Committee for Risk Assessment

RAC

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

5 December 2019

CLH-O-0000006737-63-01/F

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

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

Chemical name: **trinexapac-ethyl (ISO); ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate**

EC Number: **-**

CAS Number: **95266-40-3**

The proposal was submitted by **Lithuania** and received by RAC on **13 September 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

Lithuania has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **29 October 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 January 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

Co-Rapporteur, appointed by RAC: **Pietro Paris**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **5 December 2019** by **a simple majority of all members present and having the right to vote**.

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

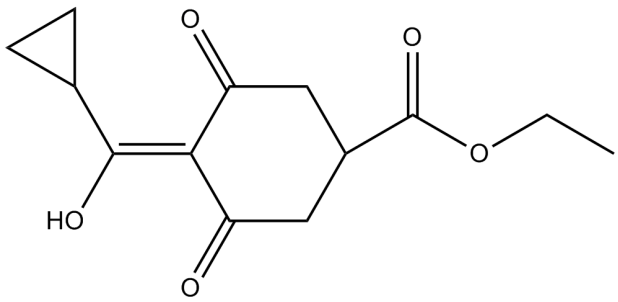
	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	trinexapac-ethyl (ISO); ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate	-	95266-40-3	Skin Sens. 1B Aquatic Chronic 1	H317 H410	GHS07 GHS09 Wng	H317 H410		M=1	
RAC opinion	TBD	trinexapac-ethyl (ISO); ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate	-	95266-40-3	STOT RE 2 Skin Sens. 1B Aquatic Chronic 1	H373 (GI tract) H317 H410	GHS08 GHS07 GHS09 Wng	H373 (GI tract) H317 H410		M=1	
Resulting Annex VI entry if agreed by COM	TBD	trinexapac-ethyl (ISO); ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate	-	95266-40-3	STOT RE 2 Skin Sens. 1B Aquatic Chronic 1	H373 (GI tract) H317 H410	GHS08 GHS07 GHS09 Wng	H373 (GI tract) H317 H410		M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Trinexapac-ethyl is a plant growth inhibitor of the cyclohexadione class, its intended mode of action is by inhibition of the biosynthesis of the plant hormone gibberellin. It is an ethyl ester resulting from the formal condensation of the carboxy group of trinexapac (derived from cyclohexanecarboxylate) with ethanol and functions as a synthetic plant growth regulator. Trinexapac-ethyl is a Class-A (Gibberellic Acid or GA biosynthesis inhibitor that interferes with GA synthesis late in the biosynthetic pathway) or type II plant growth regulator (suppressor).

Trinexapac-ethyl is approved for use in the EU on cereal crops such as barley, durum wheat, oats, rye, triticale and wheat as well as grassland, amenity turf and managed turf. It is used to control the growth of these crops and various grass species. After application of trinexapac-ethyl, the amount of active Gibberellic acid in the test plants reduces due to the blocking of hydroxylation of GA20 to the hormonally active GA1. Through this inhibition the elongation of shoots is prevented to a large extent and the height or general growth of the plant, dependent on application timing, is reduced.



An initial evaluation in the renewal assessment report (RAR) provided by Lithuania (LT) as Rapporteur Member State (RMS) was submitted to EFSA in 2017. The toxicological profile of trinexapac-ethyl and its metabolites was discussed at the EFSA Pesticides Peer Review Experts' Meeting 170 (2017) and EFSA's conclusion on trinexapac-ethyl was published in 2018 (*EFSA 2018. Conclusion on the peer review of the pesticide risk assessment of the active substance trinexapac (variant evaluated trinexapac-ethyl*). Trinexapac-ethyl has no current entry in Annex VI of the CLP regulation and all hazard classes apart from respiratory sensitisation, aspiration hazard and hazardous to the ozone layer are open for assessment in this opinion document.

A number of impurities were present; EFSA noted that the impurities CGA158377 and toluene were considered relevant based on their hazard (skin sensitisation and reproductive toxicity, respectively; maximum content of 6 g/kg and 3 g/kg, respectively). RAC considers these impurities not to impact on classification. Two other metabolites had QSAR alerts for genotoxicity but there was no data to further conclude on their actual toxicity with regards to this endpoint.

There were no findings from the toxicokinetic studies that might influence the proposed classification of trinexapac-ethyl. The active substance was extensively and rapidly absorbed. Oral absorption was estimated to be greater than 96% irrespective of dose and sex. Maximum concentrations in blood occurred 15 min after oral administration. There was no evidence for tissue accumulation. Excretion of the substance was predominantly through the urine (≥ 95% 7 days after oral administration). No indication for saturation of metabolism was found after repeated low or single high dose oral administration. The main metabolic pathway showed hydrolysis to trinexapac free acid. *In vitro* metabolic patterns in rat and human microsome test systems (derived from male and female livers) were qualitatively similar but in human liver

microsomes, metabolism was slower with 57.2% of parent remaining unmetabolised (in rats there was < 1% remaining after 60 minutes incubation). No unique human metabolite was observed.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification of trinexapac-ethyl for physical hazards on the basis of the following test results:

- Trinexapac-ethyl does not contain any chemical groups associated with explosive properties as given in section 2.1.4.3(a) of the CLP Regulation. Furthermore, trinexapac-ethyl was tested using EC Method A.14 and was found not to be explosive (*CLH report 2.2.1.1.1*);
- Trinexapac-ethyl was assessed for auto-flammability using EC Method A.15 - 'Auto-Ignition Temperature (liquids and gases)'. The test material was warmed and maintained in its liquid state for the duration of this test. The lowest auto-ignition temperature was determined to be 330 ± 35°C. Trinexapac-ethyl was concluded not to be a self-heating substance (*CLH report 2.2.1.1.10*);
- Trinexapac-ethyl was tested for oxidising properties using EC Method A.21. The test mixtures failed to create a sufficient pressure increase to enable the rise time to be measured in contrast to the positive reference mixture of cellulose and 65% aqueous nitric acid. Therefore, trinexapac-ethyl was considered non-oxidising (*CLH report 2.2.1.1.12*);
- In a standard study (EEC Method A.10), a preliminary test showed that trinexapac-ethyl did not propagate combustion; it melted but did not ignite to sustain a flame. The flash point of liquefied active substance was determined to be 156 ± 8°C (EC Test A.9). Trinexapac-ethyl did not meet the classification criteria for classification as a flammable solid (*CLH report 2.2.1.1.6*).

Highly pure trinexapac-ethyl (99.6%) is an opaque white solid at room temperature with a melting point of 36.1 - 36.6°C with decomposition at > 310°C (CLH report *2.2.1.1.7*). Lower purity batches (e.g. 96.8%) are red-brown in colour. The water solubility is low but increases from 1.1 g / L at pH 3.5 to 21.1 g / L at pH 8.2 at 25°C. The DS did not consider trinexapac-ethyl to be flammable, auto-flammable, and explosive or to display oxidising properties.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC supports the DS's proposal for no classification of trinexapac-ethyl regarding physical hazards. The criteria for classification of physical hazards have not been met based on the data obtained from several key studies. RAC agrees with the DS, **no classification for physico-chemical hazards is warranted**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral route

The DS proposed no classification for trinexapac-ethyl with respect to acute oral toxicity. There were three guideline compliant (OECD TG 401, 1987) acute oral studies with trinexapac-ethyl available (one in mouse and two in rats, table 18 of the CLH report), the acute oral LD_{50} was consistently > 2000 mg/kg bw in all studies.

Study 1 (*Anon, 1987b* - RAR B.6.2.1 Study 1)

The acute oral LD_{50} was found to be >2000 and <5000 mg/kg bw for Tif: RAIf (SPF) hybrid rats (5/sex/dose) in both sexes. Symptoms of toxicity included ruffled fur, dyspnoea, hunched posture and exophthalmos at a slight to moderate level in all groups. 3/5 males and 3/5 females administered 5000 mg/kg bw died 2-4 days after initial dosing.

Study 2 (Anon, 1993 - RAR B.6.2.1 Study 2)

The acute oral LD_{50} was found to be >2000 mg/kg bw for Tif: MAG f (SPF) mice (5/sex/dose) in both sexes. Piloerection, hunched posture and dyspnoea were observed in all animals. The severity of these effects gradually decreased and the animals had recovered completely by day 6 following administration. There was no mortality.

Study 3 (Anon, 1988 - RAR B.6.2.1 Study 3)

The acute oral LD_{50} was found to be >2000 mg/kg bw and <5000 mg/kg bw for Harlan Sprague Dawley rats (5/sex/dose) in both sexes. Several signs of general toxicity were noted across all dose groups - diarrhoea, nasal discharge, polyuria, salivation, decreased activity, piloerection, ataxia, dilated pupils, haematuria, and epistaxis. Dose groups started at 3500 mg/kg bw up to a maximum tested dose of 5050 mg/kg bw. There was mortality - 3/5 females administered 4000 mg/kg bw, 1/5 males administered 4500 mg/kg bw, 5/5 males and 4/5 females administered 5050 mg/kg bw died within 2 days of exposure. The acute oral LD_{50} of the test substance was calculated to be 4610 mg/kg bw (95% confidence interval: 4450-4790 mg/kg bw) for male rats and 4210 mg/kg bw (95% confidence interval: 3450-5140 mg/kg bw) for female rats.

Dermal route

The DS proposed no classification of trinexapac-ethyl for acute dermal toxicity based on no lethalities at a top dose (4000 mg/kg bw) in a GLP and OECD TG 402 study (*Anon. 1987a)*, semi occlusive, 24-hour exposure to 5 male and 5 female Tif: RAIf (SPF) hybrid rats. Clinical signs were confined to ruffled fur, dyspnoea, hunched posture, reduced spontaneous activity, all resolved by day 9. No local signs of skin irritation were reported. There were no internal findings at necropsy. The acute dermal LD_{50} was found to be >4000 mg/kg bw (rats, both sexes).

Inhalation route

The DS proposed no classification for acute inhalation toxicity. In a GLP and OECD TG 403 guideline compliant acute inhalation study (*Anon, 1988*), groups of 5 Tif: RAIf (SPF) hybrid rats/sex were nose-only exposed for 4 h to an aerosol of trinexapac-ethyl at a concentration of 5.3 ± 0.064 mg/L (gravimetrically determined). The MMAD was 2.1 µm and a GSD of 2.7 µm. Ruffled fur, dyspnoea, hunched posture, and reduced spontaneous activity was observed in all

animals, including the control group. No rats died and there were no macroscopic findings at necropsy. The LC_{50} was > 5.3 mg/L.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Acute oral toxicity

In order to be classified with acute toxicity category 4 (oral), the lowest category for this endpoint, the LD_{50} must fall between the following range: 300 < LD_{50} ≤ 2000 mg/kg bw. All the oral studies in rats and mice consistently revealed LD_{50} values > 2000 mg/kg bw. Calculated LD_{50} values were determined from one study only (*Anon., 1988*). The oral LD_{50} of 4610 mg/kg bw for male rats and 4210 mg/kg bw for females is above the range of values warranting classification according to CLP. The substance is not classified for acute oral toxicity.

Acute dermal toxicity

In order to be classified with acute toxicity category 4 (dermal), the LD_{50} should be between 1000 < LD_{50} ≤ 2000 mg/kg bw. The dermal LD_{50} of >4000 mg/kg bw for rats is above the range of values warranting classification according to CLP. The substance is not classified for acute dermal toxicity.

Acute inhalation toxicity

In order to be classified with acute toxicity category 4 (inhalation), the LC_{50} should lie between 1.0 < LC_{50} ≤ 5.0 mg/L (dusts and mists). The 4 h inhalation LC_{50} of > 5.3 mg/L for rats is above the range of values warranting classification in the CLP Regulation and thus there is no justification to classify for acute inhalation toxicity.

Overall, RAC agrees with the DS' proposal of no classification for acute toxicity, regarding all routes of exposure.

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

Summary of the Dossier Submitter's proposal

Trinexapac-ethyl was investigated in a number of acute studies by the oral, dermal and inhalation routes (table 43 of the CLH report). There was no indication that trinexapac-ethyl caused specific toxicity to any organ after a single exposure. There was no evidence of narcotic effects from any toxicological study. No signs of respiratory irritation were observed in the acute inhalation study. Non-lethal effects were confined to very high doses and were diverse and unspecific in nature. An acute neurotoxicity study (*Anon., 2012*; RAR B.6.7.1.1) in Crl:CD(SD) rats using 10 animals/sex/dose up to 2000 mg/kg bw was similarly devoid of evidence for STOT SE. Decreased bodyweight gain and food consumption at 2000 mg/kg bw were transient. Subsequent to study day 1, there was no evidence of a treatment-related effect.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC supports the conclusions of the DS that no classification is warranted for STOT SE (Category 1, 2 or 3).

In the absence of human data and in the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, classification of trinexapac-ethyl in Category 1 or Category 2 for STOT SE is not warranted.

With regard to Category 3 for STOT SE, clinical signs following inhalation exposure to trinexapac-ethyl were indicative of non-specific, general toxicity. There was no evidence of respiratory tract irritation or narcotic effects. Therefore, classification in Category 3 for STOT SE is also not warranted.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS described a primary dermal irritation study (GLP, but non-compliant with regard to OECD TG 404 (1981), *Anon.,* 1987a) where 3 young male adult New Zealand White rabbits were exposed to 0.5 ml trinexapac-ethyl (technical grade active ingredient, 96.6% purity), applied to the intact shaved flank under a semi-occlusive dressing, for 4 hours. Skin reactions were scored at 1, 24, 48 and 72 hours after removal of the dressings. The study showed only transient signs of slight erythema at 1 hour only. No skin irritation reactions were observed in any animal at 24, 48 and 72 hours after removal of the test article. However, it was noted that the application area used in the study deviated from the technical guideline as it was about three times larger than that recommended (i.e. 20 cm^2 whereas the guideline specify 6 cm^2).

The RMS originally rejected the study before including it as supporting information in a weight of evidence approach in the 2018 RAR. The DS considered the study as supplementary information only and assessed skin irritation/corrosion using a weight of evidence approach following their interpretation of section B part 2: Weight of Evidence Analysis from OECD Guidance document No 203 (2014). The DS referred to negative dermal findings in the M&K sensitisation study and in the low dose group of a short-term dermal toxicity study in rabbits. In addition, based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited), trinexapac-ethyl did not trigger any structural alert for skin irritation.

The DS did not propose classification for skin corrosion/irritation, but considered the data conclusive.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The rabbit skin irritation study (*Anon., 1987a*) by itself is not sufficiently robust or reliable enough to assess classification for skin corrosion/irritation. The test substance was technical grade active ingredient (TGAI) with a purity of 96.6% and described as a liquid at 20°C in the original study report. This is curious because trinexapac-ethyl has been characterised as a solid with a melting point of 36.1 - 36.6°C. A gauze patch (20 cm^2) with 0.5 ml of the test substance was applied to the animal flanks; however, this exceeded the recommended application area of 6 cm^2.

Reference to other studies in a weight of evidence approach does help to support no classification; no skin irritation in the 24h dermal toxicity study at 4g/kg bw, the negative skin irritation study, lack of skin irritation at 100% in the GPMT, as well as a lack of structural alert in the (Q)SAR analysis. The results from the short-term dermal toxicity study in rabbits show skin reactions but it is unclear whether those effects were due to solvent (ethanol) or the active substance itself.

RAC agrees with the DS in that classification for skin irritancy is not warranted for trinexapac-ethyl. RAC recognises that the data from the rabbit skin irritation study is insufficient but considers that from a WoE assessment there is sufficient evidence to warrant no classification.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

One OECD TG 405 (1987) eye irritation study in the male rabbit was available to the DS (*Anon., 1987b* (RAR B.6.2.5)). Three rabbits were exposed to the test article without eye washing. Conjunctival redness was only observed in 2/3 animals at the 1 hour post application time point. No ocular changes were observed in any animal at the 24, 48 or 72 hour post installation time points. The study was terminated following the 72 hour examination.

The results of a (Q)SAR analysis on the parent substance were also reported by the DS (*Anon., 2017*). Trinexapac-ethyl did not trigger an alert for the 'Eye irritation' endpoint using Derek Nexus.

The DS did not propose classification for serious eye damage (Category 1) or for eye irritation (Category 2).

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The test substance was technical grade active ingredient (TGAI) with a purity of 96.6% and described as a liquid at 20°C in the original study report. This is again curious because trinexapac-ethyl has been characterised as a solid with a melting point of 36.1 - 36.6°C.

In the single study available (*Anon., 1987b*), findings were limited to conjunctival redness (mean score of 1) in 2/3 animals at 1 hour post application. Trinexapac-ethyl does not meet the CLP criteria for classification for serious eye damage (Category 1) or for eye irritation (Category 2).

RAC concludes that **no classification is warranted** on the basis of conclusive data.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

In the CLH report for trinexapac-ethyl, the DS proposed a classification of Category 1B for skin sensitisation based on positive results from one LLNA mouse study (2017).

Several studies were available to the DS to assess the active substance where a range of different concentrations were tested. There was a brief summary of a QSAR analysis (Derek Nexus version 5.0.2) report generated in 2017 and referenced in the confidential section of the plant protection DAR. There were **three** skin sensitisation guideline compliant and GLP tests conducted with trinexapac-ethyl and described in the CLH report (GPMT 2001; LLNA 2006; LLNA 2017). Following the CLH public commenting period the industry applicant submitted a new and **fourth** skin sensitization study (LLNA, 2019) based on a revised technical specification of trinexapac-ethyl. The DS evaluated the new study after the public consultation in the RCOM document. The evaluation of this new study by the DS is taken together with the other studies and summarised below.

(Q)SAR analysis (Anon., 2017)

Trinexapac-ethyl triggered a "PLAUSIBLE" Derek Nexus alert for 'Skin sensitisation in mammal' endpoint due to the presence of a diketone moiety (1,3-diketones have been demonstrated to be skin sensitisers in various assays), see the figure below.

