Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Trinexapac-ethyl (ISO); ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate

EC Number: -

CAS Number: 95266-40-3

CLH-O-0000006737-63-01/F

Adopted

5 December 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON TRINEXAPAC-ETHYL (ISO); ETHYL(1RS, 4EZ)4-[CYCLOPROPYL(HYDROXY)METHYLENE]-3,5-DIOXOCYCLOHEXANECARBOXYLATE

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: Trinexapac-ethyl (ISO); ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate
EC number: -
CAS number: 95266-40-3
Dossier submitter: Lithuania

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2018	Germany		MemberState	1
Comment received				
DE-CA supports the proposal of classification for environmental hazards as Aquatic Chronic 1 (H410) and chronic M-factor of 1.				
Dossier Submitter's Response				
Noted. Thank you for your support.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
09.01.2019	France		MemberState	2
Comment received				
2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development FR: The conclusion page 102 "All these findings are not considered toxicological significant effect and/or changes in the proportions of foetal variants of high concern based on weight of evidence approach" is not agreed upon. Indeed: - Rat developmental toxicity study: increased incidence of asymmetrically shaped sternebrae on both fetal and litter basis, is considered treatment related. This anomaly is classified as grey zone anomaly according to current state of the art, which does not mean that it is not adverse but that more information is required in order to consider it as a variation or a malformation. Therefore structural abnormality was observed in the absence of maternal toxicity - Rabbit developmental toxicity study: death of the developing organism (increased post-				

implantation loss and decrease in the number of live foetuses) was observed at the high dose level. Based on the data reported in Vol3CA B.6 (Table 6.6.2.2-2 and 6.6.2.2-3) effect on dam BW was minor and effect on dams BW gain in all treated groups was very variable with no dose-response relationship. No information on corrected maternal body weight and corrected maternal body weight gain for all groups was available to allow clarifying the maternal toxicity versus fetal death.
Based on the above-mentioned considerations, classification repr. cat2 H361d seems to be warranted.

Dossier Submitter's Response

Thank you for your comment.
No classification regarding adverse effects on development (Repr. 2 H361d) has been proposed based on the following considerations:
1. Rat developmental toxicity study (OECD 414):
- Although an apparently dose dependent increase of *asymmetrically shaped sternebrae* was observed (foetuses / litter incidence, %), there were not statistically significant differences in incidence of this skeletal anomalies in the test article treated groups compared to the control group and historical control groups.
- Only litter incidence of *asymmetrically shaped sternebrae* (29.2%) at the top dose level of 1000 mg/kg was outside the historical control range (15.08±11.57%) for the laboratory (1985-1987). In addition, the notifier has provided non-combined in a single package HCD (1987-1993) from separate 12 developmental toxicity studies with total 297 pregnant females. Litter incidence of *asymmetrically shaped sternebrae* at the top dose level (29.2%) was equal to maximum observed in this HCD.
- According to the updated harmonized nomenclature for developmental toxicology (Makris et al. 2009, October 2012) *asymmetric sternebra* should be considered as a "grey zone anomaly", i.e. does not fit readily into one of the two categories (malformation or variation). According with the description of the effects in the study report it is not clear the severity of the foetal effects, i.e. if this observation (asymmetrically shaped sternebra) refers to asymmetrical ossification of the sternebra that would be termed either incomplete or increased ossification in today's terminology and considered a variation, or if it refers to an asymmetrical structure of the sternebra that would be termed misshapen in today's terminology and considered a malformation. Based on the notifier information, at the time of the study (from October 1987 to June 1988) asymmetrically shaped sternebra (foetal observations) were classed by the specific laboratory as an anomaly by using the following criteria for 'anomaly' classification: '*Rare, slight to moderate, permanent or reversible structural change that is not considered to impair foetal survival, development or function*.' Other criteria were used by this laboratory for classification of foetal observations as 'Malformations' and/or 'Variations'. It is questionable if the re-evaluation of data is possible by the pathologist.
- Although there were no statistically significant differences in group mean maternal body weights, it is noteworthy that in the high dose group body weight gain was statistically significantly reduced in treatment-related manner for the period days 0 – 6 (8.1%) and for the entire study period (days 0 – 21) (5.6%). This might have affected the litters in which asymmetrically shaped sternebrae were observed, however, no evaluation of correlation was conducted. The magnitudes of mean body weight gain changes throughout the dosing period and at study termination didn't exceed 10% and therefore, mean body weight gain changes were not considered adverse.

