Committee for Risk Assessment

RAC

Annex 1
Background document
to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 September 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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**

Index Number: **607-177-00-9**

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Version number: 2 **Date: June 2017**

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

ANNEX 1 BACKGROUND DOCUMENT TO RAC OPINION ON TRIBENURON-METHYL (ISO); METHYL 2-[N-(4-METHOXY-6-METHYL-1,3,5-TRIAZIN-2-YL)-N-METHYLCARBAMOYLSULFAMOYL]BENZOATE

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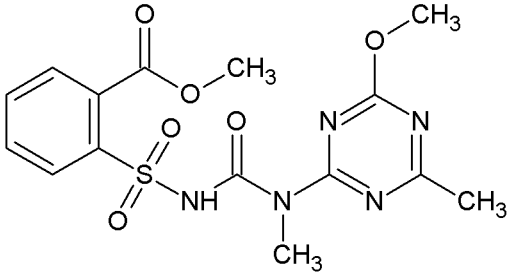
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Methyl 2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl]benzoate
Other names (usual name, trade name, abbreviation)	DPX-L5300 (development code number)
ISO common name (if available and appropriate)	Tribenuron-methyl
EC number (if available and appropriate)	401-190-1
EC name (if available and appropriate)	Methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylsulfamoyl]benzoate
CAS number (if available)	101200-48-0
Other identity code (if available)	546.201 (CIPAC No)
Molecular formula	$C_{15}H_{17}N_5O_6S$
Structural formula	
SMILES notation (if available)	Cc1nc(nc(n1)OC)N(C)C(=O)NS(=O)(=O)c2ccccc2C(=O)OC
Molecular weight or molecular weight range	395.4 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant – tribenuron-methyl does not contain any stereoisomers
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant – tribenuron-methyl is no UVCB
Degree of purity (%) (if relevant for the entry in Annex VI)	960 g/kg

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
tribenuron-methyl (ISO); methyl 2-[*N*-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-*N*-methylcarbamoylsulfamoyl]benzoate	Min. 96%	Skin Sens. 1 H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 M=100	There are four aggregated notifications with a total of 123 notifiers. The main notification comprising 66 notifiers has the same proposal as the current harmonised classification but without a M-factor. The second notification (53 notifiers) has the same proposal as the current harmonised classification. The other minor notifications proposes Skin Sens. 1 only (3 notifiers) and the current harmonised classification with the addition of the supplementary hazard statement code EUH401 (one notification).

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

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
No impurity is considered relevant for the classification (i.e. information on impurities is only presented as confidential information in the IUCLID-dossier section 1)				

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

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
Tribenuron-methyl does not contain any additives					

Table 5: Test substances (non-confidential information)

SAMPLE	ASSAY, %	STUDY	YEAR	STUDIES
L5300-9	94	HLO 369-83	1983	Primary irritation and sensitization study in guinea pigs
L5300-9	94	HLR 245-83, Revision No. 2	1988	Mutagenicity testing of INL-5300-9 in the Salmonella typhimurium plate incorporation assay
L5300-13	99	HLR 413-83 HLR 413-83, SU1	1985 2000	Ninety-day feeding and one-generation reproduction study in rats with benzoic acid, 2-[[[[N-(4-methoxy-6-methyl-1,3,5- triazin-2-yl)-N-methylamino]carbonyl]amino]sulfonyl]-, methyl ester (INL-5300)
L5300-14	96.6	HLR 627-87	1987	Primary eye irritation study with IN L5300-14 in rabbits
L5300-14	96.6	HLR 712-87	1988	Closed-patch repeated insult dermal sensitization study (Buehler method) with IN L5300-14 in guinea pigs
L5300-14	98	HLR 580-85	1985	Four-week range-finding and ninety-day feeding study in mice with INL-5300
L5300-20	96.8	HLO 514-85 HLO 514-85, SU1	1985 2000	A three-month feeding study in dogs with H-15527
L5300-20	96.8	HLR 565-84	1985	Assessment of INL-5300-20 in the in vitro unscheduled DNA synthesis in primary rat hepatocytes
L5300-20	96.8	HLR 286-85	1985	In vivo assay of INL-5300-20 for chromosome aberrations in rat bone marrow cells
L5300-20	96.8	HLR 420-85	1985	Mouse bone marrow micronucleus assay of INL-5300-20
L5300-20	96.8	HLR 61-87 HLR 61-87, SU1 HLR 61-87 SU2 HLR 61-87 SU3	1987 1987 1987 2000	Combined chronic toxicity/oncogenicity study with INL-5300
L5300-20	96.8	HLR 112-89 HLR 112-89 SU1	1989 2000	Ninety-day feeding study with IN L5300-20: Effect on estrous cycle
L5300-22	95.8	HLR 565-86	1986	One-year feeding study in dogs with IN L5300
L5300-22	94.2	HLR 60-87	1987	Oncogenicity study with INL-5300; eighteen-month feeding study in mice
L5300-22	94.2	HLR 193-86 HLR 193-86 SU1 HLR 193-86 SU2	1986 1988 2000	Two-generation reproduction study in rats with IN-L5300

L5300-22	94.2	HLO 513-85 HLO 513-85 SU1	1985 2000	Developmental toxicity study of L-5300 in the rat
L5300-22	94.2	HLR 150-86 HLR 150-86, SU1	1986 2000	INL-5300. Developmental toxicity study in rabbits dosed by gavage on days 7-19 of gestation
L5300-35	95	NOTOX 0382/510	1986	Assessment of the skin sensitization potential of L5300 in the guinea-pig (Magnusson and Kligman maximization test)
L5300-35	95	NOTOX 0382/534	1986	Assessment of the subacute oral toxicity of L5300 in the rat: 28-day study
L5300-104	95.8	HLR 376-94	1994	Primary dermal irritation study with DPX-L5300-104 in rabbits
L5300-203	97.8	DuPont-3366	1999	Tribenuron methyl technical (DPX-L5300): Acute dermal toxicity study in rats
L5300-203	97.8	DuPont-2938	2000	Tribenuron methyl (DPX-L5300): In vitro mammalian chromosome aberration test
L5300-203	97.8	DuPont-3387	2000	Tribenuron methyl (DPX-L5300): In vitro mammalian cell gene mutation (CHO/HGPRT) test with an independent repeat assay
L5300-203A	97.7	DuPont-3090	1999	Tribenuron methyl (DPX-L5300): Inhalation median lethal concentration (LC50) study in rats
L5300-271	99.3	DuPont-37516	2013	Tribenuron methyl (DPX-L5300) technical: In vitro 3T3 NRU phototoxicity test
L5300-281	98.2	Dupont-31858	2011	Tribenuron methyl (DPX-L5300) technical: 28-Day immunotoxicity feeding study in rats
Lot B1026	97.7	CIT 7611 TSL	1992	DPX-L5300 technical toxicity study for 28 days by dermal application to rabbits

SAMPLE	ASSAY, %	STUDY	YEAR	STUDIES
L5300-20	96.8	HLO 568-84	1984	An acute oral toxicity study in the bobwhite with #H 15, 527 Wildlife International Ltd (USA)
L5300-20	96.8	HLO 570-84	1984	A dietary LC50 study in the bobwhite with H#15,527
L5300-20	96.8	HLO 569-84	1984	A dietary LC50 study in the mallard with H#15,527
L5300-20	96.8	HLR 228-86 Revision No. 2	1987	Static acute 96-hour LC50 of INL-5300-20 to bluegill sunfish (Lepomis macrochirus)
L5300-20	95.8	HLR 311-89	1989	Flow-through 21-day LC50 and NOEC of DPX-L5300-20 to rainbow trout (Salmo gairdneri)
L5300-20	95.8	HLR 164-89 Revision No 1	2013	Chronic toxicity of IN L5300-20 to Daphnia magna
L5300-20	95.8	AMR 2038-91 Revision No 1	1992	Influence of tribenuron methyl on seed germination, seedling emergence, and vegetative vigor of several terrestrial plants
L5300-104	92.56 97.7	AMR 3070-94 Revision No 1	2000	Tribenuron methyl (DPX-L5300): Influence on growth and reproduction of Lemna gibba G3
L5300-104	95.8	HLO 11-95	1995	DPX-L5300-104: A reproduction study with the northern bobwhite (Colinus virginianus)
L5300-104	95.8	HLO 12-95	1995	DPX-L5300-104: A reproduction study with the mallard (Anas platyrhynchos)
L5300-143	97.4	DuPont-1222	1998	Tribenuron methyl technical (DPX-L5300): Influence on growth and growth rate of the green alga Selenastum capricornutum
L5300-143	97.4	DuPont-1221	1998	Tribenuron methyl technical (DPX-L5300): Influence on growth and growth rate of the blue-green alga Anabaena flos-aquae
L5300-152[a] L5300-153	99.7 98.9	AMR 4201-96	1996	DPX-L5300: Acute toxicity to the rainbow trout, Oncorhynchus mykiss
L5300-152[2] L5300-153	99.7 98.9	AMR 4202-96	1996	DPX-L5300: Acute toxicity to the daphnid, Daphnia magna
L5300-184	98.44	AMR 5166-98	1998	Tribenuron methyl technical: acute oral and contact toxicity to the honeybee, Apis mellifera L.
L5300-203	97.3	DuPont-4059	2000	Tribenuron methyl technical (DPX-L5300): Acute toxicity to the earthworm, Eisenia fetida (Savigny) in artificial soil

Batch	**Purity (%)**	**Study**
63B3011159	98.30	In vitro 3T3 NRU phototoxicity test 2014 (Report No 260 TBM)
Lot 03PP	96.98	Used in local nymph node assay in mice 2010 (Report No 190 TBM amdt-1)
Lot 070708	97.38	used in Ames test 2009 (Report 140965)

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2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	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	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	
Resulting Annex VI entry if agreed by RAC and COM											

Table 7: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Tribenuron-methyl has a harmonised classification in Skin sens. 1, Aquatic Acute 1 and Aquatic Chronic 1. This has been translated from the classification decided under the Dangerous Substances Directive 67/548/EEC where it was classified as Xi; R43, R50/53 by the ECB as presented in Commission Directive 2001/60/EC.

The substance was finally discussed at the Meeting of Technical Committee C&L on the Classification and Labelling of Dangerous Substances in Arona (21-24 September), 2004. In the final report (ECBI/139/04 Rev. 2) the above mentioned classification was agreed. In the discussions three member states expressed their opinion to classify for carcinogenicity (R40) but a major part of the Member States supported no classification and the TC C&L could then agree to this.

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).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Tribenuron-methyl is an active substance in the meaning of Regulation EC 1107/2009 and justification is not required (Article 36 CLP Regulation).

5 IDENTIFIED USES

Tribenuron-methyl is an active substance used in plant protection products. It is used as an herbicide on a wide range of crops in EU Member States. The representative uses currently (re)-evaluated under EC Regulation 1107/2009 includes cereals (spring and winter), pasture, sun flower and olive.

6 DATA SOURCES

Tribenuron-methyl was included in Annex I of Directive 91/414/EC by Commission Directive 2005/54/EC of 19 September 2005. Entry into Force of Annex I listing was 1st of March 2006. Annex I listing was extended to 31 October 2017 according to Commission regulation No 533/2013 of 10 June 2013. Tribenuron-methyl is currently being evaluated under the following regulations for renewal of approval as an active substance in plant protection products:

• REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market

• COMMISSION IMPLEMENTING REGULATION (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

• COMMISSION IMPLEMENTING REGULATION (EU) No 533/2013 of 10 June 2013 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of the active

substances 1-methyl-cyclopropene, chlorothalonil, chlorotoluron, cypermethrin, daminozide, forchlorfenuron, indoxacarb, thiophanate-methyl and tribenuron-methyl

The data presented in this dossier has been submitted by the two applicants (DuPont de Nemours (Deutschland) GmbH and EU Tribenuron AIR 3 Task Force) as part of the renewal process. Some of the data was submitted and evaluated during the first approval (provided by DuPont only) while other data was submitted for the first time for the purpose of renewal of approval. All data is presented in the Renewal Assessment Report (RAR) prepared by Rapporteur Member State (RMS) Sweden which has been submitted to EFSA.

The RAR currently undergoes a peer-review process among the Member states and EFSA (to be completed in March-April 2017). The peer-review process permits the applicants to provide certain requested additional data during a stop-the-clock-period. Such data when relevant for this classfication dossier has been incorporated where appropriate. In addition to this some corrections has been done to information provided for physical and chemical properties as compared to those presented in the RAR.

It is noted that there is also one active registrations under REACH. According to the file available on the ECHA website (https://echa.europa.eu/registration-dossier/-/registered-dossier/9259/5/3/2), this registration contains data on physical and chemical properties (appearance, melting point, boiling point, density, vapour pressure, partition coefficient, water solubility, solubility in organic solvents, surface tension and explosiveness), environmental fate (biodegradation in water: screening test), ecotoxicity (short-term toxicity to fish and short-term toxicity to aquatic invertebrates) and toxicity (oral, inhalative and dermal acute toxicity, repeated dose toxicity: other routes and genetic toxicity: in vitro). However, for all the data reported information on data source, materials and methods and applicant's summary and conclusion is lacking. The validity of the data can thus not be judged. Moreover, for the few tox data provided information on species is missing. In conclusion, since the reliability of the data cannot be judged and since it does not add much to the available file for tribenuron-methyl this additional data is not presented further herein.

General remark on reliability indicators

All studies referred to and used in this CLH-dossier have been submitted under the European regulatory framework for Plant Protection Products (see the references to the relevant Regulations above). Under that framework the study summaries should be submitted in the OECD format (Tier II summaries; see http://www.oecd.org/env/ehs/pesticides-biocides/oecdguidancedocumentsforpesticideregistration.htm), which does not include reporting of reliability indicators. However, the data requirements as well as the criteria for accepting studies are very strict for plant protection products. Moreover the data provided in this CLH-report has already been subject to a rigid peer-review process among the Member states and the European Food Safety Agency (EFSA). Hereby, the quality of the data used in this report corresponds to Klimish scores 1 or 2.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid (98-99%)	Jeffrey, D. J. (2000) Siripriya, G., (2014) DuPont-39970 Cowlyn, N. (2014) 288 TBM	
Melting/freezing point	Melting: 149.95°C (99.3%) Melting with decomposition: 137.9°C (98%)	Sundari, M.T (2013) DuPont-36467 Woolley, A. J., & O'Connor, B. J., (2009) 165 TBM	
Boiling point	Not applicable since the substance decomposes prior to boiling starting at 138 °C (98%) to 182.5°C (99.3%)	Sundari, M.T. (2013) DuPont-36467 Woolley, A. J., & O'Connor, B. J., (2009) 165 TBM	
Relative density	No data		
Vapour pressure	5.99 x 10^{-9} Pa (99.3%) at 20 °C 1.0 × 10^{-6} Pa (99.2%) at 25 °C	Ganesh, M.U. (2013) DuPont-36468 Cowlyn, N. (2014) 288 TBM	Extrapolated from measurements at higher temperature (40-111°C).
Surface tension	73.0 mN/m (90 % saturated solution, 19.6 ± 0.1°C, 97.4%) 71.0 mN/m (90 % saturated solution, 20°C, 99.2%)	Huntley, K. (2000) DuPont-3576 Cowlyn, N. (2014a) 288 TBM	
Water solubility	99.3%, 20 °C: Unbuffered distilled: 0.019 ± 0.002 g/L pH 5, unstable pH 7, 2.483 ± 0.600 g/L pH 9, >17.85 g/L 99.2%, 20 °C: Unbuffered distilled: 278 mg/L	Siripriya, G. (2014) DuPont-39970 Cowlyn, N. (2014) 288 TBM	

Property	Value	Reference	Comment (e.g. measured or estimated)
	pH 7, 1660 mg/L pH 10, > 250 g/L		
Partition coefficient n-octanol/water	20°C (99.3%): Distilled water: log Pow = 0.85 ± 0.03 pH 7.0 (phosphate buffer): log Pow = -0.38 ± 0.01 pH 9 (borate buffer): log Pow = -0.93 ± 0.01 20°C (99.2%): pH 4 orthophosphate buffer: logP = 2.0 pH 7.0 orthophosphate buffer: logP = -0.46 pH 10.0 borate buffer: logP = -2.22	Pakki, U.V.S. (2013) DuPont-36463 Cowlyn, N., 2014 (288 TBM)	
Flash point	Not applicable. The active substance is a solid; its melting point is > 40 °C.		
Flammability	Not highly flammable (97.8%)	Gravell, R. L. (1999) DuPont-2443	
Explosive properties	Not explosive (97.8%)	Gravell, R. L. (1999) DuPont-2443	
Self-ignition temperature	No self-ignition up to 400°C (97.8%)	Gravell, R. L. (1999) DuPont-2443	
Oxidising properties	Not oxidizing under the conditions of the test (99.3%)	Loganayagi, C. (2013) DuPont-36465	
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	pKa = 4.65 ± 0.20 at 20°C (99.3%) pKa = 4.6 at 20°C (99.2%)	Piriyadarsini, J.R. (2013) DuPont-36464 Cowlyn, N. (2014) 288 TBM	
Viscosity	Not relevant since the substance is a solid with a melting point >> 40 °C.		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A.14	Not explosive (97.8% purity)		Gravell, R. L., (1999) DuPont-2443

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

One negative study performed in accordance with EEC A.14 is available. It could also be added that the substance does not contain any functional groups known to confer explosive properties.

8.1.2 Comparison with the CLP criteria

It is not evident from the CLP-guidance that a negative result from the EEC A.14-test automatically means that it should not be classified as an explosive under CLP. Nevertheless, based on the structure, it seems that the waiving criteria for non-testing applies and a classification is thus not warranted.

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed due to lack of data.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (tribenuron-methyl is not a gas).

8.3 Oxidising gases

Hazard class not applicable (tribenuron-methyl is not a gas).

8.4 Gases under pressure

Hazard class not applicable (tribenuron-methyl is not a gas).

8.5 Flammable liquids

Hazard class not applicable (tribenuron-methyl is not a liquid).

8.6 Flammable solids

Table 10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A.10	Not highly flammable (97.8% purity)	The substance did not support combustion in the initial screening test	Gravell, R. L., (1999) DuPont-2443

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

One test performed in accordance with EEC A.10 is available. The substance did not ignite in the preliminary screening test and is thus not regarded a highly flammable in the sense of the test method.

8.6.2 Comparison with the CLP criteria

The preliminary screening test in EEC A.10 and in CLP are identical. The substance should thus not be classified as a flammable substance under CLP.

8.6.3 Conclusion on classification and labelling for flammable solids

No classification is proposed. Data is conclusive but not sufficient for classification.

8.7 Self-reactive substances

Data lacking.

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

No data has been provided addressing this property.

8.7.2 Comparison with the CLP criteria

No data has been provided that addresses this property. However, the structure of tribenuron-methyl does not contain any functional groups known to confer self-reactive properties (compared with Tables A6.1 and A6.2 in Appendix 6 to UN-MTC). It contains the structural feature S=O (Table A6.2) but none of the functional groups given in the examples. The waiving criteria in CLP therefore applies and no classification for self-reactive properties is warranted.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed due to lack of data.

8.8 Pyrophoric liquids

Hazard class not applicable (tribenuron-methyl is not a liquid).

8.9 Pyrophoric solids

Data lacking.

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

No specific data derived in accordance with the recommended test method in CLP is available. However, tribenuron-methyl has been handled in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections).

8.9.2 Comparison with the CLP criteria

Based on experience in handling of tribenuron-methyl, it is not a pyrophoric solid (compare with example in CLP guidance section 2.10.7.2).

8.9.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

8.10 Self-heating substances

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A.16	No self-ignition up to 400°C		Gravell, R. L., (1999) DuPont-2443

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

One negative study conducted in accordance with EEC A.16 is available.

8.10.2 Comparison with the CLP criteria

Since no study has been provided in accordance with the recommended test method in CLP a full assessment cannot be made.

8.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed due to lack of data (generated in accordance with the recommended test method in CLP).

8.11 Substances which in contact with water emit flammable gases

Data lacking.

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

No specific data derived in accordance with the recommended test method in CLP has been provided. However, tribenuron-methyl has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas.

8.11.2 Comparison with the CLP criteria

Based on experience in handling of tribenuron-methyl, it is not a substance which in contact with water emit flammable gases (compare with CLP guidance section 2.12.3.2).

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

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

8.12 Oxidising liquids

Hazard class not applicable (tribenuron-methyl is not a liquid).

8.13 Oxidising solids

Table 12: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A.17	Not oxidizing in the sense of the test method. The maximum burning rate of the reference (barium nitrate):cellulose mixture (3:2) was higher (0.62 mm/s) than the maximum burning of the test item: cellulose mixture (1:9; 0.42 mm/s)	The test item:cellulose 1:1 and 4:1 mixtures also burned to completion.	Loganayagi, C. (2013) DuPont-36465

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

One test performed in accordance with EEC A.17 is available. The test was negative in the sense of the test method but all tested test item:cellulose mixtures (1:9, 1:4, 3:7, 2:3, 1:1, 3:2, 7:3, 4:1 and 9:1) burned to completion.

8.13.2 Comparison with the CLP criteria

The test under EEC A.17 does not utilize the same reference standard as in the test recommended under CLP (potassium bromate). Moreover, the decision logic in CLP stipulates that the reference:cellulose mixture should also be tested in 3:7 and 2:3 ratios whereas in EEC A.17 only a 3:2 mixture could be used as in this case. Hereby, the decision logic cannot be followed and it cannot be fully concluded that the test substance is not an oxidizer under CLP.

In addition, it should be noted that the waiving criteria does not apply since the substance contains oxygen not only chemically bonded to carbon or hydrogen (i.e. it contains a sulfonyl-group).

8.13.3 Conclusion on classification and labelling for oxidising solids

No classification is proposed due to lack of data.

8.14 Organic peroxides

Hazard class not applicable (tribenuron-methyl is not an organic peroxide).

8.15 Corrosive to metals

No data has been provided addressing this property.

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

No data has been provided addressing this property. Tribenuron-methyl does contain acidic or basic functional groups (pka=4.6-4.7) and should thus be considered for classification in this class according to the CLP-guidance. However, the substance is a high melting substance which also means that it is currently difficult to test with the available test method.

8.15.2 Comparison with the CLP criteria

No data has been provided and a thorough assessment can thus not be made.

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed due to lack of data.

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.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 13: Summary table of toxicokinetic studies

Method/Route/Species/Strain/Sex/ No. per dose/Test substance/dose	Results	Remarks	Reference
OECD TG 417 Oral (gavage) Rat Crl:CD®BR 5 M, 5 F (kinetic studies) 5 M, 5 F (metabolism studies) [Phenyl-^{14}C(U)] tribenuron-methyl and [Triazine-2-^{14}C] tribenuron-methyl	Absorption is 67 % (% metabolites in the urine). Note: No bile cannulated rats. Tribenuron-methyl is rapidly metabolised. Major urinary metabolites is metsulfuron methyl, saccharin and o-demethyl triazine amine. Tribenuron-methyl did not accumulate in any tissue. 67 % was excreted via urine.	-	RAR Vol. 3 B.6.1.1/01 Key study

Method/Route/Species/Strain/Sex/ No. per dose/Test substance/dose	Results	Remarks	Reference
~20 mg/kg bw (low-dose) and ~1700-2000 mg/kg bw (high-dose) 96 and 168 hour			

M: males
F: females

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

Absorption: 67 % of the administered doses.

