

Two generation reproductive toxicity study – Anonymous (2015e)

The potential for tolpyralate to adversely affect fertility and reproduction was investigated in a 2-generation reproductive toxicity study in the rat. Groups of 24 male and female Sprague Dawley rats were administered 0, 5, 50 or 1000ppm tolpyralate in the diet for two successive generations. Parental (P) animals were administered the test item for 10 weeks before mating. Both P and F1 generations received the test item for a minimum of 18 weeks, throughout mating, gestation and lactation before termination and necropsy following weaning of their respective litters. Litters received the same diets as their parents following weaning.

Parental toxicity

No mortalities were observed in the P generation. Two F1 females in the 1000 ppm group were found dead or killed *in extremis* during the lactation period. These animals were noted to have particularly low body weights and exhibited reddish urine. A single F1 male in the 50 ppm group was killed *in extremis* following accidental trauma.

Body weight and body weight gain were unaffected by treatment in both sexes in the P generation at all dose levels. In F1 males receiving 1000 ppm, body weight was 16% lower than control animals at the start of treatment. Body weights in F1 males remained significantly lower than controls throughout the study at the top dose. Body weight gain was similarly reduced compared to controls in F1 males at the highest tested dose, throughout the treatment period.

In F1 females at 1000 ppm, body weights were significantly reduced by 14% when compared to controls at the start of the treatment period. Reduced body weight was observed in this group throughout the pre mating period, gestation and up to lactation day 7. Inconsistent reductions in body weight gains in F1 females were observed at this dose level. No other significant reductions in body weight or body weight gain were observed in parental animals.

The predominant finding at necropsy was ocular opacity. A statistically significant increased incidence was observed in P generation animals at 1000 ppm. The incidence of this lesion was 18/24 and 11/24 in males and females respectively, compared with none in controls and all other treated animals. In F1 animals the incidence of ocular opacity was 24/24 and 12/24 males and females receiving 1000 ppm respectively. Consistent with the observations in the P generation, no ocular opacity was observed in controls or other dose levels. F1 males and females at 1000 ppm were found to have macroscopic lesions in the kidney. These findings included coarse surface, cyst(s), depressed area, pale color and pelvic dilatation. These findings occurred at a relatively low incidence, affecting up to 5 animals per group.

In P males both absolute and relative liver weights were increased when compared to control animals at 50 or 1000 ppm. Relative liver weights were increased by approximately 8% and 16% at 50 ppm and 1000 ppm, respectively. Absolute and relative kidney weights were increased 1000 ppm. Relative kidney weights were approximately 13% higher in males receiving 1000 ppm than in controls. A statistically significant increase in relative testis weight (10% relative to controls) was recorded at the top dose level and a trend of increasing relative weight of both the epididymides and seminal vesicles was observed but occurred without statistical significance.

In P females there were statistically significant increases in absolute and relative liver weights in those animals given 50 or 1000 ppm. Relative liver weights were increased by approximately 8% and 13% at 50ppm and 1000ppm respectively. Relative brain weight was significantly decreased in top dose females by 6% when compared to control animals.

In F1 males there were statistically significant reductions in absolute brain and pituitary weights, but there were no corresponding significant changes when expressed relative to body weight. Absolute and relative prostate weights were decreased in those given 1000 ppm, by 29% and 21% respectively. Relative weights

of liver (22%), kidneys (16%) and testes (12%), epididymides (13%), seminal vesicles (19%) were significantly increased when compared to control animals at 1000 ppm. In F1 females there were statistically significant increases in relative liver (13%) and kidney (7%) weights at 1000ppm.

The predominant microscopic lesion was keratitis of the eye. Keratitis was observed in 21/24 P males, 23/24 P female, 24/24 F1 males and 24/24 F1 females in the 1000 ppm groups. Keratitis was also observed in 1/24 P males and 1/24 F1 males in the 50 ppm group. Keratitis was not observed in any animals in control or 5 ppm groups on in any females at 50 ppm.

Various microscopic alterations were noted in the kidneys of both sexes. In the P generation all of the observed kidney abnormalities occurred with similar incidence in the control and 1000 ppm groups and were concluded not to be treatment related. In the F1 generation, statistically significant increased incidences of basophilic changes to the renal tubule (males 10/24, females 4/24), calcification (males 7/24, females 8/24) and nephropathy (males 11/24, females 4/24) were recorded in both sexes at 1000 ppm. An increase in cortical cysts was also observed in 6/24 males at the top dose level, this effect occurred with a weak dose response in females but without statistical significance. Hyaline droplets were observed in 5/24 males. Dilatation of the renal pelvis was observed inconstantly in either gross or microscopic observations of F1 parental animals of both sexes receiving 5 or 50ppm tolpyralate, occurring without a clear dose response relationship but is consistent with an effect of the test item upon the kidney.

Reproductive toxicity

Mating, fertility and gestation indices were high and comparable to controls in all treated animals. There were no treatment related effects on the number of implantation sites. Gestation length was generally similar amongst all treated animals although a slight but statistically significant increase in gestation length was observed in F1 females at 1000 ppm (22.7 days at 1000ppm when compared to 22.2 days in controls). A similar trend was noted in P females at the top dose level, but did not achieve statistical significance. A statistically significant increase in the number of days until mating was observed in the P generation at 1000ppm, but was not apparent in the F1 generation, More detailed analysis of the data relating to number of days until mating showed that the range was large, and where the mean value was increased it was typically caused by a small number of animals. There was no clear evidence of a relevant increase in mean number of days until mating.

A reduction in both the total number of pups delivered and the mean number of pups per litter was observed in the F2 generation at 1000ppm.

Sex ratio was similar among the control and all treated groups with the exception of a slight decrease in sex ratio (male:total) in F1 pups at 1000 ppm. This effect was not observed in the F2 pups it is not considered to be a genuine treatment related effect.

Offspring toxicity

At the top dose, a decrease in pup viability on lactation day 0 was noted in the F1 and F2 generation. However, considering the individual litter data, it is noted that 9 of the 11 dead pups in the F2 generation were from the same litter (total litter loss), with the remaining deaths (1 pup each) in 2 separate litters. Therefore, given the inconsistencies between the findings in the F1 and F2 generations, the apparent decrease in viability is not considered to represent a treatment related effect. There were no consistent effects of pup viability on subsequent days. Litter data is summarised in the table below.

There was also a decrease in the total number of pups delivered in the F2 generation at the top dose. However, this was not statistically different to controls or other groups and it is noted that there were no similar findings (e.g. on post-implantations loss) in the rat developmental study, which employed doses of up to 500 mg/kg bw/day.

Table 27: Litter data (2 generation study in the rat)

Day 0 % viable pups = (no. of pups alive on LD 0/ no. of pups delivered) x 100

Lactation day(s)	Litter	Findings	Dietary level (ppm)			
			0	**5**	**50**	**1000**
0	F1	Total number of pups delivered	289	304	328	299
		Number of live pups	287	303	320	288
		Total number of dead pups	2	1	8	11
		Percent viable pups	99.4	99.6	97.6	96.3
		Number of litters with dead pups	2	1	3	8
		Dead pups per litter (no. litters affected)	1,1 (2)	1(1)	2,2,6 (3)	3,2,1,1,1,1, 1,1 (8)
	F2	Total number of pups delivered	304	311	303	239
		Number of live pups	301	307	297	228
		Total number of dead pups	3	4	6	11
		Percent viable pups	98.9	98.8	98.1	90.6
		Number of litters with dead pups	2	4	5	3
		Dead pups per litter (no. litters affected)	2,1 (2)	1,1,1,1 (4)	2,1,1,1,1 (5)	9,1,1 (3)
1-4	F1	Number of live pups	279	301	316	272
		Percent viable pups (relative to day 0)	97.2	99.3	98.8	94.4
	F2	Number of live pups	299	298	281	223
		Percent viable pups (relative to day 0)	99.3	97.1	94.6	97.8

Day 1-4 % viable pups = (no. of pups alive LD 4 / no. of pups alive on LD 0) x 100

There were no changes in relative organ weights that were representative of an adverse effect of the test item.

Of those pups subjected to necropsy on or before lactation day 4, a significant increase in kidney lesions, including pale colour (F1) , pelvic dilatation (F1 and F2) and observation of white material / substance in the pelvic space / papilla (F2) was noted in pups at the top dose only, as tabulated below.

Table 28: Gross findings - lactation day 4 (2 generation study in the rat)

Litter	Findings	Dietary level (ppm)			
		0	**5**	**50**	**1000**
F1 pup	No. of litters examined	22	22	23	21
	Kidney: Pale in color	0.0	0.0	0.0	3.3*
	Kidney: Pelvic dilatation	0.0	0.0	0.0	6.1**
	Kidney: White material in pelvic space/papilla	0.0	0.0	0.0	3.2
F2 pup	No. of litters examined	23	23	22	18

	Kidney: Pale in colour	0.0	0.0	0.0	1.7
	Kidney: Pelvic dilatation	0.0	0.0	0.0	3.6*
	Kidney: White material in pelvic space/papilla	0.0	0.0	0.0	3.9*

Values represent the number of litters examined and group mean percent litter incidences.

Data were statistically analysed by Dunnett-type test following Kruskal-Wallis test.

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

Amongst pups subjected to necropsy at 26 days of age an increase in the incidence of opacity of the eye was observed in both F1 and F2 generations from the 1000 ppm groups. This increase was statistically significant in the F2 generation. There was zero incidence of opacity of the eye in control animals or those from the 5 ppm or 50 ppm groups. Additionally, an increase in the incidence of kidney lesions was recorded in F1 and F2 pups from the highest dose groups. In F1 pups, there was a statistically significant increase in the incidence of observation of small kidneys and cysts, when compared to control animals. An increase in the incidence of white spots and pelvic dilatation was also observed at the top dose level, but without statistical significance. In F2 pups an increase in the incidence of small kidneys and cysts was noted in animals from the 1000 ppm group, but did not achieve statistical significance.

Table 29: Gross findings – 26 days (2 generation rat study)

Litter	Findings	Dietary level (ppm)			
		0	**5**	**50**	**1000**
F1 pup	No. of litters examined	22	23	23	21
	Eye: Opacity	0.0	0.0	0.0	1.9
	Kidney: small in size	0.0	0.0	0.0	4.5*
	Kidney: Spot(s) (white)	0.9	0.0	0.0	2.4
	Kidney: Cyst(s)	3.3	3.7	6.7	12.8*
	Kidney: Pelvic dilatation	0.0	1.5	1.7	4.6
F2 pup	No. of litters examined	24	24	22	20
	Eye: Opacity	0.0	0.0	0.0	5.6*
	Kidney: Small in size	0.0	0.0	0.0	1.9
	Kidney: Cyst(s)	4.7	3.3	3.5	11.3
	Kidney: Pelvic dilatation	2.1	3.1	1.7	3.1

Values represent the number of litters examined and group mean percent litter incidences.

Gross pathological examination of weanlings was performed at 26 days of age.

Data were statistically analysed by Dunnett-type test following Kruskal-Wallis test.

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

10.10.2 Additional studies relevant to reproductive toxicity

There were no additional studies in relation to fertility and reproductive function.

10.10.3 Short summary and overall relevance of the provided information on sexual function and fertility

The potential for tolpyralate to have an adverse effect on sexual function and fertility was investigated in a 2-generation reproductive toxicity study conducted in the rat.

In the parental generations the predominant adverse finding was ocular opacity and associated keratitis, this effect occurred in both P and F1 generations and in both sexes at dose levels of 50 and 1000 ppm. Other parental effects also observed at both 50 and 1000 ppm were decreased body weight and/or body weight gain and increased relative liver weight. Additionally an increased incidence of adverse histopathological findings in the kidney, including nephropathy, was seen in F1 males at ≥50 ppm and F1 females at the top dose level. Relative kidney weights were increased in both sexes at 1000 ppm. No signs of parental toxicity were observed in any animals receiving 5 ppm tolpyralate.

There were no treatment-related effects on sexual development, oestrous cycle, mating index, or sperm parameters. Gestation length and number of days until mating were slightly increased in some groups, but these effects were considered incidental and not related to administration of the test item.

In offspring, there were reductions in pup weights recorded at 1000ppm in both generations and both sexes. Other observations at the top dose level included increased incidence of ocular opacity and gross kidney lesions. Additionally an increased incidence of kidney cysts was observed at ≥50ppm in F1 animals which was not statistically significant and was not seen in F2 animals.

In an additional study using the model pharmaceutical NTBC, it was demonstrated that inhibition of tyrosine catabolism in developing rats, resulting in increased plasma tyrosine, was associated with lesions in the eyes, urinary system and kidneys to those seen with tolpyralate.

10.10.4 Comparison with the CLP criteria

The potential effects of tolpyralate on fertility and reproductive performance were investigated in a rat dietary 2-generation study. In this study, there were no adverse effects on fertility or reproductive performance up to doses causing parental toxicity. On this basis, classification of tolpyralate is not warranted.

10.10.5 Adverse effects on development

The potential for tolpyralate to have adverse effects on development has been investigated in two specific studies; one in the rat, and one in the rabbit.

Table 30: Summary table of animal studies on adverse effects on development

Study Species	Dose levels	Critical effects
Rat, SD 23-24 females / group Vehicle: 1% carboxymethyl-cellulose OECD 414 GLP Batch: 20111222-1 Purity 97.27% Anonymous (2013c) DAR B.6.6.2	0, 1, 10, 500 mg/kg bw/d Oral gavage GD 6-19	**Maternal toxicity** <u>1 and 10 mg/kg bw/d</u> No adverse effects <u>500 mg/kg bw/d</u> Reduced body weight gain (8%). Reduced food consumption until day 15, by 6-20%. **Developmental toxicity** <u>1 and 10 mg/kg bw/d</u> No adverse effects <u>500 mg/kg bw/d</u>

Study Species	Dose levels	Critical effects
		Decreased fetal weight (6.1% in both sexes) Increased total foetal incidence and litter incidence of skeletal variations: 97 foetuses/24 litters compared to 29/15 in controls. Findings occurring with a dose related incidence or at increased incidence when compared to controls were; discontinuous rib cartilage (61/23), supernumerary rib (57/18), bipartite ossification of thoracic centrum in (3/3) and 27th presacral vertebrae (3/3).
Rat, SD 12 females/ group Not guideline (limited observations – see study summary) Not GLP Anonymous (2016c) DAR B.6.6.2	1000 ppm tolpyralate in the diet (approx. 88 mg/kg bw) or 10 ppm NTBC in the diet (approx.. 0.84 mg/kg bw) or control diet Test item administered from gestation day 0 until study termination.	**Maternal toxicity** *Tolpyralate* - plasma tyrosine concentration 2016.2 µmol/L (control value 66.5 µmol/L); ocular opacity in 10/11 animals *NTBC* - plasma tyrosine concentration 2158.0 µmol/L; Opacity in the eye in 11/11 animals. **Findings in pups at termination (PND 21)** *Tolpyralate* - plasma tyrosine concentration 3197.3 µmol/L (control value 191.4 µmol/L); ocular opacitiy in 1.14% of pups Increased incidence of abnormalities in the urinary system and kidney: small kidney (2/64, 0 in controls), pale discolouration (4/64, 0), rough surface (4/64, 0), dilation of renal pelvis (3/64, 2/61) and red contents of urinary bladder (1/52, 0). *NTBC* - plasma tyrosine concentration 3651.5 µmol/L; ocular opacitiy in 7.96% pups. Decreased pup viability at PND 4 Increased incidence of abnormalities in the urinary system and kidney: small kidney (6/52, 0 in controls), pale discolouration (4/52, 0), rough surface (2/52, 0), dilation of renal pelvis (3/52, 2/61) and red contents of urinary bladder (1/52, 0).
Rabbit, (Japanese White 25 females / group Vehicle: 1% carboxymethyl-cellulose OECD 414 GLP Batch: 20111222-1 Purity 97.27% Anonymous (2013) DAR B.6.6.2	0, 0.5, 5, 500 mg/kg bw/d Oral gavage GD 6-27	**Maternal toxicity** No adverse effects at any tested dose **Developmental toxicity** <u>0.5 and 5 mg/kg bw/d</u> No adverse effects <u>500 mg/kg bw/d</u> Increased incidence of total skeletal variations affecting 98.48% of foetuses (compared to 34.85% in controls). Specific skeletal variations occurring at increased incidence when compared to controls were as follows: Supernumary rib in 197 fetuses from 22 litters (54/21 in controls) and 27th presacral vertebrae occurred (196/22 vs. 14/10) ; unossified 1st cervical centrum (3/3 vs 0), incomplete ossification of cervical centrum (1/1 vs 0), supernumary ossification (1st + 2nd cervical vertebrae) (12/4 vs 0); dumbell ossification of thoracic centrum (1/1 vs 0).

Rats

Developmental toxicity study – Anonymous, 2013c

Groups of 23 - 24 presumed pregnant Sprague Dawley rats were administered tolpyralate by oral gavage at doses of 0, 1, 10 or 500 mg/kg/day tolpyralate in 1% aqueous sodium carboxymethylcellulose on days 6 to 19 of gestation.

There were no mortalities amongst dams in any treated groups. No significant clinical signs of toxicity were observed in any treated animals. Loss of fur was observed in 2 out of 24 dams in both the 10 and 500 mg/kg bw groups. There were no statistically significant changes in body weight in any of the treated groups when compared to controls, although there were some transient reductions in body weight in the top dose group. Body weight gain was significantly reduced in dams receiving 500 mg/kg bw/day throughout the study. A statistically significant reduction in food consumption of approximately 6-20% was observed in dams receiving 500 mg/kg bw/day from the start of treatment until day 15. After day 15, food consumption was comparable to control animals for the remainder of the study.

There were no statistically significant differences in the mean numbers of corpora lutea, implantation sites or pre-implantation losses between the control and any treated groups. A statistically significant decrease in gravid uterine weight was observed in the 1 and 500 mg/kg groups, but there was no dose-response. There were no statistically significant differences in the mean number of live foetuses, resorptions or foetal deaths in any treatment group. Foetal weight in the 500 mg/kg group was statistically significantly less than those in the control group (6.1% in both sexes). Statistically significant increases in placental weight and sex ratio (male : female) were observed in the 10 mg/kg bw/day group but are regarded as incidental in the absence of a similar effect in the top dose group.

The total number of litters with malformed pups was 4/23 (17.4%), 3/24 (12.5%), 7/24 (29.2%) and 8/24 (33.3%) in the control, 1, 10 and 500 mg/kg groups, respectively. Similarly, the incidences of dams having foetuses with variations were 20/23 (87.0%), 19/24 (79.2%), 22/24 (91.7%) and 24/24 (100.0%). Although a treatment related trend was evident, no statistically significant differences in these incidences were found.

Table 31: Findings in the rats developmental toxicity study

Parameter	Dose (mg/kg bw/day)			
	0	**1**	**10**	**500**
Foetal examinations. Values represent number of foetuses. Values in parenthesis represent litter incidence.				
External examinations				
Number of foetuses examined	322	311	330	325
Total number of foetuses with external malformations	0 (0)	0 (0)	1(1)	4(2)
Short tail	0 (0)	0 (0)	1(1)	0(0)
Cleft Palate	0 (0)	0 (0)	0 (0)	1(1)
Misshapen urethral orifice	0 (0)	0 (0)	0 (0)	3(1)
Visceral observations				
Number of foetuses examined	167	163	171	169
Total number of foetuses with visceral malformations	1(1)	0 (0)	3 (3)	1 (1)
Dilated cerebral ventricle	0 (0)	0 (0)	1 (1)	0(0)
Retroesophageal subclavian	0 (0)	0 (0)	1 (1)	1 (1)
Abnormal lung lobation	0 (0)	0 (0)	1 (1)	0 (0)
Persistant truncus arteriosus	0 (0)	0 (0)	1 (1)	0 (0)
Membranous ventricular septum defect	0 (0)	0 (0)	1 (1)	0 (0)

Left subclavian artery arising from pulmonary artery	1 (1)	0 (0)	0 (0)	0 (0)
Total number of foetuses with visceral variations	13(9)	14 (9)	14(11)	14(13)
Thymic remnant in the neck	11 (7)	12 (8)	12 (9)	9 (8)
Dilated renal pelvis	1 (1)	3 (3)	1 (1)	1 (1)
Left umbilical artery	0 (0)	0 (0)	1 (1)	2 (2)
Right subclavian from aortic arch	1 (1)	0 (0)	0 (0)	2 (2)
Skeletal observations				
Number of foetuses examined	155	148	159	156
Total number of foetuses with skeletal malformations	3 (3)	3 (3)	5 (5)	6(6)
Split cartilage of ventral arch	1 (1)	0 (0)	0 (0)	0 (0)
Fused cervical arch	2 (2)	1 (1)	2 (2)	2 (2)
Dumbbell-shaped cartilage of cervical centrum	0 (0)	0 (0)	0 (0)	1 (1)
Split cartilage of thoracic centrum	0 (0)	1 (1)	1 (1)	2 (2)
Dumbbell-shaped cartilage of thoracic centrum	0 (0)	1 (1)	0 (0)	1 (1)
Rib cartilage not fused to sternum	0 (0)	0 (0)	1 (1)	0 (0)
Fused caudal centrum	0 (0)	0 (0)	1 (1)	0 (0)
Total number of foetuses with skeletal variations **	29 (15)	42 (18)	58(22)	97(24)**
Discontinuous rib cartilage (false rib)	20 (14)	20 (13)	34(20)	61(23)**
Supernumerary rib	11 (10)	14(9)	26(13)	57(18)**
Bipartite ossification of thoracic centrum	1 (0)	2(2)	2(2)	3(3)
27th presacral vertebrae	1 (0)	2(1)	1(1)	3(3)

Data were statistically analysed by Dunnett-type test following Kruskal-Wallis test.