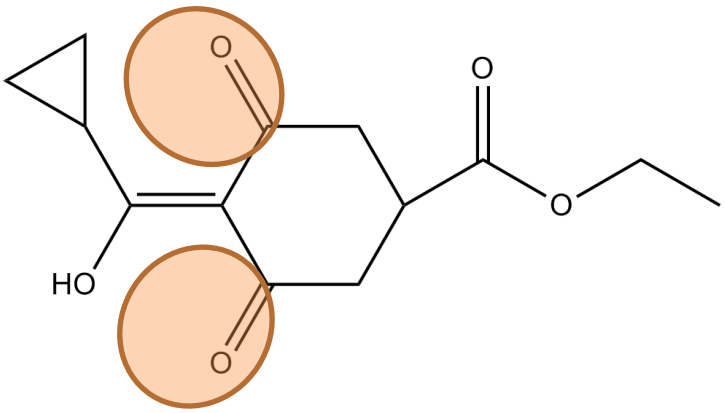


Figure: identification of the 2 ketone moieties

Guinea Pig Maximization test; OECD TG 406; GLP (Anon., 2001; RAR B.6.2.6. Study 1)

Technical trinexapac-ethyl (batch P.306042, non-spiked, purity 96.8%) was tested to a maximum intradermal concentration of 10% in the main study. This dose was based on the results of a range-finding study using 0.5, 1, 2.5, 5, 7.5 and 10% (w/v) in arachis oil for intradermal injections. The highest concentration that produced only localised reactions at the injection site was 10% (w/v). The highest concentration that produced no significant irritation by topical application was 100%. *None of the concentrations used topically in the range-finding test caused slight irritation*, and the animals in the main study were therefore pre-treated with 10% sodium dodecyl sulphate (SDS) in vaseline to increase the skin sensitivity.

Intradermal injection of 10% (w/v) and topical induction with the undiluted test substance caused no significant effects in the test animals compared with the control animals. Following challenge

with undiluted test material, 4/20 test animals and 4/10 control animals had slight erythema on the test site after 24 hours, while 1/20 test animals had well-defined erythema (single positive reaction). The erythema had cleared completely within 48 hrs. The positive control (2-mercaptobenzothiazole) confirmed the validity of the test.

This M&K test was **negative for skin sensitisation**.

The study was performed in accordance with OECD TG 406 (1992) under GLP and was considered acceptable by the RMS in the 2018 RAR under PPP Regulation (EC) No 1107/2009.

Local lymph node assay; OECD TG 429; GLP (Anon., 2006; RAR B.6.2.6. Study 2)

A sample of trinexapac-ethyl (batch SMO5D180, purity 96.6%) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The vehicle for the test substance and for the positive control was acetone in olive oil (4:1) and the positive control substance was α-hexylcinnamaldehyde (HCA). Trinexapac-ethyl was tested at 5%, 10% and 25% (w/v) and was not found to be sensitising. The DS criticised the study for failing to test higher concentrations of the active substance and for not utilising dermal irritation data in dose selection. There were reporting deficiencies, namely there was no data recorded on the dermal irritation at the site of administration for each animal.

This LLNA test was **negative for skin sensitisation**.

The study was performed in accordance with OECD TG 429 (2002) under GLP but was considered as being of limited value by the RMS in the 2018 RAR under PPP Regulation (EC) No 1107/2009.

Local lymph node assay; OECD TG 429; GLP (Anon., 2017; RAR B.6.2.6. Study 3)

A sample of trinexapac-ethyl (batch SMO5D180 Fortified, purity 93.3%) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The vehicle for the test substance and for the positive control was 1% Pluronic L92 and the positive control substance was α-hexylcinnamaldehyde. Trinexapac-ethyl was tested at 25%, 50% and neat at 100% w/v. As a stimulation index (SI) of greater than 3.0 (3.18) was observed in the treatment group with the neat test substance (100%), the test substance was considered positive for dermal sensitisation potential. No dermal irritation was observed for any of the vehicle (1% Pluronic L92) and test sites. Treatment of mice with 25%, 50% and 100% of Trinexapac-ethyl Tech. – Fortified, resulted in stimulation index values of 1.57, 1.23 and 3.18, respectively. The EC3 value calculated for the test substance was 95.4%.

This technical material was artificially spiked with several impurities up to the maximum level as detailed in the technical specification for trinexapac-ethyl outlined in the confidential section of the RAR. This sample included 6.1 g/kg of process impurity CGA158377, a substance classified as Skin Sens 1. This process impurity is included in the normal technical specification for trinexapac-ethyl up to a maximum of 6 g/kg. The positive control (25% (w/w) mixture of HCA in 1% Pluronic L92) confirmed the validity of the test.

This LLNA test was **positive for skin sensitisation**. The DS proposed skin sensitisation sub-category 1B based on this study.

The study was performed in accordance with OECD TG 429 (2010) under GLP and was considered acceptable by the RMS in the 2018 RAR under PPP Regulation (EC) No 1107/2009.

Local lymph node assay; OECD TG 429; GLP (Anon., 2019)

A new LLNA study (Anon., 2019) was submitted by the applicant after the public commenting period for trinexapac-ethyl had expired. This study was not available at the time of drafting for either the CLH report or the Jan 2018 RAR. The DS has completed an evaluation of the original study report (which is available to RAC) and commented on it in the RCOM document. A sample

of trinexapac-ethyl (batch SMO5D180_FORTIFIED-2, purity 93.2%) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The same test facility and study director as that used in the 2017 LLNA study was employed for the 2019 LLNA study. The test material was similar to that used in the 2017 LLNA study but with a new (lower) maximum content of 4.1 g/kg for the process impurity CGA158377.

It is important to note that this study was performed on a revised technical specification of the active substance (and not one taken into consideration as part of the European Commission renewal process for trinexapac-ethyl) and tested at 25%, 50% and up to a maximum of 75% w/v. The test substance (solidified melt) was dissolved in acetone/olive oil (4:1 v/v mix) as recommended in the OECD TG (OECD 429, 2010). Preliminary sample preparation testing indicated that mixtures in excess of 75% (i.e., 80-95%) were too viscous for dosing. Curiously, solubility testing indicated that the test substance was insoluble in 1% (w/w) Pluronic L92. This is in direct contrast to the situation with the 2017 study where the test material was stated to be soluble in 1% (w/w) Pluronic L92. Validity of the LLNA was confirmed via a positive response (SI = 7.40) with the concurrent positive control (25% (w/w) HCA in acetone/olive oil (4:1 v/v mix)).

This LLNA test was **negative for skin sensitisation**.

The study was performed in accordance with OECD TG 429 (2010) under GLP and was considered acceptable by the DS. Treatment of mice with 25%, 50% and 75% of Trinexapac-ethyl Tech. resulted in SI values of 1.90, 2.08 and 1.93, respectively.

Conclusion of the DS

In consideration of all four studies, the DS concluded trinexapac-ethyl is positive for dermal sensitisation potential because a stimulation index (SI) of greater than 3.0 was observed in the 2017 LLNA study using neat material (100%). Trinexapac-ethyl is a weak sensitiser but falls into the moderate skin sensitisation potency category as the EC3 value is greater than 2% (in accordance with CLP, Annex I, Table 3.4.4). The test substance EC3 value is calculated to be 95.4%. The DS considered trinexapac-ethyl to fulfil the criteria for classification with Skin Sens. 1B, H317.

Comments received during public consultation

Summary of MSCAs comments

Comments were received from 3 Member State Competent Authorities (MSCAs), and 1 Manufacturer. In all comments from the MSCAs there was agreement that there was sufficient evidence to classify trinexapac-ethyl as a skin sensitiser based on animal data from the 2017 LLNA study (SI ≥ 3). All agreed on potency and sub-categorisation, i.e. a low to moderate sensitiser under sub category 1B (EC3 value > 2%). One of the commenting MSCAs noted that the negative results of the GPMT study carried out according to OECD TG 406 (Anon., 2001) and of the first LLNA study done according to OECD TG 429 (Anon., 2006) did not contradict the results of the LLNA 2017 study because much lower concentrations of active substance were used in these older studies compared with the 2017 study.

Summary of Industry comments

The manufacturer disagreed with the proposed classification of Category 1 (sub-category 1B) for skin sensitisation potential and supplied 2 documents with comments regarding their position and a confidential note with details on a key manufacturing impurity, CGA158377.

The manufacturer argued that trinexapac-ethyl is a non-sensitiser based on the weight of evidence from the GPMT study carried out according to OECD TG 406 (Anon., 2001) and of the

first LLNA study done according to OECD TG 429 (Anon., 2006) in addition to the negative results from a new, robust 2019 LLNA study, which was also fully compliant with OECD guideline 429.

In this new 2019 LLNA study, an SI ≥ 3 was not achieved with any dose of trinexapac-ethyl (25%, 50% and 75%), whilst an SI > 3 was clearly achieved with the positive control (25% HCA in acetone/olive oil 4:1.). The maximum concentration of test item that was soluble in the acetone/olive oil vehicle was 75%. Furthermore, there was no evidence of a dose-response with trinexapac-ethyl. Industry disagreed with the classification proposal because it was based on a technically flawed study. Industry criticised the 2017 LLNA study stating:

1. Inappropriate vehicle choice: There was no robust scientific rationale to justify the use of 1% Pluronic L92 as a vehicle in favour of one of the recommended ones mentioned in the OECD TG 429, particularly since trinexapac-ethyl is soluble in organic solvents.
2. Inappropriate preparation of top dose (100%): The test laboratory heated the material to 50°C to liquefy, allowed it to cool to room temperature, and applied 25µL to each ear of the mouse. Whilst the material was initially solid at room temperature, it was reported as a liquid at room temperature following this heating/cooling regime. Despite this change in physical state, the potential impact of the heating/cooling regime on the integrity of the test item was not ascertained. They stated it was unclear whether the top dose preparation had the same chemical composition as the original test substance as supplied.
3. The LLNA conducted in 2017 used a technical specification of trinexapac-ethyl (batch number 979744) in which there was a higher concentration of an impurity that is a known sensitiser (Skin Sens. 1; H317). The newer study was conducted using a revised technical specification, which had been developed to reduce impurity CGA158377 (ethyl (1RS)-ethyl 3-hydroxy-5-oxocyclohex-3-ene-1-carboxylate), CAS 88805-65-6, EC number 441-450-4). The new 2019 study is representative of a proposed new technical specification of trinexapac-ethyl.

The DS responded during the public commenting period:

1. The DS clarified that the technical material (Bach No SMO5D180 Fortified, purity 93.3%) used in the 2017 LLNA was spiked with several impurities up to their maximum levels as proposed for inclusion in the technical specification for the active substance during the EU renewal process for registration of the plant protection product.
2. The DS noted that some impurities triggered an alert for skin sensitisation according to the results of (Q)SAR analysis by using VEGA and DEREK NEXUS. In particular they acknowledged that impurity CGA158377 (CAS No 88805-65-6) has harmonised classification as Skin Sens. 1, H317.
3. The DS does not consider the 2017 LLNA study to be deficient in any way and considers it acceptable from a regulatory point of view:
    i. The vehicle, 1% Pluronic L92 is a common, frequently used solvent in LLNA studies and OECD Guideline 429 (2010) mentions it as one of the 'appropriate solubilisers'.
    ii. The study was validated using alpha-hexylcinnamaldehyde (HCA, purity ≥ 95%) as the positive control substance. A 25% (w/w) mixture of HCA in 1% Pluronic L92 achieved an SI = 3.44.

iii. The test substance was liquefied at 50°C (melting point 36°C), thermal decomposition does not start until about 310°C, the active substance is not a readily reactive substance and therefore it is highly unlikely that there is any impact on the integrity of the test material.

iv. The technical specification of the material used in the 2019 LLNA study is not the currently supported technical specification of trinexapac-ethyl as agreed during the EU peer review and renewal process.

v. The new 2019 LLNA study was not available during the preparation of CLH Report; it was submitted after the public commenting period. The DS evaluated the study and has included the report as part of the overall data package available to RAC. The DS concluded that the test substance (Trinexapac-ethyl, Batch ID SMO5D180_FORTIFIED-2) was not sensitising at the tested concentrations of 25%, 50%, and 75% (w/v).

DS Summary of a new skin sensitization study (LLNA, 2019)

Comments and overall conclusion

The 2019 LLNA study is GLP and OECD TG 429 (2010) compliant. The study is considered to be acceptable.

The test substance (solidified melt) was dissolved in vehicle recommended by OECD TG, i.e. Acetone/Olive Oil (4:1 v/v mix) (AOO). Preliminary sample preparation testing indicated that mixtures in excess of 75% (i.e., 80-95%) were too viscous for dosing. Solubility testing indicated that the test substance was insoluble in 1% Pluronic L92 Surfactant w/w in distilled water.

Preliminary dermal irritation and ear thickness measurements with one mouse treated with the test substance at the maximum concentration suitable for application (75% dilution) did not show any dermal irritation and an increase in ear thickness of ≥ 25% (-4.0% – 4.0%).

Proper conduct of the LLNA was confirmed via a positive response (SI = 7.40) with concurrent positive control [25% w/w mixture of alpha-Hexylcinnamaldehyde (HCA), purity ≥ 95%, in AOO], a moderate contact sensitizer. Very slight erythema (score of 1) was observed at three positive controls sites on Day 2, five sites on Day 3, and five sites on Day 6.

For the tested concentrations (25%, 50%, and 75%) the test substance (Trinexapac-ethyl tech., Batch ID SMO5D180_FORTIFIED-2) was not found to be sensitising.

The latest LLNA skin sensitisation assays (2017 and 2019) have been conducted according to the OECD TG 429 (2010) in the same laboratory by the same Study Director. Both studies are acceptable, however, the results obtained are different: trinexapac-ethyl tech. (Bach No SMO5D180 Fortified) was considered to be a contact dermal sensitiser at concentration 100% in the LLNA study (2017), whereas trinexapac-ethyl tech. (Bach No SMO5D180 Fortified-2) was not found to be a dermal sensitiser at concentrations less than or equal to 75% in the LLNA (2019).

Table: *A comparison of two LLNA studies (2017, 2019), conducted with trinexapac-ethyl tech*

	Study 3 (2017) Laboratory Report No. 45617	**Study 4 (2019) Laboratory Report No. 49303**
OECD Guidelines	OECD TG 429 (2010), GLP	OECD TG 429 (2010), GLP
Test Facility	The same	The same
Test animals, sex, age **Source**	Mouse, CBA/J Female, young adult (9 weeks) The same source	Mouse, CBA/J Female, young adult (9 weeks) The same source
Test material, purity	Trinexapac-ethyl tech. 93.3% w/w	Trinexapac-ethyl tech. 93.2% w/w
Batch number:	ID 979744 or SMO5D180_FORTIFIED	ID SMO5D180_FORTIFIED-2
Physical Description	Red brown solidified melt	Red brown solidified melt
Preparation of Test Substance	The test substance as received (neat) was placed in a water bath set to 50^{O} C until liquefied, then allowed to cool to room temperature prior to use. The test substance was soluble in 1% Pluronic L92 Surfactant w/w in distilled water.	The test substance was insoluble in 1% Pluronic L92 Surfactant w/w in distilled water. Soluble in Acetone/Olive Oil (4:1 v/v mix) (AOO); Mixtures in excess of 75% (i.e., 80-95%) were too viscous for dosing.
The Vehicle	1% Pluronic® L92 Surfactant w/w in distilled water (1% Pluronic® L92)	Acetone/Olive Oil (4:1 v/v mix) (AOO)
Preliminary Toxicity Testing	One mouse was treated with the test substance (100%)	One mouse was treated with the test substance (75%)
Preliminary dermal irritation	Very slight erythema (score of 1) was observed at one site on Day 3.	None
Preliminary ear thickness measurements	↑4.0% - ↑8.33%	↓4.0% – ↑4.0%
Number of Animals; **Number of Groups;** **Number of Animals per Group**	17 5 4 per group except Preliminary Irritation (1 per group)	21 6 4 per group except Preliminary Irritation (1 per group)
Groups and the dose levels of test material that have been used for the main test	The vehicle control (1% Pluronic L92) Positive Control (25% (w/w) HCA, purity ≥ 95%, in 1% Pluronic L92) The test substance at 25%, 50% (w/w) mixtures in 1% Pluronic® L92 and the neat test substance (100%)	Vehicle Control (AOO) Positive Control (25% (w/w) HCA, purity ≥ 95%, in AOO) The test substance at 25%, 50%, and 75% (w/w) mixtures in AOO

	Study 3 (2017) Laboratory Report No. 45617	Study 4 (2019) Laboratory Report No. 49303
Observations **Clinical observation** **Body weights**	No dermal irritation was observed for any of the vehicle control and 3 test substance groups (25%, 50% & 100%). Two mice from the vehicle control and three mice in test substance groups lost or failed to gain body weight during the study.	No dermal irritation was observed for any of the vehicle control and 2 test substance groups (25% & 50%). Very slight erythema (score of 1) was observed at three 75% test group sites on Day 3 and at one 75% test group site on Day 6 One mouse from the vehicle control and seven mice in the test substance groups lost body weight during the study.
Dermal irritation **Positive Control Group**	Very slight erythema (score of 1) was evident at one site on Day 2, seven sites on Day 3 and four sites on Day 6. Slight edema (score of 1) was present at one site on Day 3. Desquamation was present at all sites on Day 6.	Very slight erythema (score of 1) was observed at three sites on Day 2, five sites on Day 3, and five sites on Day 6.
Calculated Stimulation Index (SI) value	Treatment of mice with 25%, 50% and 100% of Trinexapac-ethyl Tech. resulted in SI values of 1.57, 1.23 and **3.18**** ($p < 0.01$, by Dunnett's Multiple Comparisons Test), respectively.	Treatment of mice with 25%, 50% and 75% of Trinexapac-ethyl Tech. resulted in SI values of 1.90, 2.08 and 1.93, respectively
Calculated Stimulation Index (SI) value for the positive control	3.44	7.4*** (***- $p < 0.001$, by Dunn's Multiple Comparisons Test)
Calculated EC3 value	95.4%	-
Dose-response relationship	No clear dose-response relationship	No dose-response relationship

According to the DS trinexapac-ethyl was considered positive for dermal sensitization potential as a stimulation index (SI) of greater than 3.0 was observed in the top dose treatment group in the 2017 LLNA study. Trinexapac-ethyl has moderate skin sensitisation potency as the EC3 value is calculated to be 95.4%. Trinexapac-ethyl tech. (fortified) fulfilled the criteria for classification with Skin Sens. 1B, H317 under the conditions of the 2017 LLNA study.