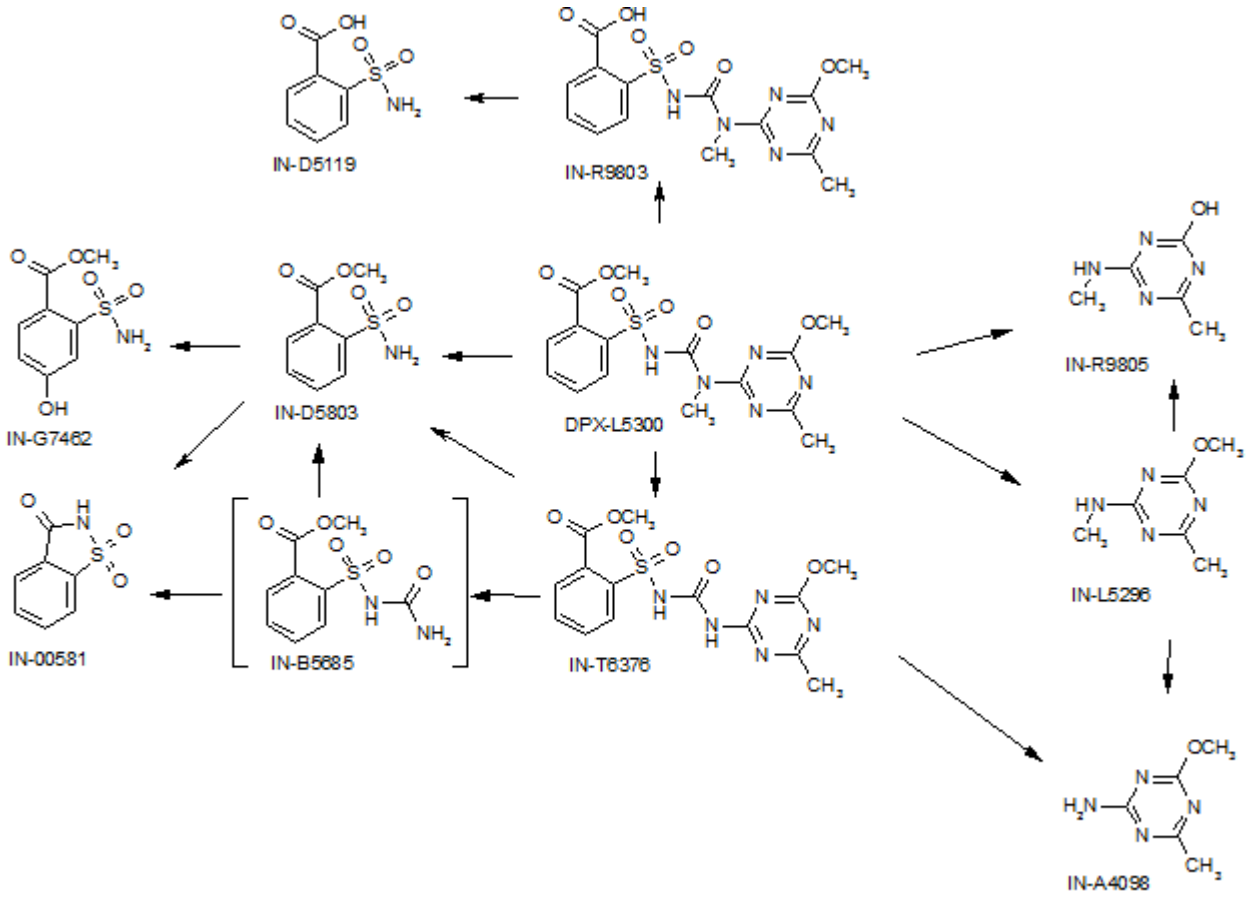
Metabolite profile: Tribenuron-methyl was extensively and rapidly metabolised in rats of both sexes and under all dosing regimens. Less than 10 percent of the total dose was recovered as intact tribenuron-methyl in the urine or faeces. Major urinary metabolites were identified as DPX-T6376 (metsulfuron methyl), IN-R9803 (tribenuron-methyl free acid), IN-00581 (saccharin), IN-G7462 (hydroxylated sulphonamide), and IN-R9805 (o-demethyl triazine amine). Minor metabolites included IN-D5119 (acid sulphonamide), IN-L5296 (triazine amine) and IN-A4098 (n-demethyl triazine amine), and IN-D5803 (sulphonamide). All metabolites were found in animals of both sexes. IN-B5685 was observed as a minor metabolite of DPX-T6376 (metsulfuron methyl) in a previous rat metabolism study (Metsulfuron methyl 7593/VI/97 – final 14 August 2000 EU review report, http://europa.eu.int/comm/food/fs/ph_ps/pro/eva/existing/list1-13_en.pdf) and is included in the proposed metabolic pathway as a plausible, but transient metabolite of tribenuron-methyl. For the major metabolites, urine profiles did not change after glucuronidase/sulfatase incubation indicating that glucuronide or sulfate conjugation was not significant.

Distribution: Tribenuron-methyl was not accumulated in any tissue. The major metabolites found in the urine were metsulfuron methyl, saccharin and O-demethyl triazine in both sexes and were the result of N-demethylation, de-esterification, hydrolysis of the sulfonylurea bridge, hydroxylation of the phenyl ring, and/or O-demethylation of the triazine ring. In the female rats hydroxylated sulfonamide was also found. Quantitatively, there were some differences between the sexes and doses and between the urinary and faecal metabolites. However, the major metabolites were found equally in both sexes and doses in urine and faeces.

Excretion: Tribenuron-methyl was primarily excreted *via* urine, with urinary excretion averaging two to four time's faecal excretion for all groups and both sexes. 67% was found in the urine and approximately 5% or less unmetabolised substance was found in faeces. The excretion rate of tribenuron-methyl was slower in the high-dose group compared to the low-dose animals, especially in female rats. The total excretion after 168 hours was greater than 97%. The estimated half-life for the high-dose was below 54 hours for males and 96 hours for females. The low-dose groups had shorter half-lives, 26 to 33 hours for males and females.

The metabolism pathway proposed in mammals is given in the following scheme. The postulated intermediates are shown in brackets.

Figure: Proposed metabolic pathway of tribenuron-methyl in mammals



10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, Vehicle	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral OECD TG 401	Rat Crl:CD®(SD)IGS BR 5/sex/dose	Tribenuron-methyl technical Purity: 97.8% Vehicle: Corn oil	5000 mg/kg bw Observations in 14 days	>5000 mg/kg bw	RAR Vol. 3 B.6.2.1/01 Key study

Table 15: Summary table of human data on acute oral toxicity

No data.

Table 16: Summary table of other studies relevant for acute oral toxicity

No data.

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

An acute oral toxicity study was conducted according to GLP and OECD TG 401. Single doses of the test substance suspended in Mazola®corn oil were administered to fasted rats at a dosage of 5000 mg/kg bw and were then observed for 14 days. Male rats exhibited no clinical signs of toxicity during the study, while one female showed a red-stained face day 2. Other clinical signs observed in the four other females started from day 8 and included red-stained head, hunched over posture, and ruffled fur. No clinical signs were observed after day 10. No body weight losses occurred in male rats. Weight loss up to 9 % from day 2 until day 11 was registered in females. However, the final body weight of all female rats surpassed their fasted body weight. No gross lesions were present in the rats at necropsy. The oral LD_{50} for male and female rats was >5000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

According to the CLP Guidance, classification in Acute Tox. 4 (the lowest classification) is required for substances with oral LD_{50} of 300-2000 mg/kg bw. The LD_{50} for oral toxicity was above 2000 mg/kg bw and tribenuron-methyl thus does not fulfil the classification criteria for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification is proposed for tribenuron-methyl.

10.2 Acute toxicity - dermal route

Table 17: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal OECD TG 402	Rat Crl:CD®(SD)IGS BR, 5/sex/dose	Tribenuron-methyl technical Purity: 97.7%	5000 mg/kg bw Observations in 14 days	>5000 mg/kg bw	RAR Vol. 3 B.6.2.2 Key study

Table 18: Summary table of human data on acute dermal toxicity

No data.

Table 19: Summary table of other studies relevant for acute dermal toxicity

No data.

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

An acute dermal toxicity study was conducted according to GLP and OECD TG 401. No treatment related mortality was observed and no clinical signs of toxicity were 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 the test substance. Six rats exhibited no erythema or oedema during the study. Mild and/or moderate erythema was observed in four rats and one rat exhibited mild oedema. Focal eschar, desquamation, hyperkeratosis and epidermal scaling were also observed during the study. No dermal effects were observed after day 13. Weight loss of approximately 4-10% of initial body weight was observed in the rats the day after application. However, these clinical signs and the weight loss were considered to be due in part to the wrapping procedure. No gross lesions were present in the rats at necropsy. The dermal LD_{50} for male and female rats was >5000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

According to the CLP Guidance, classification in Acute Tox. 4 (the lowest classification) is required for substances with dermal LD_{50} of 1000-2000 mg/kg bw. The LD_{50} for dermal toxicity was above 2000 mg/kg bw, and tribenuron-methyl thus does not fulfil the classification criteria for acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed for tribenuron-methyl.

10.3 Acute toxicity - inhalation route

Table 2010: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation OECD TG 403	Rat Crl:CD®(SD)IGS BR 5/sex/dose	Tribenuron-methyl technical, fine powder Purity: 97.7% (MMAD 2.8 or 2.7 µm)	6.0 mg/L, 4 hour, nose only 14 days observation	>6.0 mg/L	RAR Vol. 3 B.6.2.3/01 Key study

Table 21: Summary table of human data on acute inhalation toxicity

No data.

Table 22: Summary table of other studies relevant for acute inhalation toxicity

No data.

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

An acute inhalation toxicity study was conducted according to GLP and OECD TG 403. Five rats/sex/group were exposed to an atmosphere of tribenuron-methyl in air for a single 4-hours period. The rats were observed for mortality and clinical signs of toxicity immediately after they were removed from the restraints following exposure. All rats were observed for mortality daily, and were weighed and observed for clinical signs of toxicity 1-4 times a week during a 14-days post exposure period. At the end of the period, all rats were sacrificed by carbon dioxide asphyxiation and subjected to gross pathological examination. No animals died during exposure, or during the 14-days recovery period. All rats showed a slight body weight loss the day following exposure. In male rats a body weight loss of 0.35 – 4% of the initial weight was observed, and in female rats 1.0 – 2.0%. All rats showed an overall weight gain by the end of the 14-days recovery period. No clinical signs were observed during the recovery period, 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 at the end of the recovery period. The LC_{50} was considered to be greater than 6.0 mg/l for both sexes.

10.3.2 Comparison with the CLP criteria

According to the CLP Guidance, classification in Acute Tox. 4 (the lowest classification) is required for substances with an inhalation LC_{50} of 1.0-5.0 mg/L. The LC_{50} for inhalation toxicity was greater than 6.0 mg/l for both sexes, tribenuron-methyl thus does not fulfil the classification criteria for acute inhalation toxicity.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification is proposed for tribenuron-methyl.

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.

10.4 Skin corrosion/irritation

Table 23: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Dermal irritation OECD TG 404	Rabbit New Zealand White 6 males	Tribenuron-methyl technical Purity: 95.8%	0.5 g/animal, 4 hours	One of six rabbit exhibited erythema, but no oedema 1 hour after patch removal (mean score 0.17). No dermal irritation was observed throughout the remainder of the study.	RAR Vol. 3 B.6.2.4/01 Key study

Table 24: Summary table of human data on skin corrosion/irritation

No data.

Table 25: Summary table of other studies relevant for skin corrosion/irritation

No data.

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

In a study performed in accordance with GLP and OECD TG 404, 6 male New Zealand White rabbits each received dermal treatments with 0.5 g of tribenuron-methyl for 4 hours under occlusive conditions. Approximately 1 hour after removal of the test patches, the test sites were evaluated for erythema, oedema, and other evidence of dermal effects and were scored according to the Draize scale. Additional evaluations were made at approximately 24, 48 and 72 hours after removal of the patches. The adjacent areas of untreated skin were used for comparison. No dermal irritation was observed in 5 rabbits during the study. One rabbit exhibited erythema, but no oedema 1 hour after patch removal. No dermal irritation was observed throughout the remainder of the study. 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 the test substance.

10.4.2 Comparison with the CLP criteria

According to the CLP Guidance Table 3.2.2, a substance should be classified in Category 2 (Irritant) *if:*

"-mean value of ≥2.3 - ≤4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

-inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above"

In the skin irritation test conducted with tribenuron-methyl no oedema/eschar was noted, and the mean value for erythema was below 2.3. Furthermore, no inflammation persisted to the end of the observation period. Thus, tribenuron-methyl does not fulfil the classification criteria for skin irritation.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed for tribenuron-methyl.

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.

10.5 Serious eye damage/eye irritation

Table 26: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Eye irritation OECD TG 405	Rabbit New Zealand White 6 males	Tribenuron-methyl technical Purity: 96.6%	50 mg/animal Examination after 1, 24, 48 and 72 hours	Slight effects on the corneal opacity were seen in two rabbits (mean score of 0.33 at 1-hour), mild conjunctival redness (maximum score of 1) in six rabbits and slight chemosis (mean score of 0.67 at 1 hour) in four rabbits. Biomicroscopic examinations were negative for corneal injury in all treated animals throughout the study. The treated eyes of all animals were normal 72 hours after treatment.	RAR Vol. 3 B.6.2.5/01 Key study

Table 27: Summary table of human data on serious eye damage/eye irritation

No data.

Table 28: Summary table of other studies relevant for serious eye damage/eye irritation

No data.

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

In a study performed in accordance with GLP and OECD TG 405, 0.1 ml of tribenuron-methyl was placed in the lower conjunctival sac of the right eye of 6 male rabbits. The other eye served as control. Treated and control eyes remained unwashed. The eyes were examined at 1, 24, 48 and 72 hours using illumination and magnification and were scored for ocular reactions using the Draize scale. Slight effects on the corneal opacity (score of 1 at 1-hour) were seen in two rabbits, mild conjunctival redness (maximum score of 1) in six rabbits and slight chemosis (score of 1 at 1-hour) in four rabbits. No effects on iris were noted. Biomicroscopic examinations were negative for corneal injury in all treated animals throughout the study. The treated eyes of all animals were normal 72 hours after treatment.

10.5.2 Comparison with the CLP criteria

According to the CLP Guidance Table 3.3.2, a substance should be classified in Category 2 (Irritating to eyes) *" If it produces, at least in 2/3 animals, a positive response of:*

-corneal opacity ≥1 and/or

-iritis ≥1, and/or

-conjunctival redness ≥2 and/or

-conjunctival oedema (chemosis) ≥2

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days"

In the eye irritation test conducted with tribenuron-methyl mean scores for corneal opacity did not exceed 1, and mean scores for conjunctival oedema or redness did not exceed 2. Furthermore, no effects were noted in iris and no effects persisted to the end of the observation period. Thus, tribenuron-methyl does not fulfil the classification criteria for eye irritation.

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

No classification is proposed for tribenuron-methyl.

RAC evaluation of serious eye damage/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.

10.6 Respiratory sensitisation

No data.

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data.

10.6.2 Comparison with the CLP criteria

Not relevant as no data are available.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not relevant as no data are available.

10.7 Skin sensitisation

Table 29: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Buehler method OECD TG 406 except that 0.3% DNCB was used as the positive control	Guinea pig Dunkin-Hartley albino 10 animals of each sex in treatment group and 5 in control	Tribenuron-methyl technical Purity: 96.6%	Induction: 0.25 g/animal (62.5 %) Challenge: 0.25 g/animal (62.5 %)	A sensitisation rate of 20% and 60% after 24 and 48 hours, respectively after the first challenge, and of 10% after the second challenge	RAR Vol. 3 B.6.2.6/01 Key study
Magnusson and Kligman maximization test OECD TG 406 except no positive control	Guinea pig Dunkin-Hartley guinea females 10 control and 20 in treatment group	Tribenuron-methyl technical Purity: 95%	Induction: 2 % Challenge: 5-50 %	50% tribenuron-methyl administered epicutaneously in the challenge phase, induced positive skin reactions in 17 of the 19 animal. Positive reactions to the 20% concentration were seen in 9 of 19 animals.	RAR Vol. 3 B.6.2.6/03 Key study
Local Lymph Node Assay OECD TG 429 Probably a higher concentration should have been tested.	Mouse CBA/J females 5/group	Tribenuron-methyl Technical Purity: 97.1%	5, 10 and 20 % 3 days	Stimulation Indices (SI) were <1.5 for all groups. However, it seems like a higher concentration should have been tested in order to get a reliable result.	RAR Vol. 3 B.6.2.6/04

Table 30: Summary table of human data on skin sensitisation

No data.

Table 31: Summary table of other studies relevant for skin sensitisation

No data.

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

The skin sensitisation potential of tribenuron-methyl was assessed in two studies in the guinea pig (Buehler method and Magnusson and Kligman maximisation test), and in one Local Lymph Node Assay in the mouse. Tribenuron-methyl was negative in the Local Lymph Node Assay but it might be due to the fact that the concentration tested was not high enough. In the other two reliable studies tribenuron-methyl was found to be positive.

Local Lymph Node Assay:
Method:

Preliminary toxicity testing:
Concentrations of 5, 10 and 20 % were tested to determine the highest dosable level that avoids overt systemic toxicity and excessive local irritation. Each group consisted of three mice and the vehicle control was propylene glycol. The highest dosable concentration was determined to be a 20 % w/w mixture in propylene glycol, therefore, the test substance as 5, 10 and 20 % w/w mixtures in propylene glycol were selected in the main study.
Main study:
Groups of five female mice were subjected to topical applications of vehicle control (propylene glycol - PG), positive control (α-hexylcinnamaldehyde) or one of the test formulations (5, 10 or 20% in PG) to the dorsum of both ears of each mouse once a day for three consecutive days. On day 6, a 20 μCi dose of 3H-thymidine was injected intravenously into each animal. Five hours later the auricular lymph nodes were recovered from each animal, pooled for each individual mouse and processed through a scintillation counter. Test results are expressed in terms of Stimulation Indices (SI) with the threshold level to be considered a positive indicator of the potential to cause skin sensitisation being 3.0. All results were <3.0. A positive response (SI: 5.03) was observed in animals that received the concurrent positive control.

Buehler method:
Method:
A primary irritation test was run to decide what vehicle and test substance concentrations to be used. Concentrations of 5, 10 and 25% (the maximal practical test concentration based on solubility) suspensions of the test material in dimethyl phthalate were applied onto the shaved, intact skin of 2 male and 2 female guinea pigs. The main study comprised two phases: one induction phase and a challenge phase. The induction phase (dermal application) was performed once a week for 3 consecutive weeks, for a total of three 6-hour treatments with 0.4 ml test material (equivalent to approximately 0.25 g). That is a 62.5 % concentration. Following the same procedure, vehicle control animals were treated with the same amount of DMP (dimethyl phthalate) vehicle, and a positive control with DNCB (0.3% 1-chloro-2,4-dinitrobenzene). After an approximately 6-hour exposure period, the bandages and patches were removed from the animals and the test sites were washed with warm water to remove excess test material. The challenge was carried out two weeks after the last induction treatment. The same amount of test substance as used in the induction phase, was applied to unexposed and shaved skin for 6 hours. The challenge phase followed the same exposure protocol as the induction phase. The irritation responses were scored approximately 24 and 48 hours after treatment. Vehicle control animals and positive control animals were challenged after the same protocol as the treatment group. Evaluations of the skin reactions were performed 24 and 48 hours after removal of the patches. One week following the challenge phase, the test guinea pigs were rechallenged for sensitisation. The positive control and the vehicle control animals were not rechallenged.
Results:
Range finding study: Slight patchy erythema was observed in 2 of the 25% concentration sites 24 hours after treatment. No other dermal irritation was observed.
Main study: The test substance diluted in DMP and administered epicutaneously to guinea pigs in the first challenge phase, induced slight to moderate patchy response in 4/20 animals (20%) of the test group after 24 hr and in 12/20 animals (60%) after 48 hr. 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 however, showed a moderate to severe skin reaction in all animals after both 24 and 48 hr (this group was not rechallenged).

Magnusson and Kligman Maximisation Test:
Method: A primary irritation test was run to decide what vehicle and test substance concentrations to be used. Primary irritation experiments included intracutaneous injections of a 4% (w/v) dilution of the test substance in acetone, using a single animal and epicutaneous applications of dilutions of the test substance in

corn oil: 50%, 25%, 10% and 5% (w/v) using four animals. The main study comprised two phases: one induction phase and a challenge phase. Day 0 of the study, three pairs of intra dermal injections were performed (Injection 1: 0.1 ml test substance (2% w/v in acetone); Injection 2: 0.1 ml of Freunds Complete Adjuvant and water (1:1); Injection 3: 0.1 ml test substance (4% w/v) in acetone and Freunds Complete Adjuvant (1:1)) within a 2 cm x 4 cm clipped area on the animals shoulder. Control animals received the same treatment, but with injections only consisting of vehicle where test substance had been used in the treatment group. Day 6 the skin of the shoulder area was treated with 10% sodium dodecyl sulphate to create a mild irritation. Day 7 the same area was clipped again and 0.5 ml of the test substance in propylene glycol, was applied to the shoulder skin and covered by a patch for 48 hours. Again the control group were treated the same way, but only with vehicle. The challenge was carried out on day 21. The test substance was applied to the skin, and each animal received 4 different doses of test substance (0, 5, 20 and 50% w/v test substance) in propylene glycol. The test substance was covered with a patch and kept on for 24 hours. Control animals were challenged with vehicle only after the same protocol as the treatment group. Evaluations of the skin reactions were performed 24 and 48 hours after removal of the patches.
Results:
Primary irritation experiments: Intradermal administration produced necrosis to an acceptable extent, while epicutaneous application produced very slight erythema at the site treated with the 50% dilution with three of the four treated animals.
Main study: The test substance 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.

10.7.2 Comparison with the CLP criteria

According to CLP Regulation 3.4.2.2.4, a response of at least 30% of the animals is considered as positive when an adjuvant type guinea pig test method for skin sensitisation is used. In the study performed with tribenuron-methyl, where Magnusson and Kligman maximization test was used, the induction concentration of 2 % resulted in a sensitisation rate of 89% and 47% after a challenge with 50% and 20% test substance respectively. Thus, tribenuron-methyl fulfils the criteria and should be classified as a skin sensitiser.

According to CLP Regulation 3.4.2.2.4, a response of at least 15% of the animals is considered as positive when a non-adjuvant guinea pig test method is used. In the study performed with tribenuron-methyl, where Buehler method was used, the sensitisation rate was 20% and 60% after 24 and 48 hours respectively after the first challenge, and 10% after the second challenge. Thus, tribenuron-methyl fulfils the criteria and should be classified as a skin sensitiser.