* $p \leq 0.05$; ** $p \leq 0.01$

** Only selected individual skeletal variations (those deemed to be treatment related) are listed

Numbers in parenthesis represent litter incidence

There was no statistical difference in the incidence of total external malformations in any treated group. At 500 mg/kg, a single foetus had both cleft palate and local oedema. This is most likely to be a sporadic finding. Three foetuses from a single litter had a misshapen external urethral orifice. Although this is considered a rare abnormality, it is noted that these foetuses were all from the same litter. In a further study (Anonymous, 2013e), it was shown that this finding was genetic in origin and not related to treatment (see Section 10.10.5.3).

The total numbers of foetuses with visceral malformations were 1, 0, 3 and 1 at 0, 1, 10 and 500 mg/kg, respectively. In the control group, one foetus had an aberrant left subclavian artery. At 10 mg/kg, three foetuses from different litters each exhibited different visceral malformations (dilated cerebral ventricle, abnormal lung and multiple cardiovascular anomalies. At 500 mg/kg, a single visceral malformation (retroesophageal subclavian artery) was seen in one foetus. These findings are regarded to be sporadic and not related to the administration of the test item.

The total numbers of foetuses with skeletal malformations were 3, 3, 5 and 6 at 0, 1, 10 and 500 mg/kg, respectively. Findings occurring in a dose dependent manner or at increased incidence when compared to controls were split cartilage of thoracic centrum in a single foetus in the 1 and 10 mg/kg bw groups and 2 foetuses (1 each from 2 separate litters) at 500 mg/kg. Dumbbell-shaped cartilage of cervical centrum, fused cervical arch and dumbbell shaped cartilage of thoracic centrum thoracic centrum were observed in 1, 2 and 1 fetuses in the top dose group. There was no statistically significant increase in any skeletal malformations in any of the treated groups, when compared to control animals. Therefore, these findings are not considered to be related to treatment.

The total number foetuses with skeletal variations was 29, 42, 58 and 97 at 0, 1, 10 and 500 mg/kg, respectively. The most frequently observed variations were discontinuous rib cartilage and supernumerary rib. Discontinuous rib cartilage occurred at an incidence of 20, 20, 34 and 61 in the 0, 1, 10 and 500 mg/kg bw groups. Supernumerary rib occurred with fetal incidence of 11, 14, 26 and 57 in the 0, 1, 10 and 500 mg/kg bw groups. Other dose-related skeletal variations observed were 27th presacral vertebrae and bipartite ossification of thoracic centrum. These findings occurred with zero incidences in controls and are considered to be treatment related.

Overall, it is concluded that treatment of dams with relatively high exposure levels of tolpyralate have the potential to decrease foetal weight and induce various skeletal variations in rats. Although malformations were seen in this study, they were not considered to have been treatment-related.

Additional study into effect of elevated plasma tyrosine - Anonymous 2016c.

A supplementary study investigating the effect of increased tyrosine levels on the development of Sprague-Dawley rats was conducted. In this non-standard study, tolpyralate or NTBC were administered to groups of 12 pregnant dams at dietary concentrations of 1000ppm or 10ppm respectively. The mean exposure of the test items to the dams throughout the study period was calculated to be 88.87 mg/kg bw/d and 0.84 mg/kg bw/d for tolpyralate and NTBC respectively. Dams were allowed to give birth and treatment was continued until PND 21 when the study was terminated and all animals were sacrificed. A control group received the diet without either of these substances added. As discussed elsewhere in this CLH report, NTBC is a pharmaceutical used to inhibit HPPD and increase plasma levels of tyrosine. There was limited analysis, consisting of the observation of clinical signs, and measurement of body weight, food consumption, plasma tyrosine concentration and reproductive parameters, selected organ weights and histopathology in dams. Comprehensive developmental analysis, including assessment of variations/malformations was not conducted.

At the end of the treatment period, the mean plasma tyrosine concentrations in dams treated with NTBC and tolpyralate were 2158.0 µmol/L and 2016.2 µmol/L, respectively. An increased incidence of ocular opacity was evident in both groups from lactation day 4 and 7, affecting 11/11 and 10/11 dams, respectively. There were no relevant changes in body weight parameters. No effect on gestation index, gestation length, number of implantations or delivery index was reported. There were no histopathological findings that were attributed to either test item.

Percentage viability of pups at post-natal (PND) 0 was unaffected by treatment with either NTBC or tolpyralate, with 1 (0.57%), 1 (0.61%) and 3 (1.78%) pups being found dead in the control, NTBC and tolpyralate groups respectively. At PND 4 there was a decrease in percentage viability in the NTBC group when compared to controls. No such finding was evident in the tolpyralate group, 10 (6.81%), 41 (22.34%) and 13 (9.03%) pups were dead/lost on PND 4.

Ocular opacity was seen in pups in both treated groups on PND 5-21, affecting 7.96% and 1.14% of pups in the NTBC and tolpyralate, respectively, compared to 0% in controls. Mean pup body weight was reduced in both treated groups at PND21. Mean plasma tyrosine concentrations were significantly increased in both treated groups and PND4 and PND21 by approximately 35 fold in comparison to controls (> 3000 µmol/L in exposed animals, approx. 100 µmol/L in controls). Pathological and histopathological analysis was limited in this study. The predominant findings were increases in various gross and histopathological lesions in the urinary system and kidneys. These findings were minimal in both severity and incidence.

This limited study was conducted to investigate the effects of elevated plasma tyrosine on the development of rats. Serum tyrosine concentrations were increased significantly in both dams and pups of both treated groups along with increased incidence of lesions in the eyes, urinary system and kidney. Additionally, NTBC was found to cause an increase in pup death at PND 4.

Summary of developmental toxicity study in the rat

Administration of tolpyralate resulted in some signs of maternal toxicity at the highest tested dose. Dams receiving 500 mg/kg exhibited reduced body weight gain throughout the treatment period. Reductions in both body weight and food consumption compared to controls were observed in these animals periodically.

In the 500 mg/kg bw/day group a reduction in mean foetal weight (both sexes) was observed, and may be related to administration of tolpyralate.

There were no statistically significant or clear treatment related increases in skeletal malformations, visceral variations, visceral malformations or external variations. There was an increase in total skeletal variations and in the incidence of both discontinuous rib cartilage and supernumerary rib in foetuses from the 500 mg/kg bw/day group. However, these findings are considered to be of low toxicological significance.

Rabbits

Developmental toxicity study – Anonymous 2013d

Groups of 25 artificially inseminated Japanese white rabbits (Kbl:JW) were administered tolpyralate by oral gavage in a 1% solution of aqueous sodium carboxymethyl cellulose, at dose levels of 0, 0.5, 5 or 500 mg/kg bw/day on gestation days 6 to 27.

There were no treatment related increases in mortality or clinical signs of toxicity, other than small transient changes in body weight gain, at any tested dose level.

No statistically significant differences in gravid uterine weights, numbers of corpora lutea, implants and live foetuses, percent incidence of pre-implantation losses, percent incidences of resorptions and foetal deaths, foetal sex ratio, foetal body weights and placental weights were observed between treated and control groups.

Skeletal findings

There was no dose related or statistically significant increases in skeletal malformations.

A statistically significant and dose related increase in the total incidence of skeletal variations was observed, with 98.48% of foetuses in the 500 mg/kg bw/day group having at least one skeletal variation, the predominant finding was an increase in the incidence of supernumerary ribs, supernumerary ossifications site (1^{st} and 2^{nd} cervical vertebrae) and 27 presacral vertebrae. Each of these individual effects occurred with a statistically significant increased incidence at the top dose level, and is considered to be related to the administration of the test item.

Table 32: Developmental effects observed in rabbits

Parameter	Dose (mg/kg bw/day)			
	0	**0.5**	**5**	**500**
Fetal examinations. Values represent number of foetuses. Values in parenthesis represent litter incidence.				
Number of foetuses examined	**200(24)**	**184(22)**	**186(22)**	**213(22)**
Skeletal variations - Bodies	**67(23)**	**50(21)**	**74(18)**	**210(22)**
Supernumerary rib	54(21)	40(18)	67(17)	197(22)**
Unossified 1^{st} cervical centrum	0(0)	0(0)	0(0)	3(3)*
Incomplete ossification of cervical centrum	0(0)	0(0)	0(0)	1(1)
Supernumary ossification (1^{st} + 2^{nd} cervical vertebrae)	0(0)	0(0)	0(0)	12(4)**
Dumbell ossification of thoracic centrum	0(0)	0(0)	0(0)	1(1)
27th presacral vertebrae	14(10)	22(12)	25(9)	196(22)**

Summary of developmental toxicity in the rabbit

The potential for tolpyralate to have an adverse effect on development was investigated in a specific study in the rabbit. The test item was well tolerated by the parental animals with minimal clinical signs of toxicity being observed. Signs of maternal toxicity were limited to small transient reductions in body weight gain and food consumption at the top dose level.

There were no abnormalities observed in the maternal animals at necropsy which were attributed to the administration of the test item. There was no dose related or statistically significant increase in visceral abnormalities or skeletal malformations. Examination for skeletal variations showed a statistically significant increase in total skeletal variations in the 500 mg/kg bw/day group when compared to the control group. Supernumerary ribs, supernumerary ossification sites and 27th presacral vertebra were noted in 92.55, 7.08 and 91.60% of foetuses respectively, all of which were significantly increased in incidence over the control group. However, these findings are considered to be of low toxicological concern.

10.10.5.1 Supplementary studies relevant to effects on development

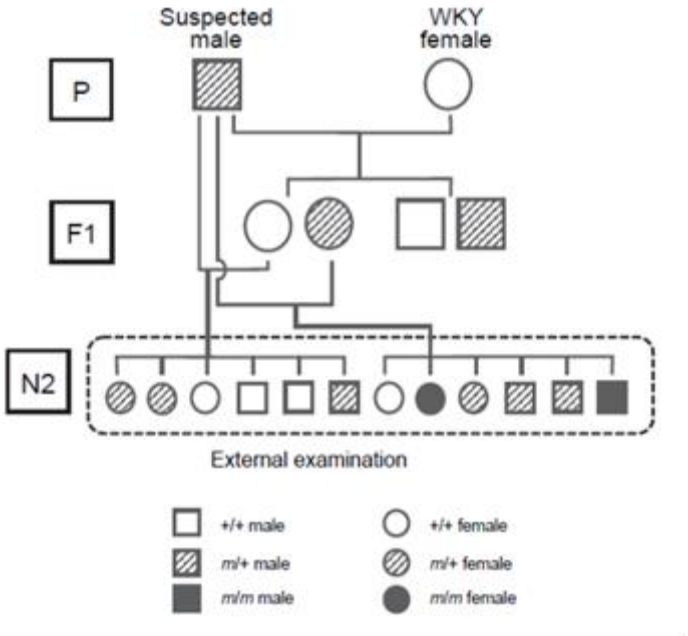
Genetic analysis of the external malformation observed in a developmental toxicity study in rats

Following the observation of an uncommon abnormality in a study of the developmental toxicity of tolpyralate performed in the rat, where a number of foetuses in the highest dose group were found to have a misshapen urethral orifice, a progeny test was conducted to determine whether the abnormality was treatment related or genetic in origin. It was hypothesised that the misshapen urethral orifice seen in the developmental toxicity study was caused by a recessive mutation carried by both the dam and the sire which produced the affected litter.

Table 33: Summary of study of genetic analysis of the external malformation observed in a developmental toxicity study in rats

Study details	Remarks
Rat, SD and WKY/NCrlCrlj 1 male (SD) mated first with 4 females (WKY/NCrlCrlj), then with 4 F1 females. Not guideline Not GLP Anonymous (2013e)	F1 offspring No pups from the F1 generation had external genital abnormalities. N2 offspring A total of 96 N2 foetuses were examined (58 males and 38 females), of these 7 female foetuses from 5 litters (25 female foetuses) were observed to have the same misshapen genital orifice as was observed in the earlier developmental toxicity study on tolpyralate. The proportion of affected female foetuses of 7 in 25 agrees with the 1:3 hypothesis for autosomal recessive inheritance. In addition to the misshapen urethral orifice, multiple malformations (cleft palate/lip, brachydactyly, anal atresia and short tail) were seen in 7 N2 fetuses (4 male and 3 female) from 4 litters.

In the developmental toxicity study in the rat described earlier, 3 fetuses from a single litter were found to have a misshapen urethral orifice. In this supplementary study, the male rat which had previously sired the affected litter was mated with a further 4 wild type (WKY/NCrlCrlj) females to produce the F1 generation. Selected females from the F1 generation were then back-crossed with the suspected male to produce the N2 generation. The offspring were examined for developmental abnormalities, particularly relating to the external genitalia. The following diagram illustrates the predicted occurrence of the abnormality if it is due to a recessive mutant allele.



The F1 generation consisted of 26 males and 27 females. No pups from the F1 generation had external genital abnormalities. A total of 96 N2 foetuses were examined (58 males and 38 females), of these 7 female foetuses from 5 litters (25 female foetuses) were observed to have the same misshapen genital orifice as was observed in the earlier developmental toxicity study on tolpyralate. The proportion of affected female foetuses of 7 in 25 agrees with the 1:3 hypothesis for autosomal recessive inheritance. In addition to the misshapen urethral orifice, multiple malformations (cleft palate/lip, brachydactyly, anal atresia and short tail) were seen in 7 N2 fetuses (4 male and 3 female) from 4 litters.

This study indicates that the misshapen urethral orifice observed in the developmental toxicity study of tolpyralate in the rat is likely to be caused by a recessive, female specific mutant allele present in both the male and female which sired the affected litter, and is not related to the administration of tolpyralate.

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

The developmental toxicity of tolpyralate was investigated in 2 standard studies conducted in rats and rabbits. Further information is also available from the 2-generation study conducted in rats and a supplementary study that was conducted to investigate the effects of increased plasma tyrosine levels on the development of rats

In the standard developmental studies in the rat and rabbit, there were increases in total skeletal variations and certain other specific variations. Notable observations were unossified/partially ossified cervical and thoracic centrum, supernumerary ribs and 27th presacral vertebrae. These findings were evident only at the highest test dose of 500 mg/kg bw/day in both studies, and occurred in the absence of any marked maternal toxicity other than small changes in body weight and body weight gain. Whilst these are considered to be related to treatment, they are considered to be of minimal toxicological significance.

In a 2-generation study, pup viability on lactation day 0 was decreased in both the F1 and F2 generations. However, the findings were not consistent across the F1 and F2 generations, with the effects in the F2 generation being due primarily to one litter. Viability on subsequent days was not affected. Whilst there was also a decrease in the total number of pups delivered at the top dose in the F2 generation, this was not found to be statistically different to controls or other treated groups. Overall, these findings are not considered to represent a treatment related effect.

In the supplementary study, in which pregnant rats were treated with 88.87 mg/kg bw/d tolpyralate or 0.84 mg/kg bw/d NTBC, the mean plasma tyrosine concentration in dams was increased to 2158.0 µmol/L and

2016.2 µmol/L respectively. Pup viability was unaffected by treatment with either substance on lactation day 0 but, by day 4, there was a decrease in the NTBC group. Mean pup body weight was reduced in both treated groups at PND21. Mean plasma tyrosine concentrations were significantly increased in both treated groups at PND4 and PND21: exceeding 3000 µmol/L in all cases in comparison to approximately 100 µmol/L in control animals. Pathological and histopathological analysis was limited in this study. The predominant findings were increases in various gross and histopathological lesions in the urinary system and kidneys. These findings were minimal in both severity and incidence; they were consistent with the repeated dose toxicity of tolpyralate. No further information on developmental parameters was provided by this study.

10.10.7 Comparison with the CLP criteria

A classification for developmental toxicity is warranted where there is evidence from humans or experimental animals of an adverse effect on the development of the conceptus either before or after birth. Such effects shall have been observed in the absence of other toxic effects, or shall not be secondary non-specific consequences of the other toxic effects.

In standard developmental studies in the rat and the rabbit there were increases in total skeletal variations and certain other specific variations, in particular, unossified/partially ossified cervical and thoracic centrum, supernumerary ribs and 27th presacral vertebrae. These effects were evident only at the highest test dose of 500 mg/kg bw/day in both studies. In accordance with section 3.7.2.3.3 of Annex I of CLP, small changes in common foetal variants (such as those observed in skeletal examinations) and/or foetal weights may be considered of low or minimal toxicological significance. Therefore, it is considered that the increases in skeletal variations are not sufficient to support classification for developmental toxicity. In conclusion, the criteria for classification are not met.

10.10.8 Adverse effects on or via lactation

Information relating to the potential for tolpyralate to have adverse effects either on or via lactation is available from the two-generation reproductive toxicity study in the rat. In this study there was a significant decrease in pup viability on lactation day 0, which was not seen subsequently. The decreased viability at the start of lactation, which was not seen later in the lactation period, is not consistent with an adverse effect on either quantity or quality of milk or an effect via lactation. Pup weights consistently decreased at the highest tested dose throughout the lactation period, most notably from day 14 onwards, in both F1 and F2 generations, by around 10% when compared to controls. Other adverse effects seen in the offspring were increased incidence of ocular opacity and gross kidney lesions at the top dose level and an increased incidence of kidney cysts at ≥50ppm in F1 animals.

10.10.9 Comparison with the CLP criteria

Classification for effects on or via lactation might be assigned where: there is human evidence that indicates a hazard to babies during the lactation period; the results of one- or two-generation studies in animals provide clear evidence of adverse effects in the offspring owing to transfer in the milk or adverse effect on the quality of the milk; toxicokinetic studies indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

In the case of tolpyralate, there is no human data to inform on this end-point. In the available two-generation study in rats, signs of toxicity in pups which may be indicative of reduced quality or quantity of milk were slightly reduced body weights (approximately 10%) in both F1 and F2 off spring, particularly from lactation days 14 onwards. Although there were some reductions in maternal body weight (particularly in F1) during the lactation period, they were minimal and inconsistent; consequently the reduced pup weights cannot be concluded to be solely secondary to maternal toxicity. The gross abnormalities observed in offspring were consistent with adverse effects observed in other studies. Gross pathological examination was performed on lactation days 4 and 26. On LD4 there were effects on the kidney but not on the eye, the effects on the kidney are likely have been present before the onset of lactation. It is not possible to conclude whether the effects on the eye at LD26 were due to exposure to tolpyralate in the milk or in the diet, as weaning is

considered to be complete by this point. In conclusion, there is no clear evidence of adverse effects in the offspring owing to transfer of tolpyralate in the milk, and classification is not warranted for this end point.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

No classification (conclusive but not sufficient for classification).

10.11 Specific target organ toxicity-single exposure

Information relating to the potential for tolpyralate to cause specific target organ toxicity is available from the acute toxicity data presented in Sections 10.1 to 10.3. In addition an acute neurotoxicity study in the rat is available.

