Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

tribenuron-methyl (ISO); methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylsulfamoyl]benzoate

EC Number: 401-190-1
CAS Number: 101200-48-0

CLH-O-0000001412-86-238/F

Adopted

14 September 2018

14 September 2018

CLH-O-0000001412-86-238/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: **tribenuron-methyl (ISO); methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylsulfamoyl]benzoate**

EC Number: **401-190-1**

CAS Number: **101200-48-0**

The proposal was submitted by **Sweden** and received by RAC on **26 June 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Sweden 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 **19 July 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 September 2017.**

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Marja Pronk**

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 **14 September 2018** by **simple majority**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	607-177-00-9	tribenuron-methyl (ISO); methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylsulfamoyl]benzoate	401-190-1	101200-48-0	Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H317 H400 H410	GHS07 GHS09 Wng	H317 H410		M=100	
Dossier submitters proposal	607-177-00-9	tribenuron-methyl (ISO); methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylsulfamoyl]benzoate	401-190-1	101200-48-0	**Retain** Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2	**Retain** H317 H400 H410 **Add** H373	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H317 H410 **Add** H373		**Retain** M=100 **Add** M=100	
RAC opinion	607-177-00-9	tribenuron-methyl (ISO); methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylsulfamoyl]benzoate	401-190-1	101200-48-0	**Retain** Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2	**Retain** H317 H400 H410 **Add** H373	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H317 H410 **Add** H373			
Resulting Annex VI entry if agreed by COM	607-177-00-9	tribenuron-methyl (ISO); methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylsulfamoyl]benzoate	401-190-1	101200-48-0	Skin Sens. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H317 H373 H400 H410	GHS07 GHS08 GHS09 Wng	H317 H373 H410		M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Tribenuron-methyl is an active substance in the meaning of Regulation EC 1107/2009. It is used as a herbicide on a wide range of crops. It has an existing entry in Annex VI of the CLP Regulation. This CLH proposal aims at modifying the existing classification based on data submitted as part of the pesticide renewal process (partly old, partly new as compared to the original application).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Tribenuron-methyl is not flammable, and is not reported (experience in handling) to self-ignite or, upon contact with water, to emit flammable gases. Therefore, the Dossier Submitter (DS) concluded that no classification is required.

Comments received during public consultation

One comment from IND was received supporting the 'no classification' proposal for physical hazards.

Assessment and comparison with the classification criteria

Tribenuron-methyl does not have flammable or pyrophoric properties and does not emit flammable gases upon contact with water. RAC therefore supports the non-classification for these physical hazards, as proposed by the DS.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity studies via the oral, dermal and inhalation routes were conducted in rats. An acute oral neurotoxicity study with tribenuron-methyl in rats was also available.

Oral

In a GLP and OECD TG 401 compliant study (RAR Vol. 3, B.6.2.1/01; DuPont-3343, 1999), fasted Crl:CD®(SD)IGS BR rats (5/sex) were treated by gavage with tribenuron-methyl in corn oil at a single dose level of 5 000 mg/kg bw. No mortalities were observed, and no gross lesions were present in the rats at necropsy. Male rats exhibited no clinical signs of toxicity during the study, while one female showed a red-stained face at day 2. In the four other females clinical signs started from day 8 and included red-stained head, hunched over posture, and ruffled fur. No

clinical signs were observed after day 10. Weight loss up to 9 % from day 2 until day 11 was registered in females, but the final body weight surpassed their fasted body weight. The oral LD_{50} value was > 5 000 mg/kg bw for both sexes.

In a GLP and OECD TG 424 compliant acute neurotoxicity study (RAR Vol. 3, B.6.7.1.1/01; DuPont-31859, 2011), Crl:CD(SD) rats (12/sex/dose) were treated by gavage with a single tribenuron-methyl dose of 0, 100, 300 or 1 000 mg/kg bw (vehicle 0.5 % methyl cellulose). No mortalities occurred. Whereas an initial depression in motor activity duration in both male and female rats given 1 000 mg/kg bw of tribenuron-methyl was observed on day 0 (2 hours after dosing), this decreased activity was not present on day 7 or day 14. Treatment was associated with transient reductions in bodyweight in high dose females and in food consumption and food efficiency in mid and high dose males and females. No treatment-related findings were noted during FOB investigation.

Dermal

In a GLP and OECD TG 402 compliant study (RAR Vol. 3, B.6.2.2/02; DuPont-3366, 1999), Crl:CD®(SD)IGS BR rats (5/sex) were exposed to a single dermal application (for 24 hours) of tribenuron-methyl (moistened in mineral oil) at a dose of 5 000 mg/kg bw. No mortalities occurred and no gross lesions were found at necropsy. Clinical signs of toxicity were not observed during the study. However, several rats exhibited wet or yellow-stained perineum, swollen face or legs, ocular or nasal discharge, or stained fur on the day of and the day after application of tribenuron-methyl. Mild and/or moderate erythema was observed in four rats, one of which also exhibited mild oedema. Focal eschar, desquamation, hyperkeratosis and epidermal scaling were also observed during the study, but all dermal effects had disappeared by day 13. Weight loss of approximately 4-10 % of initial body weight was observed in the rats the day after application. The findings were considered to be due in part to the wrapping procedure (gauze patch wrapped with stretch gauze bandage and self-adhesive bandage). The dermal LD_{50} was > 5 000 mg/kg bw for both sexes.

Inhalation

In a GLP and OECD 403 compliant study (RAR Vol. 3, B.6.2.3/01; DuPont-3090, 1999), Crl:CD®(SD)IGS BR rats (5/sex) were exposed nose-only to an atmosphere (dust) of tribenuron-methyl (6.0 mg/L; MMAD 2.8 or 2.7 µm) for a single 4-hour period. All rats showed a slight body weight loss the day following exposure, followed by an overall weight gain by the end of the 14-day recovery period. No clinical signs were observed, except for eye, nasal and/or oral discharges, irregular respiration and stained fur immediately after exposure. Gross pathological examination revealed no evidence of organ-specific toxicity, and no deaths were reported. The LC_{50} was > 6.0 mg/L for both sexes.

Conclusion

With LD_{50}/LC_{50} values above the cut-off for classification (2 000 mg/kg bw for the oral and dermal route, 5 mg/L for inhalation of dusts and mists), the DS concluded that tribenuron-methyl should not be classified for acute toxicity via any route.

Comments received during public consultation

One comment from industry was received supporting the ‘no classification’ proposal for acute toxicity.

Assessment and comparison with the classification criteria

No deaths were reported in any of the acute toxicity studies (oral, dermal and inhalation), nor in the acute oral neurotoxicity study. RAC notes though that deaths were reported in two pilot teratogenicity studies in rabbits, as described in section 10.10.3 of the CLH report as part of the main developmental toxicity study in rabbits (RAR Vol. 3, B.6.6.2.2/01; HLR 150-86, 1986). These deaths were observed at dose levels that, would the deaths have occurred within the first three days of administration, would qualify for classification for acute toxicity. However, no information is available on the time of these deaths in the original study report of the main study. As it is not possible to determine whether these could be considered early deaths, or are rather the result of repeated dosing, they are not taken into account for the acute toxicity endpoint.

Given that the LD_{50}/LC_{50} values in the standard acute toxicity studies do not fulfil the criteria for classification, RAC supports **no classification for acute toxicity (all routes)**, as proposed by the DS.

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

Summary of the Dossier Submitter's proposal

There are three acute toxicity studies (oral, dermal and inhalation) and one acute oral neurotoxicity study in rats available investigating the effects of a single dose of tribenuron-methyl. The results of these studies have been described in detail in the section on 'Acute toxicity' above.

No clear evidence of specific toxic effects on organs was reported in the acute toxicity studies. Clinical signs of toxicity were transient in nature and considered to be unspecific signs of general acute toxicity. The initial depression in motor activity duration in both male and female rats given 1 000 mg/kg bw in the acute neurotoxicity study may give some indications for neurotoxic effects. However, the effect was only transient in nature (observed on day 0, not on days 7 or 14). The DS therefore proposed no classification for STOT SE.

Comments received during public consultation

One comment from IND was received supporting the 'no classification' proposal for STOT SE.

Assessment and comparison with the classification criteria

In the acute toxicity studies, treated animals showed a variety of clinical signs, all of which considered to be indicative of general, non-specific toxicity and not fulfilling the criteria for classification with STOT SE 1 or 2. Classification for STOT SE 3 is also not warranted, as no signs of respiratory tract irritation were observed in the acute studies available, and the depression of motor activity duration observed in the acute neurotoxicity study, despite being transient, does not fulfil the criteria for narcotic effects. RAC therefore supports the conclusion of the DS that tribenuron-methyl **should not be classified for specific target organ toxicity – single exposure (STOT SE)**.

RAC evaluation of skin corrosion / irritation

Summary of the Dossier Submitter's proposal

In a GLP and OECD TG 404 compliant study (RAR Vol. 3, B.6.2.4/01; HLR 376-94, 1994), rabbits (New Zealand White, 6 male) were dermally exposed to 0.5 g of tribenuron-methyl for 4 hours. Only in one rabbit a very slight effect was observed, the animal showing grade 1 erythema (but no oedema) only at 1 hour after patch removal, not thereafter. The mean individual scores over 24/48/72 hours for erythema and oedema were 0 in all six animals. Further, no adverse clinical signs of toxicity were observed in any of the rabbits during the study. One rabbit exhibited weight loss of approximately 9 % of initial body weight by day 3 after application of tribenuron-methyl.

Given that for all animals the mean individual scores over 24-72 hours for both erythema and oedema were 0 and thus below the cut-off of 2.3 for classification, the DS concluded that tribenuron-methyl does not fulfil the classification criteria for skin corrosion/irritation.

Comments received during public consultation

One comment from IND was received supporting the 'no classification' proposal for skin corrosion/irritation.

Assessment and comparison with the classification criteria

In the one study available, tribenuron-methyl tested negative for skin irritation. RAC therefore supports the conclusion of the DS that **tribenuron-methyl should not be classified for skin irritation**.