The CLP Regulation allows classification of skin sensitisers in one hazard category, Category 1, which comprises two sub-categories, 1A and 1B. Classification into sub-categories is only allowed if data are sufficient (CLP Annex I, 3.4.2.2.1.1). Therefore care should be taken when classifying substances into Category 1B when Category 1A cannot be excluded. In such cases classification into category 1 should be considered. This is particularly important if only data are available from certain tests showing a high response after exposure to a high concentration but where lower concentrations which could show the presence of such effects at lower doses are absent (in line with some test protocols where a maximised dose should be used). The criteria for sub-categorisation based on results from Guinea pig maximisation tests and Buehler assays are given in table below:

Table 32: Criteria for sub-categorisation based on results from Guinea pig maximisation tests and Buehler assays

Sub-category	Assay	Response
1A	Guinea Pig Maximisation Test	≥30% responding at ≤0.1% intradermal induction dose or ≥60% responding at >0.1% to ≤1% intradermal induction dose
1A	Buehler assay	≥15% responding at ≤0.2% topical induction dose or ≥60% responding at >0.2% to ≤20% topical induction dose
1B	Guinea Pig Maximisation Test	≥30% to <60% responding at >0.1% to ≤1% intradermal induction dose or ≥30% responding at >1% intradermal induction dose
1B	Buehler assay	≥15% to <60% responding at >0.2% to ≤20% topical induction dose or ≥15% responding at >20% topical induction dose

According to table above, tribenuron-methyl fulfils the criteria for subcategorisation in category 1B (Guinea Pig Maximisation Test: ≥30% responding at >1% intradermal induction dose; Buehler Method: ≥15% responding at >20% topical induction dose). However, the Guidance on the application of the CLP criteria, section 3.4.2.2.2, states that subcategorization in 1B is only allowed if category 1A can be excluded. For tribenuron-methyl, there is a very high response rate (89%) following the use of an intradermal induction dose of 2%. It is therefore possible that the use of a lower dose would result in a response rate which fulfils the criteria for category 1A. Subcategorisation of tribenuron-methyl is therefore not proposed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Tribenuron-methyl has a harmonised classification as a skin sensitiser **(Skin Sens. 1, H317).** No change is proposed.

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.

10.8 Germ cell mutagenicity

Table 33: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Mutagenicity testing in the *Salmonella typhimurium* plate incorporation assay OECD TG 471 with some deviations (the number of *S. typhimurium* strains used was four instead of five). Possible oxidising mutagens and cross-link mutations were not examined in this study.	Tribenuron-methyl technical Purity: 94%	Without activation: 0, 5, 10, 50, 100 and 500 µg/plate. With activation: 0, 10, 50, 100, 500, 1000 and 2000 µg/plate.	Cytotoxicity was observed at ≥ 500 µg/plate in absence of an activation system and at >1000 µg/plate in the presence of S-9 mix. No significant increases in revertants were observed and there were no significant positive linear dose responses. Tribenuron-methyl was non-mutagenic.	RAR Vol. 3 B.6.4.1/01 Key study

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Tribenuron-methyl Reverse mutation assay "Ames Test" using *Salmonella Typhimurium* and *Escherichia Coli* OECD TG 471	Tribenuron-methyl technical Purity: 97.38%	15, 50, 150, 500, 1500, 5000 µg/plate for each tester strain in the presence and absence of S9 mix	The test material was non-toxic to TA100 and WP2uvrA, although small decreases in TA100 colony frequency were noted at the upper dose level. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains and no test material precipitate was observed on the plates, with any dose of the test material, either with or without metabolic activation. Tribenuron-methyl technical was not mutagenic.	RAR Vol. 3 B.6.4.1/02 Key study
In vitro mammalian chromosome aberration test OECD TG 473	Tribenuron-methyl technical Purity: 97.8%	0, 125, 250, 500, 750, 1000, 1250 and 1500 µg/ml for both the non-activated and S9 activated four hours exposure groups, with an additional lower dose level of 62.5 µg/ml for the S9 activated group	At the highest test concentration (1250 µg/ml) evaluated microscopically for chromosome aberrations, mitotic inhibition was 46% relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test article-treated groups were not significantly increased above that of the solvent control in neither the initial tests nor the independent repeat tests. Tribenuron-methyl was negative for structural and numerical chromosome aberrations in both non-activated and S9-activated test systems	RAR Vol. 3 B.6.4.1/03 Key study
In vitro mammalian cell gene mutation (CHO/HGPRT) test with an independent repeat assay	Tribenuron-methyl technical Purity: 97.8%	0, 250, 500, 1000, 1500 and 2700 µg/ml, in both S9 activated and non-activated system	Test article precipitate was observed at a dose level of 2700 µg/mL in treatment medium at the end of treatment. Relative cloning efficiency at the highest dose tested was 140% and 99% in the first mutation assay and 106% and 99% in the independent repeat assay in non-activated and S9-activated systems, respectively. None of the treated cultures exhibited mutant frequencies of greater than 40 mutants per 106 clonable cells. Tribenuron-methyl did not cause a positive response in the	RAR Vol. 3 B.6.4.1/04 Key study

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
			CHO/HGPRT mutation assay in S9-activated non-activated systems.	
In vitro unscheduled DNA synthesis in primary rat hepatocytes OECD TG 482	Tribenuron-methyl technical Purity: 96.8%	0, 0.1, 1, 10 and 100 µM diluted in dimethylsulfoxide (DMSO)	No cytotoxicity was observed at a medium concentration of 2500 µM of tribenuron-methyl. No concentration of test compound showed average net nuclear grain count above five, which was the definition of a positive UDS inducer. No statistically significant increases of UDS or positive linear dose-responses were observed. Tribenuron-methyl was negative in the UDS assay.	RAR Vol. 3 B.6.4.1/05 Key study

Table 34: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Chromosome aberration study in rat bone marrow cells The study generally follows the OECD TG 475 with some derivations *Deviations include sampling times of 6, 24, and 48 hours after the treatment, differing from the guideline recommended post-treatment sampling times of 12–18, and*	Tribenuron-methyl technical Purity: 96.8%	0, 50, 500 and 5000 mg/kg bw/day diluted in Mazola® corn oil Oral intubation	From 50 mg/kg bw per day: red-coloured discharge from the mouth From 500 mg/kg bw per day: wheezing, decreased weight gain and body weight loss. From 5000 mg/kg bw per day: lethargy, hunched back, sensitivity to touch. There were no significant differences in mitotic indices. Tribenuron-methyl did not induce chromosome aberrations in bone marrow cells of rats. Thus, tribenuron-methyl is considered non-clastogenic in this *in vivo* test.	RAR Vol. 3 B.6.4.2/01 Key study

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
24 hours after the first sampling time; scoring 500, not 1000, cells per animal for determination of the mitotic index; and scoring 50, not 100, cells per animal for evaluation of chromosomal aberrations.				
Mouse bone marrow micronucleus assay The study partly follows the OECD TG 475 *Deviations include conducting the test as a limit test at 5000 mg/kg, not using three dose levels for the first sampling time, even though observable toxic effects and mortality occurred; including a 72-hour sampling time of the bone marrow which exceeds the guideline recommendation of not extending the sampling beyond 48 hours after treatment; and scoring 1000, and not 2000, PCEs per animal for the*	Tribenuron-methyl technical Purity: 96.8%	Mice (Crl:CD®-1(ICR)BR) bone marrow cells Single dose of 5000 mg/kg bw per day, diluted in Mazola® corn oil, oral inturbation	Three animals were found dead 48 hours after the tribenuron-methyl treatment. Clinical signs: hyperactivity, diarrhoea, decreased activity, hyperactivity and hypersensitivity. There were no statistically significant differences in the percent of micronucleated polychromatic erythrocytes - methyl-treated groups and the concurrent negative controls. Significant depression of the PCE:NCE (normochromatic erythrocytes) ratio was detected among tribenuron-methyl treated animals at the 48-hour sampling time. This indicates that the dose tested was sufficient to cause target tissue cytotoxicity. Tribenuron-methyl did not induce micronuclei in bone marrow cells of mice.	RAR Vol. 3 B.6.4.2/02 Key study

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
presence of micronuclei				
From the open literature, Cytogenetic Effects of Technical and Formulated Tribenuron-methyl on Rat Bone-marrow Cells. Deficiencies including: absence of positive controls, repeat-dose exposures for 21 days, non-standard nomenclature for chromosome aberrations, blinding procedures not described, individual animal data not presented, historical control data not presented, dose levels not justified. *Only supportive data*	Tribenuron-methyl, technical Purity: 95 % and Formulated product Granstar® 75 % DF (components not specified)	Male albino rats (strain not specified) Single oral dose at dose: 0, 5, 25, 50 or 100 mg/kg to 8 groups of 6 male and ten repeat doses: 0, 5, 25, 50 or 100 mg/kg bw/day to 8 groups of 6 male	Technical and formulated tribenuron-methyl was not clastogenic/genotoxic following single administration up to the dose level of 100 mg/kg bw. However, clastogenic/genotoxic potential was observed following repeat dose administration at 100 mg/kg bw of technical material and at 50 and 100 mg/kg bw of formulated product.	RAR Vol. 3 B.6.4.2/03

Table 35: Summary table of human data relevant for germ cell mutagenicity

No data

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

The genotoxic potential of tribenuron-methyl was investigated in four standard *in vitro* test systems (two Ames test, mammalian chromosome aberration test, and mammalian cell gene mutation test) reinforced with a chromosome aberration study in rat bone marrow cells and an *in vivo* mouse micronucleus test. The results

from the guideline studies were consistently negative and based on these data, it was concluded that tribenuron-methyl does not possess any mutagenic or clastogenic properties. All these studies were conducted in accordance with the OECD Principles of Good Laboratory Practice (1981). However, in the non-guideline report (RAR Vol. 3, B.6.4.2/03) tribenuron-methyl was shown to cause chromosomal aberrations after repeated exposure of 100 mg/kg bw.

Testing using germ cells was not triggered as all *in vitro* and *in vivo* guideline studies were negative.

10.8.2 Comparison with the CLP criteria

The CLP Regulation allows classification of genotoxicity substances in two hazard categories, Category 1 and 2. Category 1 comprises two sub-categories, 1A and 1B. The criteria for the categories and sub-categorisation are given in table below:

Table 36: Criteria for categories and sub-categorisation for genotoxicity

Sub-category	Assay
Category 1	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.
Category 1A	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Category 1B	The classification in Category 1B is based on: -positive results(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or -positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or -positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
Category 2	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: -positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: • Somatic cell mutagenicity tests *in vivo*, in mammals; or • Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note; Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

The results from the guideline studies performed with tribenuron-methyl were consistently negative. Thus, tribenuron-methyl does not fulfil the classification criteria for germ cell mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification is proposed for tribenuron-methyl.

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.

10.9 Carcinogenicity

Table 37: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Two year combined chronic toxicity/oncogenicity long-term feeding study in rats. Crl:CD/BR rats Oral via dietary admixture OECD TG 453	Tribenuron-methyl Purity: 96.8% 0, 25, 250 and 1250 ppm corresponding in male to: 0, 0.95, 10, 55 mg/kg bw/day and in female: 0, 1.2, 13, 76 mg/kg bw/day	No mortality but general toxicity without a specific target organ. Mean body weights were decreased by 43% and 29% for female and male rats, respectively, in 1250 ppm dose groups. Body weight gains were decreased in the highest dose by 36% and 53% in the males and females, respectively. Body weight gain was also significantly decreased in 250 ppm females by 27%. Significant increase in mammary gland adenocarcinomas was noted in females of the high-dose-group. There were also several systemic non-neoplastic effects in the male 250 and 1250 ppm dose groups and in the female 1250 ppm dose group when compared to controls (mineralisation of stomach and aorta, spleen lymphoid depletion, increased pancreas polyarteritis, reduced secretion from seminal vesicles, liver fatty change, bilateral dilatation in renal pelvis, uterus dilatation and renal	RAR Vol. 3 B.6.5.1/01 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		degeneration). However, a specific target organ was not identified for non-neoplastic effects in male rats. The NOAEL of tribenuron-methyl in rat in long-term feeding was 25 ppm (corresponding to around 1 mg/kg bw per day) based on reduced bodyweight gain in females noted at ≥250 ppm and in males noted at 1250 ppm, reduced bodyweight noted in both sexes at 1250 ppm, organ weight changes, non-neoplastic histopathological findings in males at ≥250 ppm and in females at 1250 ppm, and an significant increased incidence of total mammary adenocarcinomas in female rats at the high-dose level (1250 ppm).	
Oncogenicity study, eighteen-month feeding study in mice Crl:CD®-1(ICR)BR mice OECD TG 453 *No measurements of coagulation function was done*	Tribenuron-methyl Purity: 94.2% 0, 20, 200 and 1500 ppm; corresponding in male to: 0, 2.5, 25 and 197 mg/kg/day and in female: 0, 3.1, 31 and 247 mg/kg/day, respectively	None of the clinical signs that were observed were considered to be test substance-related. The incidence of mortality was similar among the treatment and control groups. Mean body weights, mean body weight gain and food consumption in the treated group were similar to controls except that statistically significant decreased (20%) bodyweight gain was noted in females of the highest dose group. The relative liver weight increased (19%) in males at the high dose group. Histopathology data revealed several minor modifications in the normal lesions of ageing within the male and female 1500 ppm dose groups when compared to their respective control groups. In addition, secondary changes observed in a few organs (testes, thyroid, and epididymis) were considered to be directly related to the amyloidosis observed and to the slightly catabolic condition seen in these groups. No compound-related increases in the incidence of tumours were observed in this study. The NOAEL was 20 ppm (2.5 mg/kg bw/day) based on the effects seen in males; amyloidosis and bilateral oligospermia in the 200 ppm (25 mg/kg bw) dose groups and reduced bodyweight gain noted in females at 1500 ppm.	RAR Vol. 3 B.6.5.2 Key study

Table 38: Summary table of human data on carcinogenicity

No data.

Table 39: Summary table of other studies relevant for carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
90-day feeding study on oestrous cycle Guideline: not applicable Rat Crl:CD BR Female rats (20/group) *No measurements of coagulation function was done.* In the study the ability of tribenuron-methyl and its metabolites to compete *in vitro* for binding to the estrogen and progesterone receptors from uterine cytosol was also investigated.	Tribenuron-methyl Purity: 94.8% 0, 5000 ppm corresponding to 390 mg/kg bw/day	No deaths or clinical observations. Body weights and body weight gains approximately 26% and 40% lower than control values. These changes were associated with reduced food consumption (27% of control) and food efficiency (18% of control). Increased mean liver weight, increased mean relative uterine weight (consistent with the increase in uterine proliferation), increased mean relative ovarian weight, prolonged estrous, and two- to three-fold decrease uterus and mammary oestrogen receptor affinity, and two-fold increase in progesterone receptor number. *In vitro* study: Seven tribenuron-methyl metabolites can bind to the estrogen receptor (Tribenuron-methyl acid, N-Demethyl triazine amine, α-hydroxi triazine amine, N-Demethyl-6-hydroxymethyl triazine amine, Metsulfuron methyl, Sulfonamide urea, Hydroxylated saccharin).	RAR Vol. 3 B.6.8.2.1/01

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

The data available to assess this endpoint include two long-term studies (RAR Vol. 3, B.6.5). In addition a 90-day feeding study is available investigating hormonal activity for tribenuron-methyl (RAR Vol. 3, B.6.8.2.1/01). These studies were assessed in the first DAR of 2004.

For the renewal of the active substance further investigations (QSAR evaluations of tribenuron-methyl and metabolites and *in vitro* studies conducted with tribenuron-methyl) were conducted to investigate the potential for tribenuron to bind or activate estrogen receptors (RAR Vol. 3, B.6.8.3).

The conclusions from the previous assessment remain.

Rat:

Two year toxicity study:

In the long-term toxicity study in the rat (a GLP study), tribenuron-methyl was associated with increased tumour incidence. The study follows the OECD TG 453 except that no measurements of coagulation function was done. However, reconduct is unlikely to yield a significantly different result because no evidence of a bleeding disorder was observed.

The incidence of total malignant tumours in female rats was significantly increased in the high-dose level after tribenuron methyl administration. This was a result of the significant increase in mammary gland adenocarcinomas in the high-dose level.

The incidence in cancer in males increased already in the low dose but there were no dose response and no significant increase in any specific cancer type. The incidence is 21, 40, 33, 36 % in control, 25, 250, and 1250 ppm, respectively. In the 1250 ppm group (55 mg/kg bw/day) the MTD was exceeded as the mean body weight compared to control decreased with 29 %. The incidence in cancer in males was considered to be a statistical aberration and have no biological importance. Additional historical control data for tumour incidences in the male rat were submitted by the applicant during the peer-review of the active substance (see Appendix to RAR Vol. 3, Annex B.6).

The incidence in total adenocarcinoma in females increased in the middle and high dose (15, 15, 22 and 43% for control, 25, 250 and 1250 ppm respectively) but was only significant at the highest dose (the historical control incidence is 20 % with a range from 8 to 23). Additional historical control data were submitted by the applicant during the peer-review of the active substance (see table below). No increased incidence of non-neoplastic changes in the mammary gland was noted (see table below). At the two highest doses the maximum tolerated dose (MTD) seems to be reached as the body weight decreased, compared to control, with 21 and 43 % respectively for 250, 1250 ppm (see figure below). Adenocarcinoma were produced only at doses which exceeded the MTD. The dose-response for adenocarcinoma-induction, along with the demonstrated absence of genotoxicity, suggest that a non-genotoxic, threshold mechanism is responsible for the adenocarcinoma observed following exposure to a high dietary concentration of tribenuron-methyl. The specific mechanism(s) involved are not known but it seems like the cancer is secondary to general toxicity.

Irrespective of the mechanism, the relevance to humans of adenocarcinoma-induction in rat only at dose that greatly exceeded an MTD, and in a strain with high spontaneous mammary tumor rate, is questionable.

Table 40: Historical control data for female Sprague-Dawley Rat mammary adenocarcinoma incidence

Report#	**#/group**	**#examined histo.**	**#with lesions**	**% incidence**	**strain**	**dates (in life)**
HLR 61-87 (TBN study)	60 (0 ppm)	60	9 (8s/1m)	15	Crl:CD BR/KNY	8/84-8/86
HLR 61-87 (TBN study)	58 (250 ppm)	57	13 (11s/2m)	22.3	Crl:CD BR/KNY	8/84-8/86
HLR 61-87 (TBN study)	61 (1250 ppm)	61	26 (15s/11m)	42.6[a]	Crl:CD BR/KNY	8/84-8/86
1984a	69	67	7	10.4	Crl:CD BR/WMA	1/81-2/83
1985a	66	64	15	23	Crl:CD BR/KNY	3/83-3/85
1985b	33	33	2	6	Crl:CD BR/KNY	2/83-2/85
1986a	66	66	10	15	Crl:CD BR/KNY	4/83-4/85
1986b	59	58	12 (4m/8s)	20.7	Crl:CD BR/KNY	11/83-11/85

1988a	60	60	13	21.7	Crl:CD BR/KNY	9/85-10/87
1989a	62	62	14 (9s/5m)	22.6	Crl:CD BR/KNY	9/86-9/88
1989b	62	62	14 (13s/1m)	22.6	Crl:CD BR/KNY	8/86-8/88
1989c	47	47	8(4s/3m/1cs)	17	Crl:CD BR/KNY	8/87-8/89
1990a	49	49	4 (2s/2m)	8.2	Crl:CD BR/KNY	7/87-7/89
1990b	50	50	13 (9s/3m/1cs)	26	Crl:CD BR/RNC	10/88-10/90
1991	49	49	10 (10s)	20	Crl:CD BR/KNY	7/88-8/90
1992	56	56	17	30.4	Crl:CD BR/RNC	7/89-7/91
HCD Range				1.5-30.4%		

[a] outside HCD
S=single occurrence per animal
M=multiple occurrences per animal
cs=carcinosarcoma

Table 41: Incidences of neoplastic microscopic observations after two-year feeding with tribenuron-methyl in rats – number of animals affected

Dose	**Control**	**25 ppm**	**250 ppm**	**1250 ppm**
Incidence of microscopic observations				
Females				
Number of animals in group	60	60	58	61
Mammary gland:				
Adenocarcinoma	8	8	11	15
Adenocarcinoma multiple	1	1	2	11*
Total adenocarcinoma	9/60[a]	9/60	13/58	26/61*
Brain: Astrocytoma	*1*	*0*	*0*	*0*
Spinal cord: Astrocytoma	*0*	*0*	*1*	*0*
Heart: Endocardial sarcoma	*0*	*1*	*0*	*0*
Liver: Hepatocellular carcinoma	*0*	*1*	*0*	*0*
Jejunum: Neurofibrosarcoma	*1*	*0*	*0*	*0*
Kidney: Lyposarcoma	*0*	*1*	*0*	*0*
Vagina: Squamous cell carcinoma	*0*	*0*	*0*	*1*
Pituitary: Carcinoma	*2*	*4*	*0*	*4*
Thyroid: Follicular adenocarcinoma and cell carcinoma	*2*	*1*	*2*	*1*
Adrenal cortex: Carcinoma	*1*	*0*	*0*	*1*
Adrenal medulla: Pheochromocytoma	*1*	*0*	*0*	*0*
Thymus: Chemodectoma	*0*	*0*	*0*	*1*
Skin: Carcinoma and fibrosarcoma	*0*	*2*	*0*	*0*
Miscellaneous:				
head:squamous carcinoma and rhabdomysarcoma	*2*	*0*	*1*	*1*
limb: histocytic sarcoma	*0*	*0*	*1*	*0*
multiple organs: lymphoma, lymphoblastic and histocytic sarcoma	*0*	*2*	*4*	*1*

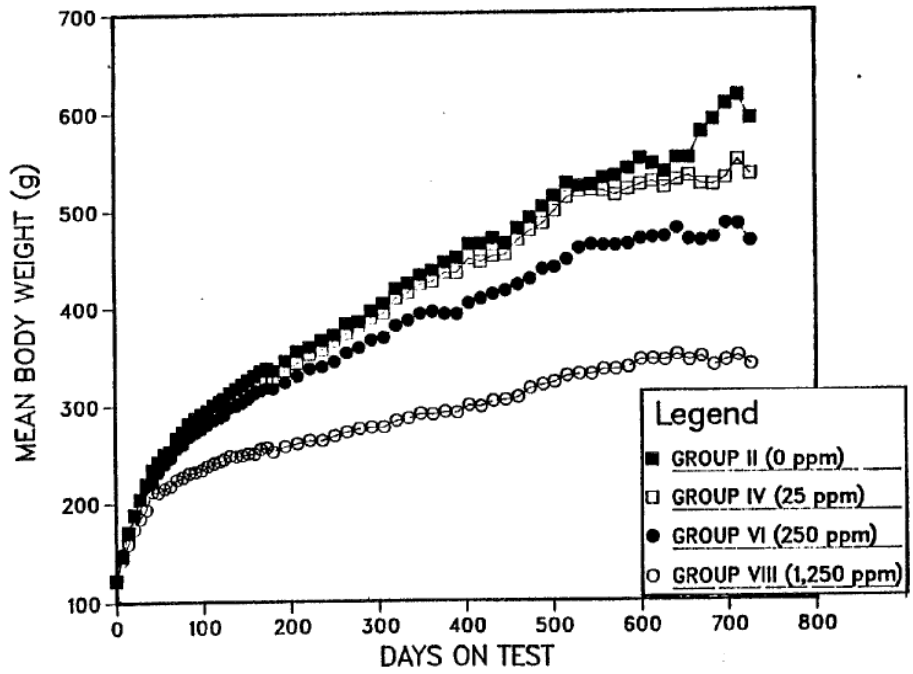
Dose	**Control**	**25 ppm**	**250 ppm**	**1250 ppm**
Peritoneal cavity: Mesothelioma	*1*	*0*	*0*	*0*
Tail:fibrosarcoma and neurofibrosarcoma	*0*	*0*	*1*	*1*
Total Miscellaneous tumours	***3***	***2***	***7***	***3***
Total malignant tumours	**20/60**	**21/60**	**23/58**	**37/61***
Males				
Number of animals in group	62	60	60	61
Brain: Astrocytoma and mixed cell glioma	*0*	*0*	*2*	*1*
Spinal cord: Astrocytoma	*0*	*0*	*1*	*0*
Nasal cavity/tubinates: Oesteosarcoma and squamous cell carcinoma	*0*	*2*	*0*	*0*
Lung: Epidermoid carcinoma	*1*	*0*	*0*	*0*
Heart: Chemodectoma	*0*	*0*	*1*	*0*
Liver: Hepatocellular carcinoma	*2*	*4*	*0*	*3*
Pancreas: Islet cell carcinoma	*0*	*2*	*0*	*0*
Salivary: Sarcoma	*0*	*1*	*0*	*0*
Kidney: Lyposarcoma and mixed tumour	*1*	*0*	*1*	*1*
Urinary bladder: Transitional cell carcinoma	*0*	*0*	*1*	*0*
Testes: Interstitial cell tumour and neoplasma	*0*	*2*	*0*	*0*
Epididymides: Adenocarcinoma and mesothelioma	*0*	*0*	*0*	*2*
Seminal vesicle: Adenocarcinoma	*1*	*1*	*0*	*0*
Thyroid: Follicular adenocarcinoma and cell carcinoma	*1*	*0*	*2*	*5*
Adrenal cortex: Cortical carcinoma	*1*	*1*	*0*	*0*
Adrenal medulla: Pheochromocytoma	*4*	*3*	*3*	*4*
Spleen: Hemangiosarcoma	*0*	*1*	*0*	*0*
Skin: Carcinoma and fibrosarcoma	*1*	*2*	*3*	*1*
Miscellaneous:				
head:squamous carcinoma	*1*	*0*	*2*	*3*
limb: fibrosarcoma	*0*	*0*	*1*	*0*
mammary gland: adenocarcinoma	*0*	*1*	*1*	*0*
multiple organs: granulocytic leukemia and histocytic sarcoma	*0*	*3*	*2*	*1*
pinna: neurofibrosarcoma	*0*	*1*	*0*	*1*
Total Miscellaneous tumours	***1***	***5***	***6***	***5***
Total malignant tumours	**13/62**	**24/60***	**20/60***	**22/61***

* Statistically significant: Fisher's Exact Test, $p<0.05$

[a] 15% compared to 12% which is the laboratory average in the historical data

For incidences of non-neoplastic microscopic observations after two-year feeding with tribenuron-methyl in rats see section 10.12.1.1.