Table 34: Summary table of animal studies on STOT SE

Study **Batch / purity** **Intakes (mg/kg bw/d)**	**Doses**	**Adverse Effects**
Acute neurotoxicity in the rat Rat, SD 10 / sex / group Oral gavage; vehicle: 1% Methylcellulose OECD 424 GLP Batch 20111222-1 Purity 97.27% Anonymous (2013f) DAR B.7.1	0, 500, 1000, 2000 mg/kg bw Single dose 14 day observation period	<u>1000 mg/kg bw</u> Reduced approach response in males on day 1. <u>2000 mg/kg bw</u> Reduced body weight in week 1 of observation period by 14% and 24% in males and females respectively. Body weight comparable to controls in week 2. Reduced activity and rearing counts and reduced approach response in males at day 1 observation, attributed to general systemic toxicity.

The potential for tolpyralate to cause acute neurotoxicity was investigated in a study performed in the rat, according to OECD Test Guideline 424 and in compliance with GLP. Groups of 10 Sprague Dawley Crl:CD (SD) rats of each sex were administered 0, 500, 1000 or 2000 mg/kg bw tolpyralate, formulated in 1% (w/v) methylcellulose as a single oral gavage dose. The animals were observed for 14 days following treatment; clinical condition, body weight and food consumption were recorded. On days 1 (2h post dosing), 8 and 15 neurobehavioural screening was performed. Following termination brain weight and anatomical measurements were taken and microscopic and histopathological examination relevant for evaluation of neurotoxicity was performed.

There were no mortalities or clinical signs of toxicity which were attributed to administration of the test item. Small reductions in body weight gain (14% and 24% in males and females respectively) were observed in animals receiving 2000 mg/kg bw tolpyralate over the first 8 days of the observation period. Body weight gain in these animals was comparable to controls over the second week of the observation period. Body weight parameters in all other treatment groups were comparable to controls. There were no treatment related changes in food consumption.

There were no changes in home cage or in-hand observations that were attributable to administration of the test item. At the day 1 observation (2h following dose administration) there was a reduction in group mean activity and rearing counts in males from all treated groups, achieving statistical significance in the 2000 mg/kg group. The reduction in activity appeared to occur in a dose dependent manner and is likely to indicate general toxicity of the test item, although it should be noted that the activity of the control group was above the historical control range for both parameters. There was no comparable effect observed at the 8 and 15 day observations.

In females there was a statistically significant reduction in mean activity at day 15 amongst animals which received 1000 and 2000 mg/kg bw. This finding did not occur in a dose dependent manner, was within the historical control range and was not observed in females at day 1 or 8 observation, consequently it is not considered to be related to administration of the test item.

The day 1 observation showed a reduced approach response in males given 1000 or 2000 mg/kg and slightly reduced touch response in males given 2000 mg/kg. These effects appeared to occur in a dose dependent manner and were still evident on Day 8. The incidence of these responses was within the historical control range and they were not observed at the day 15 observation or at any point in females. Any changes in activity or response parameters occurring at the first observation on day 1 are attributed to general toxicity as opposed to a specific neurotoxic effect of tolpyralate.

There were no treatment related changes in brain size or weight, gross pathology or histopathology.

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

Information relating to the potential for tolpyralate to cause specific target organ toxicity following a single exposure is available from the specific acute toxicity studies conducted via the oral, dermal and inhalation routes (summarised in sections 10.1-10.3) and from the acute neurotoxicity study. All studies were conducted in the rat. Based on the clinical and behavioural observations in the described studies, there were no effects that are indicative of specific target toxicity following a single dose of tolpyralate.

10.11.2 Comparison with the CLP criteria

STOT-SE categories 1 and 2 are assigned on the basis of clear evidence of significant or severe toxicity to a specific target organ that arises from a single exposure to a substance. STOT-SE category 3 is assigned for the transient effects of respiratory tract irritation and narcotic effects.

The available acute studies do not provide any indication that tolpyralate meets the classification criteria for specific target-organ toxicity category 1, 2 or 3 following a single exposure.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification (conclusive but not sufficient for classification).

10.12 Specific target organ toxicity-repeated exposure

Information relating to the potential for tolpyralate to cause specific organ toxicity following repeated exposure has been investigated in a range of studies conducted in the rat, mouse and dog. Data informing on repeat dose toxicity of tolpyralate are also available from the carcinogenicity studies conducted in both the rat and the mouse. Effects observed in these studies that are relevant to STOT-RE are summarised in this section, whilst neoplastic effects are discussed in section 10.9. Further information is also available from the 2-generation study in the rat (refer to section 10.10 for full details).

Table 35: Summary table of animal studies on STOT RE

Method Guideline, Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Dose levels duration of exposure	Relevant adverse effects
28-day oral (dietary) OECD 407 GLP Batch 20101217 Purity 98.53% Anonymous (2013g) DAR B.6.3.1 CLP guideline value for classification (mg/kg bw/d) Cat 1 = 30 Cat 2 = 300	Rat, Wistar 6/sex/group	0, 50, 500, 5000, 20000 ppm Equivalent to Males: 0, 4.49, 45.9, 447, 1799 mg/kg bw/d Females: 0, 4.98, 46.9, 496, 1907 mg/kg bw/d	No treatment-related deaths or overt signs of toxicity in any dose group 4.49/4.98 mg/kg bw/d: Hyaline deposition in kidney males (1/6). Thyroid follicular cell hypertrophy in males (2/6). Keratitis in the eye in males (1/6). 45.9/46.9 mg/kg bw/d: Increased relative liver weight in males (16%). Hyaline deposition in kidney males (3/6). Thyroid follicular cell hypertrophy in males (6/6), Keratitis in the eye in both sexes (m 4/6, f 6/6). 447/496 mg/kg bw/d: Increased relative liver weight in both sexes (males 16%, 13% females). Hyaline deposition in kidney males (2/6). Thyroid follicular cell hypertrophy in both sexes (m 4/6, f 1/6). Pancreatic single acinar cell necrosis in males (3/6). Keratitis in the eye in both sexes (m 3/6, f 3/6). 1799/1907 mg/kg bw/d: Increased relative liver weight in both sexes (males 23%, 16% females). Increased relative kidney weight in males (16%). Hyaline deposition in kidney males (4/6). Centrilobular hepatocellular hypertrophy in males (4/6). Thyroid follicular cell hypertrophy in males (4/6). Single acinar cell necrosis in both sexes (m 2/6, f 1/6). Keratitis in the eye in both sexes (m 5/6, f 6/6).
90-day oral (dietary) OECD 408 GLP Batch 20101217 Purity 98.53% Anonymous (2013h) DAR B.6.3.2 CLP guideline value for classification (mg/kg bw/d)	Rat, Wistar 10/sex/group	0, 5, 20, 2000, 20000 ppm Equivalent to Males: 0, 0.32, 1.34, 133, 1363 mg/kg bw/d Females: 0, 0.38, 1.58, 159, 1647, mg/kg bw/d	No treatment-related deaths or overt signs of toxicity in any dose group 0.32/0.38 mg/kg bw/d: No adverse effects. 1.34/1.58 mg/kg bw/d: Hyaline droplet deposition in males (2/10) 133/159 mg/kg bw/d: Increased relative liver weight in both sexes (25% in males, 8% in females). Increased relative kidney weight in males (10%). Keratitis of cornea in both sexes (m 7/10, f 10/10). Hyaline droplet deposition in males (2/10). Thyroid follicular cell hypertrophy in both sexes (m 9/10, f 4/10). Increased incidence of pancreatic single acinar cell necrosis in both sexes (m 3/10, f 1/10). 1363/1647 mg/kg bw/d: Increased relative liver weight in both sexes (22% in males, 13%

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**Relevant adverse effects**
Cat 1 = 10 Cat 2 = 100			in females). Increased relative kidney weight in both sexes (14% in males, 11% in females). Keratitis of cornea in both sexes (m 6/10, f 10/10). Hepatocellular hypertrophy in both males (5/10). Hyaline droplet deposition in males (4/10). Follicular cell hypertrophy in both sexes (m 9/10, f 2/10). Increased incidence of single acinar cell necrosis in the pancreas in both sexes (m 8/10, f 1/10).
One year repeated dose toxicity study Oral (dietary) OECD 452 Batch: 2011222-1 Purity: 97.27% Anonymous (2015i) DAR B.6.5 Guidance value for classification (mg/kg bw/d) Cat 1 = 2.5 Cat 2 = 25	Rat, Wistar 21/sex/dose	52 weeks 0, 5, 20, 2000, 10000/20000 ppm (males/females) Equivalent intake at 52 weeks: Males: 0, 0.229, 0.925, 97.0 and 482 mg/kg bw/d Females: 0,0.303, 1.18, 126 and 1336 mg/kg bw/d	No treatment-related deaths or overt signs of toxicity in any dose group. Decreased body weight, relative to controls (<20%) in mid-high and high dose groups. <u>0.925/1.18mg/kg bw/d</u> Loss of fur in males (6/21), Pancreatic acinar cell fibrosis in males (7/21). <u>97.0/126mg/kg bw/d</u> Loss of fur in both sexes (12/21 males, 21/21 females). Ocular opacity in both sexes (20/21 males, 20/21 females). Increased urinary ketones and decreased urinary pH in both sexes. Increased relative liver weight (16% in males, 10% in females), relative kidney weight (17% in males, 16% in females), incidence of fatty liver in males (11/21), incidence of basophilic change to renal tubule in males (8/9) and incidence of vacuolation of the cerebellum (males). Pancreatic acinar cell fibrosis in males (11/21). Pancreatic acinar cell necrosis in males (7/21). Thyroid follicular cell hypertrophy in both sexes (3/21 males, 2/21 females). Ocular keratitis in both sexes (20/21 males, 20/21 females). <u>482/1336mg/kg bw/d</u> Loss of fur in both sexes (17/21 males, 20/21 females). Ocular opacity in both sexes (20/21 males, 21/21 females). Increased urinary ketones and decreased urinary pH in both sexes. Increased relative liver weight (13% in males, 16% in females), relative kidney weight (23% in males, 15% in females), incidence of fatty liver in males (14/21), incidence of basophilic change to renal tubule in males, (9/21) and incidence of vacuolation of the cerebellum (both sexes). Pancreatic acinar cell fibrosis in males (12/21 males). Pancreatic acinar cell necrosis in both sexes. Thyroid follicular cell hypertrophy in both sexes (8/21 males, 12/21 females). Ocular keratitis in both sexes (20/21 males, 20/21 females).
Two-year carcinogenicity study Oral (dietary)	Rat, Wistar 51/sex/dose	104 weeks 0, 5, 20, 2000, 10000 ppm Equivalent	<u>*General toxicity*</u> No overt or serious clinical signs of toxicity. Decreased body weight, relative to controls (<20%) in mid-high and high dose groups.

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**Relevant adverse effects**
OECD 451 GLP Batch: 2011222-1 Purity: 97.27% Anonymous (2015f) DAR B.6.5 Guidance value for classification (mg/kg bw/d) Cat 1 = 1.25 Cat 2 = 12.5		intake at 104 weeks: Males: 0, 0.196, 0.765, 83.8, 426 mg/kg bw/d Females: 0, 0.255, 1.01, 108, 554 mg/kg bw/d	*Non-neoplastic effects* 0.196/0.255mg/kg bw/d No adverse effects 0.765/1.01 mg/kg bw/d No adverse effects 83.8/108 mg/kg bw/d WBC counts increased in males. Relative liver weight increased (23%) in males. Increased relative kidney weight (23%) in males. Corneal opacity and associated keratitis in both sexes (all animals), coarse surface of the kidney in males (11/51), pancreatic acinar cell fibrosis in both sexes (males 46/51, females 23/51), pancreatic fat infiltration in males (26/51), degeneration of the sciatic nerve in females (12/51), vacuolation of the cerebellum in both sexes (males 40/51, females 41/51). Colloid degeneration of the thyroid in females (16/51). 426/554 mg/kg bw/d Leukocyte count increased in males, relative liver weight increased (25%) in males, relative kidney weight increased (17%) in males, corneal opacity and associated keratitis in both sexes (all animals), increased haematopoiesis in the bone marrow in males (23/51), pancreatic acinar cell fibrosis in both sexes (males 47/51, females 22/51), pancreatic fat infiltration in males (24/51) and acinar cell necrosis in females (16/51). Colloid degeneration of the thyroid in both sexes (males 25/51, females 23/51), degeneration of the sciatic nerve (males 22/51, females 15/51) and vacuolation of the cerebellum in both sexes (males 44/51, females 44/51).
90-day oral (dietary) OECD 408 GLP Batch 20111222-1 Purity 97.27% Anonymous (2013i) DAR B.6.3.2 CLP guideline value for classification (mg/kg bw/d) Cat 1 = 10	Mouse, CD1 10/sex/group	0, 50, 500, 2000, 7000 ppm Equivalent to Males: 0, 7.17, 70.8, 284, 1056 mg/kg bw/d Females: 0, 7.94, 81.5, 331, 1176, mg/kg bw/d	No treatment-related deaths or overt signs of toxicity in any dose group 7.17/7.94 mg/kg bw/d: No adverse effects. 70.8/81.5 mg/kg bw/d: No adverse effects. 284/331 mg/kg bw/d: Hepatocellular hypertrophy in males (3/10). Thyroid follicular cell hypertrophy in males (1/10). 1056/1176 mg/kg bw/d: Hepatocellular hypertrophy in both sexes (m 10/10, f 1/10). Thyroid follicular cell hypertrophy in males (4/10). Hepatocellular necrosis (focal and single cell) in females (1/10).

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**Relevant adverse effects**
Cat 2 = 100			
18-month carcinogenicity Oral (dietary) OECD 451 GLP Anonymous. (2015) DAR B.6.5	Mouse, CD1 52/sex/group	78 weeks 0, 70, 700, 7000 ppm Equivalent intake at 78 weeks: Males: 0, 7.37, 78.5, 793 mg/kg bw/d Females: 0, 7.25, 72.6, 732 mg/kg bw/d	*General toxicity* No overt clinical signs of toxicity or increase in cumulative mortality at 78 weeks. Small decreases in body weight, relative to controls were observed in males, but were < 10%. *Non-neoplastic effects* Increased incidence of calculi in the gall bladder in both sexes in all treated groups. 78.5/72.6 mg/kg bw/d Increased incidence of hepatocellular hypertrophy in both sexes (males 8/52, females 6/52). 793/732 mg/kg bw/d Increased incidence of glomerulonephritis in females (19/52). Increased relative liver weight in both sexes (males 14%, females 21%). Increased incidence of hepatocellular hypertrophy in both sexes (males 12/52, females 17/52). Increased incidence of hypertrophy of the seminal vesicle (17/31) and coagulating gland (16/31) in terminal kill animals only).
28-day oral (dietary) Non guideline (similar to OECD 409) GLP Batch 20111222-1 Purity 97.27% Anonymous (2013j) DAR B.6.3.1 CLP guideline value for classification (mg/kg bw/d) Cat 1 = 30 Cat 2 = 300	Dog, Beagle (HRA) 2/sex/group	0, 200, 2000, 20000 ppm Equivalent to Males: 0, 7, 69, 709 mg/kg bw/d Females: 0, 7, 72, 711, mg/kg bw/d	No treatment-related deaths or overt signs of toxicity in any dose group 7 mg/kg bw/d: No adverse effects 69/72 mg/kg bw/d: No adverse effects 709/711 mg/kg bw/d: Kidney tubular regeneration and urinary cast in a single male.

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**Relevant adverse effects**
90-day oral (dietary) OECD 409 GLP Batch 20111222-1 Purity 97.27% Anonymous (2013k) DAR B.6.3.2 CLP guideline value for classification (mg/kg bw/d) Cat 1 = 10 Cat 2 = 100	Dog, Beagle (HRA) 4/sex/group	0, 200, 2000, 20000 ppm Equivalent to Males: 0, 6.47, 64.6, 699mg/kg bw/d Females: 0, 6.98, 65.3, 670 mg/kg bw/d	No treatment-related deaths or overt signs of toxicity in any dose group 6.47/6.98 mg/kg bw/d: No adverse effects. 64.6/65.3 mg/kg bw/d: No adverse effects. 699/670 mg/kg bw/d: Increased relative adrenal weight in males (34%). Ocular opacity and keratitis in a single female.
1 year oral (dietary) MAFF 12-Nousan-No. 8147, 2-1-14 (2000) GLP Batch 20111222-1 Purity 97.27% Anonymous (2014d) DAR B.6.5 CLP guideline value for classification (mg/kg bw/d) Cat 1 = 2.5 Cat 2 = 25	Dog Beagle (HRA) 4/sex/group	0, 100, 1000, 10000 ppm Equivalent to Males: 0, 2.91, 28.1, 321 mg/kg bw/d Females: 0, 2.62, 28.5, 295 mg/kg bw/d	No treatment-related deaths or overt signs of toxicity in any dose group 2.91/2.61 mg/kg bw/d: No adverse effects. 28.1/28.5 mg/kg bw/d: No adverse effects. 321/295 mg/kg bw/d: Increased relative liver weight in males (20%). Enlargement of the internal iliac lymph node in males with accompanying adverse histopatholgy (2/4).

Method Guideline, Deviation(s) from the guideline (if any)	**Species Strain Sex no/group**	**Dose levels duration of exposure**	**Relevant adverse effects**
28-d dermal OECD 410 GLP Batch 20111222-1 Purity 97.27% Anonymous (2013l) DAR B.6.3.3 CLP guideline value for classification (mg/kg bw/d) Cat 1 = 20 Cat 2 = 200	Rat, SD 10/sex/group	0, 100, 300, 1000 mg/kg bw/d 6 hours/day	No treatment-related deaths or overt signs of toxicity in any dose group <u>100 mg/kg bw/d</u>: No adverse effects. <u>300 mg/kg bw/d</u>: No adverse effects. <u>1000 mg/kg bw/d</u>: Increased relative liver weight in females (9%) Increased relative adrenal weight in males (15%). Acinar cell apoptosis and inflammatory cell infiltration in the pancreas in both sexes (m 3/6, f 1/6).

<u>Rat</u>

<u>28 day study</u>

In a preliminary range-finding study intended to determine appropriate dose levels for a subsequent 90 day study, Wistar rats were fed dietary concentrations of tolpyralate at up to 20000 ppm for a period of 28 days.

Increased relative liver weights were noted in males at dose levels ≥ 45.9 mg/kg bw/d and females at ≥496 mg/kg bw/d with associated centrilobular hypertrophy only observed in males at 1799 mg/kg bw/d. Increased deposition of hyaline droplets in the proximal tubular cells in the kidney and hypertrophy of follicular cells in the thyroid were observed in males at ≥4.49 mg/kg bw/d. Single acinar cell necrosis in the pancreas was observed in males treated at ≥447 mg/kg bw/d and females at 1907 mg/kg bw/d. Opacity and keratitis were recorded in the eye in males at ≥4.49 mg/kg bw/d and females at ≥46.9 mg/kg bw/d

<u>90-day study</u>

In a 13 week dietary study, tolpyralate was administered to the groups of Wistar rats, up to a maximum concentration of 20000 ppm. Adverse treatment related effects were observed in the eye, liver, kidney, pancreas and thyroid. Increased absolute and relative liver weight was observed in both sexes at doses ≥133 mg/kg bw/d, this was accompanied by hepatocellular hypertrophy in males receiving 1363 mg/kg bw/d, the highest tested dose. An increase in kidney weight was observed in males receiving ≥133 mg/kg bw/d and females at 1647 mg/kg bw/d. Increased incidence of deposition of hyaline droplets in the proximal tubular cells in the kidney was observed in males treated at ≥1.34 mg/kg bw/d. Follicular cell hypertrophy in the thyroid was observed in both sexes at ≥133 mg/kg bw/d. Single acinar cell necrosis in the pancreas was observed in males treated at ≥133 mg/kg bw/d and in females at 1647 mg/kg bw/d. Ocular opacity and keratitis were observed in both sexes ≥133 mg/kg bw/d.

1 year dietary

The long term toxicity of tolpyralate was investigated in a 52 week dietary study in the rat. The test item was administered to groups of Wistar rats, up to a maximum concentration 10000 (males) or 20000 ppm (females). This study was conducted concurrently to the 2 year rat carcinogenicity study, in the same species, strain and test facility. However, they were completely separate studies; this one year study cannot be viewed as a 'satellite' group within the 2 year study.

Clinical signs

There were no treatment related changes in mortality in either sex at any dose level. Treatment related clinical signs of toxicity were decreases in body weight (10-14% relative to controls) in both sexes at ≥482 mg/kg, loss of fur in males at ≥0.925 mg/kg bw/d and in females at ≥126 mg/kg bw/d. Opacity of the eye was noted in clinical observations in both sexes at doses ≥97.0 mg/kg bw/d.

