Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

EC Number: -

CAS Number: 1101132-67-5

CLH-O-0000001412-86-268/F

Adopted

15 March 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON (RS)-1-{1-ETHYL-4-[4-MESYL-3-(2-METHOXYETHOXY)-O-TOLUOYL]PYRAZOL-5-YLOXY}ETHYL METHYL CARBONATE; TOLPYRALATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: (RS)-1-{1-ethyl-4-[4-mesyl-3-(2-methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate
EC number: -
CAS number: 1101132-67-5
Dossier submitter: The United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2018	Belgium		MemberState	1
Comment received				
We thank the UK CA for this CLH report. As a general comment, tolpyralate having been demonstrated to impair the tyrosine catabolism pathway, some interrogations remain regarding the potential neurologic effects of this compound and we regret that no specific neurotoxicity study is available to clarify this concern.				
Dossier Submitter's Response				
Noted, thank you.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	France		MemberState	2
Comment received				
The proposal for classification carc.cat2 H351 is supported based on ocular tumours observed in male rats.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comments. RAC agrees that classification Carc. 2 is appropriate.				

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2018	Belgium		MemberState	3

Comment received

In a two-year carcinogenicity study (0 – 0,196/0.255 – 0.765/1.01 – 83,8/108 – 426/554 mg/kg bw/day), male rats showed eye malignant squamous cell carcinomas (3/51 and 5/51 at 83,8 and 426 mg/kg bw/day respectively). No neoplastic effects were observed in mouse but this species has been demonstrated to be less sensitive to tolpyralate. BE CA agrees with the Dossier Submitter and considers that the observed squamous cell carcinomas in the cornea of male rats are treatment-related. Although limited evidence of carcinogenicity is available in animals, the causal relation between tolpyralate and the affected tissue is quite well demonstrated.

The MoA of tolpyralate is known to be an impairment of tyrosine catabolism due to HPPD inhibition, causing an increase in tyrosine in plasma and eye. This mechanism has been confirmed by measured increases in tyrosine levels in the eye of male rats after a single dose of tolpyralate. The sustained damage to the corneal epithelium is assumed to induce regenerative hyperplasia, increased DNA synthesis and cell replication, leading to corneal tumour formation.

BE CA is of the opinion that this mode of action is considered as reliable. We would also point out that although negative results were reported in in vivo genotoxicity studies, the Comet assays did not investigated specific tissues such as cornea or bone marrow, but investigated liver, thyroid and stomach. Moreover, the in vitro gene mutation test was positive in mouse lymphoma cells with and without S9. A potential mutagenic capacity of tolpyralate, for example through its metabolites, cannot therefore be definitely excluded and might support to some extent the described mode of action.

The HPPD inhibition is observed in humans where it is associated with eye damage. For example, NTBC, a HPPD inhibitor, is used to treat patients suffering from type I tyrosinemia. The use of this compound as a therapeutic drug being quite recent, a potential relation between NTBC and eye carcinoma has not been investigated yet. However, corneal opacity and keratitis have been frequently reported in patients treated with NTBC. Therefore, this mode of action is considered to be relevant to humans.

As a general conclusion, corneal squamous cell carcinoma is a rare malignant tumour reported in male rat in a dose-dependant increase. Therefore, considering that the causal link between tolpyralate exposure and eye tumour is admitted and that this mode of action is considered to be relevant to humans, BE CA is of the opinion that a classification 1B, "presumed to have carcinogenic potential for humans", should be discussed. A Carc. 2 classification is at least warranted.

Dossier Submitter's Response

The DS is in agreement with BE CA with respect to the interpretation of the findings and the plausible mechanism of action (MoA). However, the DS considers that the available comet assay investigated standard tissues including site of first contact (stomach), point of metabolism (liver) and a tissue which is known to be a target for the test item (thyroid). The DS does not expect that genotoxicity findings in the eye would be any different to those in the tissues already tested, noting that eye specific metabolites are not anticipated.

With respect to the conclusion on classification for carcinogenicity, the DS does not agree with BE CA proposal for classification in category 1B and remains of the opinion that classification in category 2 is appropriate. Whilst the DS agrees that the mechanism of action is relevant to humans, based on the lack of evidence for genotoxicity and the observation of the carcinogenic response in a single sex and in a single species, the DS deems the substance wholly meets the criteria for classification **only** in category 2.
RAC's response
Thank you for your comments. RAC agrees that there is limited evidence of carcinogenicity in animals and that human relevance of the ocular tumours cannot be excluded. As to genotoxicity, RAC is of the opinion that two comet assays investigating the site of first contact (stomach) and the site of metabolism (liver) plus one micronucleus test are a sufficient follow-up to the positive mouse lymphoma assay. RAC agrees with the DS that the observation of a carcinogenic response in a single sex of a single species and the lack of evidence for genotoxicity make classification in Category 2 more appropriate than 1B.

Date	Country	Organisation	Type of Organisation	Comment number
29.06.2018	Germany		MemberState	4
Comment received				
Based on the data provided in the CLH-report, the DE-CA supports the proposal of the dossier submitter to classify the substance based on significant dose-dependent increase in incidences of malignant squamous cell carcinoma in the eye in a 2-year rat study. However, it should be carefully evaluated whether classification into Category 1B might even be applicable based on the following considerations (in addition to those presented in table 24 on page 26): 1) According to the definition of "sufficient" evidence (CLP Annex I, 3.6.2.2.3), a single study in one species and sex might be considered to provide sufficient evidence of carcinogenesis which is a necessary pre-requisite for classification of the substance to Category 1 B based on the data collected in animal experimental studies. 2) Dose-response is substantiated by a statistically significant trend test (incidences: 0/51, 0/51, 0/51, 3/51, 5/51, p=0.0084, Chi-square test for trend, EpiTools, available online at http://epitools.ausvet.com.au). 3) The finding is of unusual incidence rate (e.g. 0 % for HCD from the facility, 1/1 354 (0.1 %) was reported for Fisher rats by Haseman et al 1998), unusual site and unusual tumour type. It was not reported as a spontaneous tumour type. 4) Progression to malignancy was observed. 5) The type of tumour also occurs in man. 6) The oral route of exposure is relevant to humans. 7) Appearance of the tumour in one sex only is in agreement with differences in the TAT activity between male and female rats. 8) Even though malignant squamous cell carcinoma was observed at doses causing non-neoplastic ocular				