Figure: Growth curves of female rats



90-day toxicity study on estrogenous effect (supplementary study):

In a supplementary study om carcinogenicity (RAR Vol. 3, B.6.8.2.1/01), tribenuron-methyl effects on the endocrine system (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 uterus (from rats sacrificed in estrous) and mammary oestrogen receptor affinity, and two-fold increase in progesterone receptor number) were observed following administration of tribenuron-methyl. However, the marked decrements in body weight parameters at 5000 ppm makes the evaluation of the study complicated. It is known that many hormonal and reproductive endpoints are altered by caloric restriction. Therefore, 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 oestrogenic mechanism. The ability of tribenuron-methyl and its metabolites to compete *in vitro* for binding to the estrogen and progesterone receptors from uterine cytoxol was also investigated in the study. As a result, seven tribenuron-methyl metabolites can bind to the estrogen receptor (Tribenuron-methyl acid, N-Demethyl triazine amine, α-hydroxi triazine amine, N-Demethyl-6-hydroxymethyl triazine amine, Metsulfuron methyl, Sulfonamide urea, Hydroxylated saccharin). When these binding data are considered along with the increase in progesterone receptor number observed in the *in vivo* study, a hormonally mediated mechanism of mammary tumour induction cannot be ruled out.

In vitro studies conducted with tribenuron-methyl and its metabolite IN-A4089 (Vol. 3, B.6.8.3) and QSAR analyses conducted with tribenuron-methyl and ten metabolites (Vol. 3, B.6.8.3/07)

As a follow-up, an evaluation of the potential for tribenuron-methyl or its metabolite (IN-A4089) to bind or activate estrogen receptors was conducted via *in vitro* assays. Results:

Tribenuron-methyl and IN-A4089 were negative when induction or inhibition of 17β-estradiol was tested (Vol. 3, B.6.8.3/01 and 02).
Tribenuron-methyl and IN-A4089 were shown to not interact with estrogen receptor (Vol. 3, B.6.8.3/03 and 05).
Tribenuron-methyl and IN-A4089 were shown not to be agonists of human estrogen α in HeLa-9903 cell model system (Vol. 3, B.6.8.3/04 and 06).

As another follow-up, tribenuron-methyl and its metabolites were evaluated via QSAR (Toolbox v3.3.5, OASIS TIMES v2.27.16, DEREK v4.1, MedChem Studio v4.0, and ADMET Predictor v7.2 software) for structural alerts for estrogen receptor binding. The tested metabolites were: IN-D5119, IN-R9803, IN-G7462, IN-D5803, IN-R9805, IN-00581, IN-B5685, IN-T6376, IN-L5296 and IN-A4098. Tribenuron-methyl and all metabolites was all "non-binder" except IN-G7462 which seems to be false.
These data indicate that neither tribenuron-methyl nor its metabolites demonstrated potential to be endocrine active.

As a conclusion: Mammary tumors were produced only at doses which exceeded the MTD. The dose-response for mammary tumor induction, along with the demonstrated absence of genotoxicity, suggest that a non-genotoxic, threshold mechanism is responsible for mammary tumors observed following exposure to a high dietary concentration of tribenuron-methyl. Irrespective of the mechanism, the relevance to humans of mammary tumor-induction in rat only at dose that greatly exceeded an MTD, and in a strain with high spontaneous mammary tumor rate, is questionable.

A mechanistic study was completed which was inconclusive regarding the potential for an estrogenic mechanism. As a follow-up, an evaluation of the potential for tribenuron or ten metabolites to bind or activate estrogen receptors was conducted via QSAR evaluation and *in vitro* assays were completed as well. No structural alerts for estrogen receptor binding were found for the target molecules in the QSAR analyses. Furthermore, no estrogenic antagonistic activities were found in the *in vitro* studies conducted with tribenuron-methyl and one of its metabolites.

Mouse:
In the long-term toxicity study in the mouse (a GLP study) performed with tribenuron-methyl, no compound-related increases in the incidence of tumours were observed. The study follows the OECD TG 453 with exception that no measurements of coagulation function was done. However, reconduct is unlikely to yield a significantly different result because no evidence of a bleeding disorder was observed.

Histopathology data revealed several minor modifications in the normal lesions of ageing within the male and female 1500 ppm groups (197 mg/kg bw/day) when compared to controls. These effects were not considered to be of adverse character and included a slight increase in severity of amyloidosis and marginal changes in some background inflammatory lesions, an increased incidence in thyroid inflammation in the 1500 ppm dose of both sexes, a higher incidence of bilateral testicular atrophy at the highest dose. A specific target organ was not identified.

10.9.2 Comparison with the CLP criteria

According to Regulation 1272/2008 (CLP) substances are classified for carcinogenicity in Category 1 (known or presumed human carcinogens) on the basis of epidemiological and/or animal data. Category 1 is subcategorised into 1A if the substance is *"known to have carcinogenic potential for humans, classification is largely based on human evidence"* and 1B if *"presumed to have carcinogenic potential for humans classification is largely based on animal evidence."*

As there is no human data available for tribenuron-methyl that may be relevant for carcinogenicity, criteria for category 1A are not fulfilled.

For classification in category 1B evidence may be derived from *"[…]animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen) […] In addition on a case–by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals."*

Sufficient evidence from animal studies is explained as *"a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. […]"*

Tribenuron-methyl does not fulfil this criteria (mammary gland adenocarcinomas were only found in one species in one study).

The placing of substance in Category 2 (suspected human carcinogens) *"is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (2) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."*

Limited evidence from animal studies is explained as *"data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues organs"*

An increased incidence of mammary gland adenocarcinomas was noted in female rats. However, the relevance to humans of mammary tumor-induction in rat only at dose that greatly exceeded an MTD, and in a strain with high spontaneous mammary tumor rate, is questionable. The specific mechanism(s) involved are not known but it seems like the cancer is secondary to general toxicity.

10.9.3 Conclusion on classification and labelling for carcinogenicity

The relevance to humans of mammary tumor-induction in rat only at dose that greatly exceeded an MTD, and in a strain with high spontaneous mammary tumor rate, is questionable. The specific mechanism(s) involved are not known but it seems like the cancer is secondary to general toxicity. Therefore data is neither considered as sufficient evidence for category 1B nor as limited evidence for category 2. Consequently, no classification for carcinogenicity is proposed for tribenuron-methyl.

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 (10.3 %)	2 (5.4 %)	1 (1.6 %)
[hyperplasia]	1 (1.6 %)			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.

10.10 Reproductive toxicity

10.10.1.1 Adverse effects on sexual function and fertility

Table 42: Summary table of animal studies on adverse effects on sexual function and fertility

Test	Species	Doses	Results	Reference

substance **Test type** **Route of exposure** **Method** **Guideline**	**Strain** **Sex** **No./group** **Vehicle**	**Exposure Period**		
Tribenuron-methyl Purity: 94.2% Two-generation reproduction toxicity study Oral (dietary) U.S. EPA 83-4 (1982)	Rat Crl:CD®(SD)BR M, F 23/sex/dose	0, 25, 250, 1000 ppm This corresponds to 70-day mean daily intakes of 0, 1.9, 19, and 75 mg/kg bw/day and 0, 2.15, 21.3 and 88 mg/kg bw/day for male and females animals, respectively. The mean daily intakes during gestation were: 0, 2, 20 and 85, and 0, 2, 20 and 78 mg/kg bw/day for the first and second F0 animals, respectively, and 0, 1.8, 18 and 76, and 0, 2, 20 and 79 mg/kg bw/day for the first and second generation F1 animals. Throughout this study, rats were fed diets that contained the test substance. After 70 days on test, the F0 generation was bred to produce the F1A and F1B litters. At weaning, randomly selected F1A rats were fed diets containing the test substance for 80 days and then mated to produce F2A and F2B litters. 3 weeks prior to mating and during the mating period. In males treatment was continued throughout the mating period and until killing shortly thereafter. In females treatment was continued throughout pregnancy and lactation period.	<u>25 ppm:</u> No effects <u>250 ppm:</u> <u>Parental:</u> ↓ bw (F1A females: 4-11%) ↓bw gain (F0 males: Day 0-70: 7%; F1A females: premating day 0-77: 13%) ↓FC (F1A females) <u>Offsprings:</u> ↓bw (F1B pups: 8%; F2B pups 9%) -organ weight changes in F2B weanlings (↑rel testes, ↓abs spleen (M, F), ↓rel spleen (both sexes, s.s. in F only), ↓abs liver (both sexes, s.s. in M only), ↑rel lung (both sexes, s.s. in M only), and ↓abs thymus (F, n.s.), ↓abs heart (F, n.s.)) <u>1000 ppm:</u> <u>Parental:</u> ↓bw (F0 males: 16%; F1A males: 16%; F0 females: 16% (premating), 11% (1st and 2nd gestations), 6% (1st lactation), 12% (2nd lactation); F1A: 13-22%) ↓bw gain (F0 males (Day 0-70: 19%, Day 70-168: 24%); F0 females: 29% (premating); F1A females: 25% (premating Day 0-77) ↓FC (F0 and F1 males and females) -organ weight changes (↑rel testes (F0 and F1A)) <u>Offsprings:</u>	RAR Vol. 3 B.6.6.1/01 Key study

			-clinical signs (sparse fur, F2-generation) ↓bw (F1A pups: 9%; F1B pups: 16%; F2A pups: 10%; F2B pups: 12%) -organ weight changes in F2B weanlings (↓abs spleen (M, F), ↓rel spleen (both sexes, s.s. in F only), ↓abs liver (both sexes, s.s. in M only), ↑rel lung (both sexes, s.s. in M only), and ↓abs thymus (F, n.s.), ↓abs heart (F, n.s.),↓ abs kidney (F)) *NOAEL: Parental and offspring: 25 ppm (approximately 2 mg/kg bw/day)* *NOAEL: Reproductive: ≥1000 ppm (approximately 75 and 88 mg/kg bw/day in males and females, respectively)*	
Tribenuron-methyl One-generation reproduction toxicity study Oral (dietary) No Guideline *There are limitations with regard to number of animals used in the study and pathology (testes and epididymides were not weighed. No histopathology was performed).*	Rat Crl:CD®(SD)BR M, F 16/sex/dose	0, 100, 1750, 5000 ppm (correspond to 0, 7, 118, and 335 mg/kg bw/day in males; 0, 8, 135, and 386 mg/kg bw/day in females) 90 day prior to mating and throughout the study	<u>100 ppm:</u> No effects <u>1750 ppm:</u> <u>Dams:</u> ↓bw (15%) <u>Offsprings:</u> ↓bw (Day 21: 19%) <u>5000 ppm:</u> <u>Dams:</u> ↓bw (23%) <u>Offsprings:</u> ↓pup viability (Day 0-4: 19% per litter, Day 1-4: 14% per litter) ↓bw (Day 21: 36%)	RAR Vol. 3 B.6.6.1/02 Key study

M: males
F: females
s.s.: statistically significant
n.s.: not statistically significant

Table 43: Summary table of human data on adverse effects on sexual function and fertility

No data.

Table 44: Summary table of other studies relevant for toxicity on sexual function and fertility

No data.

10.10.1.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

A two-generation toxicity study (GLP) is available in the rat. Furthermore, a one-generation study (B.6.6.1/01) is available in the rat, but this non-GLP study is limited and considered as supplementary data only. The studies are thoroughly presented in Vol. 3 to RAR.

In the two-generation toxicity study (RAR Vol. 3, B.6.6.1/01), there was no adverse effects on reproduction, fertility, mating behavior or malformations in the offspring. Parental toxicity, indicated by decreased bodyweight growth and food consumption was noted from 250 ppm (approximately 20 mg/kg bw/day). At 1000 ppm, organ weight changes (increased relative testes) were noted in addition. Offspring toxicity was seen as expressed by clinical signs (sparse fur) noted in F2 generation at 1000 ppm, and decreased body weight growth noted from 250 ppm, and organ weight changes in F2B weanlings from 250 ppm. Organs that showed statistically significant weight changes were: testes, spleen, liver, kidneys (females only) and lungs. Other organs that showed weight changes were thymus (females) and heart (females) but these changes were not statistically significant.

In the one-generation toxicity study (RAR Vol. 3, B.6.6.1/02), treatment was associated with reduced pup weight noted at 1750 ppm (19%) and 5000 ppm (36%), and reduced pup viability noted at 5000 ppm. These effects on offspring weights and viability were noted at doses that also caused reduced bodyweight in dams (1750 ppm: 15%; 5000 ppm: 23%). Reproductive performance was not affected.

10.10.2 Comparison with the CLP criteria

According to CLP Guidance Annex 1: 3.7.2.4.3, "*Classification is not necessarily the outcome in the case…when there is only a small reduction in foetal/pup weight…*"

Two-generation reproduction toxicity study in the rat:
Administration of tribenuron-methyl at dietary concentrations of up to 1000 ppm (75 and 88 mg/kg bw/day in males and females, respectively) did not have any effect on mating performance or fertility. Parental toxicity, indicated by decreased bodyweight growth and food consumption was noted from 250 ppm (approximately 20 mg/kg bw/day). At 1000 ppm, organ weight changes (increased relative testes) were noted in addition. Offspring toxicity was seen as expressed by clinical signs (sparse fur) noted at 1000 ppm, and decreased body weight growth noted from 250 ppm, and organ weight changes (F2B weanlings) from 250 ppm. The observed effects were not considered of concern for a classification.

One-generation reproduction toxicity study in the rat:
Administration of tribenuron-methyl at dietary concentrations of up to 5000 ppm (335 and 386 mg/kg bw/day in males and females, respectively) did not have any effect on mating performance or fertility. Reduced bodyweight in pups were noted at maternal toxicity doses (reduced bodyweight gain). Reduced pup viability was noted at the high dose level of 5000 ppm. Because of the magnitude of effect on body weight gain in dams (23%) this effect could be secondary to maternal toxicity. The observed effects was not considered of concern for a classification.

10.10.3 Adverse effects on development

Table 45: Summary table of animal studies on adverse effects on development

Test substance **Test type** **Route of exposure** **Method** **Guideline**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Doses** **Exposure Period**	**Results**	**Reference**
Tribenuron-methyl Purity: 94.2% Developmental toxicity study Oral (gavage) U.S. EPA 83-3 (1982)	Rat Crl:COBS®CD®(SD)BR 25 dams/dose 0.5% hydroxypropyl methyl cellulose (Methocel®)	0, 20, 125, 500 mg/kg bw/day Gestation days 6-15	Maternal effects: 20 mg/kg bw/day: No effects 125 mg/kg bw/day: ↓bw (5-7%) ↓bw gain (13%) (adjusted bw gain): 31%) ↓FC (4%) -organ weight changes (↑rel liver, 7%) 500 mg/kg bw/day: - clinical signs (salivation) ↓bw (18%) ↓bw gain (53%) (adjusted bw gain): 98%) ↓FC (16%) -organ weight changes (↑rel liver, 17% ↓average uterine weight, 19%) -stomach ulceration (one animal) Developmental effects: 20 mg/kg bw/day: No effects 125 mg/kg bw/day: ↓bw (7%) ↑skeletal alterations (incomplete ossification and unossified sites, bifid thoracic vertebral centra)	RAR Vol. 3 B.6.6.2.1/01 Key study

			<u>500 mg/kg bw/day:</u> ↓bw (26%) ↑resorptions (mean no. resorptions: 1.5 compared to 1.0 in control group (n.s.); mean percentage of resorptions or dead conceptuses/litter: 13.9 compared to 5.7 in control group (n.s.)) ↑fetal deaths (no. dead fetuses: 15 compared to 0 in control group) ↑skeletal alterations (incomplete ossification and unossified sites, bifid thoracic vertebral centra, enlarged fontanelle (one animal (n.s.)) ↑oedema (n.s.) *NOAEL maternal toxicity: 20 mg/kg bw/day* *NOAEL developmental toxicity: 20 mg/kg bw/day*	
Tribenuron-methyl Purity: 94.2% Developmental toxicity study Oral (gavage) U.S. EPA 83-3 (1982)	Rabbit Hra:(NZW)SPF 22/dose group 0.5% aqueous methyl cellulose	0, 5, 20, 80 mg/kg bw/day Gestation days 7-19	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No effects <u>20 mg/kg bw/day:</u> - mortality (one dam) <u>80 mg/kg bw/day:</u> -mortality (two dams) -clinical signs (abortions, 6 animals compared to one control animal) -bw loss (-325 g) ↓FC (48%) <u>Developmental effects:</u> <u>5 and 20 mg/kg bw/day:</u> No effects <u>80 mg/kg bw/day:</u>	RAR Vol. 3 B.6.6.2.2/01 Key study

			↓ bw gain (10%, biologically significant) ↓ live fetuses, total (5.8 compared to 8.1 in control group) ↓ nidations (6.5 compared to 8.9 in control group) ↑malformations (mean % affected/litter: 13.4% compared to 0% in control group) *NOAEL maternal toxicity: 20 mg/kg bw/day* *NOAEL developmental toxicity: 20 mg/kg bw/day*	

n.s.: not statistically significant

Developmental toxicity study in the rat (RAR, Vol. 3, B.6.6.2/01):

Maternal toxicity:

The maternal toxicity consisted of reduced body weight growth noted at 125 mg/kg bw/day (reduced bw: 5-7%, reduced bw gain 13%) and 500 mg/kg bw/day (reduced bw: 18%, reduced bw gain 53%), and increased relative liver weights noted at 125 mg/kg bw/day (7%) and 500 mg/kg bw/day (17%). Stomach ulceration was also noted in one animal of the high dose group (500 mg/kg bw/day).

Effects noted in pups:

Following effects were noted in the embryo/foetus:

- increased resorptions at 500 mg/kg bw/day (highest dose level).

- increased foetal deaths at 500 mg/kg bw/day (highest dose level)

- reduced body weights at 125 mg/kg bw/day (7%) and 500 mg/kg bw/day (26%)

- increased incidence of incomplete ossification and unissified sites, bifid thoracic vertebral centra at ≥125 mg/kg bw/day

- enlarged fontanelle (one animal) at 500 mg/kg bw/day (highest dose level)

- oedema at 500 mg/kg bw/day (highest dose level)

Discussion:

- Fifteen of 16 fetuses in one 500 mg/kg bw/day litter were dead *in utero*, resulting in an increase in the average percentage of resorptions or dead conceptuses per litter. A slight increase in the average number of resorptions also occurred for the 500 mg/kg bw/day litters. Neither increase was significant compared to controls and this effect on embryo/fetal viability was not sufficiently severe to adversely affect the average number of live fetuses per litter. No dam resorbed all conceptuses, and with the exception of the previously cited dead fetuses, all fetuses were alive at day 20 delivery.

- Increased fetal deaths were noted at the highest dose level in the presence of maternal toxicity. Because of the magnitude of effect on body weight gain this effect could be secondary to maternal toxicity.

- Reduced fetal weights were noted at maternally toxicity doses. The effect could be secondary to maternal toxicity.

- Retarded ossifications were noted in the fetuses at maternally toxicity doses, and were not considered to be a major manifestation of developmental toxicity

- Enlarged fontanelle was noted in a single (high dose) animal only, and in the presence of maternal toxicity. The effect was not statistically significant

- Oedema was noted at the high dose level only (in three pups of the same litter), and in the presence of maternal toxicity. The effect was not statistically significant

As a conclusion, the effects noted in the rat developmental toxicity study were not sufficient to trigger a proposal for classification for this hazard category.

Developmental toxicity study in the rabbit (RAR, Vol. 3, B.6.6.2/02):

Maternal toxicity:

The maternal toxicity consisted of mortalities (two females), clinical signs (abortions) and body weight loss (-325 g) noted in dams at 80 mg/kg bw/day. According to study author marked maternal toxicity occurred in the high dose group (80 mg/kg bw/day). Effects included an average maternal food consumption that was 48% lower than control over the gestation day 7-19 dosing period. After the initiation of dosing at this high dose, food consumption progressively decrased from approximately 22 to 66% lower than the control values. This reduced food consumption corresponded to a mean body weight loss of 325 g (compared to a body weight gain of 114 in controls) over the gestation day 7-19 dosing period.