Ophthalmology

Corneal opacity and neovascularisation were significantly increased in both sexes at ≥97.0 mg/kg bw/d.

Urinalysis

Urinalysis showed increased urinary ketones and decreased urinary pH in both sexes at doses ≥97.0ppm, these effects are regarded as treatment related but are of unknown toxicological significance.

Organ weights

Relative liver weight was significantly increased by 10-16% relative to controls in both sexes as doses ≥97.0 mg/kg bw/d. Significant increases in relative kidney weight were observed in males at ≥97.0 mg/kg bw/d and in females at 1336 mg/kg bw/d.

Gross pathology

An increased incidence of opacity of the eye was observed in both sexes at doses ≥97.0 mg/kg bw/d. Statistically significant increases in the incidence of loss of fur were observed in both sexes at dose levels ≥97.0 mg/kg bw/d, consistent with the clinical observations. Increased incidence of soiled fur (genital) was observed in females at dose levels ≥126 mg/kg bw/d and is considered to be treatment-related, but is of unknown toxicological significance.

Histopathology

A significant increase in the incidence of fatty liver, characterised by fatty changes to centrilobular hepatocytes, was observed in males at doses ≥97.0 mg/kg bw/d. No microscopic liver effects were observed in females. A dose related increase in pancreatic acinar cell fibrosis (single cell) was observed in males at ≥0.925 mg/kg. Increased incidence of pancreatic acinar cell necrosis was observed in both sexes at ≥97.0 mg/kg bw/d. A significant increase in the incidence of adverse pathology in the kidney (renal tubule; basophilic change) was observed in males at ≥97.0 mg/kg bw/d. Increased incidence of thyroid follicular cell hypertrophy was observed in both sexes at doses ≥97.0 mg/kg bw/d. A significant increase in vacuolation of the cerebellum was observed in males at ≥97.0 mg/kg bw/d and in females at ≥1336 mg/kg bw/d. A significant increase in keratitis was observed in the eye in both sexes as doses ≥97.0 mg/kg bw/d. The effects were observed in 20/21 males and 21/21 females in both dose groups.

2 year dietary

The chronic toxicity of tolpyralate was investigated in a combined chronic toxicity / carcinogenicity study in the rat. Neoplastic findings are discussed in section 10.9. Effects relevant to STOT-RE are discussed here.

Organ Weights

Absolute brain weights were reduced by 8% and 7% in males receiving 83.8 and 426 mg/kg bw/d respectively. No decrease in relative brain weights was recorded. In the absence of an effect on relative brain weight or a comparable effect in females, this is not considered to be a relevant finding. Relative liver weight was significantly increased in males by 23% and 25% when compared to control animals at 83.8 and 426 mg/kg bw/d respectively. Relative kidney weight was significantly increased in males at 83.8 and 426 mg/kg bw/d by 11% and 17% respectively when compared to control animals. No relevant changes in organ weights were observed in females.

Haematology

There were increases in WBC counts in males in the top two dose groups, most notably in total leukocyte count which was increased by 31% and 60% relative to controls at dose levels of 83.8 and 426 mg/kg bw/d respectively. This effect is of unclear toxicological significance, but may be secondary to an inflammatory response following chronic administration of the test item and is not considered to be indicative of specific toxicity to the blood.

Gross pathology

Relevant gross findings were ocular opacity in the majority of animals at dose levels of ≥83.8 mg/kg bw/d. In 2 and 6 males in the groups receiving 83.8 and 426 mg/kg bw/d respectively, masses were observed in the eyes. A trend of increased incidence of luminal dilation of the bile duct was observed in both sexes at doses of ≥0.765/1.01 mg/kg bw/d, this effect was only statistically significant in males at 83.8/108 mg/kg bw/d and occurred with low incidence (maximum of 5/51 animals) and was not strongly dose dependent, consequently this is not considered to be a relevant finding. In males a dose related increase in the observation of coarse surface of the kidney was recorded at doses ≥83.8mg/kg bw/d, additionally a statistically significant increase in the incidence of kidney cysts was observed in males receiving 426 mg/kg bw.

Histopathology

A significant increase in the incidence of haematopoiesis in the bone marrow was observed in males at dose levels of ≥83.8 mg/kg bw/d. This effect was statistically significant in bone marrow taken from the femur when all animals (found dead, killed *in extremis* and terminal sacrifice) were considered. This effect was also observed in the bone marrow taken from the sternum, achieving statistical significance at doses ≥83.8 mg/kg bw/d, in the animals found dead or killed in extremis only, although the control incidence in this group was low. This effect was observed inconsistently and at low incidence and is not indicative of specific toxicity to the bone marrow.

Increased incidence of pancreatic acinar cell atrophy/fibrosis was observed in both sexes at doses ≥83.8/108 mg/kg bw/d, affecting 22 and 23 females and 46 and 47 males at 83.8/108 and 426/554 mg/kg bw/d respectively. A significant increase in the observation of fat infiltration in the pancreas was observed in males at ≥83.8 mg/kg bw but was not evident in females. A trend of increased incidence of pancreatic acinar cell necrosis was observed in both sexes, occurring at low incidence in males, affecting 1 and 2 animals at 83.8 and 426 mg/kg bw/d respectively compared to zero incidences in control animals. Increased incidence of acinar cell necrosis was also observed in females and was significantly increased at the highest dose level, and was observed in 16/51 animals compared to 7/51 controls.

A treatment related increase in the incidence of chronic nephropathy was observed in the kidney in both sexes. In males a dose response was evident with incidence increased over controls at doses ≥0.765/1.01 mg/kg bw/d but statistical significance was not achieved at any dose level. In females the effect was more apparent with a statistically significant increase observed at both 108 mg/kg bw/d and 554 mg/kg bw/d.

Increased incidence of colloid degeneration in the thyroid was recorded in both sexes, and was significantly increased relative to control animals at ≥108 mg/kg bw/d in females and at 426 mg/kg bw in males. An

increased incidence of follicular cell hyperplasia was recorded in males at ≥108 mg/kg bw/d but at low incidence and without statistical significance.

A clear increase in the incidence of vaculoation of the molecular layer of the cerebellum was observed in both sexes at doses ≥83.8/108 mg/kg bw/d, affecting 40 - 44 animals in each group compared to 8 and 9 incidences in male and female controls respectively.

A treatment related increase in the number of observations of degeneration of the sciatic nerve was recorded in both sexes at doses ≥83.8/108 mg/kg bw/d.

Consistent with macroscopic findings, a significant increase in the incidence of keratitis in the eye was observed in all animals at doses ≥83.8/108 mg/kg bw/d.

Table 36: Summary of non-neoplastic histopathological findings in the 90 day repeated dose toxicity study in the rat.

	Sex and dose level (mg/kg bw/d)									
	Male					Female				
Organ: Lesion	0	0.196	0.765	83.8	426	0	0.255	1.01	108	554
Bone marrow (femur):[N=]	[51]	[51]	[51]	[51]	[51]	[51]	[18a]	[15a]	[15a]	[51]
Hematopoiesis, increased	13	14	16	20	23*	18	7	4	2	17
Muscle [N=]	[51]	[51]	[51]	[51]	[51]	[51]	[18a]	[15a]	[15a]	[51]
(m. triceps surae):										
Atrophy, striated muscle										
fiber	6	2	8	9	14*	0	0	0	1	1
Pancreas: [N=]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]
Atrophy/fibrosis,										
acinar cell	14	12	14	46**	47**	10	10	6	23**	22**
Infiltration, fat	3	2	0	26**	24**	11	6	7	7	10
Necrosis, acinar cell,										
single cell	0	0	0	1	2	7	6	6	7	16*
Kidney: [N=]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]
Nephropathy, chronic	38	38	42	45	45	6	4	9	24**	18**
Thyroid: [N=]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]
Degeneration, colloid	9	11	8	15	25**	0	3	1	16**	23**
Hyperplasia, C-cell	9	11	9	4	7	14	12	10	3**	3**
Hyperplasia, follicular cell	3	2	3	8	6	1	0	0	2	2
Cerebellum: [N=]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]
Vacuolation, molecular										
layer	8	5	9	40**	44**	9	13	11	41**	44**
Nerve (sciatic): [N=]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]
Degeneration, nerve										
fiber										

	Sex and dose level (mg/kg bw/d)									
	Male					Female				
Organ: Lesion	0	0.196	0.765	83.8	426	0	0.255	1.01	108	554
	7	6	6	13	22**	4	2	3	12*	15**
Eye: [N=]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]	[51]
Keratitis	4	2	4	51**	51**	0	2	1	51**	51**

[N]: Number of animals examined at the site. Figures represent the incidence of the lesion.

a: Not subjected to statistical evaluation because the organs of terminal kill animals without macroscopic lesions were not histopathologically examined.

∗, $p \leq 0.05$; ∗∗, $p \leq 0.01$ (by Fisher's exact probability test).

28 day dermal

The repeat dose toxicity of tolpyralate via the dermal route was investigated in a 28 day study in the rat. Minimal adverse effects were observed in the adrenals liver and pancreas at the highest tested dose of 1000 mg/kg bw/day.

Mouse

90 day dietary

In a study of sub chronic toxicity, Crlj:CD1(ICR) mice were administered tolpyralate in the diet, up to a maximum concentration of 7000 ppm for a period of 13 weeks. Adverse treatment related effects were decreased platelet count and histopathological changes in the thyroid (follicular cell hypertrophy) in males at 1056 mg/kg bw/d. Centrilobular hepatocellular hypertrophy was observed in males treated at ≥284 mg/kg bw/d ppm and females treated at 1176 mg/kg bw/d, and is considered to be indicative of a non-adverse adaptive response, in the absence of significant effect on liver weight or other indication of liver damage. Total bilirubin was decreased in a dose dependent manner in both sexes at all dose levels, but is not indicative of an adverse effect of the test item. Hepatocellular necrosis was observed in a single female at 1176 mg/kg bw/d.

18 month dietary

The chronic toxicity of tolpyralate was investigated in a combined chronic toxicity / carcinogenicity study in the mouse. Neoplastic findings are discussed in section 10.9. Effects relevant to STOT-RE are discussed here.

Organ weights

A significant increase in relative liver weight of 30% was identified in females receiving 732 mg/kg bw/d. This effect was less obvious in males with relative liver weights being increased by 14% at 793 mg/kg bw/d without statistical significance.

Histopathology

An increase in the incidence of calculi in the gallbladder was evident in both sexes at dose levels ≥7.37/7.25 mg/kg bw/d.

A dose related increase in glomerulonephritis was observed in females that was statistically significant at 732 mg/kg bw/d.

An increased incidence in hepatocellular hypertrophy in the centrilobular region was recorded in both sexes at doses ≥78.5/72.6 mg/kg bw/d.

In males a dose related increase in hypertrophy of both the seminal vesicle and the coagulating gland was observed in animals which survived until study termination, achieving statistical significance at the top dose level. This was not evident when all animals were considered but may be toxicologically relevant.

Table 37: Summary of non-neoplastic findings in the 18 month carcinogenicity study (oral route)

	Sex and dose level (ppm)							
	Male				Female			
Organ/lesion	**0**	**70**	**700**	**7000**	**0**	**70**	**700**	**7000**
Number of animals examined	[52]	[52]	[51][a]	[52]	[52]	[52]	[52]	[52]
Gallbladder [N=]	[52]	[52]	[51]	[52]	[52]	[52]	[52]	[52]
Calculi	6	14*	18**	16*	4	11*	17**	20**
Kidney [N=]	[52]	[27][c]	[29] [c]	[52]	[52]	[52]	[52]	[52]
Glomerulonephritis	14	0	3	8	7	8	12	19**
Liver [N=]	[52]	[52]	[51]	[52]	[52]	[52]	[52]	[52]
Hypertrophy, hepatocyte, centrilobular	2	4	8*	12**	0	0	6*	17**
Seminal Vesicle (Terminal Kill) [N=]	[35]	[32]	[33]	[31]	-	-	-	-
Hypertrophy	9	13	15	17*	-	-	-	-
Coagulating Gland (Terminal Kill)[N=]	[35]	[32]	[33]	[31]				
Hypertrophy	10	10	14	16*				

[N=]: Number of animals examined at the site. Figures represent the incidence of the lesion.

∗, $P \leq 0.05$; ∗∗, $P \leq 0.01$ (Fisher's exact probability test).

a: One animal (Animal no. 113) was excluded from the effective number of animals because of accidental death.

c: Examined on the animals that showed macroscopic lesions at terminal kill and on all the animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.

Dog

90 day dietary

The sub chronic toxicity of tolpyralate *via* the oral route was investigated in a dietary study conducted in the dog over a period of 13 weeks. In males, both the left and total relative adrenal weights were increased by approximately 30% at the highest tested dose of 699 my/kg bw/d, when compared to control animals. Ocular opacity and keratitis was observed in a single female at 670 mg/kg bw/d.

1 year dietary

The repeated dose oral toxicity of tolpyralate was investigated in a dietary study performed in the dog over a period of 52 weeks. Animals were administered the test item in the diet at concentrations of 0, 100, 1000 and 10000ppm, corresponding to 2.91, 28.1 and 321 mg/kg/day in males and 2.62, 28.5 and 295 mg/kg/day in females. The test item was well tolerated and produced no mortalities. Clinical signs of toxicity were body weight loss and supressed body weight gain at the highest tested dose.

A significant increase in relative liver weight (approximately 20% relative to controls) was observed in males at 321 mg/kg bw/d, this effect was not seen in females. Enlargement of the internal iliac lymph node was observed in two males at 321 mg/kg bw/d, accompanied with corresponding microscopic changes to the tissue. This effect was not observed at any other dose level or in females and therefore, is not considered to represent an adverse effect of the test item.

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

Information relating to the potential for tolpyralate to cause specific organ toxicity following repeated exposure is available from a range of repeated dose toxicity studies, conducted in the rat, mouse and dog over a range of durations and *via* both the oral and dermal routes. Findings that are considered to be treatment related adverse effects and are relevant to humans are discussed below.

Eye

Adverse effects on the eye were observed in all studies conducted in the rat. Typically, ocular opacity and associated keratitis were observed in all oral studies conducted in the rat. In the 28 day oral study, adverse effects on the eye were observed at all tested dose levels (i.e., ≥ 4.49 mg/kg bw/d). In the 90 day dietary study ocular effects were recorded at doses ≥ 133 mg/kg bw/d. In the 1 year study, eye effects were observed from 97 mg/kg bw/d. In the 2 year chronic toxicity / carcinogenicity study, eye effects were observed at doses ≥ 83.8 mg/kg bw/d. Effects in the eye were also observed in both the parents and offspring in the 2-generation study. Corneal opacity and keratitis were observed in both sexes at 54.9/59.3 mg/kg bw/day in both the P and F1 parents. A single incidence of corneal opacity and keratitis was observed in P and F1 males treated with 2.7 mg/kg bw/d tolpyralate. In the offspring, corneal opacity was noted in both the F1 and F2 generations of the high dose group.

With the exception of a single incidence of ocular opacity in the 90 day study in the dog, in one female in the top dose group, there were no effects on the eye in other species.

Kidney

In the rat an increased incidence of hyaline deposition was observed in males in the 28 and 90 day repeat dose studies and in the 2 generation reproductive toxicity study, the lowest dose at which this effect was observed was 1.34 mg/kg bw/d (in the 90-day study). However, this finding was not observed in the 2-year carcinogenicity study at any dose. The hyaline deposition is likely to be associated with α2μ-globulin, although this has not been confirmed and therefore cannot be dismissed as being of no relevance to humans. In all studies in the rat, increased relative kidney weight was observed. The lowest dose at which this effect was observed was 54.9 mg/kg bw/d (in the parents of the 2-generation study). Further, in the 2-generation reproductive toxicity study, a variety of adverse kidney effects were observed in parental animals at the top dose of 54.9 mg/kg bw/d, including; basophilic change to the renal tubule, calcification, cysts, hyaline deposition and drug induced nephropathy. In both generations of offspring a variety of gross findings were observed in the kidney, including; pale colour, pelvic dilation and white material in the pelvic space/papilla also at the top dose level of 59.3 mg/kg bw/d. In the 1 year study basophilic change was observed in males from 97 mg/kg bw/d. In the 2-year carcinogenicity study, chronic nephropathy in the kidney of males was observed from 0.765 mg/kg bw/day, but was not statistically significant at any dose. The effect was more apparent in females, with a statistically significant increase observed at both 108 mg/kg bw/d and 554 mg/kg bw/d.

In the chronic toxicity study in the mouse, an increased incidence of glomerulonephritis was recorded in females at 732 mg/kg bw/d. Other than a single incidence of tubular regeneration in the 28-day range finding study in the dog which us unlikely to represent a treatment related finding, there were no signs of kidney toxicity in any other species.

Liver

The predominant adverse finding in the liver was increased relative liver weight, occurring in most studies in the rat. Increases in relative liver weight in excess of 10% were observed at dose levels of 54.9 mg/kg bw/d (in the 2-generation study). At high doses some evidence of hepatocellular hypertrophy was observed. In the mouse, hepatocellular hypertrophy was observed in both the 90 day and 18 months studies at dose levels ≥72.6 mg/kg bw/d, increased liver weight was also observed at high dose levels (732 mg/kg bw/d) and hepatocellular necrosis was observed in a single female in the 90 day study at 1176 mg/kg bw/d. Increased relative liver weight was also observed in the 1 year dog study in males at the highest dose of 321 mg/kg bw/d.

Pancreas

Pancreatic acinar cell fibrosis and/or necrosis were observed in most studies conducted in the rat. In some cases pancreatic fat infiltration was also observed. The lowest dose at which this effect was observed was seen in the 1 year study in the rat in which the start of a trend of increased incidence of pancreatic acinar cell fibrosis was observed in males from 0.925 mg/kg bw/d, affecting 7/21 males in this group, there was no evidence of necrosis in this group. In all other studies in the rat, including the 2 year study that was conducted concurrently to the 1 year study and under the same conditions, there was no evidence of this effect at dose levels relevant for classification. It is concluded that the observation of this effect at 0.925 mg/kg bw/d in the 1 year study is anomalous. In addition, the effect was marginal at this dose level, was not statistically significant and was not accompanied by any indication of necrosis.

Cerebellum

Vacuolation of the cerebellum was observed in in the rat, in both sexes in both the 1 and 2 year chronic toxicity studies, at dose levels of ≥83.8 mg/kg bw/d. This was not observed in any other studies in the rat or in any other species

Sciatic nerve

Degeneration of the sciatic nerve was observed in in the rat, in both sexes in the 2 year chronic toxicity study. This effect was recorded in females at dose levels of ≥108 mg/kg bw/d and in males at 426 mg/kg bw/d. This was not observed in any other studies in the rat or in any other species

Gall bladder

In the 18 month chronic study in the mouse, an increased incidence of calculi in the gall bladder was observed at all dose levels. This was not observed in any other studies in the mouse or in any other species and is of minimal toxicological significance.

10.12.1 Comparison with the CLP criteria

Table 38 below summarises the effects occurring at or below the relevant guidance values for classification with STOT RE.

Table 38: Summary of effects seen following repeated exposure to tolpyralate at doses relevant for classification.