Assessment and comparison with the classification criteria

Comparison with the criteria

Summary of the animal studies on skin sensitisation

Table: *Skin sensitisation studies with Trinexapac-ethyl*

Study Ref	Batch No.	Purity	Description/state	Method	Result
OECD TG 406 (1992) M&K 2001 GLP	P.306042	96.8%	Brown liquid	ID induction 10% Topical induction 100% Challenge 100% Vehicle: Arachis oil	Negative.
OECD TG 429 (2002) LLNA 2006 GLP	SMO5D180	96.6%	Yellow to red/brown solid	5, 10 and 25% Vehicle: AOO (1:):4) Limit of solubility	Negative. Stimulation index < 3.0
OECD TG 429 (2010) LLNA 2017 GLP	**979744*** SMO5D180 _FORTIFIED	**93.3%**	**Red brown solidified melt**	**25, 50, 100% Vehicle: 1% Pluronic L92**	**Positive. Stimulation index 3.18 at 100% EC3 95.4%**
OECD TG 429 (2010) LLNA 2019 GLP	SMO5D180 _FORTIFIED-2	93.2%	Red brown solidified melt	25, 50 and 75% Vehicle: acetone/olive oil Limit of solubility	Negative. Stimulation index < 3.0

* Batch ID 979744 test material is a batch of Trinexpac-ethyl technical that has been artificially spiked with impurities.

Comparison with the CLP criteria regarding skin sensitisation

Substances are classified as Category 1 skin sensitisers where data are not sufficient for sub-categorisation, if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or if there are positive results from an appropriate animal test. In this case there are positive results from an animal test and sufficient information for sub-categorisation.

Substances are classified as sub-category 1A skin sensitisers where there is evidence of a high frequency of occurrence in humans and/or a high potency in animals. The Guinea pig maximisation test was negative; the remaining 3 tests were based on the LLNA mouse assay. For the LLNA, substances are allocated to sub-category 1A where an EC3 value ≤2%. There is one positive LLNA study (2017) which clearly does not satisfy this requirement, thus sub-category 1A is not supported.

Substances are classified as sub-category 1B skin sensitisers where there is evidence of a low to moderate frequency of occurrence in humans and/or moderate potency in animals. For the LLNA study, substances are allocated to sub-category 1B where an EC3 value >2%. The criteria for classification to subcategory 1B are fulfilled for trinexapac-ethyl in the 2017 LLNA study. The classification for subcategory 1A **can be excluded** because several concentrations of the active substance were tested and all below 75% (w/v) were negative. The EC3 value was calculated to be 95.4%.

Small differences exist between the 2017 and 2019 LLNA studies and industry has questioned the validity of the positive result observed in the 2017 study. The differences mainly concern the vehicle used to suspend/dissolve the active substance (1% Pluronic L92 vs acetone/olive oil 4:1)

and the quantitative difference in the impurity profiles of the 2 tested batches of trinexapac-ethyl. One component in particular (table below) is known to be a skin sensitiser and while not explicitly stated, the implication was that this component could influence the outcome of the LLNA test.

Table: *Content of CGA158377 in the test batches used for the Local Lymph Node Assays*

Local Lymph Node Assay (LLNA)	Technical trinexapac-ethyl batch used	Content of CGA158377 (g/kg)
OECD 406 (1992) M&K 2001	P.306042	< LoD (0.5)
OECD 429 (2002) LLNA 2006	SMO5D180	unknown
OECD 429 (2010) LLNA 2017	SMO5D180_FORTIFIED	**6.1**
OECD 429 (2010) LLNA 2019	SMO5D180_FORTIFIED-2	**4.1**

The 2017 local lymph node assay was conducted with spiked technical material (with several impurities up to their maximum level as proposed in the RAR for the substance specification). This study was positive, with an EC3 value of 95.4%. The individual animal responses were positive in 3 out of 4 cases (i.e. SI > 3); 3.69; 4.16; 3.26; and 1.60.

The newer 2019 assay also used spiked technical material (with several impurities at lower levels from those originally proposed in the RAR for the substance specification). This study was negative. The older 2006 local lymph node study utilised a batch of non-spiked trinexapac-ethyl technical material and was also negative.

The level of the impurity was at the maximum specification for the active substance, i.e. 0.6% w/w in the 2017 LLNA and reduced to 0.4% w/w in the 2019 LLNA. QC data (2012-2013) from more than 800 batches and supplied in support of the 2015 renewal of the active substance showed levels up to 6 g/kg (0.63% w/w). The 2017 LLNA is a more realistic worst case as the max specification was set at 6 g/kg based on this information.

This impurity is not a potent sensitiser. It has a harmonised classification for Skin Sens 1; H317 but RAC does not have any information on what is the basis for this classification. Furthermore, an M&K study by Hagemann, 1991 (OECD TG 406, 1981; GLP, purity of CGA158377 95.6%) which was negative for sensitisation for a 0.1% intradermal induction, does not support CGA158377 as a potent sensitiser (unlike the positive control, 0.1% 1-Chlor-2,4-dinitrobenzol). RAC notes that there is no data at what level it does become sensitising. An LLNA on the impurity would have been valuable data to have but it is not available. The biggest problem with the 2019 LLNA is that it did not test to 100% of the active substance. Arguments based on a minor impurity change of 0.2% w/w are rather weak relative to the fact that it is necessary to test trinexapac-ethyl at greater than 95% before a sensitisation response could be reasonably expected based on the derived EC3 value. The data shows clearly that sensitisation only occurs when dealing with the neat or nearly neat trinexapac-ethyl (i.e. levels close to 100%). The company tested up to 100%, got a positive result and have not repeated the test to confirm or disprove the results from the 2017 study. The remaining studies tested significantly lower concentrations of technical trinexapac-ethyl. 3/4 animals had a clear SI > 3 in the 2017 LLNA study, so the mean result is not due to a spurious result amongst the individual animals. OECD test guidelines specify testing a substance at the highest concentration possible. That criterion has been satisfied in this case.

Conclusion

The alterations in impurity profile between the studies are not considered sufficient to cause the difference in the results of these studies. Rather, it is the tested substance concentration that appears critical in these cases. RAC supports the DS and agrees that the data is sufficient to warrant a **classification in Category 1B for skin sensitisation, i.e. Skin Sens 1B; H317.**

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

Summary of the Dossier Submitter's proposal

Observations from the DS

The DS summarised 13 repeated dose toxicity studies in different species (rat, dog, rabbit and mice) and of different durations, and also included sub-acute neurotoxicity, carcinogenicity, developmental and 2-generation toxicity studies in rats, developmental toxicity study in rabbits along with carcinogenicity and immunotoxicity in mice (table 46, CLH report).

Some effects were noted in the dog including cerebral vacuolisation (a high dose effect) and weight reductions in the uteri and ovaries. There were 4 additional supplementary studies evaluated by the DS that addressed these issues dating from 1994 to 2017. In addition, there was also a 2017 (Q)SAR Derek Nexus analysis report. This showed that trinexapac-ethyl did not trigger alerts for any endpoint relevant for repeated dose toxicity.

None of the studies had relevant effects at levels below the guidance value ranges for either STOT RE1 or STOT RE2 except for uterine and ovarian/testicular weight (1 year dog dietary study, Anon., 1992, not statistically significant in the low dose group) and increased mortality and retarded body weight gain (rabbit developmental study).

In general the DS reported that significant toxicity was associated with high doses of trinexapac-ethyl. Common effects were decreased body weight gain and food consumption, especially in dogs. Cerebral vacuolation and thymic atrophy were confined to high dose dogs and found at levels that did not support classification. A variety of organs were affected at high doses across most species along with effects on haematology and clinical chemistry indicative of general toxicity rather than any specific or selective adverse effect.

<u>Rat</u>

In the rat, the only significant toxic effects of relevance to humans were seen in the kidney and partly in liver; however, these occurred at dose levels well in excess of the specified guidance values for classification with STOT RE Category 2.

<u>Mouse</u>

In the mouse, no significant toxic effects occurred at any dose in any study.

<u>Dog</u>

In the dog, in general there were no treatment-related effects up to the specified guidance values for rats. Changes in oestrus cyclicity and decreased absolute/relative uterine and ovarian weight were seen from the lowest dose (1.37 mg/kg bw/day, not statistically significant) in the 1 year dietary study. This value was lower than the adjusted guidance value (when applying Haber's rule) for STOT RE1 (≤ 2.5 mg/kg bw/day for a 1 year study). At higher concentrations (greater

than the value that triggers STOT RE2), the effects become statistically significant. However, the DS acknowledged the supplementary reports that had previously been assessed in the 2017 RAR (B.6.3.2.3.2, Anon., 1999; B.6.3.2.3.4, Anon., 2017) and agreed with study authors that the reduction in mean absolute uterus weight at the two highest doses was a consequence of the physiological change occurring in the uterus at the late stages of the oestrus cycle. No histopathological effects were seen in the uterus at any dose. The DS did not rule out an adverse effect of trinexapac-ethyl on the oestrus cycle via a hormonally mediated mechanism at the highest doses and established a LOAEL of 357.1 mg/kg bw/day in females. The DS did not comment on the ovarian and testes weight effects in the 1 year dietary study.

Rabbit

In the rabbit there were no systemic effects seen in the 22-day dermal study at levels up to 1000 mg/kg bw/day. Local skin irritation was evident, possibly due to the vehicle employed (ethanol) which is a known skin irritating substance. A maternal LOAEL of 360 mg/kg bw/day was established in the rabbit developmental study. This was based on increased mortalities and retarded body weight gain to GD 15. There were no treatment-related clinical signs. At 360 mg/kg bw/day, one animal was found dead on day 13 (6 days after dosing) following a suspected convulsion and a second was killed on day 24 due to marked and continuing weight loss and was found to have haemorrhagic depressions in the stomach. The DS noted there were 4/6 and 1/6 (unverified) mortalities in a preliminary study at 800 mg/kg bw/day and at 400 mg/kg bw/day, respectively. The mortalities in the preliminary studies were attributed to substance irritation of the stomach mucosa causing haemorrhagic depressions in the stomach.

The DS also noted there were no statistically significant and/or dose related differences in mean body weights and food consumption during the treatment period (GD 7-19) and/or during gestation in all dose groups compared to controls. Body weight gain was retarded at the high dose relative to all other groups up until GD 15 but was without statistical significance.

The guidance values corrected for the duration (13 days dosing) of the exposure (according to Haber's rule) correspond to Cat 1: ≤ 69 mg/kg bw/day and Cat 2: >69; ≤690 mg/kg bw/day. The LOAEL value falls within the criteria for STOT RE 2. The DS pointed out that the use of Haber's rule in the case of rabbits should be considered with caution because it is not possible to have a clear picture of a possible recycling of active substance and consequently know what is the actual dose absorbed from the GI tract. The dietary exposure may underestimate the actual exposure. The DS stated that the weight of evidence showed (1) lethality was not a feature of trinexapac-ethyl exposure in any of the other toxicological studies, and (2) the majority of toxicologically significant effects occurred at high doses only. The DS considered mortality in rabbits to be related to substance irritation of the stomach mucosa as evidenced in those animals having haemorrhagic depressions in the stomach.

The DS did **not propose to classify** trinexapac-ethyl as STOT RE in either category 1 or 2.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Relevant repeated dose toxicity studies

Effects that may be potentially relevant for classification at the effective dose (ED) are summarised and compared with equivalent Guidance Values in Table below.

Table: *Summary of effects and classification in relevant repeated dose toxicity studies*

Study reference	Effective dose (ED) (effect relative to controls)	Length of exposure	Equivalent guidance (ED) values	Classification supported by the ED
B.6.3.2.3 OECD TG 453 (1981) / GLP	Effective Dose: 1.56/1.37 mg/kg bw/day (m/f) Critical effects[1]: Abs Uterus wt. (↓38.8%) Rel Uterus wt. (↓46.2%) Abs Ovary wt.[2] (↓18.5%) Rel Ovary wt. (↓26.7%) Abs Testes wt.[2] (↓15.8%)	1-year, oral (dietary) dog (1992)	≤ 2.5 mg/kg bw/day (Cat. 1) ≤ 25 mg/kg bw/day (Cat. 2)	Cat. 1?
B.6.6.2.2 US FIFRA 83-3 / GLP	Effective Dose: 360 mg/kg bw/day Critical effects: Mortality (2/17) Increased plasma levels of triglycerides (m)	Developmental tox, oral (gavage) rabbit (1990) equivalent to 13 days repeated dosing.	≤ 69 mg/kg bw/day (Cat. 1) >69; ≤690 mg/kg bw/day (Cat. 2)	Cat. 2?

[1] **not** statistically significant
[2] increasing dose response

Effects on reproductive organs in the 1 year dog study

This section is not about assessing sexual function and fertility, rather it is about assessing the organ weight effects observed with trinexapac-ethyl treatment in beagle dogs. In the 52-week dog study with trinexapac-ethyl (4 dogs per dose per sex), there was a decrease in uterine and ovarian weights in each of the dosed groups compared to the control animals but **not always in a clear dose responsive manner** (table below). The percent decrease relative to controls for the absolute and relative uterine weights ranged from 39 to 75% and 46 to 75%, respectively. No histopathological effects were seen in either the uteri or ovaries at any dose in this study. The percent decrease for the absolute ovarian weights ranged from 18.5 to 33% but the relative decrease was 26.7% across all the dose groups (1.37 / 39.5 / 357 / 784 mg/kg bw/day). The DS did not describe the testicular weight changes; however, it can be seen from table below that there is no clearly defined dose response and the effect may simply be related to normal variation on attainment of puberty. Testes weights in beagles typically show a large range of variation, e.g. Goedken et al., (2008) reported that at twelve to twenty-four months of age, normal testicular weights in control beagle dogs ranged from 8.3–19.1 g[1]. There is insufficient data to consider the possibility of a test article-related delay in the onset of sexual maturity and because there are only a few animals per dosage group, sexual maturity in one to two animals in a group can have a major influence on group mean organ weights. The RMS concluded in the original DAR (2005) that the change in absolute testes weights was **not considered toxicologically**

[1] Goedken, M. J., Kerlin, R. L., & Morton, D. (2008). Spontaneous and Age-Related Testicular Findings in Beagle Dogs. Toxicologic Pathology, 36(3), 465–471.

relevant, as no statistically significant change in relative weight was observed nor were there any histopathological changes.

Table: *Terminal uterine, ovarian, and testicular weights, 1-year dog study*

Dose (mg/kg/day)	0	1.56/1.37	31.6/39.5	366/357	727/784
Uterus:					
Absolute (g) Relative (g) % Abs/Rel	11.5±2.81 0.13±0.03	7.04±2.47 0.07±0.03 ↓38.8/46.2	3.55±0.63* 0.04±0.01** ↓69.1/69.9	2.87±0.21** 0.03±0.01** ↓75/75.2	3.27±0.59* 0.04±0.01* ↓71.6/67.7
Ovary:					
Absolute (g) Relative (g) % Abs/Rel	1.26±0.17 0.015±0.002	1.027±0.14 0.01±0.001 ↓18.5/26.7	0.95±0.05 0.011±0.001 ↓25/26.7	0.932±0.034 0.011±0.001 ↓26/26.7	0.85±0.08 0.01±0.001 ↓32.8/26.7
Testes:					
Absolute (g) Relative (g) % Abs/Rel	17.4±1.25 0.17±0.013	14.7±0.66 0.16±0.005 ↓15.8/4.8	13.8±0.50* 0.14±0.01 ↓20.5/18.1	12.8±1.41* 0.14±0.02 ↓26.5/16.9	13.07±0.30* 0.14±0.01 ↓24.9/13.9

***** difference with control group statistically significant $p<0.05$;

****** difference with control group statistically significant $p<0.01$

Compounds that inhibit steroidogenesis and cyclicity can cause the uterus to become small and atrophic, thereby decreasing the uterine weight. Unless uterine weight is correlated to the stage of oestrus, false-positive and false negative interpretations may result.

The status of the adult female reproductive system is subject to natural fluctuation. The ovarian and uterine structures (and other reproductive organs) change throughout the oestrous cycle. These normal fluctuations may affect or confound the evaluation of female reproductive endpoints. It is therefore important to be aware of the reproductive status of the female at necropsy, including oestrous cycle stage. This facilitates interpretation of effects with such endpoints as uterine weight and the histopathology of the ovary, uterus, and vagina. Uterine weight peaks at proestrus when the uterus is distended with watery fluid in response to increased oestrogen secretion. Similarly, ovarian weight tends to correlate well with the stage of the cycle. In Chandra & Adler (2008)[1] for example, the mean absolute ovarian weights were 0.77, 0.88, 1.81, 1.17, and 0.71 g for beagles in anoestrus, proestrus, oestrus, dioestrus and immature animals respectively.

In a supplementary report (Anon., 1999; RAR B.6.3.2.3.2), the ovaries, uterus, vagina and mammary gland were retrieved from the archives and evaluated by light microscopy to determine the stage of the oestrus cycle these female dogs were at by study termination. Results were consistent with different proportions of oestrus cycle stages amongst females in the treated

[1] Chandra, S. A., & Adler, R. R. (2008). Frequency of Different Estrous Stages in Purpose-bred Beagles: A Retrospective Study. Toxicologic Pathology, 36(7), 944–949.

groups relative to the controls (table below). However, there is insufficient data to indicate whether dosing had an effect on this distribution or not.

Table: *Distribution of animals (n = 4) at each oestrus cycle stage in each treatment group*

Stage	0 mg/kg bw/day	1.56/1.37 mg/kg bw/day	31.6/39.5 mg/kg bw/day	366/357 mg/kg bw/day	727/784 mg/kg bw/day
Pro-oestrus / oestrus	1	1			
Early metoestrus	2	1	1		
Middle metoestrus		1	2	4	3
Late metoestrus	1	1	1		1
Anoestrus					

NB: Metoestrus may be confused with dioestrus, characterised by further development and activity of the corpus luteum, which produces progesterone. The ovarian and uterine structures achieve their greatest weight during oestrus and thereafter decline.

Table above shows an apparent shift in the proportion of animals in stages of the oestrus cycle associated with reduced weight of the reproductive organs. Absolute uterine weight changes were consistent with the physiological changes of the uterus occurring at the different stages of the oestrus cycle. It is not possible to conclude if there is a substance related effect here since this is effectively a retrospective analysis of a study never designed to investigate such an effect.

In another supplementary report (Anon., 2017; RAR B.6.3.2.3.4), the authors addressed the question around the potential for trinexapac-ethyl to act as an endocrine disruptor due to observed variations in uterine weights and oestrous cycle stages. The question cannot be adequately answered due to the limited data set available and lack of historical control data. In summary, it was not possible to carry out a robust evaluation of oestrus cyclicity and hormone analysis was not performed. Inferences were made from other published studies and the same conclusions drawn as per the original study authors, i.e. the distribution of oestrus cycle stages and associated uterine and ovarian weight changes are the result of normal physiological reproductive dog biology. There is no evidence for an interaction between trinexapac-ethyl and the endocrine system.