2. Rabbit developmental toxicity study (OECD 414):
- Maternal effects. Increased treatment-related mortalities and retarded body weight gain (non-statistically significant) to Day 15 at the top dose level of 360 mg/kg bw/day were observed in dams. The mortalities were attributed to substance irritation of the stomach mucosa as the animals had haemorrhagic depressions in the stomach. Indeed, information

on corrected maternal body weight and corrected maternal body weight gain for all groups is not available for this study. - Developmental effects. There was a statistically significant decrease (26.0%) in the number of live foetuses/dam at the top dose level of 360 mg/kg bw/day. However, there was a statistically significant increase in pre-implantation loss (10%) and post-implantation loss (11.6%) in the top dose. It should be noted that treatment started at the time when implantation already had been completed. It means that the decreased number of live foetuses in the top dose group could be due to pre-implantation loss but not to post-implantation loss and therefore this is might not be a treatment related effect. Overall, the parameter of the number of live foetuses in this study is contradictory: a relationship of decrease in the number of live foetuses to treatment cannot be conclusively established on the basis of the information provided since the treatment started after unequal pre-implantation loss. Historical control data is not available. On the basis of the insufficient information to conclude, differences in litter size were considered attributable to treatment. - No teratogenic effects were observed in rabbit.
RAC's response
Agree with the DS for no classification regarding reproductive toxicity.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2019	Finland		MemberState	3
Comment received				
The FI CA considers that there is sufficient evidence to classify Trinexapac-ethyl as a Skin Sens. Category 1B; H317 - May cause an allergic skin reaction. In the LLNA study (Anon., 2017, B.6.2.6., study 3) where the concentrations of 25 %, 50 % and 100 % w/v were tested, the derived EC3 value (estimates the concentration of the test substance that induces a stimulation index of 3.0) for the test substance was 95,4 %. The EC3 value meets the criteria for sub-category 1B, according to CLP (Annex I, Table 3.4.4) as the EC3 value is >2 %. This indicates that the trinexapac-ethyl has a weak skin sensitization potency and classification into sub-category 1B is required.				
Dossier Submitter's Response				
Noted. Thank you for your support.				
RAC's response				
Agree with the DS, classification into sub-category 1B is supported.				

Date	Country	Organisation	Type of Organisation	Comment number
09.01.2019	France		MemberState	4
Comment received				
FR: The proposal for classification Skin Sens. 1B, H317 is agreed upon				
Dossier Submitter's Response				
Noted. Thank you for your support.				
RAC's response				
Agree with the DS, classification into sub-category 1B is supported.				

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Date	Country	Organisation	Type of Organisation	Comment number
11.01.2019	Sweden		MemberState	5
Comment received				
The Swedish CA supports the classification of trinexapac-ethyl as Skin Sens. 1B. The LLNA study performed in 2017 according to OECD TG 429 (Anonymous, 2017) shows clear evidence of skin sensitization potential, based upon which the observed SI was > 3 and the calculated EC3 value of 95.4%. Thus, trinexapac-ethyl is a substance that shows a low to moderate skin sensitization potency in animals and fulfills the criteria for classification under Skin Sens. 1B, according to the CLP regulation. The results of the GPMT study carried out according to OECD TG 406 (Anonymous, 2001) and of the first LLNA study done according to OECD TG 429 (Anonymous, 2006), show that trinexapac-ethyl has no skin sensitizing properties. In the CLH proposal it is stated that the acceptability and reliability of the latter are regarded as questionable since the dermal irritation data were not considered in selecting the tested concentrations and dermal irritation effects at the site of exposure were not reported. In addition, it is noted that the highest concentration tested was 25% (highest achievable concentration in the vehicle acetone / olive oil). In the LLNA study from 2017 the concentrations tested were 25%, 50% and 100% in Pluronic L92. Thus, the negative results from the LLNA study from 2006 do not contradict the findings in the 2017 study.				
Dossier Submitter's Response				
Noted. Thank you for your support.				
RAC's response				
Agree with the DS, classification into sub-category 1B is supported.				