Study	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
Rats (Wistar), 28 days Guidance values for classification *Cat 1 ≤ 30 mg/kg bw/day* *30 < Cat 2 ≤ 300 mg/kg bw/day*	4.94/4.98 mg/kg bw/day Hyaline deposition in kidney in 1/6 males. Thyroid follicular cell hypertrophy in 2/6 males. Ocular opacity and keratitis in 1/6 males.	≥45.9/46.9 mg/kg bw/day Hyaline deposition in kidney in males. Increased relative liver weight in males (16%). Thyroid follicular cell hypertrophy in males. Ocular opacity and keratitis in both sexes.
Rats (Wistar), 90 days. *Cat 1 ≤ 10 mg/kg bw/day* *10 < Cat 2 ≤ 100 mg/kg bw/day*	1.34/1.58 mg/kg bw/day Hyaline deposition in kidney in 2/10 males.	It is noted that the next dose was 133/159 mg/kg bw/day. Effects seen at this dose level included; Hyaline deposition in kidney in 2/10 males. Ocular keratitis in 7/10 males and 10/10 females Increased relative liver weight in males (25%) Increased relative kidney weight males (10%) Thyroid follicular cell hypertrophy in both sexes (males 9/10, females 4/10).
Rats (Wistar), 1 year *Cat ≤ 1 2.5 mg/kg bw/day* *2.5 < Cat 2 ≤ 25 mg/kg bw/day*	0.925/1.18 mg/kg bw/d Pancreatic acinar cell fibrosis in 7/21 males	It is noted that the next dose was 97/126 mg/kg bw/day. Effects seen at this dose level included; Ocular keratitis in 20/21 males and 21/21 females Increased relative liver weight in both sexes (16% in males, 10% in females) Fatty change in centrilobular hepatocytes in males (11/21) Increased relative kidney weight in both sexes (17% in males, 7% in females) Basophilic change to renal tubule in males (8/21) Pancreatic acinar cell necrosis in males (7/21) Pancreatic acinar cell fibrosis in males (11/21) Vacuolation of cerebellum in males (6/21)
Rats (Wistar), 2 years *Cat 1 ≤ 1.25 mg/kg bw/day* *1.25 < Cat 2 ≤ 12.5 mg/kg bw/day*	None	None

Study	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
Rats (SD), 2 generation study *Cat 1≤ 10 mg/kg bw/day* *10 < Cat 2 ≤ 100 mg/kg bw/day*	<u>P</u> Increased liver weight in both sexes (8%) <u>F1 parents</u> Ocular keratitis in one animal of each sex <u>F1 pups</u> None <u>F2 pups</u> None	<u>P</u> Ocular opacity and keratitis in both sexes Increased liver (16% males, 13% females), kidney (13% males) and testis (10%) weight. Decreased brain weight (6% females) <u>F1 parents</u> Ocular opacity and keratitis. Decreased prostate weight (21%). Increased Liver (22% male, 13% female), Kidney (16% male, 7% female), Testis (12%), Epididymides (13%), Seminal vesicle (19%) weights. Increased incidence of microscopic kidney abnormalities; Basophilic change to renal tubule (10/24 males, 4/24 females) Calcification (7/24 males, 8/24 females) Cortical cysts (6/24 males) Hyaline droplets (5/24 males) Nephropathy (11/24 males, 4/24 females) <u>F1 pups</u> Increased incidence of gross kidney lesions; Pale colour Pelvic dilation White material in pelvic space/papilla <u>F2 pups</u> Increased incidence of gross kidney lesions Pale colour Pelvic dilation White material in pelvic space/papilla
Mouse (CrljCD1), 90 days. *Cat 1 ≤ 10 mg/kg bw/day* *10 < Cat 2 ≤ 100 ≤ mg/kg bw/day*	None	None

Study	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
Mouse (CrljCD1), 18 month *Cat 1 1.7 ≤ mg/kg bw/day* *1.7 < Cat 2 ≤ 17 mg/kg bw/day*	None	Increased incidence of calculi in the gall bladder in both sexes.
Dog (Beagle- HRA), 28 days Guidance values for classification* *Cat 1 ≤ 30 mg/kg bw/day* *30 < Cat 2 ≤ 300 mg/kg bw/day*	None	None
Dog (Beagle- HRA), 90 days *Cat 1 ≤ 10 mg/kg bw/day* *10 < Cat 2 100 ≤ mg/kg bw/day*	None	None
Dog (Beagle- HRA), 1 year *Cat 1 ≤ 2.5 mg/kg bw/day* *2.5 < Cat 2 ≤ 25 mg/kg bw/day*	None	None
Rats (Wistar), 28 days (dermal) Guidance values for classification *Cat 1 ≤ 60 mg/kg bw/day* *60 < Cat 2 ≤ 600 mg/kg bw/day*	None	None

*Guidance values for the rat have been used in absence of specific values for the dog, to aid comparison

As described above, information relating to the potential for tolpyralate to cause specific target organ toxicity following repeated exposure (STOT-RE) is available from a range of repeated dose toxicity studies in the rat, mouse and dog *via* both the oral and dermal routes. In addition, information is available from combined chronic and carcinogenicity studies in both the rat and the mouse and in the two-generation reproductive toxicity study in the rat. As shown in the table, effects occurring at dose levels relevant for classification were only observed in the rat.

In the rat, a variety of changes in relative organ weights were observed in both sexes. There were no accompanying adverse histopathological findings in any of the organs in which changes in relative weights were increased, at dose levels relevant for classification. Consequently none of the effects on organ weight represent an effect sufficient for classification for STOT-RE.

In both the 28-day and 90-day studies in the rat an increase in the incidence of thyroid follicular cell hypertrophy was observed in males at dose levels relevant for classification. Whilst this finding is considered to be treatment related it does not represent a significant change affecting function or morphology and, in isolation, is not considered sufficient for classification for STOT-RE.

Ocular opacity and associated keratitis was observed consistently following repeated administration of tolpyralate to rats. These adverse effects are considered to be of significant concern and, in a number of studies, were observed at doses below the relevant guidance values for classification. As summarised at the beginning of Section 10, tolpyralate is considered to be an inhibitor of the enzyme 4-hydroxyphenylpyruvate

dioxygenase (HPPD) and as a consequence has the potential to inhibit the catabolic pathway of tyrosine. The Applicant has proposed that inhibition of 4-HPPD resulting in sustained elevation of plasma tyrosine may lead to the effects that have been seen in the eyes of rats give repeated doses of tolpyralate. As ECHA's Risk Assessment Committee has already seen when considering proposals to classify sulcotrione, mesotrione and tembotrione, these effects are common to substances that inhibit the catabolism of tyrosine and consequently cause significantly elevated levels of this amino acid in the plasma. It has been reported that a plasma tyrosine concentration of 1000 µmol/L must be exceeded before corneal lesions occur in rats (Lewis & Botham, 2013).

The following studies provide some evidence for the inhibition of HPPD by tolpyralate and that this may be related to the observed ocular toxicity. They demonstrate how a single dose of tolpyralate influences plasma tyrosine levels in rats, rabbits and mice, and tyrosine levels in the aqueous humour in rats. One study compares the effects of repeated administration of tolpyralate on the eyes and cerebellum in rats and mice. An *in vitro* study comparing the effect of tolpyralate on rat and human hepatocellular metabolism of tyrosine is also provided.

(a) Mechanistic studies on the effect of a single administration of tolpyralate

Table 39: Summary of mechanistic studies on the effect of a single administration of tolpyralate on plasma tyrosine levels

Study details	Remarks
Measurement of plasma tyrosine concentration after single-dose oral administration of SL-573 TGAI in rats A single dose (1000 mg/kg bw) of tolpyralate was administered to groups of 5 female rats (Crl:CD(SD) by oral gavage. Plasma tyrosine concentrations were determined before and at 1, 2, 4, 6, 8, 24, 48 and 72 hours after administration. One group of female rats was used per time point. Concurrent control groups of 5 female rats were performed Anonymous (2013o)	No effects on clinical condition or body weight. In control animals, plasma tyrosine concentration remained in the same range from before to 72 hours after administration. In the animals treated with 1000 mg/kg tolpyralate, plasma tyrosine concentration was increased to 261.0 µmol/L at 1 hour after administration, compared to 59.4 µmol/L in the control group. It was further increased to 414.2, 603.2, 801.4, and 977.4 µmol/L at 2, 4, 6, and 8 hours after administration, respectively. After it reached a maximum value of 2055.8 µmol/L at 24 hours, at 72 hours post-dosing plasma tyrosine levels were comparable to controls.
Measurement of plasma tyrosine concentration after single-dose oral administration of SL-573 TGAI in mice A single dose (1000 mg/kg bw) of tolpyralate was administered to groups of 5 female mice (Crl:CD1(ICR)) by oral gavage. Plasma tyrosine concentrations were determined before and at 1, 2, 4, 6, 8, 24, 48 and 72 hours after administration. One group of female mice was used per	No effects on clinical condition or body weight. In control animals, plasma tyrosine concentration remained in the same range from before to 72 hours after administration. In the animals treated with 1000 mg/kg tolpyralate, plasma tyrosine concentration was increased to 379.4 µmol/L at 1 hour after administration, compared to 87.8 µmol/L in the control group. It was further increased to 526.0, 580.4, 601.2, and 663.0 µmol/L at 2, 4, 6, and 8 hours after administration, respectively. The plasma tyrosine concentrations at 24 and 48 hours post-dosing were 502.0 and 340.8 µmol/L, respectively. At 72 hours post-dosing, plasma tyrosine levels were still higher in the

Study details	Remarks
time point. Concurrent control groups of 5 female rats were performed. Anonymous (2013p)	treated group (270.4 µmol/L) compared to the controls (107.8 µmol/L).
Measurement of plasma tyrosine concentration after single-dose oral administration of SL-573 TGAI in rabbits A single dose (1000 mg/kg bw) of tolpyralate was administered to groups of 5 female rabbits (Kbl:NZW) by oral gavage. Plasma tyrosine concentrations were determined before and at 1, 2, 4, 6, 8, 24, 48 and 72 hours after administration. One group of female rabbits was used per time point. Concurrent control groups of 5 female rats were performed Anonymous (2013q)	No effects on clinical condition or body weight. In control animals, plasma tyrosine concentration remained in the same range from before to 72 hours after administration. In the animals treated with 1000 mg/kg tolpyralate, plasma tyrosine concentration was increased to 292.6 µmol/L at 1 hour after administration, compared to 95.2 µmol/L in the control group. It was further increased to 415.0, 672.6, 811.0, and 978.8 µmol/L at 2, 4, 6, and 8 hours after administration, respectively. At 24 and 48 hours post-dosing, plasma tyrosine concentrations were 1788.2 and 1053.6 µmol/L, respectively.

Overall, a single oral administration of tolpyralate at 1000 mg/kg bw/d to rats, rabbits and mice caused a clear increase in plasma tyrosine levels compared to controls. The increase was the highest in rats (25-fold), followed by rabbits (20-fold) and then mice (13-fold). These results indicate that rats might be more sensitive to tolpyralate-induced tyrosinemia compared to mice, although it is noted that plasma tyrosine levels were similar to controls after 72 hours in the rat, but were still elevated in mice at this time point.

(b) Mechanistic study on the effect of repeated administration of tolpyralate (Anonymous, 2016a)

Tolpyralate was administered to female rats and mice at dietary concentrations of 2000 and 7000ppm respectively, corresponding to mean systemic intakes of 144 mg/kg bw/day and 1089 mg/kg bw/day in rats and mice respectively. Negative and positive control groups were included. The positive control was 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). This is a known inhibitor of HPPD, used therapeutically to treat humans that suffer from a genetic trait that causes tyrosine deficiency.

Table 40: Summary of study to investigate the effect of chronic oral administration of tolpyralate and NTBC on the cornea and cerebellum in rats and mice

Study details	Remarks
52 week dietary study 20 females per group	***Rat*** *Mortality and clinical signs of toxicity* There were no treatment related mortalities or covert clinical signs of

Study details	Remarks
  <u>Rat, Wistar</u> tolpyralate -144 mg/kg bw/day NTBC – 0.06 mg/kg bw/day <u>Mouse, CD1</u> tolpyralate 1089 mg/kg bw NTBC 14.2 mg kg bw/day Anonymous (2016a)	toxicity in either treated group. *Terminal plasma tyrosine concentrations (*µmol/L*)* Control: 57.8; tolpyralate 1732.5; NTBC 1603.3 *Gross findings* Increased incidence of rough surface of the cornea, tylosis of the hind limb and accentuated lobular pattern of the liver in both tolpyralate and NTBC treated groups. *Histopathology* Increased incidence of keratitis of the cornea (tolpyralate = 18/19 , NTBC =12/20) Increased incidence of vacuolation of the cerebellum (tolpyralate = 16/19 , NTBC =12/20) ***<u>Mouse</u>*** *Mortality and clinical signs of toxicity* There were no treatment related mortalities or covert clinical signs of toxicity in either treated group. *Terminal Plasma tyrosine concentrations (*µmol/L*)* Control 83.4, tolpyralate: 745.2, NTBC 963.4 *Gross findings* Increased incidence of abnormal contents and distention of the gall bladder in both treated groups. *<u>Histopathology</u>* No effects in the cornea or cerebellum.

Only female animals were used in this study. Treatment with both tolpyralate and NTBC caused elevated plasma tyrosine in both species when compared to controls. In the rat, control, tolpyralate and NTBC tyrosine levels were 57.8, 1732.5 and 1603.3 µmol/L, respectively. In the mouse, control, tolpyralate and NTBC tyrosine concentrations were 83.4, 745.2 and 963.4 µmol/L, respectively.

In line with the findings of the more conventional studies of repeated dose toxicity, the effects of tolpyralate in the rat were increased incidence of keratitis of the cornea (tolpyralate = 18/19, NTBC =12/20) and increased incidence of vacuolation of the cerebellum (tolpyralate = 16/19, NTBC =12/20). The potential reversibility of these effects was not studied. There were no adverse effects in the eye or brain in the mouse following chronic exposure to tolpyralate or NTBC at the tested dose levels.

Irrespective of the comparable levels of plasma tyrosine seen following tolpyralate and NTBC treatment, both the incidence and severity of the corneal keratitis appeared higher in the rats administered tolpyralate. This may indicate that the effects of tolpyralate on the eye in this study may not have been solely attributable to elevated plasma tyrosine concentration.

(c) *Determination of tyrosine concentrations in rat plasma and aqueous humour following a single oral dose of tolpyralate (Anonymous, 2016b)*

A second study investigated the tyrosine concentration in plasma and the aqueous humour of the eye in the male rat following a single oral dose of tolpyralate. The study was not conducted in accordance with a specific test guideline and does not claim GLP compliance. Groups of 8 male Wistar rats were administered 0, 1, 100 or 1000 mg/kg bw tolpyralate by oral gavage. 24 hours following dose administration the tyrosine concentration was determined in the plasma and in the eye.

Table 41: Summary of study to determine tyrosine concentration in plasma and aqueous humour of male rats – single oral dose of tolpyralate

Study details	Remarks
Rat, Wistar 8 males/group 0, 1, 100, 1000 mg/kg bw by single gavage Anonymous (2016b)	Effects on tyrosine concentration

tolpyralate dose (mg/kg bw)	Tyrosine concentration (µmol/L)	
	Plasma	Eye
0	94.1	170
1	110.3	197
100	1803.6	2039
1000	2360.4	2413

From this study, it is concluded that a single dose of tolpyralate to male rats can cause a significant increase in tyrosine levels in the plasma and in the eye at dose levels ≥100 mg/kg bw. The reversibility of these effects was not explored. A dose of 1 mg/kg bw did not significantly increase tyrosine concentrations when compared to control animals.

(d) Tyrosine metabolism in rat and human hepatocytes; effect of 4-HPPD inhibition by tolpyralate (Yokoyama H, 2016)

Additionally, an *in vitro* metabolism study was conducted in cultured rat and human hepatocytes. Levels of tyrosine and its metabolite DL-4-hydroxyphenylacetic acid (4-HLPA) were measured over the course of 4 hours following the addition 300µmol/L tolpyralate or NTBC in the presence of 100 mg/L L-tyrosine. Mean concentration of both 4-HPLA and tyrosine were measured at 2 and 4 hour time points by LC/MS/MS.

Table 42: Tyrosine metabolism in rat and mouse hepatocytes: effect of tolpyralate and NTBC

Study details	Remarks
In vitro primary human and rat hepatocytes 300µmol/L tolpyralate or NTBC Yokoyama, H. (2016) Only limited information available - number of replicate samples not known.	Mean tyrosine concentration

Species	Hepatocyte Batch Code	Time	Tyrosine concentration (µg/mg protein)		
			Control	tolpyralate (300µmol/L)	NTBC (300µmol/L)
Rat	UHU	0	58.5	-	-
		2	49.9	50.8	49.7
		4	43.2	48.4	47.0
Human	PTL	0	73.3	-	-
		2	58.4	62.1	64.3
		4	54.5	57.3	58.1
	FDX	0	97.2	-	-
		2	88.5	94.5	86.2
		4	74.9	85.9	79.7

Study details	Remarks

Mean 4-HPLA concentration

Species	Hepatocyte Batch	Time	4-HPLA concentration (µg/mg protein)		
			Control	tolpyralate (300µmol/L)	NTBC (300µmol/L)
Rat	UHU	0	-	-	-
		2	0.582	0.615	0.726
		4	0.730	0.845	0.937
Human	PTL	0	-	-	-
		2	1.49	1.60	1.93
		4	2.37	2.65	2.94
	FDX	0	-	-	-
		2	2.28	2.55	2.86
		4	3.09	3.77	3.96

In both human and rat hepatocytes the level of tyrosine decreased with time; there was no clear difference between samples treated with tolpyralate or NTBC when compared to controls or between humans and rats. The level of 4-HPLA appeared to increase over time in both species, with more 4-HPLA being detected in the human samples than in the rat. A slight trend towards increased 4-HPLA was observed in samples treated with either tolpyralate or NTBC compared to controls, possibly indicating inhibition of 4-HPPD, but this is considered inconclusive. The relevance of these findings is unclear and, owing to lack of methodological details, the study is considered to be of limited value.

Although the findings of this last study are somewhat inconclusive, the observation from the other studies that tolpyralate treatment can increase circulating tyrosine levels in rats and mice (and indeed rabbits) is consistent with the view that this substance inhibits HPPD. It appears that the rat, especially the male rat, is more sensitive than the mouse. However, these studies do not provide a clear picture of the relative sensitivity of humans to rats.

Humans who have chronically elevated plasma tyrosine levels (e.g. through HPPD inhibition) are known to exhibit ocular effects (Ahmad *et al.*, 2002; Lock *et al.*, 2006; Wisse *et al.*,2012, Schauwvlieghe *et al*, 2013). Therefore, although the rat is clearly a sensitive species, tolpyralate could cause similar effects in humans. It is generally accepted that humans are less sensitive to the effects of inhibition of 4-HPPD than rats, owing to species differences in alternative metabolic pathways (see also the introduction to this subject at the beginning of Section 10). However, there is insufficient information available to make a comparison of the relative potency of tolpyralate in rats and humans with respect to impact on plasma tyrosine levels. Consequently, it is concluded that the hazardous effects of tolpyralate observed experimentally in rats are of relevance to humans. In the rat studies, effects on the eyes were generally observed only at dose levels relevant for classification in Category 2, with only the finding of keratitis in 1/6 males in the 28-day dietary study matching the criteria for a Category 1 classification. Therefore, it is proposed that these effects most appropriately meet the criteria for classification in STOT RE Category 2.

Hyaline deposition in the kidney of males rats was noted in the 28-day, 90-day studies and in the F1 parents of the 2-generation study. Whilst it is likely this is associated with a2u-globulin, this has not been confirmed and this finding cannot be dismissed as being of no relevance to humans. In the 2 generation reproductive toxicity in the rat, a range of histopathological abnormalities were observed in the kidney in both sexes of the parents; including basophilic change to renal tubule, calcification, cortical cysts, hyaline droplets and nephropathy, all at the highest dose level of 54.9 mg/kg bw/d. Effects were also observed in the kidney of the F1 and F2 offspring and included pale colour, pelvic dilation and white material in pelvic space/papilla. The hyaline deposition was noted at doses relevant for classification with STOT-RE 1 in both the 28- and 90-day studies. However, it is noted that the effect did not occur in the 2-year carcinogenicity study at doses relevant for classification. Given the low incidence of the hyaline deposition in the low dose groups of the 28- and 90-day studies (1/6 and 2/10 males respectively), the fact that this was not seen in the 2-year study and the fact that other kidney effects were only observed at higher doses, it is proposed that these effects also support classification in STOT-RE Category 2.

10.12.2 Conclusion on classification and labelling for STOT RE

Classification in Category 2 – H373 – may cause damage to eyes and kidneys through prolonged or repeated exposure.