changes (corneal opacity, keratitis), a confounding effect appears unlikely as those occurred in
both sex while tumours were limited to males. Liver and kidney weights were increased moderately
in males (between 11 and 25 %). There were no overt or serious clinical signs of toxicity. Body
weight decreased relative to controls by < 20 % in mid-high and high dose groups.
9) The proposed mode of action is relevant to humans as the pathway of tyrosine catabolism is
conserved between rats and humans.
10) Limitations of the data presented on hepatic TAT enzyme activity (Table 13, p.13) should be
critically discussed, in particular with regard to representativeness and physiological (allometric) scaling.
11) In a structural similarity search, the herbicide Isoxaflutole was identified, which is considered
as Category 2 carcinogen by EFSA (Peer review report, 2016) and displays ocular toxicity.

Dossier Submitter's Response

See response to comment 3.

RAC's response

Thank you for your comments.
RAC agrees that there was a clear, treatment-related increase in malignant tumours. However, the occurrence of a carcinogenic response in a single sex of a single species and the non-genotoxic MoA make Category 2 more appropriate. RAC agrees that the MoA is relevant for humans; human relevance is normally assumed for all animal tumours if there is no convincing evidence to the contrary.
Limitations of the data on TAT activity are discussed in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
28.06.2018	Belgium	<confidential>	Company-Manufacturer	5

Comment received

10.9.1 - 10.9.5 (pages 22-26): The proposed classification as Carc 2 is not acceptable considering the Mode of action of HPPD inhibition. It is well known and described that the Mode of Action of HPPDi´s is not relevant to humans and that the occurence of tumors is detected in rats only. Please note the position papers that are submitted to address the HPPDi Mode of Action and its relevance to humans (Appendix documents 10.9_01, 10.9_02 and 10.9_03). In the light of scientific evidence and former decisions on HPPDi´s the carc 2 classification should not be applied to Tolpyralate.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA_Appendix ISK_Public commenting.zip

Dossier Submitter's Response

Thank you for the submission of the additional position papers. As no new data has been submitted, and the submitted papers are a re-interpretation of the existing data the DS has not performed any additional evaluation. The submitted papers should be considered by RAC, alongside all the available information. The DS remains of the opinion that classification in category 2 is appropriate, for the reasons described in the CLH report.

RAC's response

Thank you for your comments. The arguments presented in the position papers have been examined by RAC and are discussed in the RAC opinion. The evidence for human

non-relevance of the ocular tumours has not been found to be sufficient. In particular, the argument that the interspecies sensitivity in the susceptibility to ocular effects from HPPD inhibitors can be fully explained by differences in TAT activity is not convincing in the light of studies with NTBC by Lock *et al.* (2006). RAC agrees with the DS that classification in Category 2 is appropriate.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
29.06.2018	Germany		MemberState	6
Comment received				
It should be discussed more critically whether the in vivo mutagenicity assays (one MN, two Comet) do provide appropriate follow up studies for the positive mammalian cell gene mutation assay. The dossier submitter's statement "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." is not fully supported.				
Dossier Submitter's Response				
The available comet assay, including the choice of tissues, is an acceptable follow up for a positive finding in the *in vitro* MCGM assay.				
RAC's response				
RAC agrees with the DS that the *in vivo* mutagenicity tests performed, including the choice of tissues, collectively form an adequate follow-up to the positive mouse lymphoma assay.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	France		MemberState	7
Comment received				
Developmental toxicity: Rat study page 38-40 - In the absence of historical control data it cannot be dismissed that the following malformations (HCD available for left umbilical artery, right subclavian from aortic arch and split cartilage of thoracic centrum cartilage) are treatment related. - For total skeletal variations, discontinuous rib cartilage and supernumerary rib, there is a dose-related increase from 10 mg/kg bw/d onwards of both the fetal and litter incidences. In the absence of historical control data, while not statistically significant, the increase observed at 10 mg/kg bw/d cannot be dismissed. Comparison with criteria page 44 and conclusion on classification page 45 - Evidence of prenatal susceptibility was observed in the rat and rabbit developmental toxicity studies based on the increase of total skeletal variations and specific skeletal variations, in particular, unossified/partially ossified cervical and thoracic centrum, supernumerary ribs and 27th presacral vertebrae at doses below those causing maternal toxicity. Fetus weight was affected in the rat study in presence of very slight maternal toxicity. - These fetal adverse effects may result from increased maternal tyrosinaemia. While it can be expected that humans are less sensitive than rats, this mode of action has been considered relevant to humans (RAC opinion of tembotrione, 2013). - In the 2-generation study, increased number of dead pups at birth and decreased percentage viability on LD 0 in both generations are observed at the top dose.				

- It is also noteworthy that histopathological effects in kidney in the 2-generation study were only observed in F1 adults from 50 ppm onwards which can be related to more susceptible window of exposure. Indeed, F1 adults were exposed during in utero and juvenile life in contrast to P generation only exposed during adult life.

Based on the above-mentioned considerations, FR considers that classification repr.cat2 H361d is warranted.

Dossier Submitter's Response

Rat developmental study

In the rat developmental study, the incidence of the malformations left umbilical artery, right subclavian from aortic arch and split cartilage of thoracic centrum cartilage at the top dose is very low. These findings are most likely incidental.