The available data from a small group of animals is insufficient to suggest a substance mediated effect on uterine weight. Instead the data suggests that the changes, some of them statistically relevant, reflect the organ status under different stages of the oestrus cycle. RAC concludes there was insufficient evidence for a substance-related effect on uterine and testicular weights. Statistical significance was not observed at the lowest dose which is below the guidance value for consideration of STOT RE 1. RAC accepts that uterine and ovarian weight changes can plausibly be the result of normal physiological reproductive dog biology. Historical control data was not available in the original study report. The low number of animals in the 1-year study per dose group and the study design itself is insufficient for further assessment of reproductive effects under either fertility or development. RAC does not support classification for STOT RE 1 (or STOT RE 2 for that matter) based on these findings in the lowest dose group.

<u>Effects on rabbits in the developmental toxicity study</u>

Preliminary dosing study

The doses in the main study were selected based on the results of a preliminary study (CBG/493-R, Tox. No 881725) which used doses of 0, 40, 400 and 800 mg/kg bw/day in methylcellulose.

At 800 mg/kg bw/day (above the guidance value range for classification), there were 4/6 mortalities; at 400 mg/kg bw/day (below the guidance value range for classification), there was 1 mortality and transient decreased food consumption and marked weight loss to day 9; at 40 mg/kg bw/day, there were no mortalities and a transient weight loss to day 9 in 3/5 animals. The mortalities were attributed to substance irritation of the stomach mucosa as the animals had haemorrhagic depressions in the stomach.

Developmental toxicity study in rabbits

The main developmental study (Anon., 1990; RAR B.6.6.2.2) was not fully compliant with OECD TG 414 (deviations with respect to dose timing (GD 7-19) and dose intervals and numbers of animals with implantation sites at necropsy). The effective dose (LOAEL) was determined to be the 360 mg/kg bw/day group due to increased mortality (2/17 animals) and retarded body weight gain to GD 15. The two mortalities at 360 mg/kg/d were associated with treatment and the first death occurred on day 13 (6 days after dosing) following what the DS described as a suspected convulsion. The second animal was killed on day 24 due to marked and continuing weight loss and was found to have haemorrhagic depressions in the stomach.

Of the two animals from the top dose that died/were sacrificed in the main rabbit dev tox study, both had significant stomach involvement and because of the age of the study it is impossible to determine if the two events were linked. However, it could be stated that there are now two instances of 'damage to stomach mucosa' severe enough to challenge the survival of both does. The data from the necropsy report regarding stomach effects is summarized as follows:

1. Animal 403, sacrificed in extremis, day 24, stomach, haemorrhagic areas noted on necropsy.
2. Animal 412, found dead, day 13, suspected convulsion, stomach, ruptured.

The first doe that was found dead and presumed to have suffered a 'convulsion' was shown to have a ruptured stomach upon necropsy. A ruptured stomach is incompatible with survival for rabbits. But it is unknown if the ruptured stomach led to a convulsion or if the convulsion led to a ruptured stomach, though logic would indicate that damage to the wall of the stomach should first precipitate a breach of the lining before anything else.

The following summarises the data for concern in the rabbit:

1. Two rabbit deaths in the main study with serious stomach mucosal involvement at levels below the presumed GV (2/17 at 360 mg/kg vs GV: 690 mg/kg).
2. A single incidence of rabbit death with stomach mucosal involvement also below the GV (1/6 at 400 mg/kg vs GV: 690 mg/kg) in the rabbit preliminary dev tox study.
3. An apparent dose response with several more instances above the GV (4/6 at 800 mg/kg) in the rabbit preliminary dev tox study.

Conclusion

In the dog, there were effects on reproductive organs below the adjusted guidance values for a 1-year dietary study for STOT RE 1 (≤ 2.5 mg/kg bw/day). However, these effects were not statistically significant and showed a high variability from animal to animal. In addition, the low number of animals in the study precludes a robust assessment of oestrus cyclicity which can have a profound effect on the adult female reproductive system which is subject to natural fluctuation. RAC does **not propose** STOT RE 1 for these effects based on insufficient evidence for a substance-related effect.

In the rabbit there are 2 studies that support an effect on the stomach mucosa with increased mortality as the outcome. In the rabbit preliminary developmental toxicity study there was 1

mortality (out of six animals) at 400 mg/kg bw/day. In the main developmental study, there were 2 mortalities at 360 mg/kg bw/day. In both studies, 1 death either at or below 400 mg/kg bw/day was ascribed to haemorrhagic depressions in the stomach, however a second rabbit fatality in the high dose group also exhibited significant stomach involvement (stomach rupture). Substances are classified in STOT RE Category 2 based on evidence from studies in experimental animals that can be presumed to have the potential to be harmful to human health following repeated exposure. In this case mortalities from two studies were attributed to substance damage to the stomach mucosa. The effects were observed at dose levels comparable with STOT RE 2 guidance values corrected for the duration (13 days) of the exposure (according to Haber's rule).

The DS made the point that there could be uncertainties with applying Haber's Rule to correct the guidance value for a STOT RE classification in this case because the actual dose absorbed from the GI tract was itself uncertain (due to recycling of active substance in caecotrophs). However, this is speculative and there is no data to discount the effect seen in rabbits at levels lower than the adjusted guidance value for STOT RE 2. ADME studies in rats also illustrate that most of the radiolabelled material (approximately 95%) is eliminated in the urine. This suggests any potential for recycling via ingestion of caecotrophs is unlikely because the kidney is the major route of elimination and caecotrophs would not be expected to be a significant source of unchanged test substance. The parent substance is also an ethyl ester and metabolism is significant to the free acid form, a point also arguing against further ingestion of test material via caecotrophy.

The guidance values corrected for the duration of the exposure (according to Haber's rule) are Cat 2:>69; ≤690 mg/kg bw/day.

Two studies in rabbits confirm mortality due to damage to the stomach mucosa at ≤ 400 mg/kg bw/day. Several RAC members had concerns over whether the effects were local effects and questioned the use of Haber's rule in this case. Whether these were local effects or a more specific toxicity to the gastrointestinal tract, the stomach in particular, RAC could not rule out their relevance for STOT RE classification. Likewise RAC also agreed not to discount Haber's rule in this case because caecotrophy was not considered to lead to a significant effect on substance dose for the reasons outlined above. RAC concludes that **STOT RE 2; H373 (GI tract) is warranted**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that trinexapac-ethyl was tested in a range of GLP and OECD TG compliant *in vitro* and *in vivo* genotoxicity assays, details were supplied in table 50 of the CLH report.

The DS noted that based on (Q)SAR analysis (using Derek Nexus version 5.0.2 (Nexus 2.1.1 Lhasa Limited), trinexapac-ethyl did not trigger any structural alerts for genotoxicity (e.g. Mutagenicity *in vivo*/*in vitro*, Chromosome damage *in vitro*/*in vivo*, non-specific genotoxicity *in vitro*/*in vivo*).

In vitro assays included:

- 9 × *in vitro* Ames tests (reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*), 1988 - 2017; different batches of technical material including the batch fortified with process impurities (SMO5D180 (fortified) 93.3%) - all negative.

- 4 × *in vitro* mammalian cell gene mutation tests (in mouse lymphoma L5178Y cells, Chinese hamster V79 cells, Chinese hamster ovary (CHO-K1) cells), 1988 - 2017; different batches of technical material including the batch fortified with process impurities (SMO5D180 (fortified) 93.3%) – 2 × negative; 2 × equivocal.
- 3 × *in vitro* mammalian chromosome aberration test (in Chinese hamster ovary (CHO K5) cells, Human lymphocytes), 2001 – 2015; different batches of technical material – 2 × negative, 1 × equivocal.
- 2 × *in vitro* unscheduled DNA synthesis in mammalian cells (DNA Damage and Repair), using primary rat hepatocytes and human fibroblasts, both from 1988 – both negative.

In vivo assays included:

- 2 × mouse micronucleus tests (strain Tif: MAGF, SPF), single oral dose up to 4000 mg/kg bw in bone marrow, 1989 - 1992, both negative. These studies were of a limited value since it could not be shown that the target tissue was reached. No cytotoxicity was seen in bone marrow. ADME studies in rats indicate the bone marrow is reached by trinexapac-ethyl.
- 1 × rat micronucleus test (strain SD), the test article was given as two administrations, 24 hours apart, maximum dose up to 1400 mg/kg bw in bone marrow, Anon., 2010, negative.

In vitro results

1. Trinexapac-ethyl did not induce point mutations in bacteria *in vitro*. All 9 Ames assays were negative.
2. The mammalian gene mutation assays gave mixed results. Out of 4 tests in total, 2 were clearly negative. The gene mutation test in mouse lymphoma cells was positive in the **presence** of metabolic activation but only at the top concentration where marked toxicity was observed (Anon., according to OECD TG 476, 2009). The gene mutation test in Chinese hamster ovary (CHO-K1) cells using Trinexapac-ethyl tech. fortified was positive in the **absence** of metabolic activation at 175 and 1400 µg/ml with no dose response (Anon., according to OECD TG 476, 2017). A confirmatory experiment was negative. These latter 2 studies were considered equivocal by the DS.
3. Two of the mammalian chromosome aberration tests were negative. The third (Anon., according to OECD TG 473, 2010) was considered equivocal by the DS, testing positive in experiment 1 in human lymphocytes at the highest dose of 2523 µg/ml in the **presence** of S9. Two further experiments testing up to the same level in the presence of S9 were negative.
4. The results of 2 unscheduled DNA synthesis (UDS) assays were negative. However, the DS noted deviations from OECD TG 482, cells were not exposed to test substance in the presence of metabolic activation.

In vivo results

1. Two *in vivo* mouse micronucleus tests were available and showed negative results (Anon., 1992, B.6.4.2.1 study 2, and Anon., 1989, B.6.4.2.1 study 1) though the DS only considered one of them as being acceptable with minor deviations (1992 study). This study design was stated as being limited since it could not be shown that the target tissue was reached. Though the dose levels used in this study were very high (2000 mg/kg

bw/day), no cytotoxicity was seen in bone marrow. The DS also notes that ADME studies in the rat confirm that the bone marrow is indeed reached.

2. In a rat micronucleus test (Anon., 2010; RAR B.6.4.2.2) trinexapac-ethyl did not induce micronuclei in the bone marrow. Evidence of bone marrow exposure was demonstrated from the toxicokinetic studies.

Conclusion

According to the DS, in consideration of all the data, trinexapac-ethyl did not present a gene mutation hazard. There were no studies in germ cells. The DS did not propose to classify trinexapac-ethyl as mutagenic.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No human data are available for trinexapac-ethyl, therefore a classification with Muta. 1A is not warranted. Data are not available illustrating the induction of mutagenic effects in germ cells (a criterion for Category 1B). RAC concludes that classification with Muta. 1A or B is not warranted.

There were no positive *in vivo* micronucleus tests. There were some equivocal results in some of the *in vitro* assays but overall the evidence suggests there is no concern for mutagenicity. QSAR analysis also supports this conclusion with no structural alerts for genotoxicity. RAC supports the conclusion of the DS that **classification for mutagenicity is not warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Carcinogenicity: DS overview

Two guideline and GLP compliant long-term oral (dietary) carcinogenicity studies were available to the DS: a 2-year carcinogenicity study in the Sprague-Dawley [Crl:VAF/Plus CD (SD) Br] rat (Anon., 1992) and an 18-month carcinogenicity study in the CD-1 mouse (*Anon., 1991*). Study details were summarised in Table 53 of the CLH report. In addition, the results of a 2017 (Q)SAR analysis (using Derek Nexus version 5.0.2 (Nexus 2.1.1 Lhasa Limited) were also described where trinexapac-ethyl did not trigger any structural alert for carcinogenicity.

An increased incidence of rare tumours was recorded following chronic dietary exposure of Sprague-Dawley rats to trinexapc-ethyl. These tumours were evident at the highest dose of 20000 ppm (805.7 mg/kg bw for males and 1054.0 mg/kg bw for females). Three tumour types were observed;

1. Squamous cell carcinoma in the non-glandular stomach of males,
2. Thyroid follicular adenocarcinoma in males,
3. Urinary bladder papillomas in females.

The animals concerned were all subjected to the highest dose, the maximal tolerated dose (MTD) was just about achieved (based on body weight gain reduced by approximately 10% in males at the end of the study at the highest dose) and the incidence rate was low.

None of these tumours were replicated in the mouse study; there were no compound-related increases in the incidence of any tumours. Dietary administration of trinexapac-ethyl for 78 weeks to the CD-1 mouse at up to 7000 ppm (912 and 1073 mg/kg bw/day for males and females respectively), was not carcinogenic and did not cause any significant toxicity.

Rat 2-year dietary toxicity/oncogenicity study

In a rat GLP and OECD TG 453 (1981) compliant combined chronic toxicity and carcinogenicity dietary study (*Anon., 1992*), treatment with trinexapac-ethyl had no adverse compound-related effect on survival though at study termination mortality was >50% in all dose groups except the male high dose group. Sprague-Dawley rats (70/sex/dose; 50/20 for the carcinogenicity and chronic investigations, respectively) were administered trinexapac-ethyl in the diet for 104 weeks at doses of 0, 10, 100, 3000, 10000 or 20000 ppm. Table below shows the mean dose received by each group.

Table: *Mean dose received (mg/kg bw/day)*

Dietary concentration of trinexapac-ethyl (M/F ppm)	**0**	**10**	**100**	**3000**	**10000**	**20000**
Males	0	0.4	3.9	115.6	392.7	805.7
Females	0	0.5	4.9	147.4	494.0	1054

Non-Neoplastic findings

Treatment-related reductions in mean body weight and body weight gain occurred in males and females at the top dose throughout the study. Males were most affected, by approximately 10%. The main target organs for non-neoplastic effects were the liver, kidneys and mammary skin of females at ≥ 10,000 ppm. The NOAEL for long-term effects was set at 3000 ppm (115.6 mg/kg bw/day for males and 147.4 mg/kg bw/day for females), based on an increase in the incidence of bile duct hyperplasia in the livers of males and galactoceles in the mammary skin of females at the next higher dose level. In addition, following the initial 52 weeks of the study renal histopathological effects (hyaline droplets) were observed in 10000 ppm and 20000 ppm males.

Neoplastic findings

An increased incidence of rare tumours was recorded following chronic exposure of Sprague-Dawley rats to trinexapac-ethyl at the top dose.

(i) Squamous cell carcinoma in the non-glandular stomach

Neoplastic findings in the non-glandular stomach were observed only in male rats at the highest dose. The only incidence of this tumour was 2/**80** (2.5%) in males at 806 mg/kg bw/day (statistically significant).

The DS noted from the RAR that the incidence for this tumour was exclusively observed in males sacrificed before study termination and therefore could not be linked to an older age and/or the increase in survival rate of males that was observed at the highest dose. However this statement is considered rather meaningless by RAC since RAC observed one animal was sacrificed on day 433 and the other on day 716. Historical control data from the conducting laboratory (six studies conducted between September 1984 and March 1987) (0%) and other published data indicate that squamous cell carcinoma (SCC) of the forestomach is a rare spontaneous tumour (0 - 1.2% incidence as reported in the original study report). The DS commented that pre-neoplastic lesions such as basal epithelial cell hyperplasia and acanthosis of the non-glandular stomach were not considered to be compound related, there was no clear dose response though a small increase

in basal epithelial cell hyperplasia was observed in the highest dose group. The DS noted that non-glandular forestomach did not have an equivalent in humans and therefore the relevance of this tumour to humans was probably low.

(ii) Thyroid follicular adenocarcinoma

A statistically significant increase in the incidence of thyroid follicular adenocarcinoma was observed in males at the top dose of 806 mg/kg bw/day in the full 2-year study (4/80; 5%), table below. This finding was observed in the control group (1/89; 1.1%) and in the other two lower dose groups (1/80; 1.3%) of males and in 494 mg/kg bw/day females (2/80; 2.5%).

Table: *The incidence of thyroid follicular neoplasm in male rats*

Dose Level (mg/kg bw/d)	**0**	**0.4**	**3.9**	**115.6**	**392.7**	**805.7**	**Historical Control**
Number of Animals	89	79	80	80	80	80	
Follicular adenomas (number/%)	4 (4.5%)	2 (2.5%)	3 (3.75%)	5 (6.25%)	3 (3.75%)	3 (3.75%)	0-8.6%
Follicular adenocarcinomas	1 (1.1%)	0	0	1 (1.25%)	1 (1.25%)	4 (5%)	0-5%, cumulative incidence 1.8%
Combined	5 (5.6%)	2 (2.5%)	3 (3.75%)	6 (7.5%)	4 (5%)	7 (8.75%)	2-13%

The historical control range for this tumour was up to 5%, which is comparable to the incidence seen (5% - 4/80) in this study. The DS also remarked on this incidence being higher if the animals from the 12-month interim sacrifice were discounted to give 5/70 (5.7%) animals affected. All the carcinogenicity results in the RAR and CLH report included the interim sacrifice animals (10 per treatment group). There was a lack of an increased incidence of potential pre-neoplastic changes, such as follicular hyperplasia of the thyroid, at the interim and final sacrifice and in early deaths. The DS considered the increase in thyroid follicular tumours to be incidental.

(iii) Urinary bladder papilloma

A statistically significant increase in the incidence of urinary bladder papilloma was found in two females at 20000 ppm (2/**80**; 2.5%). The one female affected at 10000 ppm was from the 1-year interim sacrifice group, the original study authors had grouped the neoplastic data from both the 2 year and 1-year interim groups (table below). The DS described this benign tumour as being infrequently observed in rats and that was reflected in the HCD from the conducting laboratory (0%). The DS also remarked that the available HCD described urinary bladder polyps and seemed to imply that the HCD then showed a range of 0-1.4% if urinary bladder papilloma was considered the same as urinary bladder polyps. The incidence of pre-neoplastic lesions (e.g. a common pre-neoplastic finding such as epithelial hyperplasia in the urinary bladder) in the present study was observed in both sexes, however it was not considered to be compound-related (especially since it did not occur in the highest dose group). There was no progression to malignancy and no transitional cell carcinomas observed in any of the female dose groups.