RAC evaluation of skin corrosion / irritation

Summary of the Dossier Submitter's proposal

In a GLP and OECD 405 compliant study (RAR Vol. 3, B.6.2.5/01; HLR 627-87, 1987), rabbits (New Zealand White, 6 male) were treated with 0.1 mL (50 mg) of tribenuron-methyl in the lower conjunctival sac of the right eye. At 1 hour post exposure, corneal opacity (grade 1) was seen in two rabbits, mild conjunctival redness (grade 1) in six rabbits and slight chemosis (grade 1) in four rabbits. At later time points, only grade 1 conjunctival redness was observed, in two rabbits at 24 hours and in one rabbit at 48 hours. No effects on the iris were noted. The treated eyes of all animals were normal 72 hours after treatment. The mean individual scores over 24/48/72 hours for corneal opacity, iritis and conjunctival chemosis were 0 in all six animals, and for conjunctival redness this was 0 in four rabbits and 0.67 and 0.33 in the remaining two rabbits.

The DS concluded that tribenuron-methyl does not fulfil the criteria and should therefore not be classified for eye damage/irritation.

Comments received during public consultation

One comment from IND was received supporting the 'no classification' proposal for eye damage/irritation.

Assessment and comparison with the classification criteria

In the one study available, only slight, transient effects on the cornea and conjunctivae were observed. The mean individual scores over 24/48/72 hours for cornea opacity, iritis, conjunctival redness and conjunctival oedema were below the cut-off values for classification (1, 1, 2 and 2, respectively) in all six animals. RAC therefore supports the conclusion of the DS that **tribenuron-methyl should not be classified for eye irritation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of tribenuron-methyl was assessed in one Local Lymph Node Assay (LLNA) in the mouse and in two studies in the guinea pig (Buehler method and Magnusson and Kligman maximisation test). All three studies were GLP-compliant and basically followed OECD TG 429, 406 and 406, respectively.

LLNA in mouse (RAR Vol. 3, B.6.2.6/04; 190 TBM amdt-1, 2010)

In an OECD TG 429 compliant study, mice (CBA/J; 5 females/group) were treated topically with tribenuron-methyl (5, 10 or 20 %), vehicle control (propylene glycol) or positive control (α-hexylcinnamaldehyde). The dose of tribenuron-methyl was based on a preliminary study in which the highest tolerable concentration was determined to be a 20 % w/w mixture in propylene glycol. Treatment with tribenuron-methyl resulted in Stimulation Indices (SI) of < 3.0. A positive response (SI: 5.03) was observed in animals that received the positive control.

NB: in the RAR, the RMS questioned whether the doses tested were high enough, as the results seen with 20 % tribenuron-methyl in the preliminary test were only erythema score 1, on day 2 and 3 in two out of three mice. No higher concentration was tested.

Guinea pig – Buehler method (RAR Vol. 3, B.6.2.6/01; HLR 712-87, 1988)

Guinea pigs (Dunkin-Hartley albino; 10/sex/group in treatment group, 5/sex/group in control group, according to OECD TG 406) were treated for topical induction with 0.4 mL tribenuron-methyl (equivalent to approximately 0.25 g; corresponding to 62.5 %), vehicle control (dimethyl phthalate) or positive control (0.3 % 1-chloro-2,4-dinitrobenzene) for once a week for 3 consecutive weeks, for a total of three 6 hour treatments. A challenge was carried out two weeks after the last induction treatment using the same exposure protocol. A rechallenge, one week following the first challenge phase, was conducted for the tribenuron-methyl treated animals only. The concentrations of tribenuron-methyl and vehicle were based on a preliminary study.

Tribenuron-methyl administered epicutaneously to guinea pigs in the first challenge phase, induced a slight to moderate patchy response in 4/20 animals (20 %) of the test group after 24 hours and in 12/20 animals (60 %) after 48 hours. Following rechallenge a slight to moderate patchy response was recorded in 2/20 animals (10 %) after 24 and 48 hours. In the vehicle control group only one animal was recorded with a slight patchy response after 48 hours in the first challenge. The positive control showed a moderate to severe skin reaction in all animals after both 24 and 48 hours (this group was not rechallenged).

Guinea pig - Magnusson and Kligman maximisation test (RAR Vol. 3, B.6.2.6/03; 1986)

Guinea pigs (Dunkin-Hartley albino, female; 20/group in treatment group, 10/group in control group, according to OECD TG 406) were treated with a 2 % intradermal induction concentration

of tribenuron-methyl or a vehicle control (acetone). Before challenge, mild irritation was induced by sodium dodecyl sulphate. As challenge concentration, a 0, 5, 20 or 50 % concentration of tribenuron-methyl in propylene glycol was used. The concentrations of tribenuron-methyl and vehicle were based on a preliminary study. A positive control was not included in this study.

Tribenuron-methyl diluted to 50 % (w/v) in propylene glycol and administered epicutaneously to guinea pigs in the challenge phase, induced positive skin reactions in 17 of the 19 surviving animals (89 %) in the treatment group. Positive reactions to the 20 % concentration were seen in 9 of the 19 animals (47 %). All control animals were negative and showed no skin reactions.

NB: Neither the RAR nor the CLH-report give a result for the 5 % concentration group; supposedly no skin reactions were observed in this group.

Conclusion

The DS considered that, based on the positive results of the Guinea pig maximisation test (GPMT; ≥ 30 % responding at > 1 % intradermal induction dose) and Buehler assay (≥ 15 % responding at > 20 % topical induction dose), tribenuron-methyl fulfilled the criteria for classification as a skin sensitiser in subcategory 1B. However, since category A cannot be excluded (a high response rate of 89 % was observed in the GPMT at an intradermal induction dose of 2 %; a lower induction dose was not tested, but could possibly result in a response fulfilling the criteria for category 1A), the DS concluded that tribenuron-methyl should be classified as a skin sensitiser in category 1 without subcategorisation.

Comments received during public consultation

One MSCA and one IND commented on this endpoint, both supporting the proposal to retain the current classification for skin sensitisation 1.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the positive results of the GPMT and the Buehler assay warrant classification of tribenuron-methyl as a skin sensitiser. Although the positive results fulfil the criteria for subcategory B, RAC supports the argumentation by the DS that subcategory 1A cannot be excluded, and therefore supports the DS proposal for **Skin Sens. 1; H317**.

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

Summary of the Dossier Submitter's proposal

Eight repeated dose oral toxicity studies (almost all under GLP and guideline compliant) were available; three in rats (for 28 days, 90 days, and 2 years), three in mice (for 28 days, 90 days, and 18 months) and two in dogs (for 90 days and 1 year). Other relevant oral studies were a 28-day repeated dose immunotoxicity study in rats and a 90-day repeated dose neurotoxicity study in rats. An oral developmental toxicity study in rabbits also resulted in effects relevant for this endpoint. For the dermal route, one 28-day repeated dose dermal toxicity study was available in rabbits. The table below presents the effects in these studies at doses relevant for classification.

Table: *Summary of repeated dose toxicity studies with tribenuron-methyl*

Study	**Dose levels**	**Target organ(s)** **NOAEL/C**	**Effects at doses relevant for classification**
ORAL			
28-day (gavage) SD rat (6/sex/group) EEC Method B.7 (1984) GLP (Study RAR Vol. 3 B.6.3.1.1/01; NOTOX 0382/534, 1986)	Main study: 0, **100**, **300**, 1 000 mg/kg bw/d Parallel study: 0, **30**, **100**, **300** mg/kg bw/d Guidance value for classification **≤ 300** mg/ kg bw/day	Liver Study limited, selected as dose-range finding; no NOAEL derived	<u>30 mg/kg bw/d</u> None <u>100 mg/kg bw/d</u> - organ weight changes (↑rel. liver weight, 11 %, F) <u>300 mg/kg bw/d</u> ↓bw gain (f: 36 %) ↓food consumption (f) - changes in biochemical parameters (↑ALT (m,f), ↑Ca serum (m,f), ↓total bilirubin (m)) - organ weight changes (↑rel. liver weight, (m,f), ↑abs liver weight (f)) - histopathological changes in the liver (hypertrophy of centrilobular hepatocytes (m,f))
28-day (diet) - immunotox Crl:CD(SD) rat; female (10/group) OPPTS 870.7800 (1998) GLP (Study RAR Vol. 3 B.6.8.2.2/01; DuPont-31858, 2011)	0, 50, 150, 300 and 600 ppm equal to 0, 3.8, 11, 23 and 44 mg/kg bw/d Guidance value for classification ≤ 300 mg/ kg bw/d	None (only bw and food consumption affected) NOAEL 300 ppm	<u>50/150/300 ppm</u> None <u>600 ppm</u> - mean body weights ↓7.4 % (day 28) - body weight gains ↓24.3 % (days 0-28) - food efficiency ↓15.9 %
90-day (diet) Crl:CD(SD)BR rat (16/sex/group) Non-guideline, non-GLP (Study RAR Vol. 3 B.6.3.2.1/01; HLR 413-83, 1985/2000 (suppl.))	0, 100, 1 750 and 5 000 ppm equal to m: 0, 7, 118 and 335 mg/kg bw/d f: 0, 8, 135 and 386 mg/kg bw/d Guidance value for classification ≤ 100 mg/ kg bw/d	Liver, spleen LOAEL 100 ppm	<u>100 ppm</u> - organ weight changes (↑rel. spleen weight, f: 21 %) <u>1 750 ppm*</u> - clinical signs (coloured nasal discharge, M) - ↓bw (m: 20 %, f: 23 %); ↓bw gain (m: 27 %, f: 39 %); ↓food consumption (m,f; not stat. sign.)