Two pilot teratogenicity studies were conducted to determine the maximum tolerated dose of the test substance by gavage in the rabbit. Based on data obtained from a pilot teratogenicity study, 6 females per group were given 0, 250, 500 or 750 mg/kg body weight in the first rabbit pilot study. These dose levels were found to be extremely toxic to the female rabbit and all females in the 500 and 750 mg/kg dose groups died. The dose level for the 250 mg/kg group was reduced to 125 mg/kg after 5 doses in an effort to determine the maximum tolerated dose. Although all six females in the low dose goup survived until scheduled sacrifice, no litters were produced. In the second rabbit pilot study, 7 females per group received the test substance at dose levels of 150 or 75 mg/kg body weight. The 150 mg/kg dose group demonstrated severe weight loss and two of the females died. No deaths were observed at 75 mg/kg body weight, but moderate weight loss and decreased feed consumption were evident. Based on these data, dose levels of 0, 10, 40 and 80 mg/kg body weight were chosen for the main study.

Effects noted in pups:

Following effects were noted in the embryo/fetus:

- reduced live fetuses per litter at 80 mg/kg bw/day (highest dose level)

- decreased nidations at 80 mg/kg bw/day (highest dose level)

- reduced body weight (10% biologically significant) at 80 mg/kg bw/day (highest dose level)

- increased malformations at 80 mg/kg bw/day (highest dose level)

Table 46:

Dose (mg/kg/day)	**0 mg/kg**	**5 mg/kg**	**20 mg/kg**	**80 mg/kg**
No. examined: fetuses	**130**	**138**	**153**	**64**
Litters	**(16)**	**(17)**	**(17)**	**(11)**
External				
No. affected	0	0	1(1)	1(1)
Abdomen-Gastroschisis	-	-	-	1(1)[c]
Head-Exencephaly w/open eye	-	-	-	1(1)[c]
Paw-Clubbed	-	-	-	1(1)[c]
Umbilicus-Hernia	-	-	1(1)	-
Visceral				
No. affected	0	0	0	1(1)
Kidney-No papilla (Size 0)	-	-	-	1(1)[d]
Head				
No. affected	0	1(1)	1(1)	3(2)
Brain-Hydrocephaly	-	-	1(1)b	-
Eye-Cataract	-	1(1)	-	2(1)[d]
Skeletal				
No. affected	0	1(1)	1(1)	3(2)
Ribs-Fused	-	1(1)[a]	-	1(1)[e]
Sternebra-Fused	-	-	1(1)[b]	-
Vertebra-Hemivertebra	-	1(1)[a]	-	3(2)[e]
Total number affected	0(0)	2(2)	2(2)	6(4)
Mean percent affected per litter	0	1.6	1.3	13.4*

*Statistically significant, $p<0.05$
a-e Same fetus affected

Discussion and conclusion:

- Decreased live fetuses were noted at the highest dose level in the presence of maternal toxicity.

- Decreased nidations were noted at the highest dose level in the presence of maternal toxicity. As no significant difference in the mean number of corpora lutea per litter was observed, the reason for the decrease in nidations was not clear.

- Reduced fetal weights were noted at maternally toxicity doses. Because of the magnitude of effect on body weight (bodyweight loss:-325 g) and food consumption (48% reduction) this effect could be secondary to maternal toxicity.

-Increased malformations were noted at the highest dose level in the presence of maternal toxicity. Malformations within affected high-dose litters occurred at low incidences (1 to 2/litter) and were without a pattern of specific malformations. Furthermore, the increase in percent affected per litter was primarily due to malformations that occurred in small litters. The mean percentage of foetuses with developmental variations was significantly increased at the low dose level. No significant differences in the rates of variations due to retarded development, or in the mean percent of foetuses with variations were observed.

As a conclusion, the effects noted in the rabbit developmental toxicity study were not sufficient to trigger a proposal for classification for this hazard category.

Table 47: Summary table of human data on adverse effects on development

No data.

Table 48: Summary table of other studies relevant for developmental toxicity

No data.

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

Studies are available on developmental toxicity in rats and rabbits. The studies are thoroughly presented in Vol. 3 to the RAR.

In the rat developmental toxicity study (RAR Vol. 3, B.6.6.2.1/01), maternal findings were noted at 125 mg/kg bw/day and above. The maternal findings included reduced bodyweight growth, reduced food consumption and organ weight changes (indicating liver toxicity) noted in the dams at 125 mg/kg bw/day and above, and clinical signs (increased salivation) and stomach ulceration noted in dams at 500 mg/kg bw/day. Foetal effects consisted of reduced body weight and increased incidence of skeletal alterations noted at 125 mg/kg bw/day and above, increased resorptions and foetal deaths noted at 500 mg/kg bw/day, and oedema noted at 500 mg/kg bw/day. The increased incidence of skeletal alterations consisted of dosage-dependent increases in the litter incidences of incomplete ossification of the thoracic and caudal vertebrae, sternebrae, xiphoid and pubes noted in the middle and high dosage groups. Bifid thoracic vertebral centra was also significantly increased for the 125 and 500 mg/kg groups. In addition the observations of oedema, enlarged fontanelle (one animal), unossified supraoccipital, altered ossification of lumbar and sacral vertebrae and unossified metacarpals and metatarsals in high dosage group foetuses were also considered treatment-related, although not significantly increased. The classification of foetal alterations were reported in Supplement No. 1, to the main study (RAR Vol. 3, B.6.6.2.1/01). At the time of the report, foetal alterations were not classified as “malformations” or “variations”. Therefore, the individual fetal alterations and their classification as variations or malformations were reconducted by the applicant (additional data submitted during the peer-review of the active substance) and is presented in table below:

Table 49: Indicidual fetal alterations reported in the rat developmental toxicity study (additional data submitted by applicant during peer-review)

Dose group	**Alteration**
0 mg/kg bw/day	
Dam 28002, foetus 11	Thread-like tail (M)
Dam 21013, foetuses 5 and 11	Lung, left lobe lowest lobule absent (V)
20 mg/kg bw/day	
No foetuses reported with a malformation	-
125 mg/kg bw/day	
Dam 28051, foetus 10	Brain- lateral and third ventricles slightly dilated (V)

Dam 28069, foetus 7	Right subclavian and right carotid arteries pass to the left of the trachea and esophagus (V)
Dam 28071, foetus 1	Hemivertebra (M)
500 mg/kg bw/day	
Dam 28085, foetus 4	Body short and slightly edemoatous (V), Hemivertebra (M), fused ribs (M)
Dam 28086, foetus 1	Thread-like tail (M)
Dam 28095, foetus 10	Mulitple malformations (umbilical hernia, heart apex rounded, undescended testes, diaphragm absent, kidneys absent, ectopic adrenals, fused ribs) (M)
Dam 28099, foetuses 1, 2, 8	Edematous (V or M depending upon extent of edema)

M: malformation
V: variation

In the rabbit developmental toxicity study (RAR Vol. 3, B.6.6.2.2/01), maternal findings were noted at the highest dose level of 80 mg/kg bw/day. The maternal findings consisted of mortalities (two females), clinical signs (abortions) and reduced body weight gain noted in dams at 80 mg/kg bw/day. One middle dose female (20 mg/kg bw/day group) were also found dead. This animal had multiple mucosal haemorrhages in the stomach associated with a trichobezoar and died on gestation day 29 just prior to scheduled sacrifice. Whether this death was associated with the abortion process, disease, INL-5300 treatment or a combination of these factors could not be determined according to the study author. Foetal toxicity consisted of reduced body weight (10%, biologically significant), decreased live fetuses, nidations, and an increase in malformations noted at 80 mg/kg bw/day.

10.10.5 Comparison with the CLP criteria

According to Regulation 1272/2008 (CLP), substances are classified for reproductive toxicity in Category 1A (known human reproductive toxicant) based largely on evidence from humans or in 1B (presumed human reproductive toxicant) or 2 (suspected human reproductive toxicant) largely based on animal data. The animal data required for 1B classification *"shall provide clear evidence of an adverse effect on sexual function or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects"*.

Substances are classified in Category 2 when there is *"some evidence from humans or experimental animals… of an adverse effect on sexual function or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1"*

As there is no human data available for tribenuron-methyl, the criteria for category 1A are not fulfilled. The effects noted in the studies that are considered potentially relevant for classification are resorptions, deaths and skeletal effects noted in rats, and deaths, nidations, and malformations noted in rabbits.

10.10.6 Adverse effects on or via lactation

Table 50: Summary table of animal studies on effects on or via lactation

Test substance **Test type** **Route of**	**Species** **Strain** **Sex**	**Doses** **Exposure Period**	**Results**	**Reference**

exposure **Method** **Guideline**	**No./group** **Vehicle**			
Tribenuron-methyl Purity: 94.2% Two-generation reproduction toxicity study Oral (dietary) U.S. EPA 83-4 (1982)	Rat Crl:CD®(SD)BR M, F 23/sex/dose	0, 25, 250, 1000 ppm This corresponds to 70-day mean daily intakes of 0, 1.9, 19, and 75 mg/kg bw/day and 0, 2.15, 21.3 and 88 mg/kg bw/day for male and female animals, respectively. The mean daily intakes during gestation were: 0, 2, 20 and 85, and 0, 2, 20 and 78 mg/kg bw/day for the first and second F0 animals, respectively, and 0, 1.8, 18 and 76, and 0, 2, 20 and 79 mg/kg bw/day for the first and second generation F1 animals. Throughout this study, rats were fed diets that contained the test substance. After 70 days on test, the F0 generation was bred to produce the F1A and F1B litters. At weaning, randomly selected F1A rats were fed diets containing the test substance for 80 days and then mated to produce F2A and F2B litters. 3 weeks prior to mating and during the mating period. In males treatment was continued throughout the mating period and until killing shortly thereafter. In females treatment was continued throughout pregnancy and lactation period.	<u>25 ppm:</u> No effects <u>250 ppm:</u> <u>Parental:</u> ↓ bw (F1A females: 4-11%) ↓bw gain (F0 males: Day 0-70: 7%; F1A females: premating day 0-77: 13%) ↓FC (F1A females) <u>Offsprings:</u> ↓bw (F1B pups: 8%; F2B pups 9%) -organ weight changes in F2B weanlings (↑rel testes, ↓abs spleen (M, F), ↓rel spleen (both sexes, s.s. in F only), ↓abs liver (both sexes, s.s. in M only), ↑rel lung (both sexes, s.s. in M only), and ↓abs thymus (F, n.s.), ↓abs heart (F, n.s.)) <u>1000 ppm:</u> <u>Parental:</u> ↓bw (F0 males: 16%; F1A males: 16%; F0 females: 16% (premating), 11% (1st and 2nd gestations), 6% (1st lactation), 12% (2nd lactation); F1A: 13-22%) ↓bw gain (F0 males (Day 0-70: 19%, Day 70-168: 24%); F0 females: 29% (premating); F1A females: 25% (premating Day 0-77) ↓FC (F0 and F1 males and females) -organ weight changes (↑rel testes (F0 and F1A)) <u>Offsprings:</u> -clinical signs (sparse fur, F2-generation) ↓bw (F1A pups: 9%; F1B	RAR Vol. 3 B.6.6.1/01 Key study

			pups: 16%; F2A pups: 10%; F2B pups: 12%) -organ weight changes in F2B weanlings (↓abs spleen (M, F), ↓rel spleen (both sexes, s.s. in F only), ↓abs liver (both sexes, s.s. in M only), ↑rel lung (both sexes, s.s. in M only), and ↓abs thymus (F, n.s.), ↓abs heart (F, n.s.),↓ abs kidney (F)) *NOAEL: Parental and offspring: 25 ppm (approximately 2 mg/kg bw/day)* *NOAEL: Reproductive: ≥1000 ppm (approximately 75 and 88 mg/kg bw/day in males and females, respectively)*	
Tribenuron-methyl One-generation reproduction toxicity study Oral (dietary) No Guideline *There are limitations with regard to number of animals used in the study and pathology (testes and epididymides were not weighed. No histopathology was performed).*	Rat Crl:CD®(SD)BR M, F 16/sex/dose	0, 100, 1750, 5000 ppm (correspond to 0, 7, 118, and 335 mg/kg bw/day in males; 0, 8, 135, and 386 mg/kg bw/day in females) 90 day prior to mating and throughout the study	<u>100 ppm:</u> No effects <u>1750 ppm:</u> <u>Dams:</u> ↓bw (15%) <u>Offsprings:</u> ↓bw (Day 21: 19%) <u>5000 ppm:</u> <u>Dams:</u> ↓bw (23%) <u>Offsprings:</u> ↓pup viability (Day 0-4: 19% per litter, Day 1-4: 14% per litter) ↓bw (Day 21: 36%)	RAR Vol. 3 B.6.6.1/02 Key study

M: males
F: females
s.s.: statistically significant
n.s. : not statistically significant

Table 51: Summary table of human data on effects on or via lactation

No data.

Table 52: Summary table of other studies relevant for effects on or via lactation

No data.

10.10.7 Short summary and overall relevance of the provided information on effects on or via lactation

In the two-generation toxicity study (RAR, Vol. 3, B.6.6.1/01) decreased bodyweight was noted in pups from 250 ppm (20 mg/kg bw/day) (F1B pups: 8%; F2B pups: 9%) and 1000 ppm (F1A pups: 9%; F1B pups: 16%; F2A pups: 10%; F2B pups: 12%). Furthermore organweight changes were noted in F2 weanlings from 250 ppm. Organs that showed statistically significant weight changes were: testes, spleen, liver, kidneys (females only) and lungs. Other organs that showed weight changes were thymus (females) and heart (females) but these changes were not statistically significant. The effects on offsprings were noted at doses that also caused reduced bodyweight growth in dams (At 250 ppm: F1A females: 4-11%).

In the one-generation toxicity study (RAR Vol. 3, B.6.6.1/02), treatment was associated with reduced pup weight noted at 1750 ppm (118 mg/kg bw/day) (19%) and 5000 ppm (36%), and reduced pup viability noted at 5000 ppm. These effects on offspring weights and viability were noted at doses that also caused reduced bodyweight in dams (1750 ppm: 15%; 5000 ppm: 23%).

10.10.7.1 Comparison with the CLP criteria

According to the CLP Guidance Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:

"(a) human evidence indicating a hazard to babies during the lactation period; and /or

(b) 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

(c) absorption, metabolism, distribution and excretion studies that indicate the likehood that the substance is present in potentially toxic levels in breast milk."

No data is available to address criterias (a) and (c). A reduction in pup weight was observed. However the effect was noted in the presence of maternal toxicity. Thus the effect on bodyweight growth is not considered "provide clear evidence of adverse effect in the offspring due to transfer in the milk".

10.10.8 Conclusion on classification and labelling for reproductive toxicity

No classification is proposed for tribenuron-methyl

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

<u>Rat</u>

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.

10.11 Specific target organ toxicity-single exposure

Table 53: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute oral, OECD 401 Rat, Crl:CD®(SD)IGS BR 5/sex/dose	Tribenuron-methyl technical 5000 mg/kg bw, Observations in 14 days	>5000 mg/kg bw	RAR Vol. 3 B.6.2.1/01
Acute dermal, OECD 402 Rat, Crl:CD®(SD)IGS BR 5/sex/dose	Tribenuron-methyl technical 5000 mg/kg bw, Observations in 14 days	>5000 mg/kg bw	RAR Vol. 3 B.6.2.2
Acute inhalation, OECD 403 Rat, Crl:CD®(SD)IGS BR 5/sex/dose	Tribenuron-methyl technical, fine powder, (MMAD 2.8 or 2.7 µm) 6.0 mg/L, 4 hour, nose only 14 days observation	>6.0 mg/L	RAR Vol. 3 B.6.2.3/01
Oral (gavage) Acute neurotoxicity OECD 424 Rat Crl:CD(SD) 12/sex/dose	Tribenuron-methyl Purity: 98.2% 0, 100, 300, 1000 mg/kg bw Vehicle: 0.5% methyl cellulose	<u>100 mg/kg bw/day:</u> No effects <u>300 mg/kg bw:</u> ↓FC (M,F) ↓ food efficiency (M, F) <u>1000 mg/kg bw:</u> ↓bw (F:7%) ↓FC (M,F) ↓ food efficiency (M, F)	RAR Vol. 3 B.6.7.1.1/01

M: males
F: females

Table 54: Summary table of human data on STOT SE

No data.

Table 55: Summary table of other studies relevant for STOT SE

No Data.

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

All acute toxicity studies were conducted in accordance with the OECD Principles of Good Laboratory Practice (1981).The acute toxicity of tribenuron-methyl after oral, dermal or inhalation exposure was low.

One female showed a red-stained face on day 2 after oral exposure. Other clinical signs observed in the four other females started from day 8 and included red-stained head, hunched over posture, and ruffled fur. No clinical signs were observed after day 10. No body weight losses occurred in male rats. Weight loss up to 9 % from day 2 until day 11 was registered in females. However, the final body weight of all female rats surpassed their fasted body weight. No gross lesions were present in the rats at necropsy.

After dermal exposure 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 the test substance but no dermal effects was observed after day 13.

Eye, nasal and/or oral discharges, irregular respiration and stained fur were observed immediately after inhalation exposure. All rats showed a slight body weight loss the day following exposure. In male rats a body weight loss of 0.35 – 4% of the initial weight was observed, and in female rats 1.0 – 2.0%. All rats showed an overall weight gain by the end of the 14-days recovery period.

In the acute neurotoxicity study (RAR Vol. 3, B.6.7.1.1/01), there seems to be an initial depression in motor activity duration in both male and female rats given 1000 mg/kg bw of the test substance in the first thirty minutes of the motor activity assessment on day 0. However, this decreased activity was not present on day 7 or 14. Treatment was associated with reduced bodyweight of 1000 mg/kg bw females (7%) compared to control rat, on test day 7. Furthermore, food consumption and food efficiency were reduced in 300 and 1000 mg/kg bw males and females. No treatment-related findings were noted during FOB investigation. *Comment: The NOAEL in the study will be discussed at expert-meeting.*

10.11.1.2 Comparison with the CLP criteria

According to the CLP Guidance, specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture, which are not covered by the other hazard classes. Regulation EC No 1272/2008 (CLP), Annex 1: 8.2.1.7.3, states for STOT SE: "*…Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, and macroscipoic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, must be taken into consideration in the classification process, including but not limited to the following effects in humans and/or animals:…(b) Significant functional changes, more than transient in nature, in the respiratory system, central or peripheral nervous systems, other organs or other organ systems, including signs of central nervous system depression and effects on special senses (such as sight, hearing and sense of smell)…"*

In the acute toxicity studies performed, no specific target organ toxicity were noted. In the acute neurotoxicity study an initial depression in motor activity duration in both male and female rats given 1000 mg/kg bw of the test substance was noted on day 0. However, this decreased activity was not present on day 7 or 14. Thus the effect was transient in nature.

10.11.1.3 Conclusion on classification and labelling for STOT SE

No classification is proposed for tribenuron-methyl.

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).

10.12 Specific target organ toxicity-repeated exposure

Table 56: Summary table of animal studies on STOT RE

Test substance **Route** **Duration of study** **Guideline**	**Species** **Strain** **Sex** **No./group** **Vehicle**	**Dose levels**	**Main findings**	**Reference**
Tribenuron-methyl Purity: 96.3% Oral (gavage) 28-day EEC Method B.7 (1984) *Assessment of reactivity to stimuli of different types, grip strength and motor activity not conducted, urinalysis not performed, histopathology limited, oestrous cycle not determined, thyroid hormones not determined*	Rat Sprague Dawley (SPF quality, randomly bred) M, F 6/sex/dose Propylene glycol	Main study: 0, 100, 300, 1000 mg/kg bw/day Parallel study: 0, 30, 100, 300 mg/kg bw/day	<u>30 mg/kg bw/day:</u> No treatment-related effects <u>100 mg/kg bw/day:</u> -organ weight changes (↑rel. liver weight, 11%, F) <u>300 mg/kg bw/day:</u> ↓bw gain (F: 36%) ↓FC (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 (hyperthrophy of centrilobular hepatocytes (M,F)) <u>1000 mg/kg bw/day:</u> -clinical signs (piloerection, lethargy, emaciation, reduced defecation (M,F)) ↓bw (M: 21%, F: 23%) ↓bw gain (M: 45%, F: 58%) ↓FC (M: 18%, F: 28%) -changes in biochemical parameters (↑ALT (M,F), ↓ AST (M), ↑serum Ca (M,F), ↓ total bilirubin (M)) -organ weight changes (↑rel. liver weight (M,F), ↑abs liver weight (F),↑rel. kidney weight (M,F), ↓abs. kidney weight (F)	RAR Vol. 3 B.6.3.1.1/01

			↑rel. testes weight, ↓abs. spleen weight (F), ↓abs. adrenal weight (F)) -histopathological changes in the liver (hypertrophy of centrilobular hepatocytes (M,F)) *Study accepted for dose selection but not for NOAEL setting*	
Tribenuron methyl Purity: 99% Oral (dietary) 90-day No Guideline *Ophtalmology not performed, assessment of reactivity to stimuli of different types, grip strength and motor ativity not conducted, neurobehavioural assessment not conducted, no measurements of coagulation function, microscopical examination of spinal cord, mammary gland, peripheral nerve and skin not conducted.*	Rat Crl:CD(SD)BR M, F 16/sex/dose	0, 100, 1750 , 5000 ppm (equivalent to 0, 7, 118, and 335 mg/kg bw/day in males; 0, 8, 135, and 386 mg/kg bw/day in females)	<u>100 ppm:</u> - organ weight changes (↑rel. spleen weight, F: 21%) <u>1750 ppm:</u> -clinical signs (colored nasal discharge, M) ↓bw (M: 20%, F: 23%) ↓bw gain (M: 27%, F: 39%) ↓FC (M, F) (not stat. sign.) -changes in haematological parameters (increased 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%)) <u>5000 ppm:</u> -mortality (one female, due to cachexia) -clinical signs (colored nasal discharge, M) ↓bw (M: 30%, F: 28%) ↓bw gain (M: 40%, F: 48%) ↓FC (M, F) (not stat. sign.)	RAR Vol. 3 B.6.3.2.1/01

			-changes in haematological parameters (increased 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), ↓abs brain weight (M), ↑rel. heart weight (M,F), ↓abs heart weight (M,F) ↑rel. liver weight (M,F), ↓abs liver weight (M, F),↑rel. kidney weight (M, F), ↓abs kidney weight (M,F),↑rel testes weight, ↑rel spleen weight (M: 38%, F: 36%))	
Tribenuron methyl Purity: 98% Oral (dietary) 28-day range finding study U.S. EPA 82-1 (1982) *Limited parameters investigated (dose range finding study)*	Mouse Crl:CD®-1(ICR)BR M, F 10/sex/dose	0, 125, 500, 1250, 2500, 5000 ppm (equivalent to 0, 25, 100, 250, 500 and 1000 mg/kg bw/day using a default conversion factor of 0.2)	<u>125 and 500 ppm:</u> No treatment-related effect <u>1250 ppm:</u> -organ weight changes (↑rel liver weight (M: 11%, F:11%)) <u>2500 ppm:</u> -organ weight changes (↑abs and rel liver weights (M: 15%, F: 18%), ↑ rel spleen weight (M: 18%)) <u>5000 ppm:</u> ↓bw (M: 5%) ↓bw gain (M: 34%) -organ weight changes (↑abs and rel liver weight (M: 21%, F: 29%), (↑rel spleen weight (M: 29%)) *Study accepted for dose selection but not for NOAEL setting*	RAR Vol. 3 B.6.3.2.2/01
Tribenuron methyl Purity 98% Oral (dietary) 90-day U.S. EPA 82-1 (1982)	Mouse Crl:CD®-1(ICR)BR M, F 10/sex/dose	0, 125, 500 and 2500 ppm (equivalent to 0, 18, 70 and 350 mg/kg bw/day in males; 0, 24, 90 and 476 mg/kg	<u>125 and 500 ppm:</u> No treatment-related effects <u>2500 ppm:</u> ↓bw gain (F: 42%)	RAR Vol. 3 B.6.3.2.2/01