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2019	United Kingdom	Syngenta	Company-Manufacturer	6
Comment received				
Syngenta disagree with the classification proposal, which is based on a technically flawed study on an inappropriate specification of test material. We have conducted a more recent negative LLNA study on a more appropriate specification. See separate documents. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Syngenta Public Comments on Proposed Classification of Trinexapac-ethyl.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Trinexapac-ethyl - Syngenta Confidential Information regarding impurity level in LLNA test batches.pdf				
Dossier Submitter's Response				
Thank you for your comment. Three skin sensitisation tests conducted with trinexapac-ethyl were available (2001, 2006, and 2017) during the preparation of CLH Report. The notifier has submitted one Guinea Pig Maximization test (OECD 406) and two Local Lymph Node Assays (OECD 429). In the only reliable LLNA study (2017) trinexapac-ethyl tech. (Bach No SMO5D180 Fortified, purity 93.3%) was considered to be a contact dermal sensitiser. This technical material (Bach No SMO5D180 Fortified, purity 93.3%) was spiked with several impurities up to the maximum level they are proposed for inclusion in the technical specification proposed by the notifier. This only skin sensitisation (LLNA, 2017) study covers the technical specification regarding				

all impurities which were proposed in the notifier technical specification for the renewal of this active substance. This circumstance has a particularly importance as some impurities triggered alert for skin sensitisation according to the results of (Q)SAR by using VEGA and DEREK NEXUS. Furthermore, one of these impurities (CGA158377, CAS No 88805-65-6) is harmonised classified in Annex VI of Regulation (EC) 1272/2008 as Skin. Irrit. 2 H315 (please see CLH Report Table 78).
The CLH Report writer (RMS LT) disagrees with deficiencies (inappropriate vehicle choice and inappropriate preparation of top dose) which have been identified in the review of the 2017 LLNA study attached by the notifier. Firstly, vehicle *1% Pluronic L92* is a common, frequently used solvent in LLNA studies and OECD Guideline 429 (2010) mentions it as 'appropriate solubilisers (e.g. 1% Pluronic® L92)'. The procedures used in this study were validated using alpha-hexylcinnamaldehyde, purity ≥ 95% (HCA) as the positive control substance, namely 25% (w/w) mixture of HCA in 1% Pluronic® L92. This vehicle alone was topically applied too.
Secondly, the test substance as received (neat) was placed in a water bath set to 50°C until the test substance was liquefied. Considering that the melting point of pure trinexapac-ethyl (996 g/kg) is about 36°C, thermal decomposition starts at about 310°C and that it is not an oxidizing substance, the integrity of the test substance liquefied didn't have to change.
Other two skin sensitisation assays (2001 and 2006) have limitations and their reliability is considered to be questionable. It is noteworthy that the technical specification proposed by the notifier was not supported by the specification of the material used in these two skin sensitisation studies (Batch No P.306042 and No SMO5D180) regarding most of the impurities.
It should be noted that only after public commenting period in ECHA the Applicant submitted a new skin sensitization study (LLNA, 2019) based on new technical specification of trinexapac-ethyl. This new data was not available during the preparation of CLH Report and therefore, it was not evaluated by the CLH Report writer (RMS LT) at that time. The new study was self-evaluated (*Study Summary as well as CLH Report Writer Comments on LLNA (2019) and Conclusion (2019-06-26) are attached*) and these comments on new data and overall conclusion can be taken into account as part of the ongoing CLH process. It is noteworthy that this specific evaluation was not a part of the European Commission renewal process regarding active substance trinexapac-ethyl. It should be emphasized that at this renewal stage of the process new data on the technical specification was not taken into consideration.
Based on new study (LLNA, 2019) results, the test substance (Trinexapac-ethyl tech., Batch ID SMO5D180_FORTIFIED-2) for the tested concentrations (25%, 50%, and 75%) was not found to be sensitised.
Overall, based on four available skin sensitisation tests, trinexapac-ethyl is considered positive for a dermal sensitization potential because a stimulation index (SI) of greater than 3.0 was observed in one of the treatment group in one LLNA study (2017). Trinexapac-ethyl has moderate skin sensitisation potency as the EC3 value calculated for the test substance was 95.4%. It could be concluded that trinexapac-ethyl tech. fulfilled the criteria for classification Skin Sens. 1B, H317 under the conditions of the LLNA study (2017).