			- changes in haematological parameters (↑platelet counts at 1 and 3 months (f)) - changes in biochemical parameters (↓glucose (m,f), ↓total protein (m), ↓serum globulin (m), ↑serum cholesterol (f)) - organ weight changes (↑rel. brain weight (m,f), ↑rel. heart weight (m,f), ↓abs. heart weight (m,f), ↑rel. liver weight (f), ↓abs liver weight (m,f), ↑rel kidney weight (m,f), ↓abs kidney weight (m,f), ↑rel testes weight, ↑rel spleen weight (f:26 %))
90-day (diet) – neurotox Crl:CD(SD) rat (12/sex/dose) OECD TG 424 GLP (RAR Vol. 3 B.6.7.1.2/01; DuPont-33371, 2013)	0, 50, 200, 700 ppm equal to m: 0, 2.8, 11.3 and 40.1 mg/kg bw/d f: 0, 3.2, 12.8 and 46.6 mg/kg bw/d Guidance value for classification ≤ 100 mg/kg bw/d	None (only bw affected) NOAEL 200 ppm	<u>50/200 ppm:</u> None <u>700 ppm:</u> ↓bw (m: 12 %, f: 13 %); ↓bw gain (m: 20 %; f: 29 %); ↓food consumption (m,f)
2-year (diet) Crl:CD/BR rat (72/sex/group; interim sacrifice of 14/sex/group after 52 weeks) OECD 453 GLP (RAR Vol. 3 B.6.5.1/01; HLR 61-87, 1987)	0, 25, 250 and 1 250 ppm equal to m: 0, 0.95, 10 and 55 mg/kg bw/d f: 0, 1.2, 13 and 76 mg/kg bw/d Guidance value for classification ≤ 12.5 mg/ kg bw/d	Mammary gland (neoplastic effects; although based on histopathology several organs affected, a target organ for non-neoplastic effects was not identified) NOAEL 25 ppm	<u>25 ppm</u> None <u>250 ppm</u> - reduced bw (m:-9 %, f:-21 %) - Organ weight changes (rel ↑brain, rel ↑heart, rel ↑liver, rel ↑spleen, rel ↑kidneys) - Non-neoplastic histopathological findings: mineralisation of stomach and aorta
28-day (diet) Crl:CD®-1(ICR)BR Mouse (20/sex/group: the first 10 mice sacrificed after 28 days, the remaining 10 mice of the 0, 125, 500 and 2 500 ppm group sacrificed after 90 days; see below for 90-day data)	0, 125, 500, 1250, 2 500 and 5 000 ppm equivalent to 0, 25, 100, 250, 500 and 1 000 mg/kg bw/d Guidance value for classification ≤ 300 mg/ kg bw/d	Liver, spleen Study limited, selected as dose-range finding; no NOAEL derived	<u>125/500 ppm</u> None <u>1 250 ppm</u> - organ weight changes (↑rel liver weight (m: 11 %, f: 11 %))

U.S. EPA 82-1 (1982) Non-GLP (Study RAR Vol. 3 B.6.3.2.2/01; HLR 580-85, 1985)			
90-day (diet) CD-1 Mouse (10/sex/group) U.S. EPA 82-1 (1982) Non-GLP (Study RAR Vol. 3 B.6.3.2.2/01; HLR 580-85, 1985)	0, 125, 500 and 2500 ppm equal to m: 0, 18, 70 and 350 mg/kg bw/d f: 0, 24, 90 and 476 mg/kg bw/d Guidance value for classification ≤ 100 mg/ kg bw/d	Liver NOAEL 500 ppm	<u>125/500 ppm</u> None
18-month (diet) Crl:CD®-1(ICR)BR Mouse (80/sex/group) OECD 453 GLP (RAR Vol. 3 B.6.5.2/02; HLR 60-87, 1987)	0, 20, 200 and 1 500 ppm equal to m: 0, 2.5, 25 and 197 mg/kg bw/d f: 0, 3.1, 31 and 247 mg/kg bw/d Guidance value for classification ≤ 16.7 mg/ kg bw/d	NOAEL 20 ppm	<u>20 ppm</u> None
90-day (dietary) Beagle dog (4/sex/group) US EPA 82-1 (1982) GLP (RAR Vol. 3 B.6.3.2.3/01; HLO 514-85, 1985/2000 (suppl.))	0, 50, 500 and 2 500 ppm equal to m: 0, 1.5 15 and 73 mg/kg bw/d f: 0, 1.6, 15 and 78 mg/kg bw/d Guidance value for classification ≤ 100 mg/ kg bw/d	Thyroid, haematological system NOAEL 500 ppm	<u>50/500 ppm</u> None <u>2 500 ppm</u> - ↓bw (m: 6 % (not stat. sign.)) - haematological changes (↑mean platelets and leukocyte counts at month 3, decreased mean corpuscular haemoglobin concentration at month 2, m: 2 % (not stat. sign.)) - organ weight changes (↑absolute thyroid/parathyroid weights (m: 30 % (not stat. sign.), f: 48 % (stat. sign.), ↑relative thyroid/parathyroid weights (m: 38 % (not stat. sign.), f: 34 % (not stat. sign.))
1-year (dietary) Beagle dog (4/sex/group)	0, 25, 250 and 1 500 ppm equal to	None (only bw and serum creatinine affected) NOAEL 250 ppm	<u>25/250 ppm</u> None

US EPA 83-1 (1982) GLP (RAR Vol. 3 B.6.3.3.1/01; HLR 565-86, 1986)	m: 0, 0.79, 8.16 and 51.5 mg/kg bw/d f: 0, 0.90, 8.18 and 52 mg/kg bw/d Guidance value for classification ≤ 25 mg/kg bw/d		
Developmental toxicity study Oral (gavage) GD7-19 Rabbit (22/dose) U.S. EPA 83-3 (1982) Non-GLP (RAR Vol. 3, B.6.6.2.2/01)	0, 5, 20, 80 mg/kg bw/d Guidance value for classification ≤ 700 mg/kg bw/d	None NOAEL (*maternal toxicity*) 5 mg/kg bw/d	*Maternal effects* <u>5 mg/kg bw/d</u> None <u>20 mg/kg bw/d</u> - mortality (1 dam) <u>80 mg/kg bw/d</u> - mortality (2 dams) - abortions (6 animals vs. 1 in control group) - bw loss (-325 g) - reduced food consumption (-48 % GD7-19)
DERMAL			
28-day, 6h/d Rabbit (6/sex/group) EEC method B.9 (1984) GLP (RAR Vol.3 B.6.8.2.3/01; CIT 7611 TSL, 1992)	0, 1 000 mg/kg bw/d Guidance value for classification ≤ 600 mg/kg bw/d	Skin, kidney, haematological system LOAEL 1 000 mg/kg bw/d	<u>1 000 mg/kg bw/d*</u> - mortality (1M, 1F) - clinical signs (dermal irritation) - ↓bw (m: 12 %), ↓bw gain (m: 91 %, f:76 %), ↓food consumption (m,f)) - changes in haematological parameters (↓RBC, Hb, PCV (m)) - changes in biochemical parameters (↓inorganic phosphorus level, ↓ALT (stat. sign. in males only) (m,f)) - organ weight changes (↑rel. kidney weight (m); ↑rel. brain weight (f)) - histopathological changes in skin and kidney (skin and application site: inflammatory reaction together with epidermal or dermal degeneration/necrosis, acanthosis, hyperkeratosis, oedema, vascular ectasia and haemorrhage; kidney: bilateral multifocal marked nephrocalcinosis together with kidney tubular degeneration/necrosis)

* dose level is above the guidance value for classification, but presented here as it is relatively close to the guidance value

In rats and mice, liver was identified as a target organ for toxicity in the repeated dose studies. Effects observed at dose levels relevant for STOT RE included changes in liver weight, changes in biochemical parameters and histopathological findings (hypertrophy of centrilobular hepatocytes). Other effects observed at dose levels relevant for STOT RE in rat and mouse included bw changes and effects on spleen weight. The severity of these observed effects was not considered of concern for a classification for STOT RE according to the DS.

In dogs, effects on blood parameters and thyroid (increased (para)thyroid weights) were noticed. The DS considered the severity of these effects not of concern for a classification for STOT RE.

In the developmental toxicity study in rabbits (see also the section on reproductive toxicity below), mortality (two dams), abortions (6 dams), reduced food consumption (-48 % during GD7-19) and body weight loss (-325 g; GD 7-19 compared to a body weight gain of 114 g in controls) were noted in dams at 80 mg/kg bw/d. At a dose of 20 mg/kg bw/d, mortality was observed in one dam, most likely associated with a trichobezoar (hairball). Whether the deaths at 80 mg/kg bw/d were associated with the abortion process, disease, tribenuron-methyl treatment or a combination of these factors could not be determined according to the study author. In this study, animals were treated with tribenuron-methyl for 13 days (GD7-19) and mortality occurred on days 17 and 29. The DS considered the mortality at the high dose an effect of repeated administration of tribenuron-methyl rather than an acute effect. As the mortalities occurred at a dose falling within the range of (extrapolated) guidance values of 70-700 mg/kg bw/d for STOT RE 2, the DS concluded that classification is justified.

In addition, mortalities and histopathological changes in the kidney (nephrocalcinosis, tubular degeneration/necrosis) were noted in the 28-day rabbit dermal toxicity study using a limit dose level of 1 000 mg/kg bw/d. The DS considered the severity of the observed effects as relevant for classification as STOT RE, but noted that the limit dose is higher, albeit quite close, to the (extrapolated) guidance value of 600 mg/kg bw/d.

Overall, the DS concluded that, based on the observed mortality in the rabbit developmental toxicity study at 80 mg/kg bw/d, classification for STOT RE in category 2 is warranted for tribenuron-methyl. The DS mentioned the mortalities and histopathological changes in the kidney (nephrocalcinosis, tubular degeneration/necrosis) in the 28-day rabbit dermal toxicity study as supporting evidence.

Comments received during public consultation

One comment from IND was received agreeing that classification with STOT RE 2 may be applicable, based on the morbidity and mortality seen in the rabbit developmental toxicity study. IND however does not agree that the effects observed in the 28-day rabbit dermal repeated dose toxicity study can be used to support this classification (dose level above guidance value, limited study according to DAR).

Assessment and comparison with the classification criteria

In the available repeated dose toxicity studies, treated animals showed a variety of effects at dose levels relevant for STOT RE classification, with the rabbit being the most sensitive species. Tribenuron-methyl was not immunotoxic or neurotoxic.