One of the possible non-genotoxic modes of action, by which urinary bladder tumours in rodents may be produced, is the presence of solid aggregates or calculi within the urinary tract. The DS noted that one 20000 ppm female and one 3000 ppm male had macroscopic calculi (stones) observed in the urinary bladder at examination post-mortem. The DS considered that one of the

2 instances of papilloma in the high dose female group arose because of direct mechanical irritation.

Table: *The incidence of urinary bladder papilloma in female rats*

Dose Level (mg/kg bw/d)	0	0.5	4.9	147.4	494.0	1054	Historical Control
Number of Animals	89	80	80	80	80	80	
Epithelial hyperplasia	2	2	0	1	1	0	
Papilloma	0	0	0	0	1 (1.3%)	2 (2.5%)	0[1]

[1] HCD indicates a single incidence of urinary bladder polyp, 1/70 giving an HCD range of 0 – 1.4%

The DS considered the increased incidence of urinary bladder papilloma as incidental based on an overall weight of evidence approach.

Mouse 78-week dietary oncogenicity study

In a mouse GLP and OECD TG 451 (1981) compliant carcinogenicity dietary study (*Anon., 1991*), treatment with trinexapac-ethyl had no adverse compound-related effect on survival. CD-1 mice (70/sex/dose) were administered trinexapac-ethyl in the diet for 78 weeks at doses of 0, 7, 70, 1000, 3500 or 7000 ppm. Table below shows the mean dose received by each group which was very similar to the dosing received by rats in the 2-year study.

Table: *Mean dose received (mg/kg bw/day)*

Dietary concentration of trinexapac-ethyl (M/F ppm)	0	7	70	1000	3500	7000
Males	0	0.91	9.01	130.8	450.7	911.8
Females	0	1.08	10.66	154.1	538.7	1073.4

The MTD was not reached, the highest dose level did not induce low toxicity, but this was not a requirement in the guidelines published at that time. Dose levels were based on the results of a 13-week mouse study, in which concentrations up to 10000 ppm were used, and limited systemic toxicity (i.e. slightly lower body weight gain) was observed at that level.

There were no clinical signs of toxicity and no treatment-related effects on haematology, ophthalmology, organ weights or macroscopic findings. The observed effects on body weight, mean percent body weight gain and food consumption were not considered to be adverse.

No compound-related increases in the incidence of any tumours were observed in this study and trinexapac-ethyl was not considered to be carcinogenic in this strain of mice under the conditions of the study. In the RAR and following the final EFSA review, the NOAEL was set at the top dose of 7000 mg/kg food (911.8 mg/kg bw/d).

The DS did not propose classification for carcinogenicity based on the mouse study results.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Carcinogenicity: Introduction

An increased incidence of rare tumours was recorded following chronic exposure of Sprague-Dawley rats to trinexapac-ethyl. Males developed low incidences of squamous cell carcinoma in the non-glandular forestomach and thyroid follicular adenocarcinoma, whereas urinary bladder papilloma were increased in females. Some general points to take note of include:

- three different tumour types,
- these effects were confined to the high dose group only (805.7 mg/kg bw for males and 1054.0 mg/kg bw for females),
- there was no strong correlate or support from pre-neoplastic changes,
- the incidence rate was low but above the HCD range or cumulative incidence from the performing laboratory within the relevant time period,
- only one sex was affected for each tumour type,
- tumours were only seen in rats, no such evidence for the same tumours in mice under similar doses,
- trinexapac-ethyl is not genotoxic,
- no confounding effect by excessive toxicity

Squamous cell carcinoma in the non-glandular forestomach

A statistically significant increase in the incidence of squamous cell carcinoma in the non-glandular stomach was reported only in males at 806 mg/kg bw/day (2/70; 2.9%), table below. The incidence was above the HCD from the conducting laboratory (0%). The 2 animals were sacrificed prior (on days 433 and 716) to the scheduled end of the study (days 735-9). The pre-neoplastic lesions were not associated with the animals exhibiting carcinoma. The limited available evidence would suggest a spontaneous origin for this tumour type in the two animals that were affected.

Table: *The corrected incidence of squamous cell carcinoma of the stomach in male rats*

Dose Level (mg/kg bw/d)	0	0.4	3.9	115.6	392.7	805.7	Historical Control
Number of Animals	70	70	70	70	70	70	
SC Carcinoma (number/%)	0	0	0	0	0	2 (2.9%)	0%
Epithelial hyperplasia	0	3	0	0	1	3[$]	
Basal epithelial cell hyperplasia	1	2	0	0	1	3[$]	

[$] effects not associated with the animals found to have squamous cell carcinoma.

Thyroid follicular cell carcinoma in male rats

RAC notes *thyroid follicular adenocarcinoma is synonymous with thyroid follicular cell carcinoma*. A statistically significant increase in the incidence of thyroid follicular cell carcinoma was reported only in males at 806 mg/kg bw/day (4/70; 5.7%), at terminal sacrifice, table below. Historical control data (HCD) was available from the performing laboratory, six studies from 1983 – 1990, range 0 – 3 animals, the cumulative incidence was 1.8% and the highest incidence was 3/60 =

5%. This was in line with published data from Charles River Laboratories (1992) where a range of incidences from 0 - 6% was recorded (19 studies; initiated 1984 – 1986, 1244 animals tested, 16 positives, the highest incidence was 3/50 = 6%). The incidence in the present study was just outside the HCD range from the conducting laboratory (5.7%) but within that of the more general population from Charles River Laboratories.

RAC has re-tabulated the data for all the animal tumours, corrected to show only incidences of tumours found in those animals designated to the 104-week cohorts. The original study report and RAR and CLH reports showed data for the 104-week animals plus those animals from the 52-week cohorts designed for the 12-month interim sacrifice and chronic toxicity assessment. This would have the effect of reducing the true tumour incidence in terms of percentage of total animals affected. The true total number of animals per sex and per dose was 70 as these were designated to run the entire treatment period of up to 104 weeks. The interim necropsy group and interim recovery groups accounted for 10 animals /sex/dose in each case.

Table: *The corrected incidence of thyroid follicular neoplasm in male rats*

Dose Level (mg/kg bw/d)	0	0.4	3.9	115.6	392.7	805.7	Historical Control
Number of Animals	69	70	70	70	70	70	
Follicular adenomas (number/%)	4 (5.8%)	2 (2.9%)	3 (4.3%)	5 (7.1%)	3 (4.3%)	2 (2.9%)	0-8.6%
Follicular adenocarcinomas	1 (1.4%)	0	0	1 (1.4%)	1 (1.4%)	4 (5.7%)	0-5% 0-6%[$]
Combined	5 (7.2%)	2 (2.9%)	3 (4.3%)	6 (8.6%)	4 (5.7%)	6 (8.6%)	2-13%

$ Spontaneous neoplastic lesions and selected non-neoplastic lesions in the Crl:CD BR Rat. Published data from Charles River Laboratories (1992).

Significance of the thyroid follicular cell tumour findings

The incidence of adenomas or the combined incidence of adenoma and adenocarcinomas showed no dose-related increase. Other lesions indicating an effect on the thyroid gland such as hypertrophy have not been reported in this study or in other toxicity studies with the test substance. In the present study, pre-neoplastic lesions (such as follicular hyperplasia of the thyroid) were seen in similar incidences in all groups. There was no association with tumour incidence. Thyroid follicular cell hyperplasia was not seen in those animals in the high dose group that exhibited follicular cell carcinoma. No neoplastic effect was observed in the thyroids of females or in mice of either sex.

Table 14: *Significance of the tumour finding*

Finding	Observation	Significance
Tumor type	Rodent thyroid follicular cell carcinoma	High
Background Incidence	Medium, not an uncommon finding amongst SD rats. Stat. sig. increase observed in the high dose group. Incidence is at the upper bound limit of HCD; not sufficient evidence to establish a carcinogenic effect.	Low
Tumors at multiple sites	Yes	High
Progression of lesions to malignancy	No preneoplastic lesions associated with treatment. No evidence for progression	Low

Finding	Observation	Significance
	though the tumour type is malignant. Follicular cell adenoma does not appear to be treatment related, does not exceed HCD.	
Reduced tumour latency	No evidence.	Low
Response in both sexes	No (males only)	Low
Tumors in one or multiple species	No (rats only, mice dosed at the same level showed no response)	Low
Structural similarity to other carcinogens	No	Low
Routes of exposure	Oral, relevant to human route of exposure.	High
ADME kinetics comparable for humans	Yes	High
Confounding effect by excessive toxicity	No evidence that the MTD was exceeded.	High
Metastases	No (no evidence)	Low
Dose-related increase	Just within published upper bound limit of HCD range. Above HCD of the laboratory.	Low
Mode of Action and human relevance	Unknown MoA.	High
Genotoxicity	No	Low

At the 12-month interim sacrifice there was no substance related increase in thyroid follicular adenoma or hyperplasia. There was no further data on these tumours, and no mechanistic investigations were carried out so no comparisons with known modes of action can be made. The relevance to humans cannot be discounted, but there is no sufficient evidence to show a treatment related effect in the high dose group as the tumours occurred at levels near the HCD upper bound limit. RAC is in line with the DS and does not propose classification due to insufficient evidence of a carcinogenic effect.

Urinary bladder papilloma in female rats

A statistically significant increase in the incidence of urinary bladder papilloma was found in two females in the 1054 mg/kg bw/day (2/**70**; 2.9%) group at terminal sacrifice (table below). This was the only incidence observed amongst the animals of the dedicated 104-week carcinogenicity study dose groups. In the 12-month interim sacrifice study, one instance of urinary bladder papilloma was found in a female from the 494 mg/kg bw/day dose group. The one affected animal from the 12-month interim sacrifice showed classic signs of urinary bladder irritation and inflammation (presence of calculi with lymphocytic infiltration and epithelial hyperplasia described as severe). In contrast, the pathology report for the affected females in the high dose group of the main study **did not show** any evidence for (i) an increase in squamous metaplasia; (ii) an increase in inflammation nor (iii) an increase for epithelial hyperplasia. However, one animal did show evidence for urinary bladder calculi.

Table: *The corrected incidence of papilloma of the urinary bladder in female rats*

Dose Level (mg/kg bw/d)	0	0.5	4.9	147.4	494.0	1054	Historical Control
Number of Animals	70	70	70	70	70	70	
Papilloma (number/%)	0	0	0	0	0	2 (2.9%)	0-1.4%
Epithelial hyperplasia	2	2	0	1	0	0	
Calculi	0	0	0	0	0	0	

As stated by the DS and written in the RAR, this benign tumour is rarely observed in rats as shown by the HCD from the conducting laboratory. However, this statement relates to the fact that the HCD from the conducting laboratory does not state "urinary bladder papilloma". Instead, the HCD states only the occurrence of urinary bladder polyp. If the description of urinary bladder papilloma by Shirai & Takahashi (1998)[1] is to be believed then it is reasonable to conclude that urinary bladder papilloma = urinary bladder polyp and the HCD is not zero but it is rare and has a range (1/70 or 1.4% in one out of six studies, total of 389 females). The conducting laboratory HCD is supported by published data from Charles River Laboratories (1992) where there was also a single incidence of a urinary bladder **polyp** out of 19 studies with a total of 1249 animals. The only (maximum) study incidence rate was 1.4%.

Significance of the urinary bladder papilloma findings

Table: *Significance of the tumour finding*

Finding	Observation	Significance
Tumor type	Rodent papilloma of the urinary bladder	High
Background Incidence	Rare, an uncommon finding.	High
Tumors at multiple sites	Yes	High
Progression of lesions to malignancy	No evidence for progression, the tumour type is benign.	Low
Reduced tumour latency	No evidence.	Low
Response in both sexes	No (females only)	Low
Tumors in one or multiple species	No (rats only, mice dosed at the same level showed no response)	Low
Structural similarity to other carcinogens	No	Low
Routes of exposure	Oral, relevant to human route of exposure.	High

[1] "..tumors usually appear as single or multiple small, nodular lesions on the mucosal surface of the urinary bladder. They are exophytic and polypoid projecting from the mucosal surface, with a narrow or a broad base." Shirai T., Takahashi S. (1998) Papilloma, Urinary Bladder, Rat. In: Jones T.C., Hard G.C., Mohr U. (eds) Urinary System. Monographs on Pathology of Laboratory Animals. Springer, Berlin, Heidelberg

Finding	Observation	Significance
ADME kinetics comparable for humans	Yes	High
Confounding effect by excessive toxicity	No, questionable if the MTD was exceeded or not.	High
Metastases	No (no evidence)	Low
Dose-related increase	No.	Low
Mode of Action and human relevance	Unknown MoA. No MoA data supplied. Known MoAs include urinary bladder tumours due to crystals in the bladder, crystals were observed in one of the affected high dose females. One unexplained incidence remained. Potentially spontaneous occurrence.	Low
Genotoxicity	No	Low

Urinary bladder tumours due to crystals in the bladder (if the mechanism of crystal formation has been shown to be of no relevance), can be a mode of action with little concern for human carcinogenicity hazard and risk assessment (see CLP guidance (2017) section 3.6.2.3.2 subsection k). In the case of trinexapac-ethyl we have only a benign tumour type present at the highest dose with no progression to malignancy (i.e. no evidence of transitional cell carcinoma). There appeared to be no substance related effect on the formation of urinary bladder calculi in either females or males though the DS stated that one of the affected high dose females showed evidence of calculi (verified by RAC). The second affected female showed no evidence of calculi and it is not unreasonable to consider a spontaneous origin for the urinary bladder papilloma in this high dose female.

RAC is of the opinion that the observed papillomas may not be treatment related and are within the HCD range. One tumour appears associated with urinary bladder calculi, the second is of unknown aetiology. RAC agrees with the DS, concluding there is insufficient evidence to support classification for carcinogenicity.

Conclusions

Classification into category 1A

There is no information from studies in humans to inform on carcinogenic potential and so classification in category 1A is not supported.

Classification into category 1B

The substance was not found to be genotoxic. Tumours were restricted to high doses near or at the limit dose, to one species (rat) and one sex in each of the three cases outlined above. There was no evidence for a reduction in tumour latency. There was no evidence for progression to malignancy or of treatment related pre-neoplastic changes and there were no apparent dose response relationships below the top doses. Overall the data was considered to be insufficient to show evidence of a carcinogenic effect. The data is not sufficient to warrant classification in category 1B.

Classification into category 2

The initial presentation of three tumour types with at least two of these being rare types was of concern. However, these effects were only seen at very high doses where the MTD was hardly reached in the rat study and not achieved in the mouse study. Consideration of the tumour type, if there was evidence to suggest the tumour was substance related, significance to human carcinogenicity potential and/or low incidences with and without preneoplastic changes at very high doses, considering also incidences in HCD lowered concern for the types of tumour observed. On the weight of the presented evidence, RAC considers there is sufficient data to conclude there is no evidence of a substance-mediated carcinogenic effect to support classification in category 2.

No Classification

RAC is of the opinion that overall the data is conclusive and **there is insufficient evidence to support classification for carcinogenicity.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Trinexapac-ethyl was evaluated in a guideline (OECD TG 416; 1983) and GLP compliant two-generation study (Anon., 1991) in rats in order to assess its effects on sexual function and fertility. The effects of trinexapac-ethyl on development following exposure during pregnancy were tested additionally in pre-natal developmental toxicity studies in rats (Anon., 1988) and rabbits (Anon., 1990). These studies were guideline (OECD TG 414; 1981) and GLP compliant.

Effects on sexual function and fertility:

Rat 2-gen study

In a 2-generation reproduction study, trinexapac-ethyl (purity 96.2%) was administered to two generations of Sprague-Dawley rats (30/sex/group) at concentrations of 0, 10, 1000, 10000 or 20000 ppm for 13 weeks prior to mating (table below), during mating, throughout gestation and lactation until weaning of the F2 offspring (day 21 of lactation).

Table: *Mean dose received (mg/kg bw/day)*

Concentration (ppm)	P-males	P-females	
	Pre-mating Weeks 0-13	Pre-mating Weeks 0-13	Gestation Days 0-20
10	0.59	0.75	1
1000	60	75	65
10000	595	737	659
20000	1169	1410	1377
	F1-males	F1-females	
10	0.59	0.77	1
1000	59	77	62
10000	592	765	651
20000	1255	1560	1319

Following the RAR evaluation the average test substance intake was converted into **0.7, 106.2, 662.9 and 1293.0 mg/kg bw/day**. The average values of test substance intakes through the premating period for males as well as through the premating and gestation period for females were used to derive the relevant study NOAELs/LOAELs.

General toxicity

An early feeding error resulted in the pre-mating period being extended for 3 weeks to ensure that animals received the correct dietary levels for at least 9 consecutive weeks prior to mating (one spermatogenic cycle). This did not have any subsequent effects on the remainder of the study or any of its endpoints. There were no compound-related mortalities or clinical observations in either the P or F1 parental generation. There were small but statistically significant effects on body weight parameters in both parental generations, top dose males and females showed 10-16% lower body weights relative to controls. According to the DS the MTD was exceeded at 20000 ppm.

Effects on sexual function and fertility

There were no treatment-related changes to sexual function or fertility in males or females in both the P and F1 generations. According to the DS, the precoital interval, duration of gestation, number of implantations, mating, fertility and gestation indices were comparable among controls and all treated groups in both generations.

The DS noted that some important data endpoints were not investigated such as oestrous cyclicity, sperm parameters, the age of vaginal opening and preputial separation.

Developmental effects

Developmental effects were confined to the highest dose groups:

(1) Pup toxicity was evident with significantly reduced post-natal body weight until the end of the lactation period in two generations for both sexes (F1 pups: male -18.9%, female -20.5%, F2 pups: male -23.6%, female -24.1%) and,

(2) significantly reduced survival index under certain conditions;

- F1 pups (sexes combined, Mean % surviving days 4-21 (post-cull)) 92.4% relative to 97.8% in controls.
- F2 pups (females only, mean % surviving days 0-4 (pre-cull)) 92,1% relative to 97.6% in controls.

Conclusion

The DS concluded that the 2-generation rat study had no effects on sexual function and fertility at dietary concentrations of up to 20000 ppm (equal to 1293.0 mg/kg bw/day). The DS did not propose classification.

Developmental toxicity

As regards the two-generation study, the DS concluded (see above) that the developmental effects noted in the two-generation reproduction toxicity study (reduced body weight during and at the end of lactation period in two generations of both sexes and reduced survival index in the offspring at the highest dose level) did not provide sufficient evidence for classification for developmental toxicity.