Please see attached file "CLH - tolpyralate - historical control data" for historical control data (provided by the applicant) for the following findings: Dumbbell-shaped cartilage of thoracic centrum, Split cartilage of thoracic centrum, Dumbbell-shaped cartilage of cervical centrum, Split cartilage of cervical centrum, Left umbilical artery, Right subclavian artery arising from aortic arch, Retroesophageal subclavian artery (nb. HC data is spread over 5 worksheets within the file).

The DS can agree that there is an increase in total and specific skeletal variations (discontinuous rib cartilage and supernumerary rib) from 10 mg/kg bw/d. However, these findings are considered of minimal toxicological significance in accordance with point 3.7.2.3.3 of Annex I of the CLP Regulation and do not justify classification for developmental toxicity.

The DS maintains that the small (6.1%) decrease in fetal weight at the top dose of 500 mg/kg bw/d, whilst indicative of developmental toxicity, is secondary to maternal toxicity and does not justify classification.

Rabbit developmental study

For the rabbit developmental study, the DS agrees that total and specific skeletal variations were increased at the top dose of 500 mg/kg bw/d. However, as these findings are considered of minimal toxicological significance in accordance with point 3.7.2.3.3 of Annex I of the CLP Regulation, classification for developmental toxicity is not justified.

2-generation study

With respect to the increased pup death on LD0 at the top dose, as discussed in the CLH report, this effect occurs inconsistently between the two generations. In the F1 generation the dead pups are associated with small numbers of deaths (1-3) across 8 litters. In the F2 generation, the increased number of deaths is predominantly associated with the loss of 9 pups from a single litter. Therefore, in the F2 the effect is not considered treatment-related. As the effect on pup death at LD0 in the F1 was small and not confirmed in the F2, it was not considered related to treatment overall. Therefore, classification for developmental toxicity due to pup death is not warranted. The presence of kidney findings in F1 adults and not in P adults could indicate increased susceptibility during development. This is addressed by the risk assessment. However, as the effects in the kidney are not developmental effects, classification for developmental toxicity on the basis of these findings is not justified.

Overall, the DS maintains that classification for reproductive or developmental toxicity is not warranted.

RAC's response

Thank you for your comments.

Rat developmental study
RAC reviewed the historical control data for the three anomalies mentioned (left umbilical artery, right subclavian from aortic arch and split cartilage of thoracic centrum cartilage) and agrees with the DS that a relationship of these findings to treatment is questionable. As to the skeletal variations and the small reduction in foetal weight, RAC agrees with the DS that although treatment-related, they are not considered sufficiently severe to warrant classification.

Rabbit developmental study
RAC agrees with the DS that the skeletal variations are not sufficiently severe to support classification.

2-generation study and a 1-generation dose-range finding study
The reduction in pup viability on PND 0 in the 2-generation study and on PND 1-4 in the 1-generation range-finding study supports classification for developmental toxicity. RAC also agrees that the generational studies show increased susceptibility of developing organisms to renal toxicity compared to adults.

Overall, RAC agrees that classification in Category 2 for adverse effects on development is justified, but mainly based on reduced pup viability and renal findings in the generational studies.

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2018	Belgium		MemberState	8

Comment received

Fertility :
Some effects need to be discussed in Committee, including reduced pup viability and decreased mean number of pups. Overall, BE CA is of the opinion that no classification is warranted for fertility based on the CLH report.

Developmental toxicity :
In rat offsprings, adverse effects on target-organs have been recorded from lactation day 4, including pelvic dilatation, kidney cysts, kidney atrophy, ocular opacity and ocular keratitis in a dose-range finding investigation for reproductive toxicity. These effects are consistent with the treatment-related effects in eye and kidney reported after repeated exposure in adult rats (see section "Specific target organ toxicity - Repeated exposure" of this comment for more information). We questioned the source of exposure, which might have occurred in utero or through lactation. However, the increased incidence of absent kidney in offsprings at 20.000 ppm (2.1% pups) let us conclude that the tolpyralate exposure occurred at least in utero, although an exposure through lactation as well is not excluded.

Moreover, developmental findings in rat foetuses include increased skeletal variations, mainly discontinuous rib cartilage and supernumerary rib, at 500 mg/kg bw/day, associated with slight maternal toxicity.

Finally, 98,5% of rabbit fetuses showed in a developmental study skeletal variation without any maternal toxicity at 500 mg/kg bw/day, including supernumerary rib and 27th presacral vertebrae.

Taking into consideration the observed effects in the eye and kidney of pups, the reported malformations such as absent kidney in rat and the very high percentage of skeletal variations in rabbit without maternal toxicity, BE CA is of the opinion that a Repr. 1B H360D is warranted.

Dossier Submitter's Response

Fertility

DS notes the agreement of the BE CA that no classification for fertility is warranted – thank you for your support.

Developmental toxicity

With respect to the skeletal variations in rats and rabbits, these are considered to be of minimal toxicological significance and do not support classification for developmental toxicity in accordance with point 3.7.2.3.3 of Annex I of the CLP Regulation; also please see response to comment 7 above for further detail. It should be noted that although 98.5% of rabbit foetuses at the top dose of 500 mg/kg bw/d had one or more skeletal variations, this value should be compared with 33.5% of rabbit foetuses in controls. With respect to the observed effects on the kidneys reported in the multi-generation study, the DS is of the opinion that these are not indicative of developmental toxicity. Effects on the kidneys were seen with tolpyralate in various RDT studies in adult animals; the kidney is a primary target of toxicity of tolpyralate, and classification for STOT-RE has been proposed accordingly. The kidney effects in pups represent offspring toxicity, not specific developmental toxicity. The finding of absent kidney in the 1-generation range-finding study was observed in F1 offspring on PND26, but not on PND4; hence, it is most likely to be incidental.

Overall, the DS maintains that classification for developmental toxicity is not warranted.