In rats, mice and dogs, the effects included body weight changes, effects on liver (organ weight changes, changes in biochemical parameters and histopathological findings (liver hypertrophy)), effects on spleen (organ weight) and thyroid (organ weight) and blood parameters. With respect to the effects on liver in rat and mouse, there was however no clear evidence of organ dysfunction. The liver hypertrophy is further considered an adaptive response. With respect to the effects on

spleen in rats and (para)thyroid in dogs, it is noted that the organ weight changes were not accompanied by histopathological changes. Finally, the changes of some of the haematological parameters in the 90-day dog study were minor and not observed in the 1-year dog study. RAC agrees with the DS that the effects in rats, mice and dogs do not warrant classification as the severity of the effects does not fulfil the classification criteria.

RAC notes that in the rabbit 28-day repeated dose dermal toxicity study mortality and histopathological changes in the skin and kidney were observed at the limit dose of 1 000 mg/kg bw/d. Mortality occurred in two animals (one male and one female) on day 29 and day 24, respectively. Macroscopic abnormalities or microscopic findings that could explain the deaths were not observed. Histopathological changes of skin included inflammatory reaction together with epidermal or dermal degeneration/necrosis, acanthosis, hyperkeratosis, oedema, vascular ectasia and haemorrhage with the effects most pronounced at the application site versus non-application site skin. The histopathological kidney changes included bilateral multifocal marked nephrocalcinosis (m: 3/6, f: 6/6) and kidney tubular degeneration/necrosis (m: 1/6, f: 6/6), pointing to kidney as target organ. These kidney effects are in principle relevant for classification, but they are observed at a dose level above the extrapolated guidance value of 600 mg/kg bw/d for a 28-day dermal study. Whereas kidney effects might be anticipated to occur also at doses lower than 1 000 mg/kg bw/d, it is unclear whether the severity of the kidney effects at dose levels below the extrapolated guidance value would qualify for a classification in category 2. With respect to the observed mortality, RAC considers the occurrence of mortality below the extrapolated guidance value unlikely. Overall, RAC concludes that the effects observed in the 28-day dermal toxicity study do not exclude classification, but as stand-alone, do not warrant classification.

In the rabbit developmental toxicity study mortality was observed in one dam at a dose of 20 mg/kg bw/d and in two dams at a dose of 80 mg/kg bw/d. The dam at 20 mg/kg bw/d died on day 29 and showed multiple mucosal haemorrhages in the stomach associated with a trichobezoar (hairball). This death is not considered treatment-related. For the two dams at 80 mg/kg bw/d, the cause of death was unclear. One dam died on gestation day 17 (10 days after start of treatment) with widespread hepatisation of the lungs, stomach distended with food and no formed faeces. The other dam was found dead on gestation day 29 (10 days after treatment ended) after previously aborting one foetus and had more foetuses in utero. This dam showed rather unspecific signs (alopecia, stained tail, red discharge cageboard) and had one paw that appeared to be injured. At the dose of 80 mg/kg bw/d, there were other maternal toxic effects such as reduced food consumption and body weight loss, and abortions were noted in six dams (vs. one dam in control group).

If only for these two deaths at 80 mg/kg bw/d, RAC doubts whether these would qualify for classification, despite the dose falling within the extrapolated guidance value range for a 13-day exposure (70-700 mg/kg bw/d). This because one death may have been a consequence of pneumonia (in view of the widespread hepatisation of the lungs), leaving then only one death, with unclear relation with treatment. Together with no effects in rats, mice and dogs warranting classification, this forms insufficient evidence.

RAC however notes that mortality was also observed in two pilot rabbit teratogenicity studies (described in section 10.10.3 of the CLH report as part of the main developmental toxicity study in rabbits (RAR Vol. 3, B.6.6.2.2/01; HLR 150-86, 1986)), in a dose-related way and at doses falling within/at the upper limit of the extrapolated guidance value range of 70-700 mg/kg bw/d. In the first pilot study, where 6 females per group were given 0, 250, 500 or 750 mg/kg bw/d, all females in the 500 and 750 mg/kg bw/d dose groups died. The dose level for the 250 mg/kg group was reduced to 125 mg/kg bw/d after 5 doses, and although all females in this group survived, no litters were produced. In the second pilot study, 7 females per group received tribenuron-methyl at dose levels of 75 or 150 mg/kg bw/d. The 150 mg/kg bw/d dose group

demonstrated severe weight loss and two of the females died. No deaths were observed at 75 mg/kg bw/d, but moderate weight loss and decreased feed consumption were evident. Whether the deaths in these two pilot studies were early deaths and therefore related to acute toxicity rather than to repeated dosing, could not be determined in absence of information on the time of the deaths (and on clinical signs) in the original study report of the main study. RAC therefore assumes that they were the result of repeated rather than single dosing, also in view of the observed weight loss, an effect that generally requires multiple doses before becoming manifest.

Despite some uncertainties noted above, RAC overall considers the mortality in the rabbit developmental toxicity studies to warrant classification, given the dose-response relation observed and the doses at which they occur. RAC therefore agrees with the DS that tribenuron-methyl should be **classified as STOT RE 2; H373**. A specification of the target organ or route is considered not necessary.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In a battery of *in vitro* genotoxicity studies performed under GLP and basically following OECD guidelines, tribenuron-methyl did not cause gene mutations or chromosome aberrations and did not affect DNA repair synthesis. The *in vitro* studies included two bacterial mutation assays (RAR Vol. 3, B.6.4.1/01; HLR 245-83 rev.2, 1988 and RAR Vol. 3, B.6.4.1/02; HLR 140965, 2009), a mammalian chromosome aberration test in human peripheral lymphocytes (RAR Vol. 3, B.6.4.1/03; DuPont-2938, 2000), a mammalian cell gene mutation (HGPRT) test in Chinese hamster ovary cells (RAR Vol. 3, B.6.4.1/04; DuPont-3387, 2000) and an Unscheduled DNA Synthesis (UDS)-assay in primary rat hepatocytes (RAR Vol. 3, B.6.4.1/05; HLR 565-84, 1985).

In vivo, tribenuron-methyl did not induce chromosome aberrations in a rat bone marrow chromosome aberration test (RAR Vol. 3, B.6.4.2/01; HLR 286-85, 1985) nor micronuclei in bone marrow cells in a mouse micronucleus test (RAR Vol. 3, B.6.4.2/02; HLR 420-85, 1985). Both studies were performed under GLP and basically following OECD guidelines.

In a third *in vivo* study reported in open literature (RAR Vol. 3, B.6.4.2/03; Journal of Pharmacology and Toxicology 7(7): 330-337, 2012), male albino rats (6/group) received technical (95 % purity) or formulated tribenuron-methyl (Granstar® 75 % DF; other components not further specified), at single or multiple (10 times, with 48-hour intervals) oral dose levels of 0, 5, 25, 50 or 100 mg/kg bw. The study focussed on the evaluation of chromosome aberrations and calculation of the mitotic index and micronuclei formation. No genotoxic potential was observed following single dosing of technical and formulated tribenuron-methyl. Following repeated administration of tribenuron-methyl technical, a statistically significant increase in the frequency of total chromosome aberrations was observed in animals of the high dose group only. Following repeated administration of formulated tribenuron-methyl, a significant decrease in mitotic activity was observed in animals administered 100 mg/kg bw, and a statistically significant increase in the frequency of total chromosome aberrations and micronuclei was observed in animals administered 50 and 100 mg/kg bw. Some important deviations and limitations with respect to the experimental set-up and evaluation were noted in the RAR and CLH report, including absence of positive controls, inclusion of repeat-dose exposures, non-standard nomenclature for chromosome aberrations, blinding procedures not described, individual animal data not presented, historical control data not presented and dose levels not justified. Due to its limitations this study was considered as supportive information only by the DS.

As the results of the guideline-studies were consistently negative, the DS concluded that tribenuron-methyl does not fulfil the criteria and should therefore not be classified for germ cell mutagenicity.

Comments received during public consultation

Two comments from IND were received supporting the 'no classification' proposal for germ cell mutagenicity.

Assessment and comparison with the classification criteria

Tribenuron-methyl tested negative in various *in vitro* assays (two bacterial mutation assays, a mammalian gene mutation assay, a mammalian cytogenicity test and an UDS assay) and in two *in vivo* assays (mouse micronucleus and rat chromosome aberration studies). A third *in vivo* study pointed towards potential positive clastogenic effects of tribenuron-methyl, but only after repeated dosing, not following single dosing. RAC however notes the deficiencies identified for this study, as well as that repeated dosing is not advised for a cytogenicity test due to the limited data available on the suitability of a repeated-dose protocol. Overall, RAC supports the conclusion of the DS that **tribenuron-methyl should not be classified for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two carcinogenicity studies were available, one in rats and one in mice. Supplementary studies included a 90-day feeding study in rats focussing on estrogenous effects, as well as *in vitro* mechanistic studies and QSAR analyses.

Rat

In a 2-year chronic toxicity/carcinogenicity study (RAR Vol. 3, B.6.5.1/01; HLR 61-87, 1987) conducted under GLP and conform to OECD TG 453, tribenuron-methyl (mixed with 1 % w/w corn oil) was administered to Crl:CD/BR rats (72/sex/dose) at 0, 25, 250 or 1 250 ppm (males: 0, 0.95, 10 or 55 mg/kg bw/d; females: 0, 1.2, 13 or 76 mg/kg bw/d) in the diet for 2 years. Ten male and ten female animals of the control and high dose group were sacrificed after 52 weeks.

There was no test-substance related effect on mortality or on clinical observations. General systemic toxicity without a specific target organ was indicated by the occurrence of non-neoplastic lesions in several organs in the male 250 and 1 250 ppm groups and female 1 250 ppm group. Mean body weights and body weight gains of male and female rats in the 1 250 ppm group were significantly lower than those of their respective control groups by approximately 29 % and 36 % for males and 43 % and 54 % for females. Mean body weights and body weight gains were also reduced in the 250 ppm group (by approximately 9 and 11 % for males and 21 and 27 % for females), with statistical significance reached only for the 27 % reduction in body weight gain in females. There were no differences in food consumption between the groups. As a consequence of the reduced body weights, several organs weights were also affected.