RAC's response

Agree with the DS, classification into sub-category 1B is supported.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2019	Denmark		MemberState	7
Comment received				
Denmark agrees with the classification.				
Dossier Submitter's Response				
Noted. Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2019	United Kingdom		MemberState	8
Comment received				
Trinexapac-ethyl CAS: 95266-40-3 An ErC10 (shoot wet weight) of 0.011 mg/l (mm) is available for the growth rate (shoot wet weight) endpoint. We think this is a more appropriate basis for chronic classification than the NOEC value of ≤ 0.025 mg/l (mm) for this endpoint included in the CLH proposal. We note this is in the same 0.01 to 0.1 mg/l classification range and supports the proposed classification.				
Dossier Submitter's Response				
Thank you for your comment. CLP allows the use of both NOEC / EC_X for classification to determine long-term effects. However, recent scientific developments indicate that NOEC values have shortcomings and EC_{10} values are preferred compared to NOEC values for deriving long-term toxicity. Therefore, we agree that ErC_{10}= 0.011 mg a.s./L (the lowest ErC_{10} value) should be used for classification and labelling instead of NOEC<0.025 mg a.s./L. However, it does not change the classification proposal. The proposal of classification for environmental hazards is Aquatic Chronic 1 (H410) and M-factor of 1.				
RAC's response				
RAC agrees that the ErC_{10} is a more appropriate value where available and it is used for classification for Trinexapac-ethyl.				

Date	Country	Organisation	Type of Organisation	Comment number
09.01.2019	France		MemberState	9
Comment received				
FR agrees with the classification proposal and the chronic M factor proposed in the CLH report. However, ErC10 values (0.022 mg/L for shoot length, 0.011 mg/L for shoot wet weiht and shoot dry weight) have been estimated from the toxicity study on Myriophyllum spicatum (Kirkwood; 2015). These ErC10 values support the same chronic classification and chronic M factor than currently proposed in the CLH report. However, FR considers that it would be more reliable to base the chronic classification (H410) and the chronic M factor value of 1 on the lowest ErC10 value instead on the NOEC value < 0.025 mg/L.				
Dossier Submitter's Response				
Thank you for your comment.				

CLP allows the use of both NOEC / EC_X for classification to determine long-term effects. However, recent scientific developments indicate that NOEC values have shortcomings and EC_{10} values are preferred compared to NOEC values for deriving long-term toxicity. Therefore, we agree that ErC_{10}= 0.011 mg a.s./L (the lowest ErC_{10} value) should be used for classification and labelling instead of NOEC<0.025 mg a.s./L. However, it does not change the classification proposal. The proposal of classification for environmental hazards is Aquatic Chronic 1 (H410) and M-factor of 1.
RAC's response
RAC agrees that the ErC_{10} is a more appropriate value where available and it is used for classification for Trinexapac-ethyl.

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2018	Germany		MemberState	10
Comment received				
Page 206, point 2.9.2.4.2 Long-term aquatic hazard (Table 75) and page 207, point 2.9.2.5 Conclusion on classification and labelling for environmental hazards: Due to the lowest NOEC of < 0.025 mg a.s./L (lowest tested concentration) for growth rate from the key study of Kirkwood (2015) with Myriophyllum spicatum, DE-CA would prefer the use of ErC10 = 0.011 mg a.s./L for classification and labelling. Actually, the use of a distinct value is preferable than a range for classification. There is no influence on classification and labelling as Aquatic Chronic 1, M-factor of 1 because of this minor change.				
Dossier Submitter's Response				
Thank you for your comment. CLP allows the use of both NOEC / EC_X for classification to determine long-term effects. However, recent scientific developments indicate that NOEC values have shortcomings and EC_{10} values are preferred compared to NOEC values for deriving long-term toxicity. Therefore, we agree that ErC_{10}= 0.011 mg a.s./L (the lowest ErC_{10} value) should be used for classification and labelling instead of NOEC<0.025 mg a.s./L. However, it does not change the classification proposal. The proposal of classification for environmental hazards is Aquatic Chronic 1 (H410) and M-factor of 1.				
RAC's response				
RAC agrees that the ErC_{10} is a more appropriate value where available and it is used for classification for Trinexapac-ethyl.				

PUBLIC ATTACHMENTS
1. Syngenta Public Comments on Proposed Classification of Trinexapac-ethyl.zip [Please refer to comment No. 6]

CONFIDENTIAL ATTACHMENTS
1. Trinexapac-ethyl - Syngenta Confidential Information regarding impurity level in LLNA test batches.pdf [Please refer to comment No. 6]