RAC's response

Thank you for your comments.

Fertility
RAC agrees that the reduced litter size is not sufficient for classification. However, there was also a delay in sexual maturation (by 4 days in both sexes) that is not fully explained by the observed body weight reductions; this effect is considered sufficient for classification.

Developmental toxicity
As to the skeletal variations in the rat and rabbit PNDT studies, RAC agrees with the DS that these are not sufficiently severe to support classification.
However, the reduced pup viability, not only on PND 0 in the 2-generation study but also during PND 1-4 in the range-finding study, is considered sufficient to trigger classification for developmental toxicity. The classification is further supported by the renal effects in the offspring.

Overall, RAC is of the opinion that classification with Repr. 2; H361fd is appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
28.06.2018	Belgium	<confidential>	Company-Manufacturer	9
Comment received				
10.10.10: Please find in the Appendix a supportive review on Reproduction and Developmental Toxicities of Tolpyralate (Document 10.10_01) ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA_Appendix ISK_Public commenting.zip				
Dossier Submitter's Response				
Thank you for providing this revised position paper on the reproductive and developmental toxicity of tolpyralate, which supports our proposal for no classification. This position paper is mostly a reinterpretation of the existing data; however it does include reference to an additional developmental toxicity study (IET 13-0051, 2017) that has not previously been considered by the DS. A brief summary of the study is provided in the position paper, and the applicant has provided the DS with the full study report during this commenting period. No evidence of developmental toxicity was observed in the study. **Overall, the DS concludes that the additional information supports the proposal for no classification for developmental or reproductive toxicity.**				
RAC's response				
Thank you for your comments. RAC agrees that the developmental findings are likely to be related to tyrosinemia resulting from HPPD inhibition. However, RAC has not found the evidence for human non-relevance of tyrosinemia from HPPD inhibitors convincing (for a detailed discussion please see the carcinogenicity part of the RAC opinion) and therefore the developmental findings in the animal studies are considered relevant for humans. RAC is of the opinion that classification in Category 2 for adverse effects on development is justified based on reduced pup viability and renal findings in the generational studies. In addition, RAC proposes classification in Category 2 for adverse effects on sexual function and fertility based on delayed sexual maturation observed in both sexes in the 2-generation study.				

Date	Country	Organisation	Type of Organisation	Comment number
28.06.2018	Belgium	<confidential>	National Authority	10
Comment received				
10.12.1: Comparision with the CLP criteria. It should be pointed out that no adverse effect (including renal effects) was observed at 20 ppm in the 1-year and 2-year rat studies (longer exposure duration). ISK is providing a data sheet that shows α2u-globulin accumulation in the kidney using the tissue samples from the 90-day rat feeding study conducted in ISK (non-GLP). MOA is not human relevant. Please find the data sheet in Appendix 2, Document 10.12.1_01. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA_Appendix 2_ISK public commenting.zip				
Dossier Submitter's Response				
Many thanks for providing these additional data, which show that the kidney effects in male rats in the 90-day study are due to α2u-globulin accumulation and therefore not relevant to humans. However, as in other rat studies, including the 2-generation study,				

there are kidney findings also in females and gross findings in offspring not normally associated with α2u-globulin accumulation, not all the observed kidney findings can be regarded as not relevant to humans. In addition, there was an increased incidence of glomerulonephritis in female mice in the chronic study.
RAC's response
Thank you for this additional data showing that the increased hyaline droplet deposition in male rats administered tolpyralate was associated with α2u-globulin accumulation. Therefore, RAC does not consider this finding relevant for humans and does not propose a STOT RE classification for kidney effects. However, a relationship of the renal findings in the generational studies to α2u-globulin accumulation is unclear (effects were seen also in females) and therefore the renal findings are used to support classification for developmental toxicity.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	France		MemberState	11
Comment received				
Skin sensitisation page 21-22 - In the LLNA the highest tested concentration was 50%. According to OECD 429 TG, the highest concentration maximises exposure while avoiding systemic toxicity and/or excessive local skin irritation. The highest dose was not limited by irritation. - In the GPMT, the topic challenge concentration of 50% may not be the highest non-irritant concentration as indicated in OECD 406 TG. The chosen concentration was not limited by irritation since in the preliminary test 50% was non-irritating and no higher concentration has been tested. - Since for both LLNA and GMPT, the respective OECD guidelines were not fulfilled referring to the level of doses selected, this should be spotted as a deviation. In the absence of any justification of the tested concentration, the uncertainties on the intrinsic sensitising properties of tolpyralate due to too low selected concentrations should be mentioned also taking into account that: * Other HPPD pesticides are classified for skin sensitisation (e.g.: sulcotrione, tembotrione); * Tolpyralate highlights alert 424 (Alkyl aldehyde precursor), skin sensitisation plausible in Derek software; * The tolpyralate-based plant protection product SL-573 100OD is classified H317.				
Dossier Submitter's Response				
The DS has received justification from the company relating to the maximum tested concentration of 50%. The company states that it was not possible to test at a higher concentration as the test material was a solid that required moistening before application. Therefore, both in the LLNA and GMPT, the tested concentration was maximised. Negative results were obtained in these studies; hence classification with H317 is not required.				
RAC's response				
RAC accepts the DS's explanation and further notes that SLS pretreatment was conducted in the GPMT as required by the OECD TG for non-irritant substances. RAC agrees with the DS that no classification based on conclusive data is appropriate.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	France		MemberState	12