Table 41 in the CLH report presents an overview of the malignant neoplastic lesions found in the rats. In male rats, the incidence of total malignant tumours was statistically significantly increased at all doses (21, 40, 33 and 36 % in control, 25, 250, and 1 250 ppm, respectively)

but there was no dose-response. Moreover, there was no statistically significant increase in any specific tumour type. An apparent dose-response was seen in the combined incidences of thyroid follicular cell adenocarcinoma and C-cell carcinoma and of epididymis adenocarcinoma and mesothelioma. Additional data provided by the applicant during the pesticide peer-review process however indicated that the presentation of combined incidences is not appropriate for either the thyroid or the epididymis, because of different histogenic origins of the two tumour types in both thyroid and epididymis. Additional data by the applicant on incidences of and historical control data for the individual tumour types in the thyroid (including incidences on adenoma and hyperplasia) and epididymis showed no treatment-related increases in any of these individual tumour types. Overall, the DS did not attach biological importance to the male rat tumour profile.

In female rats, the incidence of total malignant tumours was statistically significantly increased in the high dose group only (33, 35, 40 and 61 % in control, 25, 250, and 1 250 ppm, respectively). This increase was a result of an increase in a single specific tumour type, i.e. mammary gland adenocarcinoma. The incidence in total mammary gland adenocarcinoma was dose-relatedly increased in the mid and high dose (15, 15, 22 and 43 % for control, 25, 250 and 1 250 ppm, respectively), with statistical significance only reached for the high dose. At the mid dose, the incidence was within the range of historical controls (8-23 % as reported in the DAR, 1.5-30.4 % from additional data provided by the applicant during the pesticide peer-review process and cited in the DAR Appendix), whereas at the high dose it was above. No increased incidence of non-neoplastic changes in the mammary gland was noted.

The DS considered that the increase in mammary gland adenocarcinomas was only seen at dose levels reaching the maximum tolerated dose (MTD), given the decreases in body weight (21 and 43 % for the 250 and 1 250 ppm group, respectively) and body weight gain (27 % and 54 %, respectively).

Mouse

In an 18-month chronic toxicity/carcinogenicity study (RAR Vol. 3, B.6.5.2/02; HLR 60-87, 1987) conducted under GLP and following OECD TG 453 guideline, tribenuron-methyl was administered to Crl:CD®-1(ICR)BR mouse (80/sex/group) at 0, 20, 200 and 1 500 ppm (males: 0, 2.5, 25 and 197 mg/kg bw/d, females: 0, 3.1, 31 and 247 mg/kg bw/d) in the diet for 18 months.

There was no test-substance related effect on mortality or on clinical observations. Mean body weights, mean body weight gains and food consumption were reduced in the high dose group as compared to the control group. Histopathology did not reveal a specific target organ, nor treatment-related increases in the incidence of tumours. Several minor modifications were observed in the normal lesions of ageing in the male and female high dose group and the male mid dose group, as well as some secondary changes to the amyloidosis and slightly catabolic conditions observed in these groups.

Mechanistic studies

<u>90-d feeding study in rat focussing on estrogenic effects</u> (RAR Vol. 3, B.6.8.2.1/01; HLR 112-89, 1989/2000(suppl.))

In a supplementary GLP-compliant study, female Crl:CD BR rats (20/group) were treated with 0 and 5 000 ppm tribenuron-methyl (corresponding to 390 mg/kg bw/d) via the diet for 90 days. The 5 000 ppm dose level was selected because this dose level produced similar body weight effects in a previous 90-day feeding study to those seen at 1 250 ppm in the 2-year study. The study focussed on effects on the endocrine system, investigating additionally the ability of tribenuron-methyl and its metabolites to compete *in vitro* for binding to the estrogen and progesterone receptors from uterine cytosol.

The *in vitro* part of the study showed that seven tribenuron-methyl metabolites can bind to the estrogen receptor, whereas no competition was seen for the progesterone receptor. In the *in vivo* part of the study, increased mean relative uterine weight, increased qualitative uterine cell proliferation, increased mean relative ovarian weight, increased incidence of prolonged estrous, two- to three-fold decrease in uterus (from rats sacrificed in ostrous) and mammary estrogen receptor affinity, and two-fold increase in progesterone receptor number were observed following administration of 5 000 ppm tribenuron-methyl. However, this dose also induced a marked decrease in body weight (26 %) and body weight gain (40 %).

As it is known that many hormonal and reproductive endpoints are altered by caloric restriction, the DS considered that the effects detected in this study, as variations of the estrous cycle length, organs weights and hormone levels, cannot be taken into consideration regarding a potential estrogenic mechanism. Yet, taking the *in vitro* results along with the increase in progesterone receptor number observed in the *in vivo* study, the DS considered that a hormonally mediated mechanism of mammary tumour induction cannot be ruled out. Because the mechanistic study described above was inconclusive regarding the potential for an estrogenic mechanism, additional data on the endocrine disruption potential of tribenuron-methyl were generated by the applicant for the renewal process of the pesticide.

In vitro studies conducted with tribenuron-methyl and its metabolite IN-A4089 (RAR Vol. 3, B.6.8.3/01-06; DuPont-46406/46409/45570/45571/45572/45573, 2016)

The potential for tribenuron-methyl or its metabolite IN-A4089 (i.e. triazine) to bind or activate estrogen receptors was investigated in six *in vitro* studies. These showed that tribenuron-methyl and IN-A4089 were negative for induction or inhibition of 17β-estradiol and testosterone, did not interact with estrogen receptor, and were not agonists of human estrogen receptor alpha in HeLa-9903 cell model system.

QSAR analyses conducted with tribenuron-methyl and ten metabolites (RAR Vol. 3, B.6.8.3/07; DuPont-45358, 2016)

Via QSAR analyses (Toolbox v3.3.5, OASIS TIMES v2.27.16, DEREK v4.1, MedChem Studio v4.0, and ADMET Predictor v7.2 software) tribenuron-methyl and ten metabolites were evaluated for structural alerts for estrogen receptor binding. All were “non-binder” except metabolite IN-G7462 which seems to be false based on research into the origin of this alert.

The DS considered that these data indicate that neither tribenuron-methyl nor its metabolites demonstrated a potential to be endocrine active.

Overall conclusion

The DS concluded that the mammary gland tumours in female rats were produced only at doses which exceeded the MTD. The dose-response for mammary gland tumour induction, along with the demonstrated absence of genotoxicity, suggest that a non-genotoxic, threshold mechanism is responsible for the mammary gland tumours observed in female rats following exposure to a high dietary concentration of tribenuron-methyl. The specific mechanism(s) involved are not known but it seems like the tumour-induction is secondary to general toxicity. Irrespective of the mechanism, the relevance to humans of mammary tumour-induction in rat only at a dose that greatly exceeded the MTD, and in a strain with high spontaneous mammary tumour rate, is questionable. Therefore data is neither considered as sufficient evidence for category 1B nor as limited evidence for category 2. Consequently, the DS concluded that tribenuron-methyl should not be classified for carcinogenicity.

Comments received during public consultation

Two comments from IND were received supporting the 'no classification' proposal for carcinogenicity. One MSCA commented that classification for carcinogenicity category 2 should be considered as 1) the incidences were at (mid dose) or above (high dose) the upper limit of the historical control range, 2) the increase at the high dose was hugely statistically significant, 3) they questioned whether excess toxicity has occurred given that survival rate was not affected and no increase in clinical signs was observed, and 4) there was also an increased incidence of thyroid C-cell carcinomas in high dose male rats.

In its response, the DS maintained that the mammary gland tumours do not warrant classification in view of the historical control range, the magnitude of the body weight (gain) reductions and the extensive discussions during the Pesticide Peer Review on this endpoint resulting in the same outcome. With respect to the thyroid C-cell carcinoma, the DS maintained that these were not treatment-related as 1) the increased incidence in the high dose group was slightly outside the historical control range but did not reach statistical significance, 2) there was also 1 positive control animal, whereas in the low and mid dose group there were zero, 3) the incidence of C-cell hyperplasia was higher in controls (11/62) than in high dose males (5/61), and 4) the same conclusion was drawn after extensive discussion during the Pesticide Peer Review.

Assessment and comparison with the classification criteria

In view of the absence of treatment-related increases in neoplastic effects in the mouse 18-month carcinogenicity study, RAC considers there is no evidence for carcinogenicity of tribenuron-methyl in mice.

In contrast to mice, there was evidence of carcinogenicity in rats, with female rats showing an increased incidence of mammary gland adenocarcinoma at the mid dose of 250 ppm (22 % vs 15 % in controls; not statistically significant and within laboratory historical control ranges) and at the high dose of 1 250 ppm (43 %; statistically significant and outside the laboratory historical control range). Females at these doses showed marked decreases in body weight (21 and 43 % for the 250 and 1 250 ppm group, respectively) and body weight gain (27 % and 54 %, respectively). This could indicate that the MTD was reached, although there was no treatment-related effect on mortality or clinical signs. RAC notes that general systemic toxicity was indicated by the occurrence of non-neoplastic lesions in several organs in high dose females. As noted by the DS, the specific mechanism(s) behind the mammary gland tumours are not known, although a non-genotoxic mode of action may be assumed in view of the negative results in a battery of *in vitro* and *in vivo* genotoxicity studies. An endocrine mode of action is also not likely, given the results of the mechanistic *in vitro* studies and QSAR analyses. Taken together, RAC considers the conclusion of the DS that the tumour-induction in female rats is likely secondary to general toxicity is plausible. As tumours occurring only at doses exceeding the MTD are generally more doubtful indicators for human carcinogenicity, RAC considers the mammary gland tumours not to warrant classification.