10.13 Aspiration hazard

Not relevant for solid materials.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

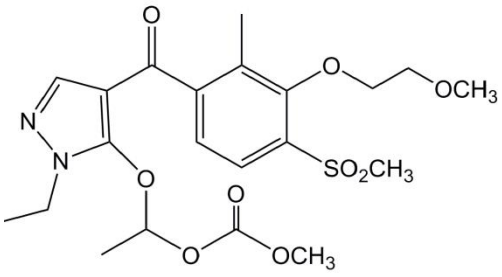
11.1 Rapid degradability of organic substances

Table 43: Summary of relevant information on rapid degradability

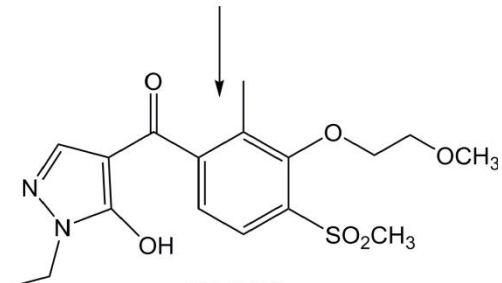
Method	Results	Remarks	Reference
Aquatic hydrolysis OECD Guideline 111, GLP	Tolpyralate hydrolysis is pH dependent. At 10 ºC DT50 for tolpyralate determined as 1050 d (pH 4), 231 d (pH 7) and 2.31 d (pH 9) One degradant identified, MT-2153 this reached max. levels at study end of 1.1, 7.8 and 86.3 % AR at pH 4.0, 7.0, and 9.0 at 10 ºC. No further evidence of degradation was observed in a degradant dosed hydrolysis study, see below.	Valid	Unsworth, R. 2013 (section B.8.2.1.1 in the DAR)
Aquatic hydrolysis (MT-2153) OECD Guideline 111, GLP	The degradation product MT-2153 was stable at pH4.0, 7.0, and 9.0 at 50 ºC. Indicated DT50 at 25 ºC are at >365d. As MT-2153 is stable to hydrolysis at 50ºC, normalising figures to 12ºC (as proposed by ECHA) would not alter the conclusion, and so this correction has not been undertaken.	Valid	Hori, K. 2014b (section B.8.2.1.1 in the DAR)

Method	Results	Remarks	Reference
Ready biodegradation OECD Guideline 301F, GLP	9% biodegradation after 25 days. Tolpyralate not classed as readily biodegradable.	Valid	Hammesfahr, U. 2013 (section B.2.2.1 in the DAR)

Proposed degradation pathway of tolpyralate under hydrolytic conditions:



SL-573



MT-2153

11.1.1 Ready biodegradability

A ready biodegradability study was conducted to GLP, according to OECD test guideline 301F and there were no significant deviations that would affect the validity of the study. The test material was non-radiolabelled tolpyralate, lot number 20111222-1 (97.27% purity).

Aliquots of washed activated sludge from a domestic waste water treatment plant equivalent to 3.5 g dry material per litre were mixed with test water. This suspension was aerated overnight and used for the test. Test water was prepared according to the description in the guideline OECD 301A; the pH was 7.6. Test flasks were prepared in duplicate for tolpyralate and the inoculum control, and single flasks were prepared for the procedure control (sodium benzoate), abiotic control ($HgCl_2$) and toxicity control (tolpyralate + sodium benzoate).

Tolpyralate was incubated with activated sewage sludge under aerobic conditions and the oxygen uptake of the microorganisms determined. Sodium benzoate was tested under the same conditions in order to give a reference value. Each flask had a volume of 500 mL and was incubated in the dark at 22 ± 1ºC with continuous stirring.

For tolpyralate, biodegradation reached a maximum of 9% of ThOD after 25 days. The extent of biodegradation of the reference substance (sodium benzoate) reached 85% of ThOD after 14 days and 94% after 28 days. The activated sludge and procedure used demonstrated the ready biodegradability of the reference substance, therefore was acceptable to test the ready biodegradability of tolpyralate. The extent of biodegradation of the reference substance and tolpyralate together in the toxicity control was 44% and 41% of ThOD within 14 days and 50% and 46% after 28 days of incubation. As degradation is

>25% based on ThOD within 14 days, it can be concluded that tolpyralate is not inhibitory to microorganisms in activated sludge. There was no oxygen demand in the abiotic control at any time during the experiment. Substances are considered to be readily biodegradable in this test if the extent of degradation reaches 60% of the theoretical value within 10 days of achieving 10% degradation.

Tolpyralate is not classed as readily biodegradable.

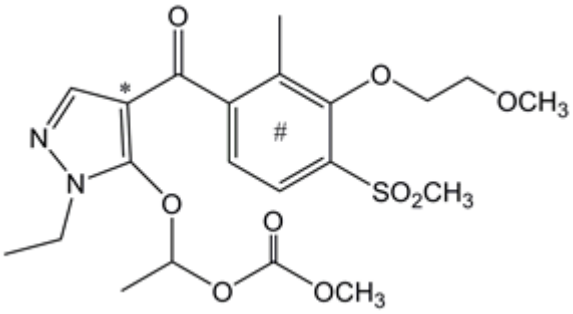
Hammesfahr, (2013)

11.1.2 BOD_5/COD

Not available.

11.1.3 Hydrolysis

The hydrolysis of tolpyralate in buffers at pH 4.0, 7.0, and 9.0 and at 10, 25, and 50ºC. The study was conducted to GLP and according to OECD test guideline 111 and there were no significant deviations that would affect the validity of the study. The test material was tolpyralate, radiolabelled in two different positions as shown below with a radiochemical purity ≥97%:



position of [14C-Ph] radiolabel * position of [14C-Pz] radiolabel

The hydrolysis of tolpyralate in sterile buffer solutions is pH and temperature dependent, and follows first order reaction kinetics. Tolpyralate hydrolyses more rapidly under conditions of higher pH and under increasing temperature. DT50 values determined for each pH and temperature tested are detailed below:

Table 44

Test pH	Temperature (ºC)	DT_{50} (days)
pH 4.0	10	1050
	25	332
	50	25.9
pH 7.0	10	231
	25	31.4
	50	1.88
pH 9.0	10	2.31
	25	0.355
	50	0.033

The only hydrolysis product measured at greater than 5% of the applied radioactivity was MT-2153. MT-2153 reached maximum concentrations of 53.9, 88.9, and 101.4 %AR at pH 4.0, 7.0, and 9.0 respectively at 50ºC and maximum levels of 1.1, 7.8 and 86.3 % AR at pH 4.0, 7.0, and 9.0 at 10 ºC. The ratio of stereoisomers (1:1) was unchanged during hydrolysis, indicating that the rate of hydrolysis of the two stereoisomers was the same.

Unsworth, (2013a)

11.1.4 Other convincing scientific evidence

Table 45. Summary of other relevant information on rapid degradation

Method	Results	Remarks	Reference
Aquatic photolysis OECD Guideline 316, GLP	DT_{50} of tolpyralate of 2.86 days in purified water (pH 6-7, 25 ºC) DT_{50} of tolpyralate of 4.41 days in natural water pH 6-7, 25 ºC)	Valid	Unsworth, R. 2014 (section B.8.2.1.1 in the DAR)
Aerobic water/sediment simulation OECD Guideline 308, GLP	DT_{50} of tolpyralate of 1.39 and 1.88 days at pH 7.4 and pH 5.7 respectively (whole system). MT-2153 reached a max. of 82 % and 79.9 % in the whole system and 19.8 % and 32.7 % in sediment. DT_{50} of MT-2153 of 206 and 176 days (whole system).	Valid	Kane, T. 2014 (section B.8.2.2.3 in the DAR)
Anaerobic water/sediment simulation OECD Guideline 308, GLP	DT_{50} of tolpyralate of 1.47 and 2.72 days at pH 7.3 and pH 5.4 respectively (whole system). MT-2153 reached a max. of 89.3 % and 93 % in the whole system and 23.6 % and 18.8 % in sediment. DT_{50} of MT-2153 of 388 and 394 days at pH 7.4 and pH 5.7 respectively (whole system).	Valid	Crowe, A. (2015) (section B.8.2.2.3 in the DAR)
Aerobic mineralization OECD Guideline 309, GLP	DT_{50} of tolpyralate of 2.43 days in surface water at an initial concentration of 10 µg/mL and 1.77 days at an initial concentration of 100 µg/mL	Valid	Van den Bosch, M.M.H. 2014 (section B.8.2.2.2 in the DAR)

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

Not available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Not available.

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

Aerobic mineralisation in surface water

The rate of degradation and mineralisation of tolpyralate in surface water under aerobic conditions was conducted to GLP and according to OECD test guideline 309, there were no significant deviations that

would affect the validity of the study. The test material was tolpyralate, radiolabelled in both the phenyl and pyrazole ring positions and with a minimum radiochemical purity of 99.2%. The surface water for this test was collected from Schoonrewoerdse Weil in the Netherlands to a depth of 1 metre and had the following characteristics:

Table 46:

Parameter	Value
pH (at sampling)	8.3
Temperature (at sampling)	5.8°C
Oxygen (at sampling)	11.5 mg/L (93%)
Total organic carbon	11.0 mg/L
Dissolved organic carbon	9.5 mg/L
Total nitrogen as N	3.7 mg/L
Nitrate	4.1 mg/L
Nitrite	<1.6 mg/L
Ammonium	<0.7 mg/L
Total hardness	151 mg/L as $CaCO_3$
Phosphate/orthophosphates	<0.3 mg/L
Total phosphorus as P	<0.1 mg/L

In microbially active surface water, at 20 ºC, tolpyralate degraded rapidly to less than 2% of applied activity after 14 days incubation. The DT50 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 was formed and increased to greater than 90% AR at 7 days and there was no clear evidence of decline at the end of the study after 62 days. The maximum amount of CO_2 formed was 3.8% AR.

Van den Bosch (2014)

Aerobic water-sediment study

The route and rate of degradation of tolpyralate in two aquatic sediment systems under aerobic conditions was determined. The study was conducted to GLP according to OECD test guideline 308 and there were no significant deviations that would affect the validity of the study. The test material was tolpyralate, radiolabelled in both the phenyl and pyrazole ring positions and had a minimum radiochemical purity of 99.5%.

The two aquatic sediments were ‘Calwich Abbey Lake’ and ‘Swiss Lake’, collected from Staffordshire and Derbyshire, UK respectively. The characterisation of sediment and water from ‘Calwich Abbey Lake’ and ‘Swiss Lake’ are detailed below:

Table 47:

Parameter	Calwich Abbey Lake	Swiss Lake
Water		
Total organic carbon (mg/L)	2.9	8.8

Parameter	Calwich Abbey Lake	Swiss Lake
Total nitrogen (mg/L)	3.1	0.9
Total phosphorus (mg/L)	<0.1	<0.1
Total suspended solids (mg/L)	20	16
Hardness as $CaCO_3$ (mg/L)	245	26.5
Sediment		
Particle Size Distribution: 0.063 mm - 2.00 mm (%) 0.002 mm - 0.063 mm (%) <0.002 mm (%) Texture Class	 26 61 13 Sandy Silt Loam	 91 6 3 Sand
pH (1:5 w/v) in water	8.0	6.3
pH (1:5 w/v) in 0.01 M $CaCl_2$	7.4	5.7
Organic carbon (%)	5.0	0.8
Total nitrogen (%)	0.48	0.07
Total phosphorus (mg/kg)	965	139
Calcium carbonate (%)	44.6	<0.1
Cation exchange capacity (meq/100 g)	22.6	4.2

Both systems remained microbiologically active throughout the study. During the incubation period oxygen levels in the water were indicative of an aerobic, oxidising water phase and a reducing sediment phase. The temperature of the incubation chamber remained within the range 20 ± 2ºC throughout. Total recoveries of applied radioactivity (AR) were in the range 90.8 - 103.9%.

The resulting data for tolpyralate and MT-2153 has been fitted according to FOCUS kinetics guidance and it was concluded that tolpyralate declined rapidly in the Calwich Abbey Lake and Swiss Lake aerobic aquatic sediment systems (DT_{50} values summarised in the table below). There was little tolpyralate in the sediment at any time (<0.1% AR). The degradant MT-2153 was formed and was present in both water and sediment, reaching a maximum 82.0% and 79.9% AR in the whole systems. Amounts of MT-2153 in the sediment increased throughout the study, reaching a maximum of 19.8% AR and 32.7% AR in the Calwich Abbey Lake and Swiss Lake sediments respectively at the end of the study.

Two other degradants (labelled Met A and Met B) and a polar fraction were also reported but were always less than 5% AR in the whole systems. The amount of bound residue in the sediment increased throughout the study and reached a maximum of 29.6% AR in the Calwich Abbey Lake system and 41.9% AR in the Swiss Lake system. Radioactivity recovered from soxhlet extraction of the bound residue was mostly MT-2153 (8.9 to 13.4% AR), with small amounts of tolpyralate, Met A, Met B and some polar components (all ≤ 0.5% AR).

Table 48: Summary of endpoints (persistence and modelling) for tolpyralate and MT-2153 in the aerobic water / sediment systems

Tolpyralate				MT-2153			
System	**Kinetic fitting**	**DT_{50} (days)**	**DT_{90} (days)**	**System**	**Kinetic fitting**	**DT_{50} (days)**	**DT_{90} (days)**

Whole system (degradation)							
Calwich Abbey	SFO	1.39	4.61	Calwich Abbey	SFO	206	683
Swiss Lake	SFO	1.88	6.24	Swiss Lake	SFO	176	585
Geomean		1.62	5.36	**Geomean**		190	632

Kane, (2014)

Anaerobic water-sediment study

A study to determine the route and rate of degradation of tolpyralate in two aquatic sediment systems under anaerobic conditions was conducted. The study was conducted to GLP and according to OECD test guideline 308 and there were no significant deviations that would affect the validity of the study.. The test material was tolpyralate, radiolabelled in both the phenyl and pyrazole ring positions and had a minimum radiochemical purity of 99.5%.The rate of degradation of tolpyralate was similar to the aerobic study, while that for MT-2153 was generally longer. A summary of endpoints is presented below.

Table 49: Summary of endpoints (persistence and modelling) for tolpyralate and MT-2153 in the anaerobic water / sediment systems

Tolpyralate				**MT-2153**			
System	**Kinetic fitting**	**DT_{50} (days)**	**DT_{90} (days)**	**System**	**Kinetic fitting**	**DT_{50} (days)**	**DT_{90} (days)**
Whole system (degradation)							
Calwich Abbey	SFO	1.47	4.89	Calwich Abbey	SFO	338	1120
Swiss Lake	SFO	2.72	9.02	Swiss Lake	SFO	394	1310
Geomean		2.00	6.64	Geomean		365	1211

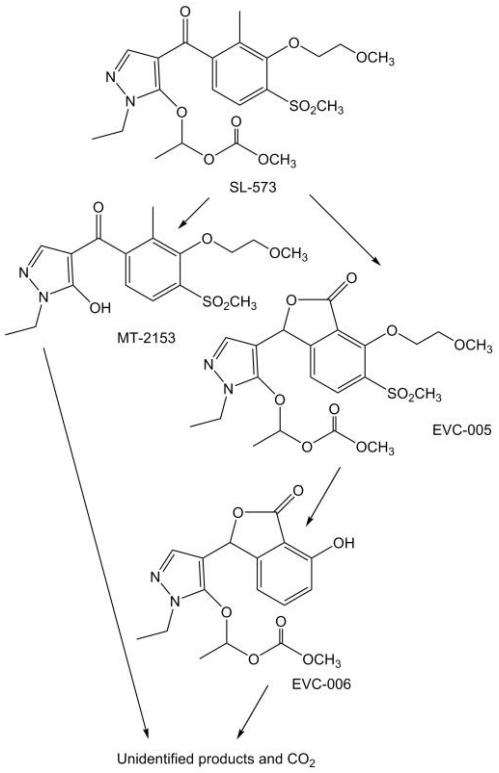
Crowe (2015)

11.1.4.4 Photochemical degradation

A photodegradation study with tolpyralate was conducted to GLP and according to OECD test guideline 316, there were no significant deviations that would affect the validity of the study. Degradation DT_{50} values and the quantum yield were determined in natural and purified water at 25 ºC with pH ranging from 5.7 – 6.72.

The data indicated that tolpyralate degrades rapidly via photolysis with DT50 values of 4.41 days in natural water and 2.86 days in purified water. The degradant MT-2153 was formed but at equal or lower amounts than in dark controls, suggesting that it was formed by hydrolysis, not photolysis. There were several photodegradation products, and there was strong evidence for the identification of EVC-005 and EVC-006 (both made up of two diastereoisomers). There was some evidence for the presence of a further set of four isomers structurally similar to EVC-005 and EVC-006, but these components were not positively identified and were all most likely below 10% of applied radioactivity.

Proposed pathway for photodegradation of tolpyralate in water:



Photolysis is of uncertain relevance as a route of degradation in typical European aquatic environments and, given the available data, there is insufficient information in this case to evaluate photodegradation in terms of mineralisation or transformation to non-classifiable substances. Therefore, aquatic photolysis is not considered further in relation to meeting the criteria for rapid degradation.

Unsworth (2014)

11.1.5 Overall summary on environmental hazard

Tolpyralate is a stereosiomer compound made up of R:S isomers at a 50:50 racemic mix. The two isomers can be considered equally biologically active with regard to herbicidal activity in addition in vivo mammalian toxicity testing indicates it is stable to chiral conversion. The ratio of stereoisomers (1:1) was unchanged during hydrolysis, indicating that the rate of hydrolysis of the two stereoisomers is the same in the aquatic environrment.

The aquatic hydrolysis study indicates that there is no ultimate degradation i.e. >70% within 28d as a DT50 of 231 d is indicated at pH 7 for tolpyralate in purified water. Hydrolysis is to the degradant MT-2153 which in turn is hydrolytically stable (DT50 >365 d at all environmental relevant pH and temperature). Tolpyralate was subject to aqueous photolysis with a DT50 of <5d in both purified and natural systems but the relevance of this as a route of degradation in typical European aquatic environments is uncertain.

A ready biodegradation study with tolpyralate indicated 9% degradation after 25 days, therefore tolpyralate is not classified as readily biodegradable.

Regarding the CLP criteria for rapid degradation, consideration has been made of other scientific evidence that may demonstrate degradation of tolpyralate in the aquatic environment to a level <70% within 28d.

The aerobic mineralisation and water sediment studies (aerobic and anaerobic) indicate that tolpyralate underwent rapid primary degradation in natural waters with DT50 values at <4.41 days. In all studies degradation was to the major degradant MT-2153 which in the aerobic and anaerobic water /sediment studies had estimated whole system DT50 values of >176 days.

The classification of the primary degradant MT-2153 is discussed at section 11.5 below and it is indicated to have significant acute and chronic toxic effects to aquatic plants and will therefore be classified as hazardous to the aquatic environment. It is concluded therefore, that although there is rapid primary degradation of the active substance, tolpyralate it does not meet the CLH 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.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant.

11.2.1 Summary of data/information on environmental transformation

Not relevant.

11.3 Environmental fate and other relevant information

Adsorption/desorption in soil

A GLP compliant adsorption/desorption study was conducted with tolpyralate in four contrasting soils according to OECD guideline 106. The soil physicochemical properties are detailed below.

Tavle 50: Soil physicochemical properties.

Soil	HDRA Biogarden	Cuckney	Fladbury	Uttoxeter Quarry
Particle size distribution[a]: Sand (%) Silt (%) Clay (%) Texture class	 68 16 16 Sandy loam	 92 4 4 Sand	 52 15 33 Sandy clay loam	 16 41 43 Clay
pH in water	7.6	6.9	6.7	5.2
pH in 0.01 M $CaCl_2$	6.8	6.4	6.1	4.9
Organic carbon (%)	5.0	0.5	3.1	4.2
Cation exchange capacity (meq/100g)	23.3	4.0	24.6	26.1

[a] USDA classification system except Uttoxeter Quarry, which is UK-ADAS system

An equilibration time of two hours was selected for both adsorption and desorption phases for tolpyralate due to its rapid degradation to MT-2153. Freundlich adsorption coefficients for tolpyralate were in the range 0.456 - 1.80 mL/g and adsorption coefficients corrected for organic carbon content (KFOC,ads) were in the range 14.9 - 91.2 mL/g, indicating the compound has the potential to be mobile in soil.

Two GLP compliant adsorption/desorption studies were conducted with MT-2153 in six contrasting soils in accordance with the OECD 106 guideline (Klaumann, S, 2015 and Sadgrove, L.2016). The soil physicochemical properties are detailed below:

Table 51: Soil physicochemical properties.

Soil	Calke	Cuckney	Fladbury	HDRA Biodynamic Garden	Uttoxeter Quarry	EVO-2	MCL-B
Particle size distribution: Sand (%) Silt (%) Clay (%) Texture class	 69.6 15.4 15.0 Sandy loam	 93.6 1.4 5.1 Sand	 50.5 28.2 21.4 Loam	 65.4 18.7 16.0 Sandy loam	 14.3 45.4 40.4 Silty clay	 17 51 32 Silty clay loam	 12 48 40 Silty clay
pH in 0.01 M $CaCl_2$	4.80	6.17	5.65	6.21	4.92	7.4	6.8
Organic carbon (%)	2.1	0.37	2.8	3.7	3.8	0.8	3.0
Cation exchange capacity (meq/100g)	11.4	2.2	23.3	23.8	20.9	31.6	43.8

Freundlich adsorption coefficients for MT-2153 were in the range 0.35 – 5.64 mL/g and adsorption coefficients corrected for organic carbon content (KFOC,ads) were in the range 40.8 – 148.1 mL/g, indicating the compound has the potential to be mobile in soil.