Comment received

STOT RE
Comparison with criteria page 59-68
- Proposal for STOT-RE in category 2 –H373, based on adverse effects in the eye and kidney is supported.
- However FR is of the opinion that thyroid, pancreas and nervous system should also be taken into consideration for classification purposes:
Thyroid: 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.
Pancreas: Pancreatic acinar cell fibrosis and/or necrosis were observed in 28-d, 90-d, 1-y and 2-y studies conducted in the rat. In all studies the lowest dose at which adverse effects on the pancreas were observed was higher that the dedicated CLP guideline value for classification. However the NOAELs for this effect were lower than the dedicated CLP guideline value for classification. Therefore, it cannot be excluded that these effects could occur at doses triggering classification especially taken into account the very large dose spacing in all the studies.
Nervous system: There was no evidence of neurotoxicity in the 90-d neurotoxicity study in rat. However, vacuolation of the cerebellum was observed in the rat, in both sexes in the 1 and 2 year chronic toxicity studies from 1000 ppm onwards. Decreased absolute brain weight in males and degeneration of sciatic nerve in both sexes were observed from 1000 ppm in the 2-y study (eq.to ≥83.8 mg/kg bw/d). The NOAEL for those effects was 20 ppm (eq. to 0.925mg/kg bw/d 0.765 mg/kg bw/d in the 1-year and 2-year rat study respectively).Taken into account the very large dose spacing in the 1 and 2 year chronic toxicity studies it cannot be excluded that effects on nervous system could occur at doses triggering classification.

Dossier Submitter's Response

The DS notes the comments from FR CA; however the DS remains of the opinion that classification for STOT-RE is only justified for effects observed in the eye and kidney.

As discussed in the CLH report, the adverse effects on the thyroid in males in the 28 and 90 rat studies at dose levels relevant for classification are limited to increased incidence of thyroid follicular cell hypertrophy. Whilst this finding is considered to be treatment related it does not represent a significant change affecting function or morphology and, in isolation (no other thyroid histopathological findings), is not considered sufficient for classification for STOT-RE.

With respect to the effects on the pancreas, no effects were observed at dose levels relevant for classification, with the exception of the observation of increased incidence of pancreatic acinar cell fibrosis in males in the 1 year study. The DS maintains that the effect in the 1 year study was an anomalous finding as it was not observed at comparable dose levels in the 2 year study. Given the large dose spacing used in some studies, the DS agrees that it cannot be excluded that the pancreatic effects may occur at doses below the guidance values for classification with STOT-RE2; however, as this remains speculative, classification with STOT-RE2 for effects on the pancreas is not justified.

With respect to the mentioned effects on the nervous system, there were no effects on the cerebellum or sciatic nerve at dose levels relevant for classification. Given the large

dose spacing used in some studies, the DS agrees that it cannot be excluded that the nervous system effects may occur at doses below the guidance values for classification with STOT-RE2; however, as this remains speculative, classification with STOT-RE2 for effects on the nervous system is not justified.

Overall the DS maintains its position on proposals for classification for STOT-RE in category 2, based on effects in the eye and kidney.

RAC's response

RAC agrees that classification with STOT RE 2 is justified for the eye. The hyaline droplet deposition in the kidneys of male rats has been shown to be associated with α2u-globulin accumulation and is not considered relevant for humans. The renal findings in the offspring in the generational studies have been used to support classification for developmental toxicity.

RAC agrees that a 100-fold interval between doses is too large and may affect the STOT RE classification. However, RAC does not attempt interpolation where an effect is only observed at doses far above the guidance values as such an interpolation would be rather uncertain.

The thyroid follicular cell hypertrophy is considered a treatment-related effect and although it is an effect of an adaptive rather than adverse nature, it may be associated with reduced T4 levels, which is an adverse effect. However, the T4 reduction was less than 20% at a dose as high as 1930 mg/kg bw/d in a 2-week study, and the 1-year and 2-year studies show that hypertrophy did not progress with increasing exposure duration, which indicates that the effect was weak and probably transient.

The incidence of pancreatic single acinal cell necrosis or fibrosis was increased mainly at doses above the GVs for classification and where the increase occurred marginally above the GVs, the incidence was relatively low. The only dose level with pancreatic effects below the GVs was approx. 1 mg/kg bw/d in the 1-year rat study; however, the increase was not statistically significant and was not replicated at the same dose level in the 2-year study. Taking a WoE approach, classification for effects on the pancreas is not justified.

The effects on the nervous system (vacuolation of the cerebellum, sciatic nerve degeneration) occurred only above the GVs for classification.

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2018	Belgium		MemberState	13

Comment received

Eye and kidney are identified as target-organs, warranting a classification for repeated toxicity. Rat is the most specific species for ocular keratitis in the available studies. However, due to a poorly designed dose-range strategy, three studies should be concluded only as supportive (90 days and 1 year repeated toxicity studies and 2 year carcinogenicity study in rat). Indeed, for both concerned studies in rat there is a 100-fold gap between the NOAEL (eye) and the LOAEL whereas almost all animals showed ocular keratitis at LOAEL. Therefore, this dose-gap might have a critical influence on the final STOT RE classification :

- 90 days OECD 408, GLP Oral, rat (STOT RE Cat 1 = 10 mg/kg ; Cat 2 = 100 mg/kg) :

Doses : 0 - 0,32/0,38 - 1,34/1,58 - 133/159 - 1363/1647 mg/kg bw/day
NOAEL for eye : 1,34/1,58 mg/kg bw/day (hyaline droplet deposition in 2/10 males)
LOAELE : 133/159 mg/kg bw/day (Ocular keratitis 7/10M and 10/10M)

- 1 year OECD452, GLP Oral, rat (STOT RE Cat 1 = 2,5 ; Cat 2 = 25)
Dose-range : 0 - 0,229/0,303 - 0,925/1,18 - 97,0/126 - 1182/1336 mg/kg bw/day
NOAEL for eye : 0,925/1,18 mg/kg bw/day (pancreatic acinar cell fibrosis reported in 7/21 M + loss of fur 6/21 M) LOAEL : 97/126 mg/kg bw/day (Ocular opacity : 20/21M + 20/21F ; Ocular keratitis : 20/21M + 20/21F)