When looking at the tumour profile in male rats, RAC supports the conclusion of the DS that there is no treatment-related increase in any specific tumour type that warrants classification. That includes the epididymis, with only single incidences (without statistical significance) of adenocarcinoma and mesothelioma in high dose males, as well as the thyroid. As can be seen in the table below, there was no dose-related increase in follicular cell tumours. The incidence of follicular cell adenocarcinoma was outside the historical control range at the mid dose, but the increase was not statistically significant, and there was no dose-response. As to C-cell tumours, none were observed in the low and mid dose group. In the high dose group the incidence of C-cell adenocarcinoma was outside the historical control range, but the increase was not statistically

significant. RAC notes that there was also one control animal with an adenocarcinoma, and that the incidence of C-cell adenoma and C-cell hyperplasia was higher in controls than in high dose males. In females, the incidence of thyroid tumours was not affected. Taken together, RAC considers the slight, not statistically significant increases in thyroid tumours in one sex of rats only not to warrant classification.

Table: *Incidences of thyroid tumours in male rats in a 2-yr study with tribenuron-methyl*

Dose (ppm)	0	25	250	1 250
No. in group	62	60	60	61
No. examined	62	29*	37*	61
Follicular cell				
- adenoma	0	1 (3.4 %)	0	0
- cystadenoma	0	2 (6.9 %)	0	0
- adenocarcinoma[1]	0	0	2 (5.4 %)	1 (1.6 %)
- combined	0	3	2 (5.4 %)	1 (1.6 %)
[hyperplasia]	1 (1.6 %)	(10.3 %)		1 (1/6 %)
C-cell				
- adenoma	3 (4.8 %)	0	0	2 (3.3 %)
- carcinoma[2]	1 (1.6 %)	0	0	4 (6.6 %)
- combined	4 (6.5 %)	0	0	6 (9.8 %)
[hyperplasia]	11 (17.7 %)			5 (8.2 %)

* histopathology was performed only on early death animals and those with a gross lesion

[1] Laboratory historical control range: 0-3.8 %
Historical control range for SD rat studies contemporary to tribenuron-methyl study: 0-8.2 % (1977-1985), 1.0-6.0 % (1984-1989)

[2] Laboratory historical control range: 0-3.3 %

Overall, RAC supports the DS conclusion that **tribenuron-methyl does not warrant classified for carcinogenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

For the endpoint reproductive toxicity, a two-generation study and a one-generation study in rats are available, as well as two developmental toxicity studies (one in rats, one in rabbits).

Adverse effects on sexual function and fertility

In the key two-generation rat toxicity study (RAR Vol. 3, B.6.6.1/01; HLR 193-86, 1986/1988 (Suppl.1)/2000(Suppl.2)), conducted under GLP and conform U.S. EPA 83-4 (1982) guideline, tribenuron-methyl was administered to Crl:CD®(SD)BR rats (23/sex/group) at 0, 25, 250 or 1 000 ppm in the diet for two generations. The achieved 70-day mean test material intakes were 0, 1.9, 19 and 75 mg/kg bw/d for males and 0, 2.15, 21.3 and 88 mg/kg bw/d for females. There were no adverse effects on reproduction, fertility and mating behaviour. Also, no malformations in the offspring were noticed. Parental toxicity was evident from 250 ppm by decreased bodyweight gain (up to 24/18 % and 29/25 % in high dose F0/F1 males and females, respectively, and up to 13 % in mid dose F1 females) and food consumption. At 1 000 ppm, also organ weight changes (increased relative testes) were noted, but there were no gross or histopathological findings in reproductive tissues. Offspring toxicity included clinical signs (sparse fur) in F2 generation at 1 000 ppm, decreased body weight growth from 250 ppm (8 % and 9 % in 250 ppm F1B and F2B pups, respectively, and 9 %, 16 %, 10 % and 12 % in 1 000 ppm F1A,

F1B, F2A and F2B pups, respectively), and several organ weight changes in F2B weanlings from 250 ppm.

In the one-generation toxicity study (RAR Vol. 3, B.6.6.1/02; HLR 413-83, 1985; non-GLP and non-guideline), tribenuron-methyl was administered to Crl:CD(SD)BR rats (16/sex/group) at 0, 100, 1 750 or 5 000 ppm in the diet for one generation. The mean achieved test material intakes were 0, 7, 118 and 335 mg/kg bw/d for males and 0, 8, 135 and 386 mg/kg bw/d for females. There were some limitations to this study with regard to the number of animals used and pathology (i.e. testes and epididymides were not weighed, no histopathology was performed). This one-generation study was considered as supplementary data only by the DS. Treatment was associated with reduced pup weight at 1 750 ppm (19 %) and 5 000 ppm (36 %) and with reduced pup viability at 5 000 ppm (19 % day 0-4 and 14 % day 1-4; not statistically significant; primarily the result of the death of nine pups in the litter of one rat). These effects on the offspring were observed at doses that also caused reduced bodyweight in dams (15 and 23 % at 1 750 and 5 000 ppm, respectively). Reproductive performance was not affected.

Given the lack of adverse effects on fertility, the DS concluded that tribenuron-methyl does not warrant classification for fertility.

Adverse effects on development

Rat

In the rat developmental toxicity study (RAR Vol. 3, B.6.6.2.1/01; HLO 513-85, 1985/2000 (Suppl.)), compliant with U.S. EPA guideline 83-3 (1982), tribenuron-methyl (in 0.5 % hydroxypropyl methyl cellulose) was administered to female Crl:COBS®CD®(SD)BR rats (25/dose) at 0, 20, 125 or 500 mg/kg bw/d by gavage, on days 6-15 of gestation. Maternal toxicological findings were noted at 125 mg/kg bw/d and above and included reduced body weight (gain) at 125 mg/kg bw/d (bw 5-7 %, bw gain 13 %, corrected bw gain 31 %) and 500 mg/kg bw/d (bw 18 %, bw gain 53 %, bw gain–adjusted 98 %), increased relative liver weights (7 % and 17 % at 125 and 500 mg/kg bw/d, respectively), stomach ulceration (one animal at 125 mg/kg bw/d) and increased salivation in dams at 500 mg/kg bw/d.

The incidence of corpora lutea, pregnancy and implantation was similar for all dosage groups. Fifteen of 16 foetuses in one high dose litter were dead in utero, resulting in an increase (not significant) in the average percentage of resorptions or dead conceptuses per litter. A slight increase (not significant) in the average number of resorptions also occurred for this dose group. The average number of live foetuses per litter was not adversely affected. Foetal effects consisted of reduced body weights at the mid dose (9 %) and high dose (26 %) and increased foetal deaths at the high dose (limited to one litter). No treatment-related malformations were observed, but incidences of skeletal alterations (mainly incomplete/altered ossifications) were increased from 125 mg/kg bw/d. Enlarged fontanelle was noted in a single high dose foetus, as well as oedema (in three foetuses of one high dose litter), but both effects were without statistical significance.

The DS noted that the foetal effects were observed in the presence of maternal toxicity (i.e. reduced bw gain). The DS did not consider the retarded ossifications to be a major manifestation of developmental toxicity. The reduced foetal weights and increased foetal deaths (one litter only)/resorptions could be secondary to maternal toxicity. Besides, the effect on the embryo/foetal viability was not severe enough to adversely affect the number of live foetuses per litter.

As a conclusion, the DS considered that the effects noted in the rat developmental toxicity study were not sufficient to trigger classification.

Rabbit

In the rabbit developmental toxicity study (RAR Vol. 3, B.6.6.2.2/01; HLR 150-86, 1986/2000 (Suppl.)), compliant with U.S. EPA guideline 83-3 (1982), tribenuron-methyl (in 0.5 % aqueous methyl cellulose) was administered to female New-Zealand White rabbits (22/dose) at 0, 5, 20 or 80 mg/kg bw/d by gavage, on days 7-19 of gestation. The dose levels were selected based on two pilot teratogenicity studies. In the first pilot study, where 6 females per group were given 0, 250, 500 or 750 mg/kg bw/d, all females in the 500 and 750 mg/kg dose groups died. The dose level for the 250 mg/kg bw/d group was reduced to 125 mg/kg bw/d after 5 doses, and although all females in this group survived, no litters were produced. In the second pilot study, 7 females per group received tribenuron-methyl at dose levels of 75 or 150 mg/kg bw/d. The 150 mg/kg bw/d dose group demonstrated severe weight loss and two of the females died. No deaths were observed at 75 mg/kg bw/d, but moderate weight loss and decreased feed consumption were evident. On the basis of these pilot studies, the highest dose for the main study was set at 80 mg/kg bw/d.

In the main study, mortality was observed in one mid dose dam and in two high dose dams. The mid dose dam died on day 29 just prior to scheduled sacrifice and showed multiple mucosal haemorrhages in the stomach associated with a trichobezoar (hairball). One high dose dam died on gestation day 17 with widespread hepatisation of the lungs, the other dam was found dead on gestation day 29 after previously aborting one foetus and had more foetuses in utero. At the high dose, other maternal toxic effects were noted such as reduced food consumption (48 % over the treatment period), body weight loss (-325 g vs 114 g weight gain in controls) and abortions (in 6 dams vs 1 control dam). Whether the deaths at the high dose were associated with the abortion process, disease, tribenuron-methyl treatment or a combination of these factors could not be determined according to the study author.

In the high dose group, the number of corpora lutea was not affected, but the number of nidations (implantations) was significantly reduced and in conjunction with that also the number of live foetuses per litter. Foetal effects consisted of reduced body weight (10 %, not statistically significant) and an increase in malformations at 80 mg/kg bw/d. The malformations observed within affected high dose litters occurred at low incidences (1 or 2/litters) and were without a pattern of a specific malformation. Furthermore, the increase in percent affected per litter was primarily due to malformations that occurred in small litters. No significant differences in the rates of variations due to retarded development, or in the mean percent of foetuses with variations were observed. The mean percentage of foetuses with developmental variations was significantly increased only at the low dose level.

The DS noted that all developmental effects were observed in the presence of maternal toxicity, and that some effects could be secondary to that. As a conclusion, the DS considered that the effects noted in the rabbit developmental toxicity study were not sufficient to warrant classification.