Unsworth, (2013b)

11.4 Bioaccumulation

Table 52: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water	Results determined at 25±1 °C applying the HPLC method. pH 6.4: log K_{ow} = 1.9	Valid	Furutami, E. 2014

11.4.1 Estimated bioaccumulation

11.4.1.1 Bioaccumulation estimation

As tolpyralate has a log K_{OW} of 1.9, it is considered to be below the CLP cut-off value of log K_{OW} ≥4 intended to identify substances with a potential to bioaccumulate.

11.4.1.2 Measured bioaccumulation data

No experimental aquatic study to determine the bioconcentration potential (BCF) of tolpyralate was submitted.

11.4.2 Summary and discussion of aquatic bioaccumulation

The log K_{ow} value of 1.9 for tolpyralate is below the log K_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate under CLP. No experimental data are available, so the results of the bioaccumulation estimation will be used to classify tolpyralate. To conclude, tolpyralate does not meet CLP criteria as a bioaccumulative substance.

11.5 Aquatic hazard

A summary of the suitable aquatic toxicity studies for tolpyralate, as reviewed under EU Regulation 1107/2009, is presented in Table 53 below. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes. All studies below conformed to GLP certification and were valid according to the criteria of the respective test guidelines (apart from the studies with *Anabaena flos-aquae* and *Crassostrea virginica* which failed some of the validity criteria, these did not highlight particular sensitivity to tolpyralate however). Additional information on the studies supporting tolpyralate has been presented in the subsections below.

Although valid and reliable studies are available on the formulated product "SL-573 100 OD" containing 103 g tolpyralate/L, studies using technical tolpyralate on the same species and endpoints are available. Therefore the studies using technical tolpyralate will take precedence over those of the formulated product, and consequently, studies using "SL-573 100 OD" have not been included in the hazard classification.

The degradants MMTA, MT-2153 and TAT-2049 were also tested in aquatic toxicity studies that were reviewed under EU Regulation 1107/2009. As MMTA and TAT-2049 are major degradants in soil studies only and were not sufficiently toxic to pass any of the hazard criteria, they are not considered any further for hazard classification. While MT-2153 exhibited high acute and chronic toxicity to aquatic plants with an E_rC_{50} of 0.0315 mg/L and a NOE_rC of 0.0009 mg/L respectively, as MT-2153 reached maximum concentrations of 53.9, 88.9, and 101.4 % AR at pH 4.0, 7.0, and 9.0 respectively at 50ºC and maximum levels of 1.1, 7.8 and 86.3 % AR at pH 4.0, 7.0, and 9.0 at 10 ºC, and a DT_{50} at 25 ºC of >365d, MT-2153 must be considered in the classification of tolpyralate. This is discussed further in Section 11.7.2.

Table 53: Summary of relevant information on aquatic toxicity of technical tolpyralate

Guideline	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./L)	
OECD 203 (1992)	*Oncorhynchus mykiss*	Mortality	Semi-static	96h	LC_{50}	>21 (mm)	Anonymous 2013m
OECD 203 (1992)	*Cyprinus caprio*	Mortality	Flow-through	96h	LC_{50}	>19 (mm)	Anonymous 2013n
OECD 203 (1992)	*Pimephales promelas*	Mortality	Flow-through	96h	LC_{50}	>19.7 (mm)	Anonymous 2014f
OECD 203 (1992)	*Cyprinodon variegatus*	Mortality	Semi-static	96h	LC_{50}	>11.6 (mm)	Anonymous 2015h
OECD 210 (2013)	*Pimephales promelas*	Hatchability, average days to hatch, rate of developmental abnormality, survival rate, **body weight**, total length	Flow-through	28d	NOEC	0.30 (mm)	Anonymous 2014g
OECD 202 (2004)	*Daphnia magna*	Immobility	Static	48h	EC_{50}	>19 (mm)	Yoshikawa, M. 2013
OPPTS 850.1035 (1996)	*Americamysis bahia*	Immobility	Semi-static	96h	EC_{50}	0.66 (mm)	Brougher, D.S., Siddiqui, A.I., Gallagher, S.P. and Krueger, H.O. 2015b
OPPTS	*Crassostrea*	Mortality, **shell**	Flow-	96h	EC_{50}	6.8 (mm)	Brougher,

850.1025 (1996)	*virginica*	**deposition**	through				D.S., Siddiqui, A.I., Gallagher, S.P. and Krueger, H.O. 2015c
OECD 211 (2012)	*Daphnia magna*	Immobility of parent, time to production of first brood, offspring survival, offspring immobilized, condition of parent, appearance of ephippium, body length of parent, dry weight of parent	Semi-static	21d	EC_{10} & NOEC	≥ 8.94 (mm)	Yoshikawa, M. 2014b
OECD 201 (2011)	*Pseudokirchneriella subcapitata*	**Growth rate**, yield, biomass	Static	96h	96h E_rC_{50} 96h NOE_rC 72h E_rC_{10}	12.3 (mm) 1.4 (mm) 4.44(mm)	Yoshikawa, M. 2013b
OECD 201 (2011)	*Anabaena flos-aquae*	**Growth rate**, yield, biomass	Static	96h	E_rC_{50} E_rC_{10}	>16.7 (mm) >16.7 (mm)	Arnie, J. R.,Martin, K. H. and Porch, J. R. 2013a
OECD 201 (2011)	*Navicula pelliculosa*	**Growth rate**, yield, biomass	Static	96h	E_rC_{50} E_rC_{10}	>20.9 (mm) 14.8 (mm)	Arnie, J. R., Martin, K. H. and Porch, J. R. 2013b
OECD 201 (2011)	*Skeletonema costatum*	**Growth rate**, yield, biomass	Static	96h	E_rC_{50} E_rC_{10}	1.8 (mm) 0.15 (mm)	Arnie, J. R., Martin, K. H. and Porch, J. R. 2013c
OECD 221 (2006)	*Lemna gibba*	Growth frond number, growth dry weight, yield frond number, Yield dry weight	Semi-static	7d	E_rC_{50} E_rC_{10} NOE_rC	0.0353 (nom) 0.00142 (nom) 0.00102 (nom)	Kuhl, R. and Wydra V. 2013
Proposed OECD test method for the rooted aquatic macrophyte, *Myriophyllum sp.* in a water-sediment system	*Myriophyllum aquaticum*	Growth rate total shoot length, yield total shoot length, growth rate wet weight, yield wet weight, growth rate dry weight, yield dry weight	Static	7d	E_rC_{50} NOE_rC E_rC_{10}	>0.244 (mm) <0.000304* n.d.	Seeland-Fremer, A. and Wydra, V. 2014

(2013)							

Bold words indicate which type of data the endpoints were calculated from for where there multiple endpoints calculated in a study
(mm) Endpoint based upon mean measured concentrations
(nom) Endpoint based upon nominal concentrations
n.d. Endpoint not determinable
* Uncertainty in test concentration

11.5.1 Acute (short-term) toxicity to fish

11.5.1.1 Short-term toxicity to fish

Four acute toxicity to fish studies are available for tolpyralate, all following GLP and according to OECD 203 (1992). The toxicity endpoints are driven by the solubility limit of tolpyralate.

Anonymous, 2013m

The acute toxicity to juvenile rainbow trout was determined in an aerated, semi static, 96-hour test. The study encompassed 7 treatment groups, 5 dose rates of the test substance corresponding to mean measured values of 21.0, 9.68, 4.47, 1.96 and 0.89 mg a.s./L, a control and N,N-dimethylformamide (DMF) solvent control (100 µL DMF/L) each containing 7 individuals. The samples of the test medium collected from freshly prepared (test start and day 2) and in aged (day 2 and test end) were analysed *via* HPLC-UV-method. Since some analysed values were below 80%, all reported endpoints were related to the mean measured concentrations. All the validity criteria were met, no mortalities occurred, and no sub-lethal effects were observed. Therefore, the 96-hour LC_{50} of tolpyralate was >21.0 mg a.s./L based on mean measured concentrations.

Anonymous, 2013n

A 96-hour limit test with common carp (*Cyprinus carpio*) was performed with a nominal tolpyralate concentration of 22 mg/L under semi-static conditions. Seven fish were exposed to the test material, with further groups of seven fish in control and vehicle control groups (0.10 mL DMF/L). All measured concentrations of tolpyralate throughout the test were within the range of 80 - 120% of the nominal concentration. No mortalities occurred and no sub-lethal effects were observed during the 96-hour period of the test and all the validity criteria were met. Therefore, the 48-hour and 96-hour LC_{50} of tolpyralate is >19 mg a.s./L based on mean measured concentrations.

Anonymous. 2014f

A 96-hour limit test with fathead minnow (*Pimephales promelas*) was carried out at a tolpyralate concentration of 22 mg/L under semi-static conditions. Seven fish were exposed to the test material, with further groups of seven fish in control and vehicle control groups (0.10 mL DMF/L). All measured concentrations of tolpyralate throughout the test were within the range of 80 - 120% of the nominal concentration. No mortalities occurred and no sub-lethal effects were observed during the 96-hour period of the test and all the validity criteria were met. Therefore, the 48-hour and 96-hour LC_{50} of tolpyralate is >19.7 mg a.s./L based on mean measured concentrations.

Anonymous, 2015h

Sheepshead minnows (*Cyprinodon variegatus*) were exposed to a single limit test of nominal concentration 26.5 mg a.s./L and a negative control (dilution water) for 96 hours under static-renewal conditions. Three replicate test chambers of ten fish were maintained in each treatment and control group. As analysis of the test concentration was outside the 80-120% range of the nominal, all results were based on the mean measured concentration after centrifugation. All the validity criteria were met, no mortalities occurred, and no sub-lethal effects were observed. Therefore, the 96-hour LC_{50} of tolpyralate was ≥11.6 mg a.s./L based on mean measured centrifuged concentrations.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Three acute toxicity to aquatic invertebrate studies using tolpyralate are available for hazard classification, all GLP compliant.

Yoshikawa, M. 2013a

A 48-hour limit test with *Daphnia magna* was carried out at a tolpyralate concentration of 22 mg/L under static conditions. Twenty daphnids (four replicates of five organisms) were exposed to the test material, control and vehicle control (0.10 mL DMF/L). Measured concentrations of tolpyralate at test start and end were within the range of 80 - 120% of the nominal concentration and all the validity criteria were met. No immobility occurred and no sub-lethal effects were observed during the 48-hour period of the test at 22 a.s. mg/L. Therefore, the 24-hour and 48-hour EC_{50} of tolpyralate is >19 mg a.s./L based on mean measured concentrations.

Brougher, D.S., Siddiqui, A.I., Gallagher, S.P. and Krueger, H.O. 2015b

Saltwater mysids (*Americamysis bahia*) were exposed to five nominal test concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./L and a negative control (dilution water) for 96 hours under static-renewal conditions. Two replicate test chambers were maintained in each treatment and control group, with 10 mysids in each test chamber. All validity criteria were met. Test concentrations were measured in samples of test water collected from each treatment and control group at the beginning, prior to and after renewal and at 96 hours of the test. Measured concentrations of the un-centrifuged samples ranged from approximately 71.7 to 93.1% of nominal, and recovery decreased upon centrifugation. Consequently, the results were based upon mean measured centrifuged concentrations. The 96-hour LC_{50} value was 0.66 mg a.s./L, with a 95% confidence interval of 0.41 to 8.4mg/L a.s./L. The confidence interval is large due to the flat dose response, therefore there is some uncertainty in the endpoint derived.

Brougher, D.S., Siddiqui, A.I., Gallagher, S.P. and Krueger, H.O. 2015c

Eastern oysters (*Crassostrea virginica*) were exposed to a geometric series of six test concentrations (nominal 0.19, 0.43, 0.94, 2.1, 4.5 and 10 mg a.s./L), a negative control (dilution water) and a solvent control (0.1 mL DMF/L) for 96 hours under flow-through conditions. One test chamber of 20 oysters was maintained in each treatment and control group. Test concentrations were measured in samples of test water collected from each treatment and control group at the beginning, the approximate mid-point and the end of the test. While measured concentrations of the samples ranged from approximately 88 to 101% of nominal, after centrifugation, mean measured recovery ranged from 68-91% of the nominal. Consequently, the results of the study were based on the mean measured concentrations for the centrifuged samples.

However, not all of the validity criteria were met as the criteria specifies that a minimum of 2 mm of shell growth should occur in the controls over the course of the test. Both the water control and the solvent control were below 2 mm (1.81 and 1.49 mm), with a pooled control value of 1.65 mm. Whilst this might reduce the sensitivity of the test to detect effects, a significant growth decrease was observed in the highest treatment solution and no clear dose-response was observed at lower concentrations. Consequently, the difference in control growth does not appear to have had any adverse impact on the study. Therefore this must be considered with the 96-hour EC_{50} value for inhibition of shell deposition of 6.8 mg a.s./L (based upon mean measured concentrations, with a 95% confidence interval of 3.6 to 8.6 mg a.s./L).

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

Six studies testing toxicity to algae and aquatic plants have been evaluated for tolpyralate, all according to GLP certification. Four of these studies tested species of algae (OECD 201), one tested *Lemna* (OECD 221) and one study tested *Myriophyllum* (conducted prior to the OECD 239 guideline). Five of the studies are considered suitable for hazard classification. The endpoints derived from the algal study conducted with *Anabena flos-aquae* (Arnie *et al*. 2013) are not considered valid as two of the OECD 201 (2011) validity criteria were not met and there were issues regarding concentrations at test end for the two lowest test concentrations.

Algae:

Yoshikawa, M. 2013b

A 96-hour test with *Pseudokirchneriella subcapitata* was carried out at nominal tolpyralate concentrations of 0.94, 2.1, 4.5, 10 and 22 mg/L under static conditions. The initial cell density was 0.5×10^4 cells/mL and there were four replicates at each concentration, with similarly constituted control and vehicle control (0.10 mL DMF/L) groups. All the validity criteria were met. As the measured concentrations ranged beyond ±20% from nominal (range at test end was 49.9-65.3% of nominal and the overall geomean measured concentrations were in the range 66.5-76% of nominal), analysis of the results was based on geometric mean concentrations. The 96-hour E_rC_{50} was 12.3 mg a.s/L and the 96-hour NOE_rC was 1.40 mg a.s./L based on mean measured concentrations. The E_rC_{10} was only calculated for 72 hours with a value of 4.44 mg a.s./L. Endpoints were based on statistical comparisons between treatment and solvent control data.

Arnie, J. R., Martin, K. H. and Porch, J. R. 2013a

The freshwater alga (cyanobacterium), *Anabaena flos-aquae*, was exposed to five test concentrations, a solvent control (0.1 mL DMF/L) and a negative control (culture medium) for 96 hours. Measured concentrations were determined from samples of test medium collected from each treatment and control group at 0, 72 and 96 hours of the test. Four replicate test chambers were maintained in each treatment and control group for the whole test, and an additional replicate was included in each control and treatment group to provide test solution for analytical sampling at 72 hours. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 10,000 cells/mL.

Not all the validity criteria were met as the coefficient of variation of both the whole period and section-by-section were not met. Cell density data from tests conducted with this species are known to be highly variable, although sonification was performed. The results of the study are based on geometric mean, measured concentrations of 0.961, 1.61, 4.37, 8.36, 16.7 mg a.s./L, representing 58, 59, 66, 63 and 63% of the target nominal test concentrations. Therefore the 96-hour E_rC_{50} and E_rC_{10} value was determined to be >16.7 mg a.s./L for both endpoints based on statistical comparisons between treatment and medium control data. There is uncertainty regarding the geometric mean measured concentrations at the 2 lowest test concentrations, as the measured values were < LOQ on day 4 and a value of ½ the LOQ (0.50 mg a.s./L) was used to calculate the geometric mean. Given that two of the OECD validity criteria were not met and the issue regarding concentrations at test end for the two lowest test concentrations, the endpoints from this study are not considered reliable for hazard classification.

Arnie, J. R., Martin, K. H. and Porch, J. R. 2013b

The freshwater diatom, *Navicula pelliculosa*, was exposed to five test concentrations (geometric mean measured concentrations 0.737, 0.937, 2.25, 9.32, and 20.9 mg a.s./L), a solvent control (0.1 mL DMF/L) and a negative control (culture medium) for 96 hours. Samples of test medium collected from each treatment and control group at 0, 72 and 96 hours of the test to analyse tolpyralate concentration. Four replicate test chambers were maintained in each treatment and control group for the whole test, and an additional replicate was included in each control and treatment group to provide test solution for analytical sampling at 72 hours. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 10,000 cells/mL. All of the validity criteria were met. The decline in measured concentrations at the end of the exposure period was outside ±20% of the nominal (range at test end was <LOQ-70% of nominal and the overall geometric mean measured concentrations were in the range 28-79% of nominal). Consequently, the results of the study are based on geometric mean, measured concentrations. The 96-hour E_rC_{50} and E_rC_{10} values were determined to be >20.9 and 14.8 mg a.s./L respectively based on statistical comparisons between treatment and pooled control data. There is uncertainty in the geomean measured test concentrations for the lowest 3 test concentrations – as measured concentrations were < LOQ on days 3 and/or 4 (value of half LOQ was used when the measured concentration was < LOQ). The output from EFSA Peer Review Meeting 133 (2015) states that "the experts considered that this approach could be used when intermediate measurements (e.g. more than one intermediate point or other information) are available. This information may allow using the LOD or half of the LOQ, to calculate a geometric mean concentration". The approach is considered acceptable for the 6.63

mg/l (nominal) test concentration as measured concentrations were obtained on day 3 and therefore it is known that concentrations declined below LOQ between days 3 and 4. However, it is not deemed appropriate for the lowest 2 concentrations. Nevertheless, since measured concentrations were obtained at all time points for the 2 highest concentrations, the E_rC_{50} endpoint is > than the highest concentration and the E_yC_{50} is between the 2 highest test concentrations – the EC_{50} endpoints from this study based on geometric mean measured concentrations are deemed reliable.

Arnie, J. R., Martin, K. H. and Porch, J. R. 2013c

A 96-hour study had the marine diatom, *Skeletonema costatum*, exposed to five test concentrations (mean measured concentrations 0.038, 0.092, 0.25, 0.61, 1.6 and 4.3 mg a.s./L), a solvent control (0.1 mL DMF/L) and a negative control (culture medium). Samples of test medium collected from each treatment and control group at 0, 72 and 96 hours of the test to analyse tolpyralate concentration. Four replicate test chambers were maintained in each treatment and control group for the whole test, and an additional replicate was included in each control and treatment group to provide test solution for analytical sampling at 72 hours. At test initiation, an inoculum of the algal cells was added to each test chamber to achieve a nominal concentration of approximately 10,000 cells/mL. All of the validity criteria were met. The decline in measured concentrations at the end of the exposure period was outside ±20% of the nominal (range at test end was <LOQ-73.1% of nominal and the overall geomean measured concentrations were in the range of 71-86% of nominal). Consequently, the results of the study are based on geometric mean measured concentrations. The 96-hour E_rC_{50} and E_rC_{10} values were determined to be 1.8 and 0.15 mg a.s./L respectively based on statistical comparisons between treatment and pooled control data. It should be noted that there is some uncertainty in the mean concentration calculated for the lowest test concentration (0.038 mg/L) as an extrapolated value was used for day 4 as the measured concentration was < LOQ. The study report does not make it clear how the value was derived. However, as this is a measurement for a single time point at the lowest test concentration, whereas the E_rC_{50} endpoint relates to a much higher concentration, it is not considered to have a major impact upon the E_rC_{50} value derived from this study.