Developmental toxicity was also investigated in the rat and the rabbit in GLP and OECD TG 414 (1981) guideline compliant studies.

Rat developmental toxicity (teratogenicity) study

In the developmental toxicity study (Anon., 1988), mated female Sprague-Dawley, RAIf (SPF) hybrids of RII/1 × RII/2 rats (24/group) were orally (gavage) administered dose levels of 0, 20, 200 and 1000 mg/kg bw/day on day 6 to day 15 of gestation (period of major organogenesis only) as a suspension in peanut oil. Dose levels were based on the results of a range-finding study, in which dose levels of 100 and 1000 mg/kg bw/day were administered on gestation days 6-15 where no maternal or foetal toxicity was observed. Dams were sacrificed on GD21.

No animals were found dead or killed in extremis during the study period. No treatment-related clinical signs or effects on body weight, adjusted body or body weight gain were observed even on gestation days 6-16. There were no treatment-related gross findings in the females at necropsy. There was no indication of maternal toxicity up to and including the top dose.

The only adverse effect observed was a statistically significantly decreased number of corpora lutea in the high dose group compared to the control group. Due to the smaller number of corpora lutea in the high dose group, the number of implantation sites was also reduced. The mean no. corpora lutea / dam (17.0) at the top dose level was just at the lower bound limit of the historical control range (min. 17.0, max. 19.1). HCD: median = 17.5, 13 control groups with a total of 297 pregnant females were examined from 1985 to 1987 from the same laboratory. Since ovulation occurs before the start of dosing, this finding was not considered experimentally relevant.

The number of live foetuses per dam and the sex ratio of the foetuses was not affected by the administration of trinexapac-ethyl. The number of early resorptions and late resorptions was comparable for all experimental groups, dead or aborted foetuses were not detected in any dose group. There were no other remarkable observations from the laparohysterectomy investigation at terminal sacrifice.

Foetal anomalies

Visceral findings: no test article related effects, though one foetus of the high dose group showed hypoplasia of the left testicle.

External findings: no test article related effects. In the high dose group, one foetus showed fibrous adhesion of the right forelimb and the tail. Since there was a small haemorrhagic area and the skeletal examination revealed no abnormality of the skeletal elements, the adhesion was attributed to external accidental injury.

Skeletal findings: there were some non-statistically significant differences in the type and incidence of skeletal anomalies in the test article treated groups compared to the control group and historical control groups. There was a single foetal incidence of fragmentary sternebra seen in the top dose group, this was not considered to be a significant finding. There were two main types of anomalies of interest:

i. A dose dependent increase of asymmetrically shaped sternebrae.
ii. A statistically significant increase in unossified cervical vertebral centres.

(i) Asymmetrically shaped sternebrae

This structural abnormality was observed in the absence of maternal toxicity. Although an apparently dose dependent increase of asymmetrically shaped sternebrae was observed (table below; foetal / litter incidence, %), there were no statistically significant differences between test article treated groups compared to the control group. Comparisons with the HCD showed that the litter and foetal incidence (%) of asymmetrically shaped sternebrae (29.2% and 3.3%, respectively) at the top dose level of 1000 mg/kg bw/day were outside the historical control range (15.08±11.57% and 2.28±2.09%, respectively) for the performing laboratory (10 studies;

1985-1987). RAC noted that the HCD range was not clear because the HCD was incompletely reported in the original study. The DS stated that the notifier had also provided a separate set of generic HCD (1987-1993) from 12 developmental toxicity studies with a total of 297 pregnant females of the same strain. According to the DS, the litter incidence of asymmetrically shaped sternebrae at the top dose level (29.2%) was below the incidence mean for this finding only in three studies out of twelve (this conclusion is unverified by RAC). This skeletal anomaly was considered by the DS as a "grey zone anomaly".

Table: *Details of sternal findings in the rat prenatal developmental tox. study*

		Dose (mg/kg bw/d)				
		0	**20**	**200**	**1000**	**HCD**
No. foetuses examined		234	248	245	239	2093
No. litters		22	24	24	24	234
Sternebra(e)						
Asymmetrically shaped sternebra:	Foetuses	2	4	5	8	48
	% of total	0.9	1.6	2.0	3.3	2.3
Asymmetrically shaped sternebra:	Litters	2	4	4	7	35
	% of total	9.1	16.7	16.7	29.2	14.9

(ii) Unossified cervical vertebral centres

The incidences of unossified cervical vertebral centres (CVC) (described as "still absent ossification for cervical vertebral centres") were statistically significantly (CHI-square test) increased (CVC 3, CVC 4, CVC 5) at both the low and high dose groups (table below). There was no dose response. Similar numbers of litters were affected across all doses. The high dose group foetal incidences were within the supplied HCD range (10 inhouse control groups with a total of 234 pregnant females, 1985 – 1987).

Table: *Details of unossified cervical vertebral centres in the rat prenatal developmental tox. study – foetal incidence (%)*

		Dose (mg/kg bw/d)				
		0	**20**	**200**	**1000**	**HCD**
No. foetuses examined		234	248	245	239	2093
No. litters		22	24	24	24	234
CVC 3:	Foetuses	83	**123****	90	**113****	
	% of total	35.5	49.6	36.7	47.3	50.7±6.7
CVC 4:	Foetuses	53	**76***	68	**87****	
	% of total	22.6	30.6	27.7	36.4	38.5±7.5
CVC 5:	Foetuses	37	**63****	51	**70*****	
	% of total	15.8	25.4	20.8	29.3	28.7±6.7

Relevance of findings: The DS stated in the CLH report that asymmetric sternebrae (asymmetric ossification in sternabrae?) in rats and other species should be considered as a "grey

zone anomaly" according to revised IFTS terminology (Makris et al. 2009)[1]. A grey zone anomaly means that this anomaly does not fit readily into one of the two categories (malformation or variation). Ultimately, characterization of anomalies as variations or malformations is contingent upon their adversity to health and in some cases postnatal persistence.

According to the DS cervical centrum unossified should also be considered as a "grey zone anomaly" (Makris et al. 2009). Originally described as "still absent ossification" for cervical vertebral centres, the data shows this anomaly to have a high foetal incidence. The DS did not characterise the effect further. Development and ossification of the rodent skeleton occurs from the perinatal period (i.e., near the time of birth) and extends through the early postnatal period. Observations of reduced ossification are not malformations, because they are transient and typically catch up during the lactation period. RAC notes the fact that there is a high incidence of cervical centrum unossified which does not show any particular dose trend and a lack of other more serious and extensive effects that would be expected from a substance that is a true developmental toxicant. This would indicate this effect to be more correctly termed a variation rather than a malformation or a grey zone anomaly.

Rabbit developmental toxicity (teratogenicity) study

Range-finding study

Dose levels were based on the results of a range-finding study, in which dose levels of 0, 40, 400 and 800 mg/kg bw/day in methylcellulose were administered. At **800** mg/kg bw/day, there were **4/6 mortalities**; at **400** mg/kg bw/day there was **1 mortality** and a transient decreased food consumption and marked weight loss to GD 9; at 40 mg/kg bw/day, there were no mortalities and a transient weight loss to GD 9 in 3/5 animals. No treatment-related effects were seen in the foetuses at any of the dose levels.

Main Study

In the developmental toxicity study (Anon., 1990), mated female New Zealand White rabbits (16-17/group) were orally (gavage) administered at dose levels of 0, 10, 60 and 360 mg/kg bw/day from day 7 to day 19 of gestation (period of major organogenesis only) in 2% methylcellulose. On day 29 of pregnancy, the does were sacrificed, litter values determined, and foetuses examined for external, visceral and skeletal abnormalities.

There were no treatment-related clinical signs in dams. The maternal NOAEL was set at 60 mg/kg bw/day, based on increased mortalities and retarded body weight gain to day 15 at 360 mg/kg bw/day dose. The two mortalities at 360 mg/kg bw/day were considered to be associated with treatment. The first death occurred on day 13 (6 days after dosing) following a suspected convulsion (but RAC noted the necropsy indicated a ruptured stomach). A second dam was killed on day 24 due to marked and continuing weight loss. The mortalities from both the main and range-finding studies were attributed to substance related irritation of the stomach mucosa as the animals had haemorrhagic depressions in the stomach.

There were small effects on bodyweight relative to controls as well as a small reduction in food consumption across all dose groups with no apparent dose response. The DS noted that information on corrected maternal body weight and corrected maternal body weight gain for all groups was not available for this study.

[1] Makris et al., (2009), Terminology of developmental abnormalities in common laboratory mammals (Version 2). Birth Defects Research Part B: Developmental and Reproductive Toxicology, 86: 227-327.

Developmental effects

There were 4 total litter losses (1 abortion each at 0 (control), 60 and 360 mg/kg bw/day, plus 1 total resorption in controls). These litter losses were therefore considered not to be treatment related.

There was a statistically significant decrease in the number of live foetuses and increase in pre-implantation loss (%) and post-implantation loss (%) in the top dose group compared to controls. Historical control data was not available. The developmental NOAEL was 60 mg/kg bw/day, based on increased post-implantation loss and the decrease in the number of live foetuses at 360 mg/kg bw/day.

The DS reported that there were no teratogenic effects observed in the rabbit. In addition, they noted that based on a (Q)SAR analysis (using Derek Nexus version 5.0.2 (Nexus 2.1.1 Lhasa Limited), trinexapac-ethyl did not trigger any structural alert for developmental toxicity and/or teratogenicity. The DS did not propose classification based on the rabbit developmental toxicity study.

Conclusion

The DS concluded that the adverse effects on development noted in the two-generation reproduction toxicity study in rat and the developmental toxicity studies in rats and rabbits were not sufficient to trigger a proposal for classification for this hazard category.

Adverse effects on or via lactation

The DS stated offspring effects from the two-generation reproductive study were associated with reduced maternal body weight and were not considered to be a direct effect of trinexapac-ethyl exposure via lactation. Offspring effects were limited to reduced body weight during and at the end of lactation period (F1 pups: male 18.9%, female 20.5%, F2 pups: male 23.6%, female 24.1%) as well as reduced survival index (post-cull, days 4-21) in F1 pups (sexes combined) and (pre-cull, days 0-4) in F2 female pups at the highest dose level.

Comments received during public consultation

One comment was received from a Member State in support of classification with Repr. 2; H361d.

Two points were made:

1. Rat dev tox study: the increased incidence of asymmetrically shaped sternebrae on both a foetal and litter basis is a structural abnormality observed in the absence of maternal toxicity.
2. Rabbit dev tox 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 in the absence of maternal toxicity.

Assessment and comparison with the classification criteria

Assessment of sexual function and fertility

There was limited parental toxicity (no evidence of treatment-related mortality or clinical signs, only slightly reduced body weight gain of 10 to 18% for males and females, respectively as compared to controls, in association with minor reductions in food consumption at the highest dose of 1293 mg/kg bw/day) observed in the 2-generation study in rats.

There were no treatment-related changes to sexual function or fertility in males or females in both the P and F1 generations. RAC notes however, that because of the age of the study, some important data endpoints were not investigated such as oestrous cyclicity, sperm parameters, the age of vaginal opening and preputial separation. This raises the issue of whether the fertility endpoint has been fully investigated. Developmental effects and effects on or via lactation are assessed by RAC under the respective headings.

There were adverse effects noted on the reproductive organs in dogs after 1-year exposure (↓ weight of uterus, ovaries and testis, reaching statistical significance for uterus and testis, see section 2.2.1 and the table under STOT RE). The study design and number of animals do not provide sufficient information for a robust assessment of sexual function and fertility. Changes in uterine weight have to be assessed with caution because they can be associated with different stages of oestrus cycling and not just substance exposure. The available data and retrospective analysis suggests that the changes, some of them statistically relevant, reflect the organ status under different stages of the oestrus cycle. The testicular weights are also not thought to be due to substance treatment. There was no clear dose response and variability in testes weights are a common feature of young beagle dogs. The study had no information with regard to fertility indices or developmental milestones.

Summary of the dog data:

- Adverse effects on reproductive organs in dogs after 1-year exposure (↓ weight of uterus, ovaries and testis, reaching statistical significance for uterus and testis).
- Retrospective analysis suggested treatment groups were in different phases of the oestrus cycle, this may account for ↓ uterine weights.
- Testes weights are considered highly variable in young dogs → insufficient evidence for a treatment related effect.
- Not possible to carry out a robust evaluation of oestrus cyclicity, and hormone analysis was not performed.
- The dog study was never designed with conclusive reproductive parameters to be assessed, and no HCD was available.

The effects on reproductive organs in the dog were inconclusive and were not considered sufficient to support classification for reproductive effects.

On the basis of the data available, no classification for effects on fertility and sexual function is warranted. RAC however notes that the available 2-generation study does not fully inform on all endpoints.

Assessment of developmental toxicity

Rat developmental toxicity (teratogenicity) study

In a guideline-compliant rat developmental toxicity study (0, 20, 200, 1000 mg trinexapac-ethyl/kg bw/day from GD6-15), presumed treatment-related effects included:

1. Decreased number of corpora lutea.
2. A dose dependent increase of asymmetrically shaped sternebrae.
3. A statistically significant increase in unossified cervical vertebral centres.

(i) Decreased number of corpora lutea.

There was a statistically significantly decreased number of corpora lutea in the high dose group compared to the control group (table below). This incidence was at the lower bound limit of the

historical control data from the performing laboratory. Consultation of the 1993 Charles River Laboratories report on Repro HCD in the CD BR rat shows a mean corpora luteal number of 16.99 per dam, with a range of 13.80 – 20.00 from 1860 pregnant females from 96 studies.

Table: *Summary of pregnancy data – uterine findings*

dose (mg/kg)	group 1 0	group 2 20	group 3 200	group 4 1000
pregnant females on day 21	22	24	24	24
no. corpora lutea	420	448	448	408
mean no./dam	19.1	18.7	18.7	17.0**
no. impl.sites[1]	365	382	386	376
mean no./dam	16.6	15.9	16.1	15.7
pre-implantation loss[2] (%)	12.4	13.7	12.9	8.3
implantation efficiency (%)	87.6	86.3	87.1	91.7
resorptions				
no. ER[3]	14	10	19	17
mean no./dam	0.6	0.4	0.8	0.7
% of impl.sites	3.8	2.6	4.9	4.5
no. LR[4]	0	0	0	0
mean no./dam	-	-	-	-
% of impl.sites	-	-	-	-
combined resorptions	14	10	19	17
mean no./dam	0.6	0.4	0.8	0.7
% of impl.sites	3.8	2.6	4.9	4.5
no. dead fetuses[5]	0	0	0	0
mean no./dam	-	-	-	-
% of impl.sites	-	-	-	-
aborted fetuses	0	0	0	0
mean no./dam	-	-	-	-
% of impl.sites	-	-	-	-
post-implantation loss[6] (%)	4.0	2.8	4.6	4.9

** Statistically significant difference from control group mean, $p<0.01$

The number of corpora lutea were not reported in the rat 2-gen study and there was no evidence of that effect in the rabbit developmental toxicity study. Since ovulation occurs before the start of dosing and there were no pre-implantation losses in the top dose group, and also taking into account that the effect was just at the limit of the HCD, RAC considers the decrease in number of corpora lutea at the high dose group in this case insufficient to warrant concern.

(ii) A dose dependent increase of asymmetrically shaped sternebrae

Foetal external and visceral examinations revealed no treatment-related or toxicologically significant findings. Sternebral alterations are a frequent finding in prenatal toxicity studies, while in humans they are seldom observed. RAC notes the calcification of the bone in the rat starts from gestation day (GD) 16 and increases rapidly until GD20-21 (i.e., when the dams are euthanized, and litters are examined and sampled, GD21 in the present study).

Skeletal assessment in this study was accomplished following staining according to the Dawson's technique from 1926. After clearing with potassium hydroxide, the specimens were stained with alizarin red S and cleared with glycerol. This is still recognised as the "gold standard" for staining of the skeleton though in recent years the double staining, Alcian Blue- Alizarin Red, method, has become recognised to better distinguish calcification delays from actual alterations of the bone and cartilaginous structures (e.g., unossified from missing structures).

The DS stated in the CLH report that asymmetric sternebrae in rats and other species should be considered as a "grey zone anomaly", i.e. more information is required in order to characterise it as either a variation or a malformation. Implicit in this is the recognition that a malformation may be determined by considering postnatal persistence and adversity to health. This anomaly may be better described as a variation. The original description of this anomaly in the pathologist's report is ambiguous, it simply states "asym. asymmetrically shaped" and occurrences in sternobrae give reference to the location and are labelled "A" for anomaly. There is no distinction made between the two possible descriptions this labelling implies: does it refer to asymmetrical ossification of the sternabra (incomplete or increased ossification and therefore considered a variation) or does it refer to an asymmetrical structure of the sternabra (and therefore considered a malformation)? What is clear from the laboratory data is that 'asymmetrical sternabrae' are a fairly common finding in this strain of rat at this laboratory and that would suggest a variation, i.e. a generally transient unsymmetrical or incomplete and therefore delayed ossification. If there was a true developmental effect on the maturation of the sterno-vertebral axis, then RAC would expect to see more relevant skeletal defects and the involvement of many more ossification centres throughout the axial skeleton.

RAC agrees with the DS that the asymmetric sternal findings seen at 1000 mg/kg bw/day are likely to represent small deviations from normal sternal development. The delay in ossification at one site over another in the sternum is considered by RAC to be indicative of a delay in development; this may be an adverse effect, but it is probably not a predictor of teratogenic potential in this case. In agreement with the DS, RAC considers this effect is not sufficient to warrant a classification.

(iii) A statistically significant increase in unossified cervical vertebral centres

The incidences of unossified cervical vertebral centres (described as "still absent ossification for cervical vertebral centres") did not show any dose dependency. Very high background levels were observed across all dose groups. The high dose group foetal incidences were within the supplied HCD (10 inhouse control groups with a total of 234 pregnant females, 1985 – 1987). These variant findings were ultimately considered unrelated to treatment. RAC is in agreement with the DS and does not propose classification due to no evidence of a developmental malformation.