Adverse effects on or via lactation

The DS did not consider classification for effects on or via lactation warranted because the chemical does not meet the criteria, for two out of three criteria due to lack of data (i.e., there is no human evidence available indicating a hazard to babies, and the ability of tribenuron-methyl to distribute into the breast milk has not been investigated). For the third criterion, the two- and one-generation studies in rats showed a reduction in pup weight. But since this was observed in the presence of maternal toxicity (reduced bw (gain)), the DS considered the effect on body weight growth not clear evidence for an effect of tribenuron-methyl on lactation performance.

Overall, the DS therefore concluded that tribenuron-methyl does not warrant classification for reproductive toxicity.

Comments received during public consultation

Two comments from IND were received supporting the 'no classification' proposal for reproductive toxicity.

Assessment and comparison with the classification criteria

Fertility

In view of the absence of findings on fertility parameters in the key two-generation study and in the supportive one-generation study in rats and the absence of adverse effects on the reproductive organs in the two-generation study and the repeated dose studies, RAC supports the conclusion of the DS that tribenuron-methyl does not need to be classified for effects on fertility and sexual development.

Developmental toxicity

In the rat developmental toxicity study, reduced foetal body weight and significantly increased incidences of skeletal alterations (incomplete ossification, unossified sites) were observed. These effects point towards delayed ossification or are indicative of developmental delay; they were observed at maternally toxic doses. Other significant effects observed in this study included increased foetal deaths at the highest (maternally toxic) dose of 500 mg/kg bw/d, but this was limited to one litter (with 15 out of 16 foetuses being dead in utero). The average percentage of resorptions or dead conceptuses per litter, the average number of resorptions or the average number of live foetuses were not statistically significantly affected. Overall, RAC agrees with the DS that the effects observed in the rat developmental toxicity study do no warrant classification.

RAC notes that in the two- and one-generation studies treatment with tribenuron-methyl was associated with decreased body weight growth of the pups, but only at dose levels that were also maternally toxic. The effect on growth is probably secondary to the maternal toxicity, and not sufficient to warrant classification. The same is true for the additional effect on pup viability in the one-generation study, which was not statistically significant and mainly due to one nest.

In the rabbit developmental toxicity study, a reduced number of nidations was noted. But as the number of corpora lutea per litter was not affected, the reason for the reduced number of nidations was not clear. Other effects included reduced foetal body weight (indicative of developmental delay), decreased live foetuses per litter (as a consequence of the reduction in nidations) and an increase in malformations at the high dose of 80 mg/kg bw/d. This dose was clearly maternally toxic, with mortality in two dams, abortions in six dams, significantly reduced food consumption and body weight loss (-325 g). RAC notes that dietary restriction to feed levels that produce substantial reductions in maternal body weight gain, or even weight loss, in pregnant rabbits can result in developmental toxicity expressed by abortion, reduced foetal weight, and alteration in ossification (Cappon *et al.,* 2005). In the rabbit developmental toxicity study with tribenuron-methyl, the dams with abortions presented in general with the highest body weight loss over the treatment period. And the malformations induced were of low incidence and without a specific pattern. Overall, given the clear maternal toxicity, RAC considers that the effects observed in the rabbit developmental toxicity study do no warrant classification.

Based on the available data, RAC support the conclusion of the DS that tribenuron-methyl does not need to be classified for effects on development.

Lactation

RAC supports the analysis of the DS that tribenuron-methyl does not need to be classified for effects on or via lactation.

Overall, RAC supports the DS conclusion that tribenuron-methyl **does not warrant classification for reproductive toxicity**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Tribenuron-methyl has a current entry in Annex VI to the CLP regulation as Aquatic Acute 1 and Aquatic Chronic 1 with a generic M-factor of 100. Based on the available data on aquatic toxicity the dossier submitter (DS) proposed to update the environmental classification to Aquatic Acute 1 (M=100) and Aquatic Chronic 1 (M=100) according to CLP Regulation.

Degradation

Two hydrolysis studies were performed. The first one was carried out according to OPPTS 835.2120, SETAC Europe, OECD TG 111 and in compliance with GLP at pH 4, 7 and 9 for 30 days at temperatures ranging from 10 to 40 °C. The second one was conducted according to OECD TG 111 at pH 4, 7 and 9 at 25, 50 and 65 °C. Both studies showed that tribenuron-methyl is rapidly hydrolysed under pH 4 (< 1 day at all temps), moderately at pH 7 (0.72 – 63.5 days from 50 – 15 °C, respectively) and nearly stable at pH 9 (1.6 – 743 days at 65 – 15 °C, respectively). Three metabolites were formed: IN-00581 (≥ 92.8 % 0.42 days at pH 4), IN-L5296 (≥ 88 % 0.1 days at pH 4), both of which decreased with increased formation time as pH increased, and IN-D5803 (6.9 % 7 days at pH 7).

The photodegradation of radio-labelled tribenuron-methyl in water was examined in one study performed according to OECD TG 316 and GLP. The test was only performed at pH 9 in order to minimise the hydrolysis. All irradiated samples were exposed to artificial sunlight of a xenon arc lamp for approximately 15 days at ca 25 ± 2 °C, equivalent to ca. 30 days of mid-summer sunlight (at latitude of 40° N), assuming a 12 h light/ 12 h dark cycle. The photodegradation is not an important degradation route for tribenuron-methyl since the DT_{50} was 120 days.

A ready biodegradability study was carried out according to OECD TG 301E and GLP. A mixture of tribenuron-methyl technical and inorganic nutrient medium was added to activated sludge from a sewage treatment plant for domestic sewage and incubated for 28 days at 21 °C. The test concentration used was 32 mg/L. Degradation of the substance after 28 days was 11-24 %. The reference compound was 100 % degraded. The inhibition check attained 71 % biodegradation, indicating a slight inhibitory effect. The DS concluded that the test substance is not readily biodegradable.

An aerobic mineralisation in surface water study was performed with pond water (pelagic conditions) according to OECD TG 309 and GLP for 60 days at 20±2 °C. The test substance was added to the surface water at two different concentrations (500 and 50 µg/L). In the surface water, levels of tribenuron-methyl decreased from 98.8 and 99.1 % at Day 0 to 66.0 and 62.0 % by study termination for the phenyl- and triazine-label, respectively. The only identified metabolite for the phenyl-label was IN-00581 which increased up to 28.3 % by day 46, after

which it declined to 0.6 % at study termination. Unassigned radioactivity reached 19.7 % by day 60, when it was deemed by the study authors to consist of 17.9 % of dissolved carbonates based on the elution properties in the HPLC and the removal of this region of radioactivity upon acidification. For the triazine-label, IN-L5296 was the only identified metabolite and it reached 26.5 % by day 46 (25.8 % by study termination). Unassigned radioactivity added up to 1.8 % by study termination. The kinetic evaluation, done based on the radioactivity measured as tribenuron-methyl at the high dose level (0.5 mg/L) and applying SFO-kinetics, results in DT_{50} = 86.2 days.

The route and rate of degradation of tribenuron-methyl was also studied in natural water from a river in a water only test (pelagic conditions) and in a suspended sediment test according to OECD TG 309 for 110 days at 20±2 °C. For the water only test, the radiolabelled substance was applied at two concentrations (5 and 50 µg/L). For the suspended sediment test, tribenuron-methyl was applied at 50 µg/L. In the water only samples treated at the low concentration (5 µg/L), a slight degradation of tribenuron-methyl could be observed between days 28 and 61 (after a lag phase of about 14 days). The analysis of the water phase after 28 days showed that 84 % (phenyl-label) and 93 % (triazine-label) remained as parent compound. About 68-76 % was observed as tribenuron-methyl in the samples analysed after 61 days of incubation whereas on the last sampling day (110 days after application) the concentration of parent apparently increased again (90 %). In total, four transformation products were observed. IN-00581 was detected at 5.3 % at one sampling point (14 days). IN-L5296 was observed at two sampling points (14 and 61 days) and reached 10.4 % (61 days). IN-R9805 was also only observed at day 61 at 8.9 %. In addition one unknown metabolite was detected at a low amount (2.7 %) after 61 days of incubation. In the water only samples treated at the high concentration (50 µg/L), similar results were observed after a lag phase of 28 days. The analysis of the water phase after 61 and 110 days of incubation showed 85 % and 69 %, respectively, remaining as parent compound. Up to five known metabolites could be detected in water samples. IN-L5296 and IN GK521 reached maximums of 7.0 % AR and 10.8 % AR, respectively at the last sampling interval. IN-00581 reached a maximum of 5 % AR 61 days after application but could not be detected in the water sample of the last sampling day. The metabolites IN-A4098 and IN-D5119 were only detected on the last sampling day and accounted for 6 % AR and 10 % AR, respectively. In addition, low amounts of two unidentified metabolites were determined (max 3.3 %) at the last two sampling intervals. In the suspended sediment samples (50 µg/L), a slightly faster degradation could be observed after a lag phase of about 14 days. 73 % remained as tribenuron-methyl after day 28 whereas only 49 % remained unchanged by study termination (110 days). The main metabolites detected were IN-L5296 (8.3 %), IN-00581 (20.4 %) and IN-GK521 (12.1 %). Additionally, IN-D5803 (day 28, 5 % AR), IN-A4098 (day 61, 1 % AR) and IN-D5119 (day 110, 7 % AR) were detected at single intervals. A calculation using first order kinetics gives a half-life of 139 days for the suspended sediment test system.

Degradation in aerobic water-sediment systems was studied according to SETAC Europe, U.S. EPA 162-4, for 135 days in two different systems; one collected from the Brandywine River (anaerobic conditions in the sediment), the other originated from the Lums Pond (aerobic conditions in sediment). When the systems were treated with triazine-labelled test material, the majority of the applied radioactivity remained in the sediment (maximum 64 % in the river system and 102 % in the pond system). In the systems treated with phenyl-labelled material, the majority of the radioactivity was transformed to CO_2 with 60 and 65 % formed by study termination for the river and pond systems, respectively. For the triazine-label, the CO_2 formation reached 18 and 1.4 % (day 105) for the river and pond systems respectively. Bound residues accounted for max 16 % (day 71) and 26 % (day 105) in the river sediment and 11 % (day 71) and 16 % (day 105) in the pond sediment for the triazine- and phenyl-labelled substance, respectively. For tribenuron-methyl, a DT_{50} geometric mean at 20 °C of 18.5 days were calculated

and for the metabolite IN-L5296 of 227.8 days, IN-00581 of 10.8 days and IN-GN815 of 47.6 days.