Ophtalmology not performed, assessment of reactivity to stimuli of different types, grip strength and motor ativity not conducted, neurobehavioural assessment not conducted, no measurements of coagulation function, clinical biochemistry not determined, no microscopical examination of spinal cord, mammary gland, peripheral nerve		bw/day in females)	-organ weight changes (↑abs. and rel. liver weight (F), ↑rel. liver weight (M)) *NOAEL both sexes: 500 ppm (70 and 90 mg/kg bw/day in males and females, respectively)*	
Tribenuron methyl Purity: 96.8% Oral (dietary) 90-day U.S. EPA 82-1 (1982) *Opthlamology not evaluated, no measurements of coagulation function*	Dog Beagle M, F 4/sex/dose	0. 50, 500 and 2500 ppm (equivalent to 0, 1.5, 15 and 73 mg/kg bw/day in males, and 0, 1.6, 15 and 78 mg/kg bw/day in females)	<u>50 and 500 ppm</u> No treatment-related effects <u>2500 ppm:</u> ↓bw (M: 6% (n.s.)) -haematological changes (↑mean platelets and leukocyte counts at Month 3, decreased mean corpuscular haemoglobin concentration at Month 2, M: 2% (n.s)) -organ weight changes (↑ absolute thyroid/parathyroid weights (M: 30% (n.s), F: 48% (s.s), ↑relative thyroid/parathyroid weights (M: 38% (n.s), F: 34% (n.s)) *NOAEL both sexes: 500 ppm (15 mg/kg bw/day)*	RAR Vol. 3 B.6.3.2.3/01
Tribenuron methyl Purity: 96.8% Oral (dietary) 1-year U.S. EPA 83-1 (1982)	Dog Beagle M, F 5/sex/dose	0, 25, 250 and 1500 ppm (equivalent to 0, 0.79, 8.16 and 51.5 mg/kg bw/day in males; 0, 0.90, 8.18 and 52 mg/kg bw/day in females)	<u>25 and 250 ppm:</u> No treatment-related effects <u>1500 ppm:</u> ↓bw (M: 2-11%, F: 4-8%) (not statistically significant) ↓bw gain (M: 20%, F: 18%) (not statistically significant) - changes in biochemical parameters (↑serum creatinine concentrations (M, F)) *NOAEL both sexes: 250 ppm (8 mg/kg bw/day)*	RAR Vol. 3 B.6.3.3.1/01

Tribenuron-methyl Purity: 97.7% Dermal 28-day EEC Method B.9 (1984) *No measurement of haematocrit in the haematology examination, no measurement of ornithine decarboxylase and gamma glutamyl transpeptidase in the blood biochemistry examination*	Rabbit New Zealand White M, F 6/sex/dose	0, 1000 mg/kg bw/day	<u>1000 mg/kg bw/day:</u> -clinical signs (dermal irritation) ↓bw (M: 12%) ↓bw gain (M: 91%; F:76%) ↓FC (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). *NOAEL: not established* *LOAEL: 1000 mg/kg bw/day*	RAR Vol 3 B.6.8.2.3/01
Tribenuron-methyl Purity: 98.2% Immunotoxicity feeding study 28-day OPPTS 870.7800 (1998) *Parameters evaluated limited to bodyweight gain, food consumption, food efficiency, clinical signs, gross pathology, humoral immune response, and organ weights*	Rat Crl:CD(SD) F 10/dose	0, 50, 150, 300, and 600 ppm 0, 3.8, 11, 23, and 44 mg/kg bw/day	No test substance-related effects were observed on 1) gross pathology; 2) absolute and relative brain, spleen, and thymus weights; or 3) humoral immune response. <u>44 mg/kg bw/day</u> mean body weights ↓7.4% body weight gains ↓24.3% food efficiency ↓ 15.9% *NOAEL systemic toxicity: 300 ppm (23 mg/kg bw/day)* *NOAEL immunotoxicity: ≥600 ppm*	RAR Vol 3 B.6.8.2.2/01
Tribenuron-methyl Purity: 98.2%	Rat Crl:CD(SD)	0, 50, 200, 700 ppm (correspond to 0, 2.8, 11.3,	<u>50 and 200 ppm</u> No effects	RAR Vol. 3 B.6.7.1.2/01

Oral (dietary) Subchronic neurotoxicity OECD 424	12/sex/dose	40.1 mg/kg bw/day in males; 0, 3.2, 12.8, 46.6 mg/kg bw/day in females)	<u>700 ppm:</u> ↓ bw (M: 12%, F:13%) ↓ bw gain (M: 20%; F: 29%)	
Tribenuron-methyl Purity: 94.2% Developmental toxicity study Oral (gavage) U.S. EPA 83-3 (1982)	Rabbit Hra:(NZW)SPF 22/dose group 0.5% aqueous methyl cellulose	0, 5, 20, 80 mg/kg bw/day	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No effects <u>20 mg/kg bw/day:</u> - mortality (one dam) <u>80 mg/kg bw/day:</u> -mortality (two dams) -clinical signs (abortions, 6 animals compared to one control animal) -bw loss (-325 g) ↓FC (48%) <u>Developmental effects:</u> <u>5 and 20 mg/kg bw/day:</u> No effects <u>80 mg/kg bw/day:</u> ↓ bw gain (10%, biologically significant) ↓ live fetuses, total (5.8 compared to 8.1 in control group) ↓ nidations (6.5 compared to 8.9 in control group) ↑malformations (mean % affected/litter: 13.4% compared to 0% in control group)	RAR Vol. 3 B.6.6.2.2/01
Tribenuron-methyl Purity: 96.8% Two year combined chronic toxicity/oncogenicity long-term feeding study in rats. Oral via dietary admixture OECD TG 453	Crl:CD/BR rats	0, 25, 250 and 1250 ppm corresponding in male to: 0, 0.95, 10, 55 mg/kg bw/day and in female: 0, 1.2, 13, 76 mg/kg bw/day	No mortality but general toxicity without a specific target organ. Mean body weights were decreased by 43% and 29% for female and male rats, respectively, in 1250 ppm dose groups. Body weight gains were decreased in the highest dose by 36% and 53% in the males and females, respectively. Body weight gain was also significantly	RAR Vol. 3 B.6.5.1/01

			decreased in 250 ppm females by 27%. Significant increase in mammary gland adenocarcinomas was noted in females in of the high-dose level group. There were also several systemic non-neoplastic effects in the male 250 and 1250 ppm dose groups and in the female 1250 ppm dose group when compared to controls (mineralisation of stomach and aorta, spleen lymphoid depletion, increased pancreas polyarteritis, reduced secretion from seminal vesicles, liver fatty change, bilateral dilatation in renal pelvis, uterus dilatation and renal degeneration). However, a specific target organ was not identified for non-neoplastic effects in male rats. The NOAEL of tribenuron-methyl in rat in long-term feeding was 25 ppm (corresponding to around 1 mg/kg bw per day) based on reduced bodyweight gain in females noted at ≥250 ppm and in males noted at 1250 ppm, reduced bodyweight noted in both sexes at 1250 ppm, organ weight changes, non-neoplastic histopathological findings in males at ≥250 ppm and in females at 1250 ppm, and an significant increase incidence of total mammary adenocarcinomas in female rats at the high-dose level (1250 ppm).	
Tribenuron-methyl Purity: 94.2% Oncogenicity study, eighteen-month feeding study in mice OECD TG 453	Crl:CD®-1(ICR)BR mice	0, 20, 200 and 1500 ppm; corresponding in male to: 0, 2.5, 25 and 197 mg/kg/day and in female: 0, 3.1, 31 and 247 mg/kg/day, respectively	None of the clinical signs that were observed were considered to be test substance-related. The incidence of mortality was similar among the treatment and control groups. Mean body weights, mean body weight gain and food consumption in the treated group were similar to controls except that statistically significant decreased (20%) bodyweight gain was noted in females of the highest dose group.	Vol. 3 B.6.5.2

			The relative liver weight in liver increased (19%) in males at the high dose group. Histopathology data revealed several minor modifications in the normal lesions of ageing within the male and female 1500 ppm dose groups when compared to their respective control groups. In addition, secondary changes observed in a few organs (testes, thyroid, and epididymis) were considered to be directly related to the amyloidosis observed and to the slightly catabolic condition seen in these groups. No compound-related increases in the incidence of tumours were observed in this study. The NOAEL was 20 ppm (2.5 mg/kg bw/day) based on the effects seen in males; amyloidosis and bilateral oligospermia in the 200 ppm (25 mg/kg bw) dose groups and reduced bodyweight gain noted in females at 1500 ppm. No compound-related increases in the incidence of tumours were observed in this study. The NOAEL was 20 ppm (2.5 mg/kg bw/day) based on the effects seen in males; amyloidosis and bilateral oligospermia in the 200 ppm (25 mg/kg bw) dose groups and reduced bodyweight gain noted in females at 1500 ppm	

M: males
F: females

Table 57: Summary table of human data on STOT RE

No data.

Table 58: Summary table of other studies relevant for STOT RE

No data.

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

All studies are thoroughly presented in Volume 3 to the RAR.

Two short-term oral toxicity studies with tribenuron-methyl are available with rats (one 28-day toxicity study and one 90- day toxicity study. A 90-day oral toxicity and a dose-range finding study are available with mice. Short-term oral toxicity was also tested in dogs (one 90-day toxicity study and 1-year toxicity study). Furthermore a 28-day immunotoxicity feeding study in rats and 28-day dermal toxicity study in rabbits are available.

In the 28-day oral toxicity study in rats, the liver appeared to be the primary target organ as evidenced by a dose related increase of its weight noted in females at ≥100 mg/kg bw/day and in males at ≥300 mg/kg bw/day, combined with elevated blood serum alanine aminotransferase levels (ALT) noted in both sexes at ≥300 mg/kg bw/day. Moreover, histopathological examination of the liver tissue showed a slight to well-defined hypertrophy of centrilobular hepatocytes in the 300 and 1000 mg/kg bw/day dose groups. Clinical signs (piloerection, lethargy, emaciation, reduced defecation) were also noted in the study (both sexes at 1000 mg/kg bw/day). Furthermore, effects on body weight growth (reduced bodyweight/bodyweight gain) and reduced food consumption were noted in females at ≥300 mg/kg bw/day and in males at 1000 mg/kg bw/day. In addition changes in organweights were noted for the kidney, testes, spleen and adrenal at 1000 mg/kg bw/day. There was also an increase in serum calcium concentrations noted in both sexes at ≥300 mg/kg bw/day and decreased bilirubin concentrations noted in males at ≥300 mg/kg bw/day.

In the 90-day oral toxicity study in rats, treatment was associated with mortality (one female due to cachexia) noted at 5000 ppm (386 mg/kg bw/day). Effects on the liver consisted of changes in biochemical parameters noted in both sexes at ≥1750 ppm and increased liver weights noted in females at ≥1750 ppm and in males at 5000 ppm. Changes in biochemical parameters noted at ≥1750 ppm consisted of decreased total protein concentrations (in males), decreased serum globulin (in males), decreased glucose (in both sexes) and increased serum cholesterol (in females). Clinical signs (coloured discharge from the nose) were noted in males at 1750 ppm and 5000 ppm. Furthermore effects on body weight growth (reduced bodyweight and bodyweight gain) were noted in both sexes at ≥1750 ppm. Changes in organweights were also noted for the spleen (at ≥100 ppm), and the brain, heart, kidney and testes (at ≥1750 ppm). In addition, changes in haematological parameters were noted in females at ≥1750 ppm and consisted of increased platelet counts at 1 and 3 Months. *The relevant NOAEL in the study will be discussed at expert-meeting.*

In the 28-day range finding study in mice, treatment was associated with effects on body weight growth (reduced body weight and bodyweight gain) noted in males at 5000 ppm and organ weight changes noted in both sexes at ≥1250 ppm (250 mg/kg bw/day). The organweight changes consisted of increased liver weights noted in both sexes at ≥1250 ppm, and increased spleen weights noted in males at ≥2500 ppm.

In the 90-day oral toxicity study in mice, treatment was associated with effects on body weight growth (reduced bodyweight gain) noted in females at 2500 ppm (476 mg/kg bw/day), and organ weight changes noted in both sexes at 2500 ppm (increased liver weights) (350 and 476 mg/kg bw/day in males and females, respectively).

In the 90-day feeding study in dogs, treatment was associated with reduced body weight noted in males at 2500 ppm (73 mg/kg bw/day). Changes in haematological parameters were also noted in males at 2500 ppm and consisted of increased mean platelets at Month 3, increased mean total leukocyte counts at Month 3, decreased mean corpuscular haemoglobin concentration at Month 2 (n.s). Organ weight changes were noted at 2500 ppm (73 and 78 mg/kg bw/day in males and females, respectively) (in males: increased absolute and relative thyroid/parathyroid weight (n.s.); in females: increased absolute thyroid/parathyroid weight (s.s.), increased relative thyroid/parathyroid weight (n.s.))

In the 28-day dermal toxicity study in rabbits (limit dose study), treatment at the limit dose of 1000 mg/kg bw/day was associated with clinical signs of dermal irritation (scabs, necrosis and pustulae). One male and one female were found dead (on day 29 and day 24, respectively) without clinical signs preceding the death and no macroscopic abnormalities or microscopic findings were noted that could explain the death.

Furthermore, effects on bodyweight growth (reduced bodyweight amd bodyweightgain), and reduced food consumption were noted in both sexes. Changes in haematological parameters (reduced RBC, Hb, PCV) were noted in males. Changes in biochemical parameters were noted in both sexes and consisted of reduced inorganic phosphorus level, reduced ALP activity (s.s. in males only). Organ weight changes were also noted (males: increased relative kidney weight; females: increased relative brain weight). Macroscopic findings of skin effects consisted of scabs, induration, haematomas, necrosis abscess formation at the application site and histopathological changes (skin and application site) consisted of inflammatory reaction together with epidermal or dermal degeneration/necrosis, acanthosis, hyperkeratosis, oedema, vascular ectasia and haemorrhage. Histopathological changes in the kidney were noted and consisted of bilateral multifocal marked nephrocalcinosis together with kidney tubular degeneration/necrosis.

Table 59: Histopathological findings in 28 days dermal toxicity study in rabbit

	0 mg/kg bw/day		**1000 mg/kg bw/day**	
Number of rats/group	**6**		**6**	
	M	**F**	**M**	**F**
Skin				
Degen./Necr./Epider.	0	0	1	1
Degen./Necr./Dermis.	0	0	1	1
Acanthosis	0	0	1	1
Oedema	0	0	1	1
Haemorrhage	0	0	1	0
Inflammatory Cell Aggregation	0	0	1	1
Application site				
Ancanthosis	0	2	4	3
Hyperkeratosis	0	2	3	3
Degen./Necr./Epider.	0	0	2	3
Degen./Necr./Dermis.	0	0	0	3
Oedema	0	0	1	2
Haemorrhage	0	0	2	2
Vascular Ectasia	0	0	1	0
Inflammatory Cell Aggregation	0	1	4	2
Kidneys				
Multifocal Nephrocalcinosis	0	0	3	6
Focal Nephrocalcinosis	1	1	0	0
Tubular Degeneration/Necrosis	0	0	1	6

In the 28-day immunotoxicity study in rats, tribenuron-methyl did not show any immunotoxic effect. Effects on bodyweight growth (decreased bodyweight and bodyweight gain) and reduced food consumption were noted at 600 ppm (44 mg/kg bw/day).

In the developmental toxicity study in the rabbit, the maternal toxicity consisted of mortalities (two females) noted at 80 mg/kg bw/day. One high dose female found dead on gestation day 17 had wide spread hepatisation of the lungs. The other high dose female was found dead on gestation day 29 after previously aborting a fetus and had more fetuses *in utero*. Both of these females had severely decreased food consumption prior to death. One middle dose female (20 mg/kg bw/day group) were also found dead. This animal had multiple mucosal haemorrhages in the stomach associated with a trichobezoar and died on gestation day 29 just prior to scheduled sacrifice. Since no effects were observed for the remaining animals of the mid-dose group this single death was not given further significance for the purpose of classification. Clinical signs (abortions) and body weight loss (-325 g) were also noted in dams at 80 mg/kg bw/day.

In the subchronic neurotoxicity study in the rat, treatment was associated with effects on bodyweight growth (reduced bodyweight and bodyweight gain) and reduced food efficiency noted in both sexes at 700 ppm. No treatment-related findings were noted during the FOB investigation. *The NOAEL in the study will be discussed at expert-meeting.*

In the long-term toxicity study in the rat, treatment was associated with effects on bodyweight growth (reduced bodyweight and bodyweight gain). The incidence of total malignant tumours in female rats was significantly increased in the high-dose level (1250 ppm, corresponding to 76 mg/kg bw/day) after tribenuron-methyl administration. This was a result of the significant increase in mammary gland adenocarcinomas in the high-dose level. In males there were a statistical increase in total tumours already in the lowest dose group. However, there were no dose response and the study director concludes that this should be considered to be a statistical aberration and have no biological importance because there was no significant increase in any specific tumour type. There were also several systemic non-neoplastic effects in the male 250 (10 mg/kg bw/day) and 1250 ppm (55 mg/kg bw/day) dose group and in the female 1250 ppm dose (76 mg/kg bw/day) group when compared to controls (see table below). However, a specific target organ was not identified for non-neoplastic effects in male rats.

Table 60: Incidences of non-neoplastic microscopic observations after two-year feeding with tribenuron-methyl in rats

Dose	0 ppm	25 ppm	250 ppm	1250 ppm
Number of rats/group:	62a	60	60b	61
Males:				
Stomach: Mineralisation	3	2	8*	11*
Aorta: Mineralisation	2	2	7*	9*
Spleen: Lymphoid depletion	-	2	3	8*
Pancreas: Polyarteritis increase	4	8	4	15*
Seminal Vesicles: Reduced secretions	4	7	7	20*
Liver: Fatty Change, Focal/Multifocal	17	14	12	8*
Females:				
Kidneys:Bilateral dilatation of renal pelvis	2	4	5	8*
Uterus: Dilatation	10	7	4	27*
Eyes: Bilateral retinal degeneration	27	4	8	41*
Liver:Fatty Change, Focal/Multifocal	29	28	21	6*

[a] Number of rats/group = 60 in females.

[b] Number of rats/group = 58 in females.

[*] $p < 0.05$ according to Fisher's Exact test

Table 61: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
RAR Vol. 3, B.6.6.2.2/01 Developmental toxicity study in rabbits	80 mg/kg bw/day	13 day	560 mg/kg bw/day	STOT RE Cat 2

10.12.1.2 Comparison with the CLP criteria

Regulation EC No 1272/2008 (CLP), Annex 1: 3.9.2.7.3, states for STOT RE:

"All available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell):

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver);

(g) evidence of appreciable cell death (including degeneration and reduced cell number) in vital organs incapable of regeneration.

According to the CLP Guidance Table 3.9.3, a substance should be classified in Category 2 when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the guidance value ranges as indicated in table below:

Table 62: Guidance values for acute toxicity categories

Route of Exposure	Units	Guidance Values Ranges: (dose/concentration)

Oral (rat)	mg/kg bw/day	10<C≤100
Dermal (rat or rabbit)	mg/kg bw/day	20<C≤200
Inhalation (rat) gas	ppm V/6h/day	50<C≤250
Inhalation (rat) vapour	mg/litre/6h/day	0.2<C≤1.0
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	0.02<C≤0.2

According to Annex 1 3.9.2.9.8, the guidance values in table above is increased by a factor of three for a 28-day study.

Rat:

In the 28-day oral toxicity study in the rat (RAR Vol. 3, B.6.3.1.1/01), histopathological findings in the liver (hypertrophy of centrilobular hepatocytes) was noted at ≥300 mg/kg bw/day. The severity of the observed effects was however not considered of concern for a classification as STOT-RE, also taken into consideration that no histopathological changes in the liver were noted in the 90-day feeding study at doses up to 5000 ppm (335 and 386 mg/kg bw/day in males and females, respectively).

In the 90-day oral toxicity study in the rat (RAR Vol. 3, B.6.3.2.1/01), effects on the liver (changes in biochemical parameters and liver weights) were noted at ≥1750 ppm (118 and 135 mg/kg bw/day in males and females, respectively). In addition effects on blood parameters (increased platelet counts) were noted at ≥1750 ppm. Furthermore, effects on the spleen (increased organ weights) were noted at 100 ppm (8 mg/kg bw/day). The severity of the observed effects was however not considered of concern for a classification as STOT-RE.

In the 28-day immunotoxicity study in female rats (RAR Vol 3, B.6.8.2.2/01), effects on body weight growth and food consumption were noted at 300 ppm (44 mg/kg bw/day). The severity of the observed effects was however not considered of concern for a classification as STOT-RE.

In the subchronic neurotoxicity study in the rat (RAR Vol 3, B.6.7.1.2/01), treatment was associated with effects on bodyweight growth (reduced bodyweight and bodyweight gain) and reduced food efficiency noted in both sexes at 700 ppm. No treatment-related findings were noted during the FOB investigation. The severity of the observed effects was however not considered of concern for a classification as STOT-RE.

In the two-year rat study (RAR Vol. 3, B.6.5.1/01), treatment was associated with non-neoplastic changes in the male 250 (10 mg/kg bw/day) and 1250 ppm (55 mg/kg bw/day) dose groups and in the female 1250 ppm (76 mg/kg bw/day) dose group (mineralisation of stomach and aorta, spleen lymphoid depletion, increased pancreas polyarteritis, reduced secretion from seminal vesicles, liver fatty change, bilateral dilatation in renal pelvis, uterus dilatation and renal degeneration). However, a specific target organ was not identified for non-neoplastic effects in male rats. Thus, no changes of concern for classification were noted within the critical range of doses (i.e. 1.25<C≤12.5 mg/kg bw/day) (Habers rule considered, exposure 8 times the concentration limits for 90 day study).

Mouse:

In the 28-day range finding study in mice (RAR Vol. 3, B.6.3.2.2/01), effects on the liver (changes in organ weights) were noted in both sexes at ≥1250 ppm (250 mg/kg bw/day in males and females). The severity of the observed effects was however not considered of concern for a classification as STOT-RE.

In the 90-day feeding study in mice (RAR Vol. 3, B.6.3.2.2/01), effects on the liver (changes in organ weights) were noted in both sexes at 2500 ppm (350 and 476 mg/kg bw/day in males and females, respectively). The severity of the observed effects was however not considered of concern for a classification as STOT-RE. Furthermore, no changes of concern for classification were noted within the critical range of doses (i.e. 10<C≤100 mg/kg bw/day).