<u>Rabbit developmental toxicity (teratogenicity) study</u>

In a guideline-compliant rabbit developmental toxicity study (Anon., 1990), the maternal NOAEL was set at 60 mg/kg bw/day, based on increased mortalities and retarded body weight gain to day 15 at 360 mg/kg bw/day dose. At 360 mg/kg bw/day, 2/17 dams died (1 was found dead, another was killed). One dam at 60 mg/kg bw/day was killed on day 8 due to an intubation error. The mortalities were associated with substance irritation/damage to the stomach mucosa as the animals had either a haemorrhagic depression in the stomach or rupture of the stomach. The cause of death in the animal found on day 13 is uncertain, the study authors suggested a suspected convulsion, but a ruptured stomach is incompatible with survival in rabbits and could precipitate a convulsion.

Regarding developmental effects there was a statistically significant decrease in the number of live foetuses due to an increase in pre-implantation loss (%) and post-implantation loss (%) in

the top dose group compared to controls. The significance of this is uncertain; it is not assessed in a coherent way in the CLH report. Again, pre-implantation loss, as it occurs prior to treatment, is of low concern in this case. Without HCD (which has proven difficult to search for in the open literature) interpreting these findings is difficult.

Table: *Summary of pregnancy data – laprohysterectomy observations*

	Dose (mg/kg bw/d)				
	0	**10**	**60**	**360**	**HCD**
No. litters (viable)	12	16	14	14	none
Corpora Lutea/Doe:	10.5	10.9	10.9	10.2	
Pre-implantation Loss/Doe	1.7	1.7	1.8	2.6 (+52.9%)	
Pre-implantation Loss (%)	14.3	16.5	16.2	**24.3* (+70%)**	
Implantations/Doe	8.8	9.2	9.1	7.6 (-13.6%)	
Live Foetuses/Doe	7.7	8.4	7.0	**5.7* (-26%)**	
Total embryonic deaths/Doe	1.2	0.8	2.1	1.9	
Post-implantation Loss (%)	13.2	8.1	21.4	**24.8***	
Mean Foetal Weight (g)	44.4	43.8	47.0	45.2	
Sex Ratio (% Males per litter)	40.9	56.9	53.5	56.7	

* Statistically significant different trend from control group mean, $p<0.05$ (Jonckheere "J" statistic)

The effect on post-implantation loss (and therefore litter size) was statistically significant. However, in rabbits this parameter is subject to wide variability and the lack of HCD makes this value difficult to interpret. Looking at the raw data confirms that two does presented with extremely high values for post-implantation loss and that the resulting standard deviation for the high dose group is also quite large. It is difficult to appreciate how the original study achieved statistical significance.

Control group: mean = [13.18 ± 14.45] %

High dose group: mean = [24.75 ± 22.67] %

There were no treatment-related effects on total litter loss, sex ratios, litter weight or mean foetal weight. There were no treatment-related differences in the incidence or the type of malformations, grey zone anomalies or variants. Skeletal variants were looked at in more depth in the RAR but as can be seen from the results (table below), the incidence of 12 or 13 ribs was essentially similar among all dose groups. Although there was an apparent tendency for a slightly higher incidence of variant sternebrae, none of the differences obtained statistical significance. Because of the high variability and spontaneous incidence of these variants in rabbits, they should not be considered biologically significant. There were some signs of developmental toxicity, i.e., embryo lethality, found only at the highest dose tested. A statistically significant decrease in the number of live foetuses/doe was observed; 5.7 vs 7.7 in controls due to an increase in the % post-implantation loss, i.e. 24.8% vs 13.2% in controls. There was no HCD for comparison. RAC does not consider the observed effects on post-implantation loss to be sufficient for classification in this case. RAC concludes there is insufficient evidence to propose classification for developmental effects in rabbits.

Table 22: *Summary of Rabbit skeletal variants*

Group	Foetuses examined +	Foetuses with							
		12 Ribs		13 Ribs		Normal sternebrae		Variant sternebrae	
		No.	%	No.	%	No.	%	No.	%
1	88	44	45.8	44	54.2	79	84.8	9	15.2
2	131	58	44.1	73	55.9	110	81.7	21	18.3
3	91	46	50.0	45	50.0	73	81.6	18	18.4
4	78	38	53.0	40	47.0	63	79.3	15	20.7
Kruskal-Wallis 'H' statistic			NS		NS		NS		NS
Jonckheere 'J' statistic			NS		NS		NS		NS

NS Not significant, P>0.05
\+ Excludes malformed foetuses

RAC conclusion on classification for adverse effects on development

In the 2-generation study pup toxicity was evident with significantly reduced post-natal body weight until the end of the lactation period in two generations for both sexes (F1 pups: male -18.9%, female -20.5%, F2 pups: male -23.6%, female -24.1%). There was a reduced survival index in the offspring (F1, F2 pups) noted at the top dose (1293.0 mg/kg bw/day). These effects were not sufficient to support classification for development. There was no loss in body weight in pups and they continued to thrive post weaning to give rise to the parental F1 generation.

In a guideline-compliant rat developmental toxicity study treatment-related effects included a dose dependent increase of asymmetrically shaped sternebrae and a statistically significant increase in unossified cervical vertebral centres. These effects were not considered to constitute malformations and did not support classification for developmental toxicity.

In a guideline-compliant rabbit developmental toxicity study there was a statistically significant increase in post-implantation loss in the top dose group compared to controls. There was also an apparent tendency for a slightly but statistically non-significantly higher incidence of variant sternebrae. No malformations were evident and the increase in post implantation loss was not considered sufficient to support classification for developmental toxicity.

Assessment of effects on or via lactation

Post-natal body weight retardation.

It is important to remember that the rat 2-generation study is an old one and as such cannot be compared with the current guidelines. However, it is also important to note the study did not include endpoints such as oestrous cyclicity, sperm parameters, the age of vaginal opening and preputial separation so it is not possible to know if the reductions in body weight gain affected

the time of attainment of puberty or had some other effect on the normal reproductive cycle of the rat or if there were effects on these parameters independent of body weight development.

The DS did not comment in depth on effects by trinexapac-ethyl at the highest dose which included adverse developmental delays (reduced body weight) and reduced survival index in both the F1 and F2 offspring. At birth, the mean weight of pups in all treated groups was comparable to that of the controls so that there was no in-utero effect exerted by treatment with trinexapac-ethyl (also supported by results from the rat developmental toxicity study where there were no effects on foetal body weight at the time of laprohysterectomy, GD 21). But, from LD4 onwards body weight gain was statistically significantly decreased, (table below), i.e. growth retardation with decreased post-natal body weight at all measured time points during and at the end of the lactation period (F1 pups: male -18.9%, female -20.5%, F2 pups: male -23.6%, female -24.1%).

Table: *Rat Post-natal F1 and F2 pup % body weight changes with treatment*

Postnatal Day	Equivalent dose (mg/kg bw/day)									
	Males					Females				
	0	0.	106	663	1293	0	0.	106	663	1293
	F_1					F_1 Pups				
Birth	Ref.	+1.3%	+2.5%	+2.2%	-6.0%	Ref.	+0.3	+2.5	+2.0%	-5.2%
4 (pre-cull)	Ref.	+3.5 %	+0.6 %	--	**-24.2%***	Ref.	+2.9 %	+5.2 %	+1.4%	**-22.7%****
7	Ref.	+2.7%	+4.2%	+0.3%	**-24.3%***	Ref.	+3.1%	+6.9%	+2.0 %	**-23.2%****
14	Ref.	+1.3%	+2.8%	+0.6%	**-17.5%***	Ref.	+3.9%	+4.4%	+2.3 %	**-18.1%****
21	Ref.	+4.8%	+5.9%	+1.9%	**-18.9%***	Ref.	+4.2%	+5.1%	+1.7 %	**-20.5%****
	F_2					F_2 Pups				
Birth	Ref.	+5.8 %	+1.4 %	+3.0 %	-5.0%	Ref.	+3.5 %	-1.5%	-1.3%	**-6.3%***
4 (pre-cull)	Ref.	+8.1%	+2.8 %	+3.2 %	**-17.2%***	Ref.	+0.7 %	-2.2%	-3.1%	**-19.4%****
7	Ref.	+1.4%	+0.3%	-1.2%	**-20.8%***	Ref.	-0.4%	+0.3%	-2.5%	**-21.2%***
14	Ref.	+0.8%	+0.9%	-4.0%	**-19.7%***	Ref.	-0.8%	-1.1%	-5.8%	**-20.7%***
21	Ref.	+1.6%	-0.5%	-4.3%	**-23.6%***	Ref.	-1.5%	-2.2%	-6.5%	**-24.1%***

* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

In the highest dose group at all timepoints after birth the body weight reduction is statistically significant and consistent over both sexes and both generations, with effect sizes up to 24%, which is considered adverse. The DS believed there was no evidence from the two-generation reproductive study (Anonymous, 1991 (B.6.6.1.1)) for specific effects of trinexapac-ethyl treatment on lactation or via lactation on offspring. However, RAC notes significant effects were observed during that time in which the only nutritional source for the pups was via maternal lactation. The effect was consistent, across two generations and biologically and statistically significant.

Concern for the effect in the pups is reduced based on pup toxicity not being that much worse than maternal toxicity (decreased bwg 19-24% vs 10-18%). Maternal toxicity is demonstrated

in that the dams also show reductions in bwg relative to controls (table below), but the effect is greatest at PND0 or LD0 when there are small effects observed in the high dose pups and the situation steadily improves for the dams after LD7 while the reverse is true for the pups, their bwg reduction is near maximal from LD4 – LD21.

Table: *Reductions in maternal body weight during lactation*

	Postnatal Day[1]							
	LD0	**LD7**	**LD14**	**LD21**	**LD0**	**LD7**	**LD14**	**LD21**
	P generation				**F_1 Parents**			
High dose group	-18 %	-14 %	-12 %	-7 %	-14 %	-11 %	-9 %	-7 %

[1] There was no data for LD 4

The reductions in maternal body weight may have a bearing on the reductions in post-natal pup body weight.

It is important to consider whether the effects on pup body weight qualifies for classification for effects on or via lactation. When assessing the effect, the following observations were noted:

1. There was a reduced body weight in the F0 and F1 generation females (F0: premating -16.6%, gestation -14.2%, 7-day lactation -14.1%; F1: premating -16.0%, gestation -10.9%, 7-day lactation -14.2%).
2. In contrast, mean maternal corrected body weight and corrected body-weight gain for all test article treated groups were comparable to the control group at (gavage) doses up to 1000 mg/kg bw/day in the rat developmental toxicity study.
3. It does not seem to be a specific in-utero developmental effect, as there was a little effect on pup body weight at birth in the 2-generation study. There was also no *in utero* effect on mean foetal body weight at (gavage) doses up to 1000 mg/kg bw/day in the rat developmental toxicity study.
4. There was no loss in body weight amongst pups. All pups continued to thrive throughout PND 1-21.
5. The survival of pups at the top dose was only slightly significantly reduced under certain conditions - F1 pups (sexes combined, mean % surviving, days 4-21 (post-cull)) 92.4% relative to 97.8% in controls - F2 pups (females only, mean % surviving, days 0-4 (pre-cull)) 92,1% relative to 97.6% in controls.

Classification for developmental toxicity is not warranted, as the significant adverse effect on rat F1 and F2 postnatal pup bodyweight does not appear to affect later reproductive potential and body weight gain recovers. There appear to be no specific, long lasting developmental effects.

There was a small reduced survival index in the offspring (F1, F2 pups) noted at the top dose (table below). Reductions were apparent at all timepoints and in both sexes for F1 pups, being statistically significant only for sexes combined on LD 4-21. In the second generation F2 pups only females on LD 0-4 had a significant reduction in pup survival. There was no evidence of an effect on milk intake, necropsy results showed no substance related effect on pups presenting with no milk in stomach.

Table: *Rat Post-natal F1 and F2 pup mean % surviving*

Postnatal Day	Equivalent dose (mg/kg bw/day)									
	Males					Females				
	0	0.	106	663	1293	0	0.7	106	663	1293
	F_1 Pups					F_1				
0 - 4	96.9	96.8	93.8	99.3	87.1	96.8	96.2	97.6	98.0	92.7
4 - 21	97.8	100.0	98.9	97.0	93.0	97.8	100.0	97.7	99.0	93.0
Sexes combined 0 – 4 / 4 - 21	96.9	96.3	93.4	98.5	90.1	97.8	100.0	98.3	98.0	**92.4***
	F_2 Pups					F_2				
0 - 4	96.1	95.7	95.3	100.0	94.6	97.6	97.5	97.5	93.5	**92.1***
4 - 21	100.0	98.3	100.0	98.9	96.7	96.4	100.0	99.4	100.0	99.1
Sexes combined 0 – 4 / 4 - 21	96.9	96.5	96.9	97.5	93.6	98.3	99.2	99.4	99.5	97.7

* Statistically different from control, $p<0.05$

In summary, biologically relevant effects were seen in the pups from the top dose group. The effect on body weight gain reduction was consistent across two generations, affected both sexes and was accompanied by a small but significantly reduced survival index in the offspring.

<u>For classification for effects on or via lactation, the CLP criteria require:</u>

(i) Human evidence indicating a hazard to babies during the lactation period…and/or…
No such data from humans are available for trinexapac-ethyl.

(ii) Results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk…and/or…

There is no indication of behavioural changes in dams that could have affected weight gain development of the pups, as dams of all dose groups successfully reared their litters to weaning on day 21 postpartum. No information is available on the quantity or quality of the milk produced by the dams, nor was the rat milk analysed for the presence of trinexapac-ethyl or metabolites. The RAR studies in ruminants showed trinexapac-ethyl and its metabolites are not excreted in milk to an appreciable extent (in goats 0.01-0.02% of the totally administered dose; in cows approximately at the limit of quantification of 0.01 mg/kg). Given these results, it seems unlikely that trinexapac-ethyl or its metabolites would be transferred into the milk of rats. RAC notes that relative to the doses given to rats, the doses in the ruminant studies were rather low (goats were administered 0.2-20 mg/kg bw trinexapac-ethyl for 4 days, cows received 2, 5.6 or 20 mg/kg feed trinexapac-ethyl for 28-29 days).

(iii) Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk

The ADME studies in rats clearly show that trinexapac-ethyl is rapidly eliminated mainly via the urine and then faeces. The log P_{OW} of - 0.29 at neutral pH, indicates a low potential of

bioaccumulation and limited lipophilicity. There is no data to indicate how much trinexapac-ethyl or its metabolites would be transferred into the milk of rats.

The available data is non-conclusive with respect to linking the effects in the post-natal rat pup to lactation. Other possibilities may include a direct effect of pups consuming treated diet (or avoiding it, because of palatability reasons), as solid food intake starts from around LD14. While this may contribute to the reduced body weight development during the later phase of the lactation period, it does not fully explain the effect seen at LD4-7 when the pups are suckle-fed only. Another possibility is that the retarded body weight development is a secondary effect of maternal toxicity.

The cause of the reduced body weights in pups during the lactation period is not entirely clear. However, RAC does not consider the two-generation study in animals to provide clear evidence of a treatment related, adverse effect in the offspring. Transfer of the active substance into milk has not been demonstrated. The significant adverse effect on rat F1 and F2 postnatal pup bodyweight seen in the 2-generation study may be viewed in the context of an important postnatal growth delay but without significant impact on later maturation and fertility. A small reduction in survival index in the offspring (F1, F2 pups) was also noted at the top dose. There was no evidence that treatment with trinexapac-ethyl affected nursing behaviour; there was no indication of a lack of milk delivery to pups during the lactation phase as necropsy results indicated no treatment related increases in pups without milk in their gut. There is no information as to the cause of the reduction in survival index but the effect was small and possibly not biologically significant. RAC does not propose classification for adverse effects on or via lactation.

Conclusions on Classification of toxicity to reproduction

Sexual function and fertility

RAC notes that the available 2-generation study does not fully inform on all relevant reproductive endpoints (oestrous cyclicity, sperm parameters, the age of vaginal opening and preputial separation). RAC proposes **no classification** based on the available data.

Developmental toxicity

RAC concludes there is insufficient evidence to propose classification for developmental effects in rats and rabbits. RAC proposes **no classification** for adverse effects on development.

Effects on or via lactation

Classification for effects on or via lactation is not considered justified. RAC proposes **no classification** for adverse effects on or via lactation.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Trinexapac-ethyl is a synthetic compound belonging to the chemical group of ciclohexanediones and acts as a plant growth regulator to prevent lodging and brackling (crop leaning) in field crops, like cereals, oil seed rape, pulses and grass seeds for seed production. It is taken up by

plants, almost exclusively through the green portions and the growth regulatory activity is expressed in these tissues as an inhibition of internode elongation.

Trinexapac-ethyl does not currently have a harmonised classification. Based on the available data on aquatic toxicity, the dossier submitter (DS) proposed an environmental classification as Aquatic Chronic 1 (H410), M-factor=1.

Degradation

A hydrolysis study was conducted according to EPA Guideline No. 161-1, in compliance with GLP.

Trinexapac-ethyl is stable at pH 7 and quickly hydrolyses to trinexapac (CGA179500) under basic conditions (pH 9) with half-life values of 7.2 and 11.3 days. Under acidic conditions, trinexapac-ethyl slowly hydrolyses with half-life values of 514 and 221 days at pH 5 and 188 days at pH 4 at 25°C.

Two hydrolysis studies on the metabolite trinexapac are presented. Study results indicate that trinexapac is hydrolytically stable under neutral and alkaline conditions (pH 7 and pH 9). However, under acidic conditions, three metabolites have been observed over 10% AR: CGA113745 forms up to 18.6% AR (pH 4), CGA313458 forms up to 36.8% AR (pH 4) and CGA224439 (not unequivocally identified) up to 35% (pH 5). WaterM3Hydrolys (SYN549299) forms up to 23% AR after 64 days at pH = 4 and 24.7°C. The metabolite WaterM3Hydrolys is hydrolytically stable under acidic pH.

Aquatic photolysis was studied in sterile and natural buffered water under artificial light. In sterile water, trinexapac-ethyl is readily degraded with half-life value of 5.4 days (natural light, 50ºN). In natural water at irradiation equivalent to sunlight at latitude of 35°N, the half-life for trinexapac-ethyl was 15.3 days. One major photodegradant (3-ethoxycarbonylpentanedioic, acid CGA300405) was produced and progressively increased throughout the irradiation period, reaching a maximum of 83.4% of applied radioactivity by day 7.

In natural water at irradiation equivalent to sunlight at latitude of 30°N, two degradants were observed over 10% AR: 3-ethoxycarbonylpentanedioic acid up to 61% AR and citric acid (or isocitric acid) up to 11% AR. Citric acid and/or isocitric acid were observed using a protocol not in line with the current guidelines and which presented some major technical deficiencies (irradiation time and number of samples analysed). Therefore, citric acid and/or isocitric acid were not considered further.