- 2 year carc. study OECD451, GLP Oral, rat (STOT RE Cat 1 = 1,25mg/kg ; Cat 2 = 12,5)
Dose-range : 0 - 0,196/0,255 - 0,765/1,01 - 83,8/108 - 426/554 mg/kg bw/day
NOAEL for eye : 0,765/1,01 mg/kg bw/day (No other observed effect)
LOAEL : 83,8/108 mg/kg bw/day (Corneal opacity and associated keratitis in all animals)

Moreover, the levels reported for the 90 days rat study in Table 36 (p. 55 ; 0 – 0,196/0,255 – 0,765/1,01 – 83,8/108 – 426/554 mg/kg bw/day) are not the same as the dose-range for the same study as presented in the Table 35 of CLH report (p.47 ; 0 - 0,32/0,38 - 1,34/1,58 - 133/159 - 1363/1647 mg/kg bw/day). However, these dose levels are consistent with the two-year carcinogenicity study. Please provide further clarification for Table 36.

BE CA would also know if the cornea and the cerebellum were specifically investigated in all mouse and rat repeated toxicity studies, as no mention of negative results were reported for the these endpoints for some studies and the study protocols were not described in the CLH report. In particular, we noticed that vacuolation of the cerebellum was observed in rat during the mechanistic studies, this endpoint seems therefore of high concern.

Overall, BE CA is of the opinion that the 28-day study is the only reliable rat study for STOT RE classification based on information provided in the CLH report. In the 28-day rat study (0 – 4,49/4,98 – 45,9/46,9 – 447/496 and 1799/1907 mg/kg bw/day), ocular keratitis was observed in 1/6 male at 4,46 mg/kg and increased at higher doses, warranting a STOT RE 1 classification for the eye. Moreover, supportive information for the STOT RE 1 classification for eye might be found in the rat reproductive toxicity studies.

Indeed, in a two-generation reproductive toxicity study in rat, ocular keratitis was reported in 1/24 P males after exposure to 2,70 mg/kg tolpyralate. The duration of exposure needs to be clarified, but according to OECD 416 guideline, we assume that it would be comprised between 12 and 20 weeks. The guidance value for a STOT RE 1 classification after 20 weeks of exposure is 6 mg/kg bw/day when applying Haber's rule, and this might therefore support this classification.

Moreover, in the preliminary range-finding study in rat, parents showed ocular opacity (1/8M and 1/8F) in the 200 ppm group (corresponding to 10,7-19,6 mg/kg bw/day). The exposure duration for this range-finding study is assumed to be 11 weeks, but this point needs to be clarified as well.

The ocular keratitis are clearly related to the treatment, as demonstrated by the MoA and the measured increase in tyrosine in the eye. Moreover, this MoA is considered to be relevant to human and there is no reason to believe that the potency in human would be

lower. Therefore, BE CA is in favour of a STOT RE 1 (eye) classification for tolpyralate.

Finally, the relevance of the kidney effects have been questioned by the DS because hyaline deposition and kidney nephropathy are likely to be associated with α2μ-globulin, although this has not been confirmed. BE CA noticed that no tubular neoplasms have been reported after tolpyralate exposure in the rat carcinogenicity studies. Moreover, kidney tubular effects have been observed in dog on the 28-day oral study, which suggest that this effect is not species-specific. Therefore, this hypothesis is not supported.

Dossier Submitter's Response

Thank you for your detailed comments. With respect to the dose spacing, the DS agrees that this was very large in some studies; however, this does not invalidate them. Although it cannot be excluded that some effects may occur at doses below the guidance values for classification with STOT-RE2 and, for the eye and kidney, at doses below the guidance values for STOT-RE1, this is only speculative. Therefore, in the absence of evidence of an adverse effect at dose levels relevant for classification, no classification is warranted.

The DS can confirm that the eyes and nervous system were investigated in the 90 day studies in both the rat and mouse, in the 1 year and 2 year studies in the rat, and in the 18 month study in the mouse.

With respect to the dose levels quoted in table 36, there is a typographical error. The correct dose levels are those quoted in table 35.

With respect to classification for STOT-RE for eye effects, the DS considered all the available information, and as described in the CLH report , because effects relevant for classification in category 1 were only seen in the 28 day study (shortest study) whereas in all the other longer studies the effects only occurred at guidance values relevant for classification in category 2, the DS applied a weight of evidence approach and concluded the appropriate proposal was for classification in category 2. In addition, although the eye findings in the 2-generation and 1-generation range-finding studies may support classification with STOT-RE1, the incidence was very low and, again, taking a WoE approach, classification with STOT-RE2 appears more appropriate.

With respect to the kidney effects, the DS agrees with the BE CA that these are relevant to humans and warrant classification with STOT-RE2 as kidney findings were also seen in rat females and gross kidney findings not normally associated with α2u-globulin accumulation were seen in rat offspring. In addition, there was an increased incidence of glomerulonephritis in female mice in the chronic study and tubular effects in the dog 28-day study.

RAC's response

RAC agrees that a 100-fold interval between doses is too large and is not in line with the OECD recommendation. Although this may have impact on STOT RE classification, it does not invalidate the studies. Nevertheless, RAC does not attempt interpolation where an effect is only observed at doses far above the guidance values as such an interpolation would be rather uncertain.

As to the classification for ocular effects, RAC acknowledges that these occurred at doses below the GV for Category 1 in two studies (28-day and 2-generation) and the MoA is relevant for humans (which is the default assumptions for all effects seen in the animal studies if there is no convincing evidence to the contrary). However, the incidence below the GVs for Cat. 1 was relatively low and rats are generally more sensitive than humans

to the ocular effects from exposure to HPPD inhibitors. Therefore, RAC agrees with the DS that classification in Category 2 for effects on the eyes is appropriate.

Vacuolation of the cerebellum occurred only above the GVs and therefore is not considered to support classification.