In the long-term toxicity study in the mouse (RAR Vol. 3, B.6.3.2.2/02) histopathology data revealed several minor modifications in the normal lesions of ageing within the male and female 1500 ppm (197 mg/kg bw/day) groups when compared to controls. These effects were not considered to be of adverse character and included a slight increase in severity of amyloidosis and marginal changes in some background inflammatory lesions, an increased incidence in thyroid inflammation in the 1500 ppm dose of both sexes, a higher incidence of bilateral testicular atrophy at the highest dose. A specific target organ was not identified. No changes of concern for classification were noted within the critical range of doses (i.e. 1.67<C≤16.7 mg/kg bw/day) (Habers rule considered, exposure 6 times the concentration limits for 90 day study)

Dog:

In the 90-day feeding study in dogs (RAR Vol. 3, B.6.3.2.3/01), effects on blood parameters (increased platelets (40%) at Month 3, increased leukocyte counts (30%) at Month 3, decreased mean corpuscular haemoglobin (2%, n.s.) at Month 2 were noted in males at 2500 ppm (78 mg/kg bw/day). The severity of the observed effects was however not considered of concern for a classification as STOT-RE.

In the 1-year feeding study in dogs (RAR Vol. 3, B.6.3.3.1/01), effects on body weight growth and changes in biochemical parameters (increased serum creatinine concentrations) were noted in both sexes at 1500 ppm (52 mg/kg bw/day in males and females). The severity of the observed effects was however not considered of concern for a classification as STOT-RE.

Rabbit:

In the 28-day dermal toxicity study in rabbits (RAR Vol.3, B.6.3.3.1/01), histopathological changes in the kidneys (nephrocalcinosis, tubular degeneration/necrosis) were noted in both sexes at 1000 mg/kg bw/day. Furthermore, one male and one female were found dead (on day 29 and day 24, respectively) without clinical signs preceding the death and no macroscopic abnormalities or microscopic findings were noted that could explain the death. The severity of the observed effect is considered relevant for a classification as STOT-RE. No NOAEL was determined in this limit dose study. It could however be noted that the LOAEL of 1000 mg/kg bw/day is close to the higher limit value for the critical range of doses (i.e. 28-day study: 60<C≤600 mg/kg bw/day) for a classification as STOT-RE cat 2.

In the developmental toxicity study in the rabbit (RAR Vol. 3, B.6.6.2.2/01), mortalities (two animals) and body bw loss (-325 g) were noted in dams at 80 mg/kg bw/day. According to study author marked maternal toxicity occurred in the high dose group (80 mg/kg bw/day). Effects included an average maternal food consumption that was 48% lower than control over the gestation day 7-19 dosing period. After the initiation of dosing at this high dose, food consumption progressively decrased from approximately 22 to 66% lower than the control values. This reduced food consumption corresponded to a mean body weight loss of 325 g (compared to a body weight gain of 114 in controls) over the gestation day 7-19 dosing period. Exposure in this study was for 13 days (GDs 7-19) and mortality occurred on days 17 and 29. It is therefore considered an effect of repeated administration of the test substance rather than an acute effect. As exposure was for 13

days i.e. 1/7 of 90 days, it is proposed to multiply the Guidance Values of 10 for Cat 1 and 100 for Cat 2 classification of 10 and 100 mg/kg bw/day, respectively, by a factor 7, giving adjusted Guidance Values for 13 days of 70 and 700 mg/kg bw/day for STOT RE 1 and 2, respectively. The mortalities occurred above 70 mg/kg bw/day but below 700 mg/kg bw/day and therefore justify classification in STOT RE-2.

Two pilot teratogenicity studies were conducted to determine the maximum tolerated dose of the test substance by gavage in the rabbit. Based on data obtained from a pilot teratogenicity study, 6 females per group were given 0, 250, 500 or 750 mg/kg body weight in the first rabbit pilot study. These dose levels were found to be extremely toxic to the female rabbit and all females in the 500 and 750 mg/kg dose groups died. The dose level for the 250 mg/kg group was reduced to 125 mg/kg after 5 doses in an effort to determine the maximum tolerated dose. Although all six females in the low dose goup survived until scheduled sacrifice, no litters were produced. In the second rabbit pilot study, 7 females per group received the test substance at dose levels of 150 or 75 mg/kg body weight. The 150 mg/kg dose group demonstrated severe weight loss and two of the females died. No deaths were observed at 75 mg/kg body weight, but moderate weight loss and decreased feed consumption were evident. Based on these data, dose levels of 0, 10, 40 and 80 mg/kg body weight were chosen for the main study.

10.12.1.3 Conclusion on classification and labelling for STOT RE

Classification in **STOT RE 2**, H373 is proposed based on the mortalities seen in rabbits at 80 mg/kg bw/day in a developmental toxicity study with 13 days exposure to tribenuron-methyl. Furthermore mortalities and histopathological changes in the kidney (nephrocalcinosis, tubular degeneration/necrosis) were noted in the 28-day dermal toxicity study using a limit dose level of 1000 mg/kg bw/day.

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)	Main study: 0, 100, 300, 1 000 mg/kg bw/d	Liver Study limited, selected as dose-	<u>30 mg/kg bw/d</u> None

EEC Method B.7 (1984) GLP (Study RAR Vol. 3 B.6.3.1.1/01; NOTOX 0382/534, 1986)	Parallel study: 0, 30, 100, 300 mg/kg bw/d Guidance value for classification **≤** 300 mg/ kg bw/day	range finding; no NOAEL derived	<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) U.S. EPA 82-1 (1982) Non-GLP (Study RAR Vol. 3 B.6.3.2.2/01;	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 %))

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	125/500 ppm 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	20 ppm 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	50/500 ppm None 2 500 ppm - ↓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) US EPA 83-1 (1982) GLP	0, 25, 250 and 1 500 ppm equal to m: 0, 0.79, 8.16 and 51.5 mg/kg bw/d	None (only bw and serum creatinine affected) NOAEL 250 ppm	25/250 ppm None

(RAR Vol. 3 B.6.3.3.1/01; HLR 565-86, 1986)	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.

10.13 Aspiration hazard

10.13.1.1 Short summary and overall relevance of the provided information on aspiration hazard

No data is available.

10.13.1.2 Comparison with the CLP criteria

No relevant as no data is available.

10.13.1.3 Conclusion on classification and labelling for aspiration hazard

No classification is proposed for tribenuron-methyl.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 63: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD 301 E (1992)	11-24% degradation of the test substance in 28 days. The degradation of the reference compound was 100% in the same time span.	The test substance is not readily biodegradable	Douglas, M.T. & Halls, R.W.S. (1993) AMR 2422-92
OPPTS 835.2120 (2008), SETAC Europe (1995), OECD 111 (2004)	First order half-lives: pH 4: 0.41 days at 10°C 0.19 days at 15°C 0.10 days at 20°C pH 7: 63.5 days at 15°C 31.0 days at 20°C 14.9 days at 25°C pH 9: 743 days at 15°C 266 days at 30°C 41.1 days at 40 °C pH 4: 0.04 days at 25°C pH 7: 8.0 days at 25°C 0.72 days at 50°C pH 9: 157 days at 25°C 9.4 days at 50°C 1.6 days at 65°C Max formed metabolites (>5% of applied radioactivity): IN-00581: 92.8% at pH 7 (0.42 days, 20°C), 45.6% at pH 7 (30.0 days, 20°C), 9.3% (30.0 days, 25°C) IN-L5296: 90.5% at pH 4 (0.42 days, 20°C), 50.2% at pH 9 (30.0 days, 20°C), 9.7% at pH 9 (30.0 days, 25°C). IN-D5803: 6.9% at pH 7 (7.0 days, 20°C)	Rapid hydrolysis under pH 4, moderate at pH 7 and nearly stable at pH 9	Liu, X. (2013) DuPont-36471 Hein, W. (2015) Report No: AS343 Report No. 315 TBM
OECD 316, OPPTS 835.2240	First order half-lives: Irradiated: 120 days Dark control: 315 days Net Photo-degradation: 195 days Max formed metabolites (>5% of applied radioactivity): IN-00581: 7.5% (15 days), IN-L5296: 5.62% (15 days)	Tribenuron-methyl was photolytically stable (i.e. net photodegradation DT_{50} is >190 days)	Wen, L. (2014) DuPont-36470
OECD 309 (2004), OPPTS 835.3190 (2008)	12.1-18.2% mineralisation of test substance in 60 days at low dose (50 µg/L) 7.7-28.2% mineralisation of	Study performed with pond water (pelagic conditions)	Allan, J. (2014) DuPont-36661

Method	Results	Remarks	Reference
	test substance in 60 days at high dose (500 µg/L) 91.6-93.0% mineralisation of reference compound ([^{14}C]sodium benzoate) at high dose in the same time span. First order half-life of test substance: 86.2 days Max formed metabolites (of applied radioactivity): IN-00581: 28.3% (46 days) IN-L5296: 26.5% (46 days)		
OECD 309 (2004)	Min 68% remaining as the test substance in 110 days at low dose (5 µg/L) in water only test. Min 60% remaining as the test substance in 110 days at high dose (50 µg/L) in water only test. Min. 49% remaining as the test substance in 110 days at high dose (50 µg/L) in suspended sediment test. Full degradation of the positive control ([^{14}C]-benzoic acid) in 48 hours. Max formed metabolites (>5% of applied radioactivity): IN-00581: 5.3% (14 days, 5 µg/L water only), 5.0% (61 days, 50 µg/L water only), 20.4% (28 days, suspended sediment test). IN-L5296: 10.4% (61 days, 5 µg/L water only), 7.0% (110 days, 50 g/l water only), 8.3% (110 days, 50 µg/l water only) IN-A4098: 6.3% (110 days, 50 µg/L water only) IN-R9805: 8.9% (61 days, 5 µg/L water only) IN-GK521: 13.6% (110 days, 50 µg/L water only), 14.8% (110 days, suspended sediment test) IN-D5119: 10% (110 days, 50 µg/L water only test), 6.8%	Study performed with river water (pelagic conditions and suspended sediment test). Too low mineralisation to accurately calculate half-lives. A rough calculation using first order kinetics gives a half-life of 139 days for the suspended sediment test system.	Moendel M (2015) Project No: AS344 Report No. 328 TBM

Method	Results	Remarks	Reference
	(110 days, suspended sediment test) IN-D5803: 5.1% (28 days, 50 µg/L water only test)		
SETAC Europe (1995), U.S. EPA 162-4 (1982)	Half-lives: <u>Brandywine river:</u> Total system: 26.2 days (SFO) Water column: 22.5 days (SFO) Sediment: 18.8 days (FOMC) <u>Lums pond:</u> *Triazine label:* Total system: 7.4 days (SFO) Water column: 6.5 days (SFO) Sediment: 5.3 days (SFO) *Phenyl label:* Total system: 21.5 days (SFO) Water column: 17.6 days (FOMC) Sediment: 19.1 days (SFO) Overall geometric half-life in total system: 18.2 days (SFO) Max formed metabolites (>5% of applied radioactivity): IN-L5296 (max 89% in total system, max 42% in water, max 86% in sediment) IN-D5119 (max 26.5% in total system, max 19% in water, max 7.5% in sediment) IN-00581 (max 38.4% in total system, max 32% in water, max 6.4% in sediment) IN-R9805 (max 14.7% in total system, max 9% in water, max 5.7% in sediment) IN-GN815 (max 13% in total system, max 5.7% in water, max 9.2% in sediment)	Study performed with two water systems: Brandy wine river (anaerobic conditions in the sediment) and Lums pond (aerobic conditions in sediment). For Lums pond there was significant differences in the half-lives for triazine and phenyl-labbelled test substance. In the overall geomean of the half-life for the water systems the geomean of Lums pond is first calculated	Pedersen, C.T., Fetterman, D.E. (2001) DuPont-2286, Revision No. 1 Kinetic calculations in Snyder *et al* (2012) and Mamaouni & Callow (2015b)

All the information on rapid biodegradability are taken from the RAR and list of endpoints for tribenuron-methyl, June 2016. The tests that were available were mostly water/sediment studies and hydrolysis studies with tribenuron-methyl and its metabolites IN-L5296, IN-00581, IN-D5119, IN-GN815, and IN-R9805.

There was also a degradation test studying the aerobic mineralisation of tribenuron-methyl in surface water.

11.1.1 Ready biodegradability

Material and methods

A ready biodegradable test was performed with tribenuron-methyl according to OECD-guideline 301 E (modified OECD screening test) and GLP.

A mixture of test substance (tribenuron-methyl technical) and inorganic nutrient medium was added to activated sludge from a sewage treatment plant for domestic sewage at a rate of 0.5 ml/litre, and incubated for 28 days at 21°C. The test concentration used in the test was 32 mg/l (equivalent to 15 mg carbon/litre). The reference substance used was sodium benzoate (26 mg/l, equivalent to 15 mg carbon/litre). Duplicate test beakers were prepared for the test and standard substance, and for a blank test. An additional beaker was set up containing both test substance and reference substance in order to determine if tribenuron-methyl exhibits any bacterial-inhibiting properties.

Measurements of dissolved organic carbon (DOC) was conducted using a TC/TOC analyser, and percent biodegradation was determined by comparing residual DOC value with the DOC value measured at the start of the study.

Results

Degradation of the test substance in 28 days was 11-24 %. The reference compound was 100 % degraded. The inhibition check attained only 71 % biodegradation, indicating a slight inhibitory effect.

Summary

The test substance is not readily biodegradable in the sense of the test method (OECD 301). RMS comments was that the study was accepted in the DAR (2004) and is still deemed valid for the purpose of renewal.

11.1.2

11.1.3 BOD_5/COD

No test provided according to OECD guidelines or other relevant guidelines.

11.1.4 Hydrolysis

Reference: Liu, X. (2013); Annex III

Guideline: OPPTS 835.2120 (2008), SETAC Europe (1995), OECD 111 (2004)

GLP: Yes

Test material: Label 1: [phenyl(U)-^{14}C]tribenuron-methyl, Label 2: [triazine-2-^{14}C]tribenuron-methyl

Test system: The tier 1 test at 50°C was not performed since the previous hydrolysis study showed that there was at least some hydrolysis at all three pH.

Hydrolysis of radiolabelled tribenuron-methyl at 1.02–1.16 mg/L (1 ppm) was studied in the dark at 10, 15, and 20°C (pH 4); 15, 20, and 25°C (pH 7); and 20, 30, and 40°C (pH 9). Hydrolysis was carried out in sterile aqueous buffered solutions at pH 4 (0.01 M potassium hydrogen phthalate), pH 7 (0.01 M potassium phosphate), and pH 9 (0.01 M potassium borate) for up to 30 days.

Results:

First order half-lives:
pH 4: 0.41 days at 10°C
0.19 days at 15°C
0.10 days at 20°C

pH 7: 63.5 days at 15°C
31.0 days at 20°C
14.9 days at 25°C

pH 9: 743 days at 15°C
266 days at 30°C
41.1 days at 40 °C

Max formed metabolites (>5% of applied radioactivity; toxicological profile of metabolites described in the other parts of the report):
IN-00581: 94.6% at pH 7 (0.42 days, 20°C), 45.6% at pH 7 (30.0 days, 20°C)
IN-L5296: 90.5% at pH 4 (0.42 days, 20°C), 50.2% at pH 9 (30.0 days, 20°C)
IN-D5803: 6.9% at pH 7 (7.0 days, 20°C)

Reference: Hein, W. (2015); Annex III

Guideline: OECD 111 (2004)

GLP: Yes

Test material: Label 1: [phenyl(U)-^{14}C]tribenuron-methyl, Label 2: [triazine-2-^{14}C]tribenuron-methyl

Test system: A preliminary hydrolysis test was done at 50°C and pH 4, pH 7 and pH 9. The main test was then done at 25°C for pH 4 and 7 and at 25 °C, 50°C and 65°C at pH 9. The following buffers were used:

pH 4: citric acid buffer adjusted to pH 4 by addition of 0.1 N NaOH

pH 7: potassium dihydrogen phosphate adjusted to pH 7 by addition of 0.1 N NaOH

pH 9: potassium borate adjusted to pH 9 by addition of 0.1 N NaOH

Results:

First order half-lives:
pH 4: 0.04 days at 25°C

pH 7: 8.02 days at 25°C
0.72 days at 50°C
14.9 days at 25°C

pH 9: 157 days at 25°C
9.37 days at 50°C
1.6 days at 65 °C

Max formed metabolites (>5% of applied radioactivity; toxicological profile of metabolites described in the other parts of the report):
IN-00581: 92.8% at pH 4 (0.10 days, 25°C), 82.4% at pH 7 (21 days, 25°C), 9.3% at pH 9 (30 days, 25°C)
IN-L5296: 88.0% at pH 4 (0.10 days, 25°C), 78.8% at pH 7 (21 days, 25°C), 9.7% at pH 9 (30 days, 25°C)
IN-D5803: 6.9% at pH 7 (7.0 days, 20°C)

11.1.5 Other convincing scientific evidence

No information.

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

Not relevant

11.1.5.2 Inherent and enhanced ready biodegradability tests

No information

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

The available data on tribenuron-methyl and its metabolites on degradation in surface water and water-sediment studies are summarised in the tables below. Individual studies are further summarized in the List of Endpoints, RAR tribenuron-methyl, June 2016.

Aerobic mineralisation in surface water (Regulation (EU) N° 283/2013, Annex Part A, point 7.2.2.2 and Regulation (EU) N° 284/2013, Annex Part A, point 9.2.1)

Parent										
System identifier (indicate fresh, estuarine or marine)	pH water phase	pH sed [a]	t. °C[b]	DT_{50} /DT_{90} whole sys. (suspended sediment test)		St. (χ2)	DT_{50} /DT_{90} Water (pelagic test)		St. (χ2)	Method of calculation
				At study temp	Normalised to x °C		At study temp	Normalised to x °C		
pond water	8.0	-	20	-	-	-	86.2[c]	-	3	SFO
river water	8.3	-[d]	20-24	139	-	2	-[e]	-	-	SFO

a) Measured in [medium to be stated, usually calcium chloride solution or water].

b) Temperature of incubation.
c) Based on degradation of parent into transformation products (not full mineralisation).
d) pH of sediment not reported.
e) Due to long lag phase and too few data points no degradation half-life could be calculated.

Mineralisation and non extractable residues (for parent dosed experiments)					
System identifier (indicate fresh, estuarine or marine)	pH water phase	pH sed	Mineralisation x % after n d. (end of the study).	Non-extractable residues. max x % after n d (suspended sediment test)	Non-extractable residues. max x % after n d (end of the study) (suspended sediment test)
Pond water	8.0	-	28% after 60 d	--	--
river water	8.3	-	2.2% after 110 days (pelagic) 3.8% after 110 days (susp. sed.)	2.7% after 14 days	1.6% after 110 days

Parent										
System identifier (indicate fresh, estuarine or marine)	pH water phase	pH sed [a]	t. °C[b]	DT_{50} /DT_{90} whole sys. (suspended sediment test)		St. (χ2)	DT_{50} /DT_{90} Water (pelagic test)		St. (χ2)	Method of calculation
				At study temp	Normalised to x °C		At study temp	Normalised to x °C		
pond water	8.0	-	20	-	-	-	86.2[c]	-	3	SFO
river water	8.3	-[d]	20-24	139	-	2	-[e]	-	-	SFO

a) Measured in [medium to be stated, usually calcium chloride solution or water].

b) Temperature of incubation.

c) Based on degradation of parent into transformation products (not full mineralisation).

d) pH of sediment not reported.

e) Due to long lag phase and too few data points no degradation half-life could be calculated.

Summary

Tribenuron-methyl and its metabolites are slowly mineralized with a DT50 for the whole system 139 days in river water (suspended sediment test) and DT50 in pond water 86.2 days based on degradation of parent into transformation products in water (pelagic test).

Water / sediment study (Regulation (EU) N° 283/2013, Annex Part A, point 7.2.2.3 and Regulation (EU) N° 284/2013, Annex Part A, point 9.2.2)

Parent	Distribution; mainly distributed to water phase									
Water / sediment system	pH water phase	pH sed [a]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Brandywine river	6.8-8.3	6.5-7.5	20	26.7/ 88.6	7-11[b]	23.3/ 77.3	6-8[b]	18.8/87.6[c]	4	SFO
Lums pond	4.9-7.7	4.9-5.4	20	12.9/ 42.8	7-21[b]	11.4/ 40.9	8-20[b]	10.1/33.6	31-35[b]	SFO
Geometric mean at 20°C[b]				**18.5**		-		-		-

a) Measured in water.

b) Range of χ2-error from the fittings in two kinetic studies and/or from the fitting of data derived with two different radiolabels.

c) Dissipation from sediment in Brandywine river; FOMC kinetic model.

Mineralisation and non extractable residues (from parent dosed experiments)					
Water / sediment system	pH water phase	pH sed[a)]	Mineralisation x % after n d. (end of the study).	Non-extractable residues in sed. max x % after n d	Non-extractable residues in sed. max x % after n d (end of the study)
Brandywine river	6.8-8.3	6.5-7.5	60% (135 d, phenyl(U)-^{14}C) 18% (135 d, triazine-2-^{14}C)	26% after 105 d (phenyl(U)-^{14}C) 16% after 71 d (triazine-2-^{14}C)	22% after 135 d (phenyl(U)-^{14}C) 14% after 135 d (triazine-2-^{14}C)
Lums pond	4.9-7.7	4.9-5.4	65% (135 d, phenyl(U)-^{14}C) 1.4% (105 d, triazine-2-^{14}C)	16% after 105 d (phenyl(U)-^{14}C) 9.3% after 105 d (triazine-2-^{14}C)	15% after 135 d (phenyl(U)-^{14}C) --

a) Measured in water.

IN-L5296	Distribution; up to 88.9% (total system, 56 d), max 42% in water (14 d), max 86% in sediment (56 d)									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Brandywine river	6.8-8.3	6.5-7.5	20	168/558	10	not avail.	-	not avail.	-	SFO-SFO
Lums pond	4.9-7.7	4.9-5.4	20	309/1026	10	3.2/ 33.6[c)]	7	not avail.		SFO-SFO
Geometric mean at 20°C[b)]				**227.8**		-		-		-

a) Measured in water.

b) Normalised using a Q10 of 2.58.

c) Dissipation from water column in Lums pond; FOMC kinetic model.

IN-00581	Distribution; up to 38.4% (total system, 14 d), max 32% in water (14 d), max 6.4% in sediment (14 d)									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Brandywine river	6.8-8.3	6.5-7.5	20	not avail.	-	not avail.	-	not avail.	-	
Lums pond	4.9-7.7	4.9-5.4	20	5/35.8[c)]	10	2.3/20.5[c)]	7	not avail.	-	FOMC
Geometric mean at 20°C[b)]				**10.8[d)]**		-		-		-

a) Measured in water.

b) Normalised using a Q10 of 2.58.

c) Decline fit; FOMC kinetic model.

d) Modelling input - FOMC DT_{90} / 3.32.

IN-D5119	Distribution; up to 26.5% (total system, 56 d), max 19% in water (56 d), max 7.5% in sediment (56 d)									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Brandywine river	6.8-8.3	6.5-7.5	20	not avail.	-	not avail.	-	23.3/77.5[c)]	18	SFO

Lums pond	4.9-7.7	4.9-5.4	20	not avail.	-	not avail.	-	not avail.	-	-
Geometric mean at 20°C[b)]				**not avail.**		-		-		-

a) Measured in water.

b) Normalised using a Q10 of 2.58.

c) Dissipation from sediment in Brandywine river.