Aquatic plants:

Kuhl, R. and Wydra V. 2013

Cultures of *Lemna gibba* were exposed to 6 dose rates of the test substance (nominal concentrations 100, 40.0, 16.0, 6.40, 2.56 and 1.02 µg a.s./L), a solvent control (0.1 mL DMF/L) and a medium control for 7 days with four replicates per test concentration and control. At test start, 12 fronds were introduced in each replicate and incubated for 7 days under semi-static conditions. All the validity criteria were met. Test solutions were renewed on days 3 and 5. Analytical samples were taken from the freshly prepared solutions on days 0, 3, 5, and from the aged test media on days 3, 5 and 7. Over the test duration, the test substance was within ±20% of the nominal at all times, and the *Lemna* were exposed to a mean of 99 % of nominal (measured concentrations were in the range 85-115% of nominal). Consequently, all the endpoints have been expressed as nominal concentrations. The 7-day E_rC_{50} and E_rC_{10} were calculated to be 0.0353 (dry weight) and 0.00142 mg a.s./L (frond number) respectively. The 7-day NOE_rC was 0.00102 mg a.s./L (dry weight and frond number).

Seeland-Fremer, A. and Wydra, V. 2014

It should be noted that this *Myriophyllum* study was conducted prior to the OECD 239 guideline. Nevertheless, the study meets the validity criteria outlined in the guideline apart from there is no reporting of whether or not sub-lethal effects occurred in control plants. However, the study was conducted over 7 days of exposure rather than the guideline duration of 14 days.

Plants of *Myriophyllum aquaticum* were exposed in a static water-sediment test to 5 dose rates of the test substance (geometric mean measured concentrations 244, 43.8, 8.36, 1.52 and 0.304 µg test substance/L) and a control, with three replicates per test concentration and six replicates for the control. The sediment was prepared according to OECD 219. Each treatment group was spiked with a defined volume of the stock solution in order to obtain the desired test concentrations. Therefore these particular volumes were removed from the test water in each replicate before adding the stock solution. The overlying water was then mixed. After a pre-rooting phase of 3 days, 3 shoot tips per replicate were incubated for 7 days under static conditions. Over the test duration, the test substance declined outside ±20% of the nominal, with an average recovery from the water phase of 66% at test initiation and 28% at test termination. Consequently, all the endpoints have been expressed as geometric mean measured concentration. Therefore, the 7-day E_rC_{50} was calculated to be >0.244 mg a.s./L (for growth rate based on shoot length, wet weight and dry weight). Due to > 10% effects on growth rate (wet weight and shoot length) at the lowest test concentration it is not possible to derive a NOEC from this study. In addition, it has not been possible to derive an EC_{10}. It should be noted that there is uncertainty in the lowest test concentration (0.304 µg test substance/L), as measured concentrations were below the LOQ at test start and end. The study authors used the geometric mean recovery of 38% obtained from the next highest test concentration (nominal test concentration of 4.0µg/L) for the statistical evaluation of the endpoints. This approach is not deemed appropriate and is considered likely to overestimate the amount of test substance as it is apparent that recovery at test end declined with lowering test concentration. However, this does not impact upon the EC_{50} endpoints as these are >0.244 mg a.s./L (the highest concentration tested). In addition, it is noted that the guideline states that concentrations in sediment and sediment pore-water should be determined at test initiation and test termination, at least in the highest test concentration, unless the test substances are known to be stable in water (> 80% of nominal). As tolpyralate does not appear to be stable in water – further monitoring should ideally have taken place.

Table 54: EC_{10}, NOEC values and % inhibition for growth rate parameters

Geometric mean measured concentration (µg/L)	Growth rate (total shoot length, 1/day) and percentage inhibition 7 days	Endpoint	Growth rate (based on wet weight, 1/day) and percentage inhibition 7 days	Endpoint	Growth rate (based on dry weight, 1/day) and percentage inhibition 7 days	Endpoint
Control	0.104		0.101		0.058	
0.304**	0.082 20.7%*	E_rC_{10} n.d. NOE_rC <0.304	0.089 12.1%*	E_rC_{10} n.d. NOE_rC <0.304	0.059 -2.6%	E_rC_{10} n.d. NOE_rC 0.304

* mean value significantly different from the control

** uncertainty in test concentration

n.d. = not determined

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

No further relevant aquatic toxicity data are available.

11.6 Long-term aquatic hazard

11.6.1 Chronic toxicity to fish

There is one suitable long-term toxicity to fish study for tolpyralate to be used for hazard classification. The fish early life stage test is GLP compliant and is according to OECD 210 (2013).

Anonymous, 2014g

An early-life stage study was conducted with fathead minnow (*Pimephales promelas*) at tolpyralate concentrations of 0.0300, 0.0949, 0.300, 0.949 and 3.00 mg/L under flow-through conditions. Eighty fish embryos per test concentration (four replicates of 20 embryos per concentration) were exposed to the test material with the same number of embryos in similarly constituted control and vehicle control (0.10 mL DMF/L) groups. All measured concentrations of tolpyralate throughout the test were in the range ±20% of the nominal concentration and all the validity criteria were met. There were no statistically significant differences between any of the exposure groups and the vehicle control with regard to hatchability, average days to hatch, rate of developmental abnormalities, survival rate after hatching, body weight and total body length. However, there were > 10% effects (16.3-17.7%) on body weight at the two highest test concentrations. Therefore, the NOEC was considered to be 0.3 mg/L based on effects on body weight and mean measured concentrations. A reliable EC_{10} value could not be derived (the lower confidence limit was a negative value).

11.6.2 Chronic toxicity to aquatic invertebrates

There is one suitable long-term toxicity to aquatic invertebrate study for tolpyralate to be used for hazard classification. The *Daphnia magna* reproductive test is GLP compliant and conducted according to OECD 211 (2012).

Yoshikawa, M. 2014b

> A 21-day reproduction study was conducted with *Daphnia magna* at tolpyralate concentrations of 0.625, 1.25, 2.50, 5.00 and 10.0 mg/L under semi-static conditions. Ten daphnids (as ten replicates) were tested per test concentration, with similarly constituted control and vehicle control (50 µL DMF/L) groups. All measured concentrations of tolpyralate throughout the test were within ±20% of nominal, and all the validity criteria were met. No test substance-related parental immobility occurred and no sub-lethal effects (parameters considered were size, swimming behaviour, abnormal appearance, sex distinction, time to production of first brood and presence of ephippium or aborted brood) were observed during the 21-day period of the test. Therefore the 21 day NOEC was >8.94 mg a.s./L based on mean measured concentrations.

Two acute aquatic invertebrate studies have been provided, one with *Daphnia magna* (EC_{50}> 19 mg/l) and one with *Americamysis bahia* (EC_{50} 0.66 mg/l). The data requirements (283-2013) state "*If acute toxicity tests have been conducted on two aquatic invertebrate species the acute endpoints shall be taken into account in order to determine the appropriate species to be tested in the chronic toxicity study*". In addition, the EFSA 2013 aquatic guidance states that "*The PPR Panel proposes to select the more sensitive species in case the difference in acute toxicity is more than a factor of 10.*" *Americamysis bahia* is clearly the most sensitive aquatic invertebrate species tested in the acute studies (> factor of 10 difference). Therefore, this mysid species should have been tested in the chronic study, rather than *Daphnia magna* and as such this is considered by the RMS to be a data gap in the DAR.

11.6.3 Chronic toxicity to algae or other aquatic plants

See Section 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No further relevant aquatic toxicity data are available.

11.7 Comparison with the CLP criteria

The active substance, tolpyralate does not meet the criteria for 'rapidly degradable', as it remains > 70% after 28 days. It does meet the primary criteria with a ½ life of < 16 days. The major aquatic metabolite MT-2153 is persistent and is toxic to aquatic organisms. As a consequence, within the classification criteria, tolpyralate is not considered 'rapidly degradable'. Information presented in Annex II indicates the major aquatic degradant, MT-2153, has acute and chronic aquatic toxicity similar to, or slightly greater than parental toxicity (although the endpoints for algae and *Lemna* are slightly lower than parent, the differences are considered to be within the realms of inter-study variability). Therefore, this degradant, along with the active substance will be considered further for hazard classification.

MMTA and TAT-2049 are major degradants in soil studies only and were not sufficiently toxic to pass any of the hazard criteria; therefore they are not considered further for hazard classification.

Tolpyralate has a log K_{OW} value of 1.9, which is lower than the CLP cut-off Log K_{OW} value of ≥4. Therefore, tolpyralate is not considered to have the potential to bioaccumulate.

Acute and chronic aquatic toxicity data on tolpyralate are available for fish, invertebrates, algae and aquatic plants. Aquatic plants are considered the most sensitive trophic group over both acute and chronic timescales which is not unexpected given the herbicidal activity of tolpyralate. Toxicity data is also available for the degradant MT-2153 (acute fish and invertebrates, algae and aquatic plants). Again, aquatic plants are the most sensitive group. Therefore, the classification proposals will focus on the toxicity to aquatic plants.

11.7.1 Acute aquatic hazard

Endpoints are available for tolpyralate and the degradant MT-2153 for the aquatic plants *Myriophyllum aquaticum* and *Lemna gibba*. The endpoints for *Myriophyllum aquaticum* are 7-day E_rC_{50} >0.244mg tolpyralate/L and 14-day E_rC_{50} 0.0611 mg MT-2153/L. The endpoints for *Lemna gibba* are 7-day E_rC_{50} 0.0353 tolpyralate/L and a 7-day E_rC_{50} 0.0315 mg MT-2153/L. The lowest overall acute endpoint is a 7-day mean measured E_rC_{50} of 0.0315 mg MT-2153/L for *Lemna gibba* (dry weight). This endpoint is > 0.01 but ≤ 0.1 mg/L, therefore tolpyralate should be classified as Aquatic Acute category 1 with an acute M-factor of 10 based on the data for the major degradant MT-2153.

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

The lowest chronic endpoint is a 7-day mean measured NOE_rC of <0.000304 mg tolopyralate/L for *Myriophyllum aquaticum*. No EC_{10} endpoints could be derived for this study. It should be noted that the study duration (7 days) was shorter than the recommended 14 days, there was no analytical monitoring of the sediment, and there is uncertainty in the lowest test concentration due to the manner in which the geometric mean measured concentration was derived (via extrapolation from a higher test concentration). In terms of the E_rC_{50} endpoints *Lemna gibba* appears to be more sensitive than *Myriophyllum aquaticum*, however it is less clear in terms of the NOEC values. The NOE_rC for *Lemna gibba* is 0.00102 mg a.s./L (dry weight and frond number), whereas the NOE_rC for *Myriophyllum* is <0.000304 mg a.s./L. In addition, the results from the *Myriophyllum* study with the major aquatic degradant MT-2153 indicate a NOE_rC of < 0.000606 mg a.s./L (again uncertainty in this the lowest test concentration as measured concentrations were < LOQ at test start and end), indicating that *Myriophyllum* is potentially more sensitive. An EC_{10} could not be derived for the most sensitive growth rate parameter (dry weight). As a consequence, there is no clear reliable endpoint that can be used for classification purposes for *Myriophyllum*.

In the absence of a reliable *Myriophllum* endpoint (for either the active substance or degradant MT-2153) it is proposed to use the toxicity data derived from the studies with *Lemna gibba* (as an interim measure). As

the degradant MT-2153 is not 'rapidly degradable' and appears to be marginally more toxic than the active substance to *Lemna gibba*, the chronic classification is based upon the 7 d NOEC of 0.0009 mg/l (based on dry weight) for MT-2153. This endpoint is between >0.0001 to ≤ 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.

It is acknowledged that there is an element of uncertainty in the chronic M-factor due to the absence of a reliable NOE_rC or EC_{10} endpoint for *Myriophyllum*. Should further reliable chronic data become available on this species or other sensitive aquatic plants (e.g. during the pesticide renewal process), then this classification should ideally be reconsidered. It is understood that a further study on *Myriophyllum* with the active substance will be provided during the current EU review of tolpyralate. Although, a further study with the degradant MT-2153 may also be useful.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M-factor = 10

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M-factor = 100

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not applicable.

12.1 Hazardous to the ozone layer

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

Not applicable. Based upon its molecular structure tolpyralate would not qualify as a controlled substance in the Annexes to the Montréal Protocol. Furthermore, it is not expected to enter in contact with stratospheric ozone molecules given its physico-chemical parameters.

12.1.2 Comparison with the CLP criteria

Not applicable. Based upon its molecular structure tolpyralate would not qualify as a controlled substance in the Annexes to the Montréal Protocol. Furthermore, it is not expected to enter in contact with stratospheric ozone molecules given its physico-chemical parameters.

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

This section contains the non-confidential references. The confidential references are included in separate Annex (Annex III)

Human Health References:

Author	Year	Study
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		experimental animals and its relationship to corneal injury. Toxicology Appl. Pharmacol., 215, 9-16.
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DAR Section	Author	Date	Study title
B.9.2.7.1/1	Arnie, J.R., Martin, K.H. and Porch, J.R.	2013a	SL-573 TGAI: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*). Study no. 272P-108. GLP, unpublished.
B.9.2.7.1/3	Arnie, J.R., Martin, K.H. and Porch, J.R.	2013b	SL-573 TGAI: A 96-hour toxicity test with the freshwater diatom (*Navicula pelliculosa*). Study no. 272P-109. GLP, unpublished.
B.9.2.7.1/4	Arnie, J.R., Martin, K.H. and Porch, J.R.	2013c	SL-573 TGAI: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*). Study no. 272P-110. GLP, unpublished.
B.9.2.4.1/2	Brougher, D.S., Siddiqui, A.I., Gallagher, S.P. and Krueger, H.O.	2015b	SL-573 TGAI: A 96-hour static-renewal acute toxicity test with the saltwater mysid (*Americamysis bahia*). Study no. 272A-133C. GLP, unpublished.
B.9.2.4.1/3	Brougher, D.S., Siddiqui, A.I., Gallagher, S.P. and Krueger, H.O.	2015c	SL-573 TGAI: A 96-hour shell deposition test with the eastern oyster (*Crassostrea virginica*). no. 272A-132. GLP, unpublished.
B.8.2.2.3	Crowe, A.	2015	SL-573: Anaerobic aquatic metabolism Study number: JSM0409 GLP / Unpublished
B.8.2.2.1	Hammesfahr, U.	2013	Ready biodegradability of SL-573 TGAI in a manometric respirometry test. Study number 72805163 . GLP/Unpublished
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B.9.2.7.2/1	Handlos, F. and Erk, T.	2014c	Toxicity of MT-2153 to *Pseudokirchneriella subcapitata* in an algal growth inhibition test. Study no. 85651210. GLP, unpublished.
B.9.2.8.2/1	Hermes, H. and Frank, C.	2015a	Toxicity of MT-2153 to the aquatic plant *Lemna gibba* in a static growth inhibition test. Study no. 85651240. GLP, unpublished.
B.9.2.8.2/2	Hermes, H. and Frank, C.	2015b	Toxicity of MT-2153 to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test with a prior rooting phase. Study no. 85651215. GLP, unpublished.
B.8.2.1.1	Hori, K.	2014b	Hydrolysis test for MT-2153 (SL-573 metabolite). Study Number: 84072 GLP/Unpublished
B.8.2.2.3	Kane, T.	2014	SL-573: Aerobic aquatic metabolism Study Number: JSM0408 GLP / Unpublished
B.9.2.8.2/4	Kosak, L. and Emnet, P.	2016	TAT-2049: Toxicity to the aquatic plant *Lemna gibba* in a static growth inhibition test. Study no. 114161240. GLP, unpublished.
B.9.2.6/1	Kuhl, R. and Frank, C.	2015	Effects of MT-2153 on the development of *Chironomus riparius* in a sediment-water system - exposed via spiked sediment. Study no. 85652250. GLP, unpublished.
B.9.2.8.1/1	Kuhl, R. and Wydra, V.	2013	Toxicity of SL-573 TGAI to the aquatic plant *Lemna gibba* in a semi-static growth inhibition test. Study no. 72801240. GLP, unpublished.
B.9.2.7.2/2	Seeland-Fremer, A. and Mosch, W.	2014b	Toxicity of MMTA to *Pseudokirchneriella subcapitata* in an algal growth inhibition test. Study no. 93291210. GLP, unpublished.
B.9.2.8.2./3	Seeland-Fremer, A. and Mosch, W.	2014c	Toxicity of MMTA to the aquatic plant *Lemna gibba* in a static growth inhibition Test. Institut für Biologische Analytik und Consulting IBACON GmbH. Laboratory no. 93291240. Ishihara

DAR Section	Author	Date	Study title
			Sangyo Kaisha, Ltd. GLP, unpublished.
B.9.2.8.2.1/2	Seeland-Fremer, A. and Wydra, V.	2014	Toxicity of SL-573 TGAI to the aquatic plant *Myriophyllum aquaticum* in a static growth inhibition test with a prior rooting phase. Study no. 72809215. GLP, unpublished.
B.8.2.1.1	Unsworth, R.	2013	SL-573: Hydrolysis in water Study Number: JSM0399 GLP / Unpublished
B.8.2.1.1	Unsworth, R.	2014	SL-573: Photodegradation in water and determination of the quantum yield. Study Number. JSM0400 GLP/Unpublished
B.8.2.2.2	Van den Bosch, M.M.H.	2014	Aerobic mineralisation of SL-573 in surface water. Study Number: 504338 GLP/Unpublished
B.9.2.4.1/1	Yoshikawa, M.	2013a	A 48-hour acute immobilization study of SL-573 TGAI in *Daphnia magna*. Study no. 96015. GLP, unpublished.
B.9.2.4.2/2	Yoshikawa, M.	2014a	A 48-hour acute immobilization study of MMTA in *Daphnia magna*. Study no. 96787. GLP, unpublished.
B.9.2.5/1	Yoshikawa, M.	2014b	*Daphnia magna* reproduction study of SL-573 TGAI. Study no. 96530. GLP, unpublished.
B.9.2.7.1/1	Yoshikawa, M.	2013b	Algae growth inhibition study of SL-573 TGAI in *Pseudokirchneriella subcapitata*. Study no. 96014. GLP, unpublished.

15 ANNEXES

Annex I – Robust Study Summaries

Not provided as part of this report.

Annex II - Summary of the aquatic toxicity of degradants of Tolpyralate

Table 1 Summary of toxicity of Tolpyralate degradants to fish and invertebrates

Test spp./substance	Study type & duration	L/EC50 mg/L	NOEC mg/L	Reference
Oncorhynchus mykiss				
MT-2153	static acute (96h)	> 100 (n)	100 (n)	Anonymous (2014f)
MMTA	static acute (96h)	> 100 (n)	100 (n)	Anonymous (2014g)
Daphnia magna				
MT-2153	static acute (48h)	>100 (n)	100 (n)	Handlos, F. and Erk, T. (2014b)
MMTA	static acute (48h)	>100 (n)	100 (n)	Yoshikawa, M., (2014a)

(n) = nominal

Table 2 Summary of toxicity of Tolpyralate degradants to sediment dwellers, algae and plants

Test spp./substance	Study type	Endpoint mg/L	References
Chironomous riparius			
MT-2153	Chronic sediment study (spiked sediment)	28d EC10 & 28d NOEC = ≥ 1000 mg/Kg (n)	Kuhl, R and Frank, C (2015)
Pseudokirchneriella subcapitata			

MT-2153	Static chronic	72h E_rC50 = 8.88 (n) 72 h E_rC10 = 2.94 72 h NOE_rC = 1.0	Handlos, F. and Erk, T. (2014c)
MMTA	Static chronic	72h E_rC50 = > 32 (n) 72 h E_rC10 = 17.5 72 h NOE_rC = 10	Seeland-Fremer, A & Mosch, W. (2014b)
***Lemna gibba* G3**			
MT-2153	Static chronic	7d E_rC50 = 0.0315 7d E_rC10 = <0.0009 7d NOE_rC= 0.0009 Endpoints based on dry weight (mm)	Hermes, H. and Frank, C. (2015a)
MMTA	Static chronic	7d E_rC50 = > 100 (n) 7d NOE_rC= > 100	Seeland-Fremer, A & Mosch, W. (2014c)
TAT-2049	Static chronic	7d E_rC50 = > 100 (n) 7d NOE_rC= > 100	Kosak, L. & Emnet, P. (2016)
Myriophyllum spicatum			
MT-2153	Static chronic sediment study (spiked aqueous medium)	14d E_rC50 = 0.115 EC_{10} = nd EC_{20} = 0.00103 NOE_rC = < 0.000606* Endpoints based on dry weight (mm)	Hermes, H. and Frank, C. (2015b)

(n) = nominal

(mm) = mean measured

nd = not determined (for dry weight the most sensitive growth parameter in this study)

*uncertainty in test concentration

Annex III – Confidential references

See separate document.