The hyaline droplet deposition in the kidneys of male rats has been shown to be associated with α2u-globulin accumulation and is not considered relevant for humans. Alpha2u-globulin accumulation does not always result in tumours in rats. The renal findings in the offspring in the generational studies have been used to support classification for developmental toxicity. The kidney effects in the mouse occurred only at a dose far above the GV. The renal finding in the dog occurred only above the GV, was limited to a single animal in a short-term study and was not reproduced in studies of longer duration.

Date	Country	Organisation	Type of Organisation	Comment number
29.06.2018	Germany		MemberState	14
Comment received				
Specific target organ toxicity – repeated exposure: Classification as STOT RE2 is supported. However, an unusually high dose spacing was noted for the 90-days, 1-year and 2-year studies in rats (100X) versus recommended by the OECD 6-10 fold dose spacing that potentially affected threshold-based allocation to STOT RE categories.				
Dossier Submitter's Response				
Comment from DE CA is noted. Please see responses from the DS to comments 12 and 13 above. The DS maintains that the proposed classification is appropriate.				
RAC's response				
RAC agrees that classification with STOT RE 2 is justified for the eye. The hyaline droplet deposition in the kidneys of male rats has been shown to be associated with α2u-globulin accumulation and is not considered relevant for humans. The renal findings in the offspring in the generational studies have been used to support classification for developmental toxicity. RAC agrees that a 100-fold interval between doses is too large, not in line with the OECD recommendations, and may affect threshold-based allocation to STOT RE categories. However, RAC does not attempt interpolation where an effect is only seen at doses far above the guidance values as such an interpolation would be rather uncertain.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	France		MemberState	15
Comment received				
FR would agree with the classification and the M-factor values proposed for Environmental hazards if the proposals of classification and M-factor could be based on data from a metabolite and not only on data of the substance. If the classification proposal has to be based only on toxicity data of the substance, the current classification proposed in the CLH report would have to be updated. FR agrees that the proposal for the chronic M-factor would have to be reconsidered when further data giving more reliable chronic toxicity data would be available.				

Dossier Submitter's Response
We had originally based the chronic classification and M factor on the a.s. data for *Lemna gibba*. However, the ECHA accordance check report proposed that the classification should be revised and instead based on the degradant MT-2153 (as the degradant is forming very fast and in significant amounts). A new *Myriophyllum* study with the a.s. has been provided – however, it is not possible to obtain a reliable NOEC from this study –please see response to comment 16 for further details.
RAC's response
Classification of substances is primarily based on their intrinsic properties. However according to the CLP guidance the classification of the parent compound should take due account of the hazard of the degradation product, and the rate at which it can be formed under normal environmental conditions. Since major degradant MT-2153 forming in significant amounts and rather fast under normal environmental conditions, as well it cannot be demonstrated that it does not fulfil the criteria for classification as hazardous to the aquatic environment, RAC is of opinion that major degradant MT-2153 should be considered for classification purposes of tolpyralate. A new 14-day *Myriophyllum spicatum* study conducted according to OECD 239 (GLP) with tolpyralate has been provided during the public consultation. Although the DS indicated some uncertainty in the lower test concentrations and pH between the different test solutions RAC is of the opinion that this uncertainty does not compromise the reliability of the study. Therefore RAC is of the opinion that classification should be based on endpoints from this study, however that will not change classification and M-factors proposed by the DS as all relevant endpoints from others relevant studies are slightly different but still in the same classification range.

Date	Country	Organisation	Type of Organisation	Comment number
29.06.2018	Germany		MemberState	16
Comment received				
Summary of relevant information on aquatic toxicity of technical tolpyralate (page 78 table 53): The study design of the acute toxicity studies to the fish species Cyprinus carpio and Pimephales promelas were semi-static instead of flow-through. It would be helpful to give a better overview of the acute and chronic toxicity studies of the relevant degradant MT-2153. Comparison with CLP criteria for long-term aquatic hazard (page 86ff point 11.7.2): It is recommended to use data for the substance itself (intrinsic toxicity) for classification and labelling purposes. Therefore the chronic data for aquatic plant Myriophyllum aquaticum (NOErC ≤ 0.000304 mg/L) as the lowest long term value of tolpyralate should be used. The DE-CA agrees to the recommendation that a further study with the sensitive species Myriophyllum according to OECD 239 with duration of 14 days instead of 7 days would be useful and preferable.				
Dossier Submitter's Response				
Thank you for highlighting the error in table 53; the acute toxicity studies for *Cyprinus carpio* and *Pimephales promelas* were indeed semi-static instead of flow through. If required, further information can be provided regarding the aquatic studies with the degradant MT-2153.				