One study was performed to determine the ready biodegradability of trinexapac-ethyl in a carbon dioxide evolution test in activated sludge in accordance with the Guideline 92/69/EEC C.4-C, ready biodegradability carbon dioxide evolution test (Baumann, W., 1993) and in conformity with GLP. Biodegradation of the test substance was 10% after 29 days, therefore trinexapac-ethyl is considered as not readily biodegradable.

In an aerobic surface water simulation study, carried out according to OECD TG 309 and GLP, trinexapac-ethyl mineralisation to CO_2 was low (did not exceed a 4% AR) and no other volatiles were detected (< 0.1% AR). Calculated DT_{50} values for trinexapac-ethyl in surface water were 21.2 - 25.9 days. In the sterile system, the DT_{50} for trinexapac-ethyl was 69.9 days.

An aerobic water/sediment study according to BBA IV (5-1, 1990) Guidelines and Pesticide Assessment Guidelines, Subdivision N. (1982) and GLP was conducted with 14C-trineaxpac-ethyl (on the applied radioactivity) in two water/sediment systems: one with Rhine water (pH 8.2) and sand sediment, and another with pond water (pH 8.5) and loam sediment. The maximum formation rate for the metabolite trinexapac was 48% and 64% in the pond and river systems, respectively. No other metabolites were detected in water/sediment systems above 5% AR. Adsorption to sediments is minimal with levels not being observed above 6.9% of AR for both trinexapac-ethyl and trinexapac. For trinexapac-ethyl, the single first order DT_{50} in water were

3.3 and 5.0 days (average 4.0 days) and the single first order DT_{50}, whole system were 3.7 and 5.1 days (average 4.4 days). For trinexapac, the first order DT_{50}s in the whole system were 14 and 18 days (average 16 days).

Aerobic degradation in soil of trinexapac-ethyl was investigated in four studies at 20°C. Trinexapac-ethyl readily degrades to trinexapac in aerobic soil degradation studies, with levels up to 93% AR. Trinexapac was subsequently mineralised to carbon dioxide and bound residues. Other than trinexapac, no metabolites have been detected over 5% AR. Under anaerobic conditions in soil dosed with the [cyclohexanedione-1,2,6-14C]-labelled trinexapac-ethyl, trinexapac was the major transformation product, with formation rate of 87% AR at 121 day, which in turn is stable under anaerobic conditions. Other metabolites were not detected at >5% of the applied radioactivity.

Based on the available information, the DS concluded that trinexapac-ethyl is not rapidly degradable.

Bioaccumulation

To evaluate the bioconcentration of trinexapac-ethyl in aquatic organisms, two different studies were conducted on the bluegill sunfish (*Lepomis Macrochirus*) using test guidelines equivalent to OECD TG 305. In the first study, the fish were exposed to 14C-ring-labeled trinexapac-ethyl in an aerated flow-through system for 28 days. Trinexapac-ethyl depurated rapidly from all tissues, with a half-life between 1 and 3 days, while complete elimination of the 14C-residues from the whole body tissues occurred after 7 days of depuration. BCFs (based on total radioactive residue) of 2.5, 11 and 6 were calculated in edible parts, non-edible parts and the whole fish, respectively.

In the second study, similar results were obtained using 14C-ring-labeled trinexapac-ethyl and the same experimental conditions. Extracted tissue fractions showed a predominant presence of the parent trinexapac-ethyl and its metabolite trinexapac. After 14 days, BCFs of 1.9, 9.9 and 5.5 were calculated in edible parts, non-edible parts and the whole fish, respectively, while after 28 days the BCF values from the same tissues were 1.4, 6.2 and 3.5, respectively. The BCF values were not normalised to the fish lipid content. However, even by using lipid contents from 1 to 10% the resultant BCFs are expected to range from 1.3 to 11, which is far below the CLP criterion of 500. This further indicates that trinexapac-ethyl has a low potential for bioaccumulation.

The log P_{ow} of the active metabolite trinexapac was determined by using the test OECD TG 107, while for trinexapac-ethyl both the OECD TG 107 and the OECD TG 117 methods were used. The octanol-water partition coefficient of trinexapac-ethyl was pH-dependent and at environmentally relevant pH-values, was far below 4 (log P_{ow} = -0.29 at pH 6.9). Similarly, the log P_{ow} for its metabolite trinexapac was below 4 (log P_{ow} = 1.8 at pH 1.8) and decreased at higher pH values. Apparently, the calculated log P_{ow} values indicate a low bioaccumulative potential in aquatic organisms (in all the cases the log P_{ow} is below the threshold of 4). However, since the substance is surface active (surface tension is <60 mN/m), the shake flask method is not adequate for log P_{ow} determination and this parameter cannot be used to evaluate the bioaccumulative potential of trinexapac. Despite this limitation, the studies on BCF determination are sufficient to ascertain that trinexapac has a low bioaccumulative potential in aquatic organisms. The summary of relevant information for bioaccumulation are listed in the table below.

Table : *Available bioaccumulation information*

Method	Species	Results	Remarks	Reference
Test freely adapted after: Subpart N, Environmental Chemistry Guideline Reference No. 165-4 and Laboratory Studies of Pesticide Accumulation in Fish (1982).	Bluegill sunfish (*Lepomis macrochirus*)	BCF is 6 L/kg wwt for whole fish tissue Uptake/depuration kinetics BCF is 100% after 14 days	BCFs in *Lepomis macrochirus* were 6 L/kg wwt for whole fish, 2.5 L/kg wwt for edible parts and 11 L/kg wwt for non-edible parts. Trinexapac-ethyl was demonstrated to have a low BCF in bluegill	Anonymous, 1990 CA8.2.2.3/01
Test freely adapted after: FIFRA 165.4	Bluegill sunfish (*Lepomis macrochirus*)	BCF is 3.5 L/kg wwt for whole fish tissue	Accumulation potential in aquatic organisms is considered to be low	Anonymous, 1991 CA8.2.2.3/02
OECD TG 117		at pH 6.9: log P_{ow} =- 0.29	Since the substance is surface active (surface tension <60 mN/m), the test is not adequate to determine Log Pow values	Kettner, 1999 Study no. 77863 (KCA 2.8/01)

Aquatic toxicity

Studies on acute and chronic aquatic toxicity of trinexapac-ethyl for all three trophic levels are available. The test results are summarised in the following table. Based on the acute aquatic toxicity studies for trinexapac, this metabolite is less toxic (48h LD_{50} >100 mg/L in the fish and invertebrates) than the parental trinexapac-ethyl and is not discussed further. The key tests forming the basis for classification are reported in bold.

Table : *Available aquatic toxicity information*

Method	Test organism	Test system	Results			Test conc.	Reference
			Endpoint	LC_{50}/EC_{50} [mg/L]	NOEC [mg/L]		
Fish							
US EPA/FIFRA Guideline 72-1	*Oncorhynchus mykiss*	Semi-static 96h	mortality	68		Nominal	Anonymous (1990)
US EPA/FIFRA Guideline 72-1	*Lepomis macrochirus*	Semi-static 96h	mortality	>130		Nominal highest concentration tested	Anonymous (1990a)
US EPA/FIFRA Guideline 72-1	*Cyprinus carpio*	Flow-through 96h	mortality	57		Nominal	Anonymous (1991)
US EPA/FIFRA Guideline 72-1	***Ictalurus punctatus***	**Flow-through 96h**	**mortality**	**35**		**Mean measured**	Anonymous (1991a)
EPA guideline No. 72-3	*Cyprinodon variegatus*	Flow-through 96h	mortality	180		Mean measured	Anonymous (1991b)
EPA guideline No. 72-4	***Pimephales promelas***	**Flow-through 35d**	**Egg hatchability, survival and growth**		**0.41**	**Mean measured**	Anonymous (1991)
Aquatic invertebrates							
US EPA/FIFRA Guideline 72-2	*Daphnia magna*	Semi-static 48h	Mortality (immobilization)	>142.5		Nominal concentration highest concentration tested	Smith *et al*. (1990)
EPA Guideline No. 72-3	*Crassostrea virginica*	Flow-through 96h	mortality	89		Mean measured	Dionne (1991)
EPA Guideline No. 72-3	***Americamysis bahia***	**Flow-through 96h**	**mortality**	**6.5**		**Mean measured**	**Sousa (1991)**
US EPA/FIFRA Guideline 72-4	*Daphnia magna*	Flow-through 21d	Mortality, Reproduction and growth		11	Mean measured	Putt (1991)
US EPA/FIFRA Guideline 72-4	***Daphnia magna***	**Flow-through 21d**	**Mortality, Reproduction and growth**		**2.4**	**Mean measured**	**Putt (1994)**
Algae and aquatic plants							
OECD TG 201	*Pseudokirchneriella subcapitata*	Static 72h	Growth rate	60	9.4	Mean measured	Maetzler (2001)
OECD TG 201 O.J. L383A, Part C.3: Algal inhibition test (1992) **US EPA Guideline OPPTS 850.5400 Algal Toxicity, Tiers I and II, (1996)**	***Pseudokirchneriella subcapitata***	**Static 96h**	**Growth rate**	**24.9 (72h)**	**8**	**Nominal**	**Cartee *et al*. (2009)**

Method	Test organism	Test system	Results			Test conc.	Reference
			Endpoint	LC_{50}/EC_{50} [mg/L]	NOEC [mg/L]		
OECD TG 201 (2006) EU Commission Directive 92/69/EEC	*Pseudokirchneriella subcapitata*	Static 72h	Growth rate	61	10	Nominal	Bätscher (2008) Adama study no. B93014
OECD TG 201 (2006)	*Pseudokirchneriella subcapitata*	Static 72h	Growth rate	41.6	10	Nominal	Scheerbaum (2008) Cheminova Report Doc. No.: 77 TPE
OECD TG 201 (2006)	*Anabaena flos-aquae*	Static 72h	Growth rate	>100	46	Nominal	Liedtka (2010) Adama study no. B92867
OECD TG 201 (2006)	*Anabaena flos-aquae*	Static 72h	Growth rate	214	100	Nominal	Scheerbaum (2008b) Cheminova Report Doc. No.: 76 TPE
FIFRA Guideline 122-2 and 123-2, ASTM E 1415-91 and OECD (draft December 1999)	*Lemna gibba*	Static 7d	Frond number Growth rate	27.4	2.3	Mean measured	Grade (2001)
OECD TG 221 (2006)	*Lemna gibba*	Static 7d	Frond number Growth rate	65	0.95	Mean measured	Bätscher (2008b) Adama study no. B92891
OECD TG 221 (2006)	*Lemna gibba*	Static 7d	Frond number Growth rate	36.1	1	Nominal	Scheerbaum (2008c) Cheminova Report Doc. No.: 78 TPE
OECD TG 239 (2014)	***Myriophyllum spicatum***	**Static 14d**	**Growth rate**	**1.2**	**<0.025 (NOEC) 0.011 (EC_{10})**	**Mean measured**	**Kirkwood (2015)**

Acute aquatic toxicity

For trinexapac-ethyl, five acute fish toxicity studies are available and included in the CLH report as reliable and adequate data for acute classification purposes.

The lowest reliable LC_{50} result for fish, the 96h LC_{50} of 35 mg a.s./L (mean measured concentrations), was determined in the acute toxicity study (Anonymous, 1991a) conducted on fresh water species *Ictalurus punctatus* according to US EPA/FIFRA Guideline 72-1. This study is provided in the CLH report as acceptable with fulfilled validity criteria and used as relevant key data for classification on acute aquatic toxicity.

In addition, some acute fish toxicity studies are also available with metabolites of trinexapac-ethyl (such as the major metabolite trinexapac), showing to be of lower toxicity (48h LD_{50} >100 mg/L) than the active substance.

Three acute aquatic invertebrates toxicity tests are available and reported as relevant, reliable and adequate for classification purposes. One study was performed on *Daphnia magna,* the other

studies were performed on the marine species *Crassostrea virginica* and *Americamysis bahia* (formerly *Mysidopsis bahia*).

The lowest reliable LC_{50} result, the 96h LC_{50} of 6.5 mg a.s./L, based on mean measured concentrations, was determined in a GLP-compliant flow-through test performed to EPA Guideline 72-3 in *Americamysis bahia* (Sousa, 1991).

For aquatic invertebrates, low levels of acute toxicity (48h LD_{50} >100 mg/L) are also detected on the metabolites trinexapac and 3-ethoxycarbonylpentanedioic acid, as reported in Draft Assessment Report vol.3-B.9

For algae and aquatic plants, six algal studies are available, four of which are for *Pseudokirchneriella subcapitata* and two for *Anabaena flos-aquae*. Four aquatic plant studies are available, three for *Lemna gibba* and one for *Myriophyllum spicatum*.

The lowest E_rC_{50} for algae was the 72h E_rC_{50} of 24.9 mg a.s./L in *Pseudokirchneriella subcapitata*, based on nominal concentrations.

The lowest 14d $E_rC_{50\ for}$ aquatic plants was observed in *Myriophyllum spicatum* with a value 1.2 mg a.s./L, based on shoot length and mean measured concentrations.

Based on the available information, which is all above 1 mg/L, the DS proposed no classification for acute aquatic hazard.

Chronic aquatic toxicity

A single chronic toxicity study to fish (Anonymous, 1991) performed with trinexapac-ethyl is provided in the CLH Report with a chronic 35d NOEC value of 0.41 mg a.s/L (mean measured concentration), based on development and growth parameters. This was determined in a fish early life-stage toxicity study with the Fathead minnow (*Pimephales promelas*) according to EPA/FIFRA Guideline No. 72-4 and GLP. This study is regarded as reliable and adequate.

For aquatic invertebrates, two chronic toxicity studies are available in the CLH report, both performed on freshwater *Daphnia magna* and regarded as relevant, reliable and adequate for classification purposes. The lowest chronic result, 21d NOEC value of 2.4 mg a.s/L (mean measured concentration) based on mortality, reproduction and growth parameters was obtained in a flow-through test performed according to US EPA/FIFRA Guideline 72-4 and GLP compliant (Putt A.E., 1994).

The most sensitive chronic endpoint for algae and aquatic plants was a 14 days NOEC of <0.025 mg/L obtained in aquatic macrophyte *Myriophyllum spicatum* for growth rate. The result is based on the mean measured concentrations.

Based on the NOEC of <0.025 mg/L for *Myriophyllum spicatum* and trinexapac-ethyl being not rapidly degradable, the DS proposed a classification of Aquatic Chronic 1, with an M-factor of 1 ($0.01 < NOEC \leq 0.1$).

Comments received during public consultation

During Public Consultation, four Member States (MSs) commented on the proposals for Aquatic classification. One of these agreed with the proposed environmental classification. The other three MSs agreed to the proposed classification but supported the use of the E_rC_{10} of 0.011 mg/L instead of the NOEC value of <0.025 mg/L for the growth rate endpoint of the chronic study on *Myriophyllum spicatum.* As outlined in the CLP guidance, this E_rC_{10} value is more appropriate and supports the same chronic classification and chronic M-factor than currently proposed in the CLH report. The DS agreed that the proposed classification should be based on the more appropriate E_rC_{10}, noting that the proposed classification is not altered.

Assessment and comparison with the classification criteria

Degradation

The substance is hydrolytically stable at pH 7, it quickly hydrolyses to trinexapac (CGA179500) under basic conditions (pH 9), while it slowly hydrolyses under acidic conditions. The substance is not readily biodegradable and it is not ultimately degraded to a level greater than 70% over 28 days in surface water, water/sediment and soil simulation studies. Although hydrolysis occurred rapidly in the surface water simulation test, the study was performed under alkaline conditions, which facilitates hydrolysis. Therefore, it is expected that an aerobic water/sediment simulation test performed under neutral pH values (which are the most relevant for the environment) would have resulted in a significantly reduced degradation rate of trinexapac-ethyl and a half–life > 16 days cannot be excluded. The only metabolite formed under these experimental conditions, is trinexapac. Based on the acute ecotoxicological studies, this metabolite is less toxic (48h LD_{50} >100 mg/L in the fish and invertebrates) than the parental trinexapac-ethyl.

RAC agrees with the DS proposal to consider trinexapac-ethyl as not rapidly degradable.

Bioaccumulation

The BCF_{fish} of trinexapac-ethyl is below the threshold limit of 500. Available log P_{ow} values are also below the CLP criterion of 4, although as trinexapac-ethyl is surface active and the values were obtained using the shake flask method they cannot be used for determining the bioaccumulation potential.

Therefore, based on the available BCF data RAC agrees with the DS proposal that trinexapac-ethyl has a low bioaccumulation potential.

Aquatic toxicity

Acute aquatic hazard

Valid and reliable data are available for fish, invertebrates, algae, and aquatic plants. The lowest acute aquatic toxicity values for each trophic level are all above 1 mg/L. The lowest endpoint is a 14d shoot length E_rC_{50} = 1.2 mg a.s./L for *Myriophyllum spicatum*. This is above the trigger value of 1 mg/L for acute classification. Therefore, **trinexapac-ethyl does not warrant classification for acute aquatic hazard**.

Chronic aquatic hazard

The substance is considered to be not rapidly degradable and has a low potential for bioaccumulation.

Valid and reliable data are available for fish, invertebrates, algae, and aquatic plants. The lowest chronic endpoint is the E_rC_{10} of 0.011 mg/L for *Myriophyllum spicatum*. As outlined in the PC comments, the DS accepts that this is a more appropriate value on which to base their proposal, which is not altered as a consequence. RAC agrees that the E_rC_{10} is a more appropriate value and it is used for the classification of trinexapac-ethyl.

A surrogate approach can be applied for aquatic invertebrates using a LC_{50} of 6.5 mg a.s./L for *Americamysis bahia; in this case, the resulting chronic classification is Aquatic chronic 2, which is less stringent than the Aquatic Chronic 1 proposal outlined above.*

Therefore, based on the E_rC_{10} of 0.011 mg/L for *Myriophyllum spicatum* and considering that trinexapac-ethyl is not rapidly degradable, it warrants classification as Aquatic Chronic 1, with an M-factor of 1 ($0.01 < ErC_{10} \leq 0.1$ mg/L).

In conclusion, **RAC agrees with the DS that trinexapac-ethyl warrants classification as Aquatic Chronic 1 (H410), M=1**.

ANNEXES:

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

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