Based on the information above, the DS concluded that the substance is not considered to be rapidly degradable.

Bioaccumulation

No BCF studies on fish were available for tribenuorn-methyl

Two studies on n-Octanol/water partition coefficient were performed, according to OECD TG 117/shake flask method and HPLC analysis (GLP).

In the first study (Pakki, U.V.S., 2013 DuPont-36463) at 20°C, Log K_{ow} in distilled water was 0.85, at pH 7.0 Log K_{ow} was -0.38 and at pH 9.0 was -0.93. In the study by Cowlyn (2014) (288 TBM) at 20°C, Log K_{ow} at pH 4, 7.0 and 10.0 was 2.0, -0.46 and -2.22. The result at pH 4 (Log K_{ow} = 2.0) is most likely somewhat underestimated as tribenuron-methyl undergoes hydrolysis at acidic pH.

Based on the information above, tribenuron-methyl has a Log K_{ow} < 4, therefore, in the absence of experimental data, the DS concluded that the potential for bioaccumulation is low.

Ecotoxicity

Several acute and long-term aquatic toxicity data for all three trophic levels were available.

The test results were summarized in the following table:

Method	Test organism	Test system	Results			Test conc.	Reference
			Endpoint	LC_{50} / EC_{50} [mg/L]	NOEC [mg/L]		
OECD TG 203	*Oncorhynchus mykiss*	Static 96h	Mortality	738		mean measured	DUP, Anonymous, 1997, AMR 4201-96
OECD TG 204	*Oncorhynchus mykiss*	flow-through 21d	Length, weight survival		560	mean measured	DUP, Anonymous, 1989, HLR 311-89
OECD TG 210	*Cyprinodon variegatus*	flow-through 28d Early life stage (ELS)	Larval length weight, survival		11.9	mean measured	DUP, Anonymous, 2012, DuPont-33943
OECD TG 202	*Daphnia magna*	Static 48h	Immobility	> 894		mean measured	DUP, Boeri, R.L., et al, 1997 AMR 4202-96
OECD TG 202	*Daphnia magna*	Static-renewal 21d	Adult length (NOEC), Reproduction (EC10)		NOEC = 120, EC_{10} = 52	mean measured	DUP, Hutton, 1989 Hoke, 2013 HLR 164-89, Revision No. 1
OECD TG 211	*Daphnia magna*	Static-renewal 21d	Adult length		NOEC = 114	nominal	DUP, Rebstock, M., 2013, DuPont-35848

							Revision No. 1
OECD TG 211	*Daphnia magna*	Static-renewal 21d	Mortality, reproduction		41	nominal	TTF, Zawadsky, C., 2015 329 TBM
OECD TG 201	*Pseudokirchneriella subcapitata*	Static 72h	Growth rate	0.068	NOEC = 0.004 E_rC_{10} = 0.011	nominal	DUP, Sloman, T.L., Leva, S.E., 1998, DuPont-1222
OECD TG 201	*Anabaena flos-aquae*	Static 72h	Growth rate	ErC50 > 100	NOEC = 10 E_rC_{10} = 13.2	nominal	TTF: Hermes, H., Sonntag, F. (2016) Ibacon Project 118631218
US EPA guideline 123-2 OECD TG 221 (2006)	*Lemna gibba*	Static 14d Recalculation to 7d	Growth rate	**0.0047**	NOE_rC = 0.001 E_rC_{10} = 0.0024	nominal	DUP, Kannuck, R.M., Samel, A., 2000, AMR 3070-94, Revision No. 1 Scown, T., et al 2014 DuPont-41634
OECD TG 239	*Myriophyllum spicatum*	Static 14d	Growth rate (fresh weight)	0.0065	**NOEC = 0.000954** **E_rC_{10} = 0.00081**	nominal	TTF, Gonsior , G., 2015, 297 TBM amdt-1
	Myriophyllum spicatum	Static 14d	Growth ra	ErC50 >94	NOEC = 94 Necrosis NOEC = 0.87 E_rC_{10} = 3.5 Apical bud damage, NOEC = <0.0022	mean measured	DUP, Kirkwood, A., 2015 DuPont-32239, Revision No. 2
	Elodea canadensis	Static 14d			Chlorosis NOEC = 1.0 Growth rate (biomass), NOEC = 10 Shoot dry weight, NOEC = 0.1	nominal	DUP, Kirkwood, A., 2013 DuPont-32243, Revision No. 1

Acute toxicity

A single acute toxicity study to fish performed with test substance tribenuron-methyl was provided according to OECD TG 203 and GLP criteria. In this study, rainbow trout (*Oncorhynchus mykiss*) were exposed in a 96h static test system. Mean measured test concentrations were between 83-88 % of nominal concentrations. The 96h LC_{50} value was calculated to be 738 mg/L based on mean measured concentrations. This study fulfilled the validity criteria is considered relevant and acceptable.

In the one acute toxicity study with invertebrates, *Daphnia magna* were exposed in a 48h static study according to OECD TG 202. The mean measured concentrations during the test were 89-92 % of nominal concentrations. According to the results of the test, the LC_{50} after 24 and 48 h was determined to be > 894 mg/L. The test fulfilled the validity criteria.

Both algae and aquatic plants were sensitive to tribenuron-methyl. The lowest EC_{50} is 0.0047 mg/L derived with *Lemna gibba*. The study, in accordance to US EPA guideline 123-2 (DUP, Kannuck, R.M., Samel, A., 2000, AMR 3070-94, Revision No. 1 Scown, T., *et al*. 2014), covers both acute and long-term endpoints. Concentrations measured at day 14 ranged between 94 and 102 % of the values measured at day 0.

In the revised study by Scown (2014), the EC_{50} and NOEC values were calculated after 7d of exposure from the raw data. The 7d E_rC_{50} was 0.00473 mg/L based on growth rate. The study is relevant and acceptable.

Chronic toxicity

Two chronic toxicity studies with fish were available and included in the CLH Report; *DUP Anonymous* (*2012*) following OECD TG 210, reported as the key study, and *DUP Anonymous* (*1989a*) following OECD TG 204, regarded as supplementary information, which is not acceptable for hazard classification purposes (Guidance on the information Requirements and Chemical Safety assessment, R.7.8.4.1).

In the only valid study (*DUP Anonymous, 2012)* the chronic effect of tribenuron-methyl was examined in an early life-stage toxicity test with sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions, following OECD TG 210 and GLP criteria. Based on mean measured concentrations of tribenuron-methyl, the NOEC value for all tested endpoints (egg hatchability, post-hatch survival, standard length and blotted wet weight) was 11.9 mg a.s./L.

Three studies were available on aquatic invertebrates. The lowest value was the NOEC of 41 mg/L (nominal concentrations) with *Daphnia magna* in a 21 days static-renewal study according to OECD TG 211.

Several studies were available on algae and aquatic plants. Plants were more sensitive than algae. The lowest chronic endpoint was derived with *Myriophyllum spicatum,* following to OECD TG 239. The mean analytically determined concentration at test start was 98 % of the nominal concentration and at test end this concentration was 82 % of the nominal concentration. Consequently, all study results were based on nominal concentrations. Tribenuron-methyl had a significant inhibitory effect in growth rate based on shoot length and fresh weight at test item concentrations of 3.05 µg/L and higher. Therefore, the 14-day NOEC based on these endpoints was determined to be 0.000954 mg/L and **the 14-day EC_{10} based on fresh weight was 0.00081 mg/L**. The study is relevant and acceptable.

Comments received during public consultation

One MS and a company commented on environmental classification proposal. Both of them agreed with the proposed environmental classification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider tribenuron-methyl as not rapidly degradable.

The substance is rapidly hydrolysed under pH 4, moderately at pH 7 and nearly stable at pH 9, photochemically stable and not readily biodegradable. Moreover, the tribenuron-methyl is not ultimately degraded to a level greater than 70 % over 28 days in aerobic water and water/sediment simulation studies.

Bioaccumulation

RAC agrees with the DS that tribenuron-methyl has a low potential to bioaccumulate in aquatic organisms. The basis for this is that the log K_{ow} values are < 4.

Acute aquatic toxicity

Both algae and aquatic plants are sensitive to tribenuron-methyl. The most sensitive organism was *Lemna gibba* with the 7d E_rC_{50} = 0.0047 mg/L based on nominal concentrations. This value is below the cut -off of 1 mg/L for aquatic acute category 1, with an M-factor of 100 (0.001 < $L(E)C_{50}$ ≤ 0.01).

Chronic aquatic toxicity

The most sensitive species tested was *Myriophyllum spicatum*. The study is acceptable and relevant *because Myriophyllum spicatum*, a rooted macrophyte species, may be considered a target aquatic plant species for a herbicide. Therefore it is adequate to use macrophyte data for the classification of tribenuron-methyl. The result for the 14d static condition test is an E_rC_{10} = 0.00081 mg/L, based on nominal concentrations. This value is lower than the classification criterion for aquatic Chronic Category 1 (0.1 mg/L) for not rapidly degradable substances in the aquatic environment. The appropriate M-factor is 100, since the toxicity is within the range of 0.0001 < EC_{10} (NOEC) ≤ 0.001.

In conclusion, RAC in agreement with the DS recommends that tribenuron-methyl should be classified as:

Aquatic Acute 1; H400, M-factor of 100;

Aquatic Chronic 1; H410, M-factor of 100.

Additional references

Cappon G.D., Fleeman T.L., Chapin R.E., Hurtt M.E. (2005). Effects of feed restriction during organogenesis on embryo-fetal development in rabbit. Birth Defects Research (Part B) 74, 424-430.

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).

Annex 3 Minority opinion