A new *Myriophyllum* study (14 d duration) has been submitted. However, there are issues with the new study. The test design was semi-static with renewal of test solutions every 2-3 days. Despite this, test concentrations were not maintained at the 3 lowest concentrations tested. In the lowest test concentration – the majority of samples were < LOQ or < LOD in the aged media. In the second lowest concentration, half the analytical samples were < LOQ or < LOD in the aged media. In the third lowest concentration, 2 of the analytical samples were < LOD in the aged media. The study report has used ½ LOQ or the LOD (when concentrations were < LOQ or < LOD, respectively) to calculate geomean measured test concentrations. Therefore, there is uncertainty in the 3 lower concentrations –two of which are key concentrations for deriving a NOEC from this study. The non-maintenance of test concentrations in the lower 3 test concentrations appears to be linked to pH. SI-573 appears to be more stable under acidic conditions. The pH in the control and lowest 3 concentrations drifted by > 3 pH units over some of the 2-3 day renewal periods. Although the pH was 5.9-6.5 in the fresh media, in the control aged media it drifted to a maximum of pH 9.5 and reached a maximum of pH 9.8 in the lowest test concentration. This large drift in pH was not seen in the 4 highest test concentrations (maximum pH was7.4- 7.8 in the 4 highest test concentrations), and test concentrations were maintained much better. There is no explanation within the study report regarding the large difference in pH between the different test solutions. It is noted that the OECD guideline specifies that the pH of the test media (water phase) at test initiation should be between 7.5 and 8.0 for optimum plant growth. It appears that the pH in the study varied from well below to well above the recommended level over the renewal periods. Nevertheless, the study report states that all validity criteria were met. However, it is not possible to currently check two of the criteria as the raw data does not appear to have been included within the study report. Overall, the dossier submitter does not deem the study to be reliable to derive a NOEC given the uncertainty in the lower test concentrations i.e. due to recovery at the relevant test concentrations being < LOQ or < LOD in the aged media and therefore the endpoint based on geomean measured concentrations may underestimate the toxicity of the test substance. In addition, there is the issue of the large difference in pH between the different test solutions. As no reliable NOEC for *Myriophyllum* can be derived from the new or previous study, the chronic M factor will remain based on the data for *Lemna gibba* for the degradant MT-2153.
RAC's response
Classification of substances is primarily based on their intrinsic properties. However according to the CLP guidance the classification of the parent compound should take due account of the hazard of the degradation product, and the rate at which it can be formed under normal environmental conditions. Since the major degradant MT-2153 formed in significant amounts and rather fast under normal environmental conditions, and it cannot be demonstrated that it does not fulfil the criteria for classification as hazardous to the aquatic environment, RAC is of opinion that major degradant MT-2153 should be considered for classification purposes of tolpyralate. A new 14-day *Myriophyllum spicatum* study conducted according to OECD 239 (GLP) with tolpyralate has been provided during the public consultation. Although the DS indicated some uncertainty in the lower test concentrations and pH between the different test solutions RAC is of the opinion that this uncertainty does not compromise the reliability of the study. Therefore RAC is of the opinion that classification should be based on endpoints from this study, however that will not change classification and M-factors

proposed by DS as all relevant endpoints from other relevant studies are slightly different but still in the same classification range.

Date	Country	Organisation	Type of Organisation	Comment number
28.06.2018	Belgium	<confidential>	Company-Manufacturer	17
Comment received				
11.5.3: A new Myriophyllum study was performed addressing a duration of 14 days according to guideline OECD 239. Please find it in the Appendix, document 11.5_01). This study will as well be submitted to EFSA peer review. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA_Appendix ISK_Public commenting.zip				
Dossier Submitter's Response				
The new *Myriophyllum* study has been evaluated in the context of the CLH report. Please see response to comment 16 regarding the overall reliability of this study.				
RAC's response				
A new 14-day *Myriophyllum spicatum* study conducted according to OECD 239 (GLP) with tolpyralate has been taken into consideration. It should be noted that relevant endpoints were slightly different however still in the same classification range as proposed by the DS.				

Date	Country	Organisation	Type of Organisation	Comment number
28.06.2018	Belgium	<confidential>	National Authority	18
Comment received				
11.6.2 (page 85): The study on Mysid chronic toxicity was finished and is submitted to adress the data GAP identified by the RMS and applicant of the CLH Dossier. Please find the document in Appendix 2, 11.6.2_01 Data Point 11.5: New study: ErC50 from Myriophyllum is 0.0102 mg/L and NOEC is 0.00063 mg/L. This means aquatic acute category 1 (M=10) and aquatic chronic category 1 (M=100) can be proposed based on new Myriophyllum study with Tolpyralate, which is the same as the ones already proposed in CLH dossier. The uncertainty on the M factor should therefore be addressed. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA_Appendix 2_ISK public commenting.zip				
Dossier Submitter's Response				
The chronic mysid study has been evaluated in the context of the CLH report. NOECs can be derived from this study. However, the aquatic plant endpoints are clearly lower and therefore this study does not have any impact on the M factors already proposed.				
RAC's response				
A new chronic toxicity study with Mysid (*Americamysis bahia*) conducted according to U.S. EPA OPPTS 850.1350 (GLP) with tolpyralate has been taken into consideration. RAC notes that this study eliminates the data GAP related to the chronic toxicity with the invertebrates. However as aquatic plants are clearly more sensitive than invertebrates the results of this study do not effect classification or M-factors. A new 14-day *Myriophyllum spicatum* study conducted according to OECD 239 (GLP) with tolpyralate has been taken into consideration. It should be noted that relevant endpoints were slightly different however still in same classification range as proposed by DS.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	France		MemberState	19
Comment received				
Page 6 and page 88 - Log Pow: Reference to Okuzuno (2016) should be preferred to Furatani (2014), as it refers to the pure active substance. See the RAR of Tolpyralate for the exact reference of the study.				
Dossier Submitter's Response				
Thank you for bringing this to our attention. You are correct that the partition co-efficient study by Okuzuno (2016) should be used in preference to Furatani (2014). The study by Okuzuno (2016) is conduction on PAI (99.9%) and gives a Log Pow of 1.9. The full reference for this study is: Okuzono K (2016) Measurement of 1-octanol/water partition coefficient for SL-573 (PAI) (HPLC Method), ISK BIOSCIENCES EUROPE NV, Report No : 84849 NB., the study by Furatani (2014) also gave a Log Pow of 1.9, therefore this does not have any impact on the rest of the CLH proposal.				
RAC's response				
Noted.				

CONFIDENTIAL ATTACHMENTS
1. ECHA_Appendix ISK_Public commenting.zip [Please refer to comment No. 5, 9, 17]
2. ECHA_Appendix 2_ISK public commenting.zip [Please refer to comment No. 10, 18]

Dossier submitter's attachments:
1. CLH - tolpyralate - historical control data [please refer to response to comment No.7]
2. Tolpyralate - Position paper – mean number of days until mating [this does not relate to a specific comment, but was submitted to us by the applicant following comments made during the EFSA process. It is included here for completeness]