IN-GN815	Distribution; up to 13% (total system, 29 d), max 5.7% in water (42 d), max 9.2% in sediment (29 d)									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Brandywine river	6.8-8.3	6.5-7.5	20	47.6/158[c)]	10	32.1/107[c)]	7	28.5/94.6[c)]	17	SFO
Lums pond	4.9-7.7	4.9-5.4	20	not avail.	-	not avail.	-	not avail.	-	-
Geometric mean at 20°C[b)]				**47.6**		-		-		-

a) Measured in water.

b) Normalised using a Q10 of 2.58.

c) Decline fit; SFO kinetic model.

IN-R9805	Distribution; up to 14.7% (total system, 71 d), max 9% in water (71 d), max 5.7% in sediment (71 d)									
Water / sediment system	pH water phase	pH sed [a)]	t. °C	DT_{50} /DT_{90} whole sys.	St. (χ2)	DT_{50} /DT_{90} water	St. (χ2)	DT_{50} /DT_{90} sed	St. (χ2)	Method of calculation
Brandywine river	6.8-8.3	6.5-7.5	20	not avail.	-	not avail.	-	not avail.	-	-
Lums pond	4.9-7.7	4.9-5.4	20	not avail.	-	not avail.	-	not avail.	-	-
Geometric mean at 20°C[b)]				**not avail.**		-		-		-

a) Measured in water.

b) Normalised using a Q10 of 2.58.

Summary

Tribenuron-methyl and its metabolites IN-L5296 are not rapidly degradable in the water sediment study. For Tribenuron-methyl with a DT50 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. The metabolites IN-D5519 and IN-R9805 were not available.

Summary of the degradation tests

Tribenuron-methyl and its metabolites are not rapidly degradable.

11.1.5.4 Photochemical degradation

Reference: Wen, L. (2014); Annex III

Guideline: OECD 316, OPPTS 835.2240

GLP: Yes

Test material: Label 1: [phenyl(U)-^{14}C]tribenuron-methyl, Label 2: [triazine-2-^{14}C]tribenuron-methyl

Test system: The rate and route of photochemical degradation of [^{14}C]tribenuron-methyl was determined in sterile 0.01 M pH 9 borate buffer solution. The test was conducted at concentrations of 1.13 mg/L for both the phenyl nd triazine-label under continuous irradiation using a xenon arc lamp for approximately 15 days at ca 25 ± 2°C. This was equivalent to *ca* 30 days of mid-summer sunlight (at latitude of 40° N), assuming a 12 h light/ 12 h dark cycle. The radiolabelled material was applied to autoclaved 0.01 M, pH 9 borate buffer prepared in HPLC-grade water in individual quartz glass photolysis vessels.

The test was only performed at pH 9 in order to minimise the hydrolysis as prescribed by OECD 316. All irradiated samples were exposed to artificial sunlight of a xenon arc lamp fitted with a filter designed to mimic natural sunlight (nominal UV cut-off of 295 nm). Non-irradiated (dark control) samples were also prepared for each radiolabel and test system and maintained in the dark at ca 25 ± 2°C.

Results:

Treatment	First-order rate constant (day^{-1})	DT_{50}[1] (days)	DT_{75}[1] (days)	r^2
Irradiated	0.005758	120	241	0.9554
Dark Control	0.002203	315	629	0.7489
Net Photo-degradation	0.003555	195	390	NA

Max formed metabolites (>5% of applied radioactivity):

IN-00581: 7.5% (15 days), IN-L5296: 5.62% (15 days)

Summary of the photolytical degradation

Not an important route for tribenuron-methyl.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable

11.3 Environmental fate and other relevant information

No further relevant information

11.4 Bioaccumulation

Since tribenuron-methyl has a Log Kow < 4 (actual value is -0.46 to 2.0 in the pH range of 4 to 10) the potential for bioaccumulation is low.

Table 64: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD 117 / shake flask method and HPLC analysis	All results are given at 20°C. distilled water: Log Kow = 0.85 ± 0.03 acidic pH not measured due to rapid hydrolysis pH 7.0 phosphate buffer: log Kow = - 0.38 ± 0.01 pH 9.0 borate buffer: log Kow = -0.93 ± 0.01	**Key study**	Pakki, U.V.S., 2013 DuPont-36463

Method	Results	Remarks	Reference
OECD 117 / shake flask method and HPLC analysis	All results are given at 20°C. pH 4 orthophosphate buffer: Log Kow = 2.0 pH 7.0 orthophosphate buffer: Log Kow = -0.46 pH 10.0 borate buffer: Log Kow = -2.22	**Key study** It should be noted that tribenuron-methyl undergo hydrolysis at acidic pH, and the result at pH 4 i.e. logKow = 2.0 is most likely somewhat underestimated.	Cowlyn, N., 2014 (288 TBM)

11.4.1 Estimated bioaccumulation

Since tribenuron-methyl has a Log Kow < 4 (actual value is -0.46 to 2.0 in the pH range of 4 to 10) the potential for bioaccumulation is low.

11.4.2 Measured partition coefficient and bioaccumulation test data

No data available

11.5 Acute aquatic hazard

Table 65: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results [mg/L]	Remarks	Reference
OECD 203 96h (static)	*Oncorhynchus mykiss* Rainbow trout	Tribenuron-methyl	Mortality, LC_{50} = 738 (mean measured)	Key study	DUP, Boeri, R.L., Kowalski, P.L., Ward, T.J., 1997, AMR 4201-96
OECD 202 48h (static)	*Daphnia magna* Water flea	Tribenuron-methyl	Immobility, EC_{50} > 894 (mean measured)	Key study	DUP, Boeri, R.L., Kowalski, P.L., Ward, T.J., 1997 AMR 4202-96
OECD 201 72h (static)	*Pseudokirchneriella subcapitata* Green algae	Tribenuron-methyl	Growth rate, ErC_{50} = 0.068 (nominal)	Key study	DUP, Sloman, T.L., Leva, S.E., 1998, DuPont-1222
OECD 201 72h (static)	*Anabena flos-aquae* Cyanobacteria	Tribenuron-methyl	Growth rate, ErC_{50} >100 (nominal)		TTF: Hermes, H., Sonntag, F. (2016) Ibacon Project 118631218
US EPA guideline 123-2 14d (static)	*Lemna gibba* Duck weed	Tribenuron-methyl	Growth rate, ErC_{50} = 0.0047 (nominal)	Key study	DUP, Kannuck, R.M., Samel, A., 2000, AMR 3070-94, Revision No. 1 Scown, T., et al 2014 DuPont-41634
14d (static)	*Myriophyllum spicatum*	Tribenuron-methyl	Growth rate, ErC_{50} >94		DUP, Kirkwood, A., 2015 DuPont-32239, Revision No. 2
OECD 239 14d (static)	*Myriophyllum spicatum*	Tribenuron-methyl	Growth rate (fresh weight), ErC_{50} = 0.0065 (nominal)		TTF, Gonsior , G., 2015, 297 TBM amdt-1

11.5.1 Acute (short-term) toxicity to fish

Tribenuron-methyl shows a low acute toxicity to fish with an LC_{50} of **738 mg/L**.

Reference: **DUP: Boeri et al., 1997a** DPX-L5300: Acute toxicity to the rainbow trout, *Oncorhynchus mykiss*

Company Report No.:	AMR 4201-96
Guideline:	EEC Method C.1. (1992), OECD 203 (1992), U.S. EPA 71-2 (1988)
GLP:	Yes
Material and methods:	
Test material:	Tribenuron methyl technical
Lot/Batch #:	L5300-152, L5300-153
Purity:	99.7% (L5300-152) 98.9% (L5300-153)
Test system/ test conditions:	The acute toxicity of tribenuron-methyl to rainbow trout (*Oncorhynchus mykiss*) was tested in a static test system. Twenty fish per test concentration (two replicates of 10 fish each) were exposed for 96 hours to the following nominal test concentrations: 130, 220, 360, 600, and 1000 mg/l, along with a water control. The mean weight of the test fish was 1.2 g and the mean length was 51 mm. The water temperature during the test was 12 - 13°C, the pH was 7.2-7.6 and the oxygen content was 7.0-9.5 mg/l. Hardness and alkalinity of the dilution water was 44-48 mg/l and 29-31 mg/l, respectively.

Results:

Mean measured concentrations were 83-88 % of nominal concentrations. The 96-hour LC_{50}-value was calculated to be 738 mg/l. Changes in coloration were observed in several of the test organisms at test concentration 302 mg/l at 48, 72 and 96 hours. Changes in coloration, lethargy and/or distended abdomen were observed in several organisms at test concentrations 522 and 878 mg/l at 24, 48, 72 and 96 hours, and the 96 hour NOEC was therefore set to 183 mg/l. All values are based on mean measured concentrations.

There were no mortality or sublethal effects observed in the control for the duration of the study.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Tribenuron-methyl shows a low acute toxicity to aquatic invertebrates with a LC_{50} of **>894 mg/L**.

Reference:	**DUP: Boeri et al 1997b** Acute toxicity to the daphnid, *Daphnia magna*
Company Report No.:	AMR 4202-96
Guideline:	OECD 202 (1992)
GLP:	Yes
Material and methods:	
Test material:	Tribenuron-methyl
Lot/Batch No:	DPX-L5300-152
Purity:	98.9% (w/w)
Test system/test conditions:	A 48-hour static test with tribenuron-methyl was conducted with *Daphnia magna* at the following nominal test concentrations: 130, 220, 360, 600, and 1000 mg/l, along with a dilution water control. The study was conducted with twenty daphnids (two replicates of ten daphnids each) per test concentration. The water temperature during the test was 20 - 21°C, the pH was 7.5-8.0, and the

	oxygen content was 7.1-8.6 mg/l. Hardness and alkalinity of the dilution water were 172 mg/l and 116 mg/l, respectively. The pH of the dilution water was adjusted with phosporic acid.

Results:

Mean measured concentrations during the test were 89-92 % of nominal concentrations. One out of 20 daphnids was immobilised in the control, in the 120 and the 537 mg/l test concentrations, respectively. No sublethal effects were observed. The 24- and 48-hour EC_{50} was >894 mg/l. NOEC was set to 894 mg/l. The values are based on measured concentrations. The validity criteria of OECD 202 (2004) are fulfilled.

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

Both algae and aquatic plants are sensitive to tribenuron-methyl, which is expected for a herbicide. Higher plants are more sensitive than algae. The lowest EC_{50} is **0.0047 mg/L** derived with *Lemna gibba*.

Reference:	**DUP: Sloman 1998b** Tribenuron-methyl technical (DPX-L5300): Influence on growth and growth rate of the green alga *Selenastrum capricornutum*
Company Report No.:	DuPont 1222
Guideline:	OECD Guideline 201, EU Commission Directive 92/69/EEC, Method C3
GLP:	Yes
Material and methods:	
Test material:	Tribenuron-methyl
Lot/Batch No:	DPX-L5300-143
Purity:	97.4% (w/w)
Test system/test conditions:	The influence of technical tribenuron-methyl on growth rate of *Selenastrum capricornutum* was tested in a static system at measured concentrations (at the end of the study) of 4.0, 8.0, 16, 23 and 32 µg/l in synthetic AAP nutrient medium, along with control cultures. The test vessels (triplicate) were incubated for 120 hours at 24°C under continuous illumination. The pH was in the range 7.0-8.0. The algal cell densities were determined using a hemacytometer and a compound microscope.

Results:

The measured concentrations at day 5 were 96-100 % of nominal concentrations.

72h NOEC = 4 µg/L

72h EC_{10} = 11.48 µg/L 95% C.I. (7.02- 18.75)

72h EC_{20} = 21.19 µg/L 95% C.I. (16.27-27.60)

72h EC_{50} = 68.50 µg/L 95% C.I. (45.02-104.2)

The following comments were made in the DAR 2004: The number of cells (3000) at test initiation was lower than what is recommended in the OECD guideline (10 000), but this will not influence the results of the test. Cell counts were not done at day 2. The duration of the study was longer than the guideline requirements. However, since exponential growth was observed throughout the test, the 120 hour data is still valid.

For the present report, the RMS re-evaluated the study in line with OECD 201 (2006) and concluded that validity criteria are met.

Reference:	**TTF: Hermes, H., Sonntag, F. (2016);** Tribenuron-methyl Technical: Toxicity to *Anabena flos-aquae* in an Algal Growth Inhibition Test
Company Report No.:	Ibacon Project 118631218
Guideline:	OECD 201 (2011).
GLP:	Yes
Material and methods:	
Test material:	Tribenuron-methyl Technical
Lot/Batch #:	Batch No.: 63B3011159
Purity:	98.3% by analysis
Control:	Reconstituted Water (20x AAP Medium)
Test vehicle:	None
Toxic reference:	None
Test species:	*Anabena flos-aquae UTEX 1444*
Initial population:	15,000 cells/mL
Source:	The University of Texas at Austin, UTEX Culture Collection of Algae, 1 University Station", Austin, TX 78712, USA.
Test chamber:	Erlenmeyer flasks of 50 mL volume with 50 mL of test medium.
Experimental treatments	- 72h static - 100, 32, 10, 3.2, 1.0, 0.32 and 0.10 mg test item/L, and a control - 3 replicates.
Growth medium:	Reconstituted Water (20x AAP Medium)
pH	at test start 7.6, at the end of the test 8.6 to 9.4
Environmental conditions (in-life period)	
Temperature:	22 to 24 °C
Photoperiod:	Continuous illumination; mean light intensity: 4203 lux (4000 to 4400 lux).
Observations	The cell density on each observation time was determined by spectrophotometric measurement (spectral photometer Specord50/Specord 50 Plus, 440 nm). Therefore, defined volumes of the algal suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The algal cell densities were calculated by subtracting the absorption of the blanks, from each of the measured absorption of the test media (with algae). Based on the counted cell densities and the absorption from an algal suspension and its dilutions, a linear regression was performed for the calculation of the cell densities of the replicates during the test. To check for any effect of the test item on the morphology of the algal cells, at least one sample from each test item concentration with a reduced cell density was taken after the test period of 72 hours. The shape of the treated algal cells compared to the control was examined microscopically
Statistics	Based on the calculated cell densities, the 72 hours ErC50 and the 72 hours EyC50 (see Definitions), the corresponding EC20 and EC10 values and where possible their 95 %-confidence limits were calculated by Probit analysis. For the determination of the 72 hours LOEC and the 72 hours NOEC, the calculated growth rates and yields at each test concentration were tested for significant differences compared to the control values by Williams t-test.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ToxRat Solutions GmbH.

Analytical Results:

At the start of the test 97% of the nominal test concentrations were found (average of all test concentrations). After 72 hours test duration, 97% of the nominal value was determined (average of all test concentrations). During the test the algae were exposed to a mean of 97% of nominal. Therefore, all reported results refer to nominal concentrations.

Conclusion:

The 72-hour EyC50 was calculated to be 62.5 mg test item/L, and the 72-hour ErC50 value was calculated to be > 100 mg test item/L. The 72-hour NOEyC was determined to be 10 mg test item/L and the associated 72-hour LOEyC was 32 mg test item/L.

The validity criteria stipulated in OECD guideline 201 (2011) are fulfilled.

Reference:	**DUP: Kannuck 2000,** Tribenuron-methyl (DPX-L5300): Influence on growth and reproduction of *Lemna gibba* G3 **DUP: Scown 2014** Tribenuron-methyl : Calculation of Lemna 7-day EC_{50} values from original exposure study data
Company	AMR 3070-94, Revision No. 1
Report No.:	DuPont-41634
Guideline:	US EPA guideline 123-2
GLP:	Yes
Material and methods:	
Test material:	Tribenuron-methyl
Lot/Batch No:	DPX-L5300-104
Purity:	92.6% (w/w)
Test system/test conditions:	The influence of tribenuron-methyl on growth and reproduction of *Lemna gibba* was tested in a static system at measured (day 0) test concentrations of 0.49, 1.02, 2.54, 4.69 and 11.4 µg/l in synthetic 20X AAP nutrient medium, along with a blank control and an abiotic control. The test vessels (triplicate) were incubated for 14 days at 24.0-26.5 °C under continuous illumination. The pH ranged from 7.5 to 9.5

Results: Concentrations measured at day 14 ranged between 94 and 102% of the values measured at day 0. The 14 day EC_{50} for frond number was 4.3 µg/l, and NOEC was 1.0 µg/l. The 14 day EC_{50} for biomass was 5.6 µg/l and the NOEC was 1.0 µg/l, based on measured concentrations (day 0). Recovery was tested for the organisms in the concentrations that resulted in 50 % or greater inhibition. Full recovery was observed in the 4.7 and 2.5 µg/l concentrations, but plants exposed to 11 µg/l did not recover after 14 days in fresh, untreated media.

The Scown 2014 paper provides the calculation of 7-day EC_{50} and NOEC values from the raw data available from the exposure study on the active substance with *Lemna gibba*. The 7-day EC_{50} and NOEC values from the raw data available from the exposure study on the active substance with *Lemna gibba* were calculated for use in environmental risk assessments for aquatic macrophytes. The EC_{50} and NOEC based on 0-7 day growth rate were 4.73 µg a.s./L and 1.02 µg a.s./L, respectively.

The study is relevant and acceptable. The RMS found the study to be valid based on criteria of OECD 221 (2006) and agrees with the conclusions made by the applicant.

The following comments were made in the DAR 2004: The nutrient medium was not renewed during the test, however, judged from the analyses it seems that the test concentration remained within acceptable levels throughout the study. The light intensity was lower than the guideline recommendations, but the validity criterion of maximum doubling time was met with regard to the exposure during 0-7 days, for which the NOEC and EC_{50} values were derived.

Reference:	**DUP: Kirkwood 2015** Tribenuron-methyl (DPX-L5300) technical - growth inhibition of the aquatic macrophyte *Myriophyllum spicatum*
Company Report No.:	DuPont-32239, Revision No. 2
Guideline:	None
GLP:	Yes
Material and methods:	
Test material:	Tribenuron-methyl technical
Lot/Batch #:	L5300-271
Purity:	99.3%
Control:	Well water fortified for hardness
Test carrier:	Well water fortified for hardness
Toxic reference:	None
Test organism:	Aquatic macrophyte
Species:	*Myriophyllum spicatum*
Initial population:	3 plants per replicate
Source:	Bayer Crop Science
Growth medium:	Well water fortified for hardness
Test chamber:	4-liter beakers with 3 cm sediment
Treatments:	Five replicates with 3 plants per replicate were initiated for each test substance concentration and the dilution water (freshwater reconstituted for hardness) control. Each 4-liter test chamber (replicate) contained one pot with 3 cm of sediment, planted with 3 plants with shoot lengths of approximately 7 cm each. Nominal exposure concentrations of tribenuron-methyl were 0.0010, 0.010, 0.10, 1.0, 10, and 100 mg a.s./L. A single test vessel containing no M. spicatum or sediment was initiated at the highest test concentration for the abiotic control.
Exposure:	14d static
Environmental conditions (in-life period):	
Temperature:	23 to 26°C
Photoperiod:	16-hour light/8 hour dark (4400 to 7300 lux; PAR: 50-93 µE * m^{-2} * s^{-1})
pH:	7.9 to 10 throughout the exposure period
Observations	Individual shoots from each replicate were cut at the sediment surface and placed into a pre-weighed aluminium pan. Shoots were then dried in an oven at approximately 70°C for a minimum of three days and individual shoot dry weights were determined using an analytical balance.
Statistics	The EC_{50} value is defined as the concentration of test substance which caused a 50% reduction in mean shoot dry weight compared to the control data, determined by linear interpolation of response using the statistical program CETIS (Ives, 2011). If <50% response was observed in the highest concentration tested, the EC_{50} was empirically estimated to be greater than the highest concentration tested

Results:

Measured concentrations 90-97% of nominal, with exemption of lowest tested concentration where measured was 220% of nominal.

At test termination, no plant mortality was observed in the control or any of treatment levels. Approximately twenty five percent of plant biomass was observed to be necrotic in 3, 6, and 9 plants in the 0.090, 8.9, and 94 mg a.s./L treatment levels, respectively. Approximately fifty percent of plant biomass was observed to be necrotic in 1, 3, and 3 plants at the 0.87, 8.9, and 94 mg a.s./L treatment levels, respectively. Approximately seventy-five percent of plant biomass was observed to be necrotic in 1 plant in the 94 mg a.s./L treatment level. Apical bud damage was observed among all 15 plants exposed to the 0.0022, 0.0097, 0.090, 0.87, 8.9, and 94 mg a.s./L treatment. Apical bud damage was observed among all 15 plants exposed to the 0.0022, 0.0097, 0.090, 0.87, 8.9 and 94 mg/L treatment levels and control. Apical bud damage refers to any effect observed on the apical bud portion of a plant. Apical bud damage was observed at 7 and 14 days exposure.

The 14 day EC_{50} value for dry shoot weight was empirically estimated to be >94 mg a.s./L, the highest concentration tested.

On request by the RMS, the applicant provided the following additional information. The statistical methods used are described in the beginning of chapter 9.2.7.

The NOEC for growth rate was statistically calculated = 94 mg/L. No useful model could be fit to the data to allow calculation of ECx endpoints for growth rate.

NOEC necrosis = 0.87 mg/L

NOEC growth rate = 94 mg/L

ErC_{10} = 3.5111 mg/L 95% C.I. (1.547-6.662)

ErC_{20} = 9.6558 mg/L 95% C.I. (4.318-18.452)

ErC_{50} >94 mg/L

If apical bud damage is considered an adverse effect, then the NOEC is <0.0022 mg/L.

This study was conducted before OECD 239 (2014) was in place. The RMS evaluated the validity of this study using the criteria set in line with OECD 239: Slight deviations are found in the used test medium (EPA hard water versus Smart Bako medium), irradiation (50-93 versus 140 (± 20) µE * m^{-2} * s^{-1}) and temperature (23-26°C versus 20°C). However, since the doubling time and coefficient of variance are within the validity limits set in the guidance, the study is valid.

Reference:	**TTF: Gonsior, G. 2015.** Tribenuron-Methyl: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System.
Company Report No.:	Report No. 297 TBM amdt-1 Project no.: S14-00454
Guideline:	OECD 239 (2014)
GLP:	Yes

Material and methods:

Test material:	Tribenuron-methyl	
	Batch:	63B3011159
	Purity:	98.23 % w/w (analysed)

	Description:	Solid, light brown
Test organism:	*Myriophyllum spicatum*	
Source:	Originally cultured by the Landesanstalt für Gewässerkunde Koblenz, Germany	
Treatment:	0, 0.298, 0.954, 3.05, 9.77, 31.3 and 100 µg test item/L	
Test units:		
Test vessels:	2 L glass beakers filled with 350 g moist test sediment and 1.5 L test medium	
Test sediment:	Artificial soil according to OECD guideline 219, containing 4 % sphagnum peat, 20 % kaolin clay, 75-76 % quartz sand, 0.2 % CaCO3, 2 % organic carbon content and 100 mg ammonium chloride and sodium phosphate per kg dry sediment	
Test medium:	Smart and Barko growth medium	
Environmental conditions:		
	Temperature:	20.1 – 21.9 °C
	pH:	mean at test start: 8.18 and mean at test end: 8.98
	Oxygen content:	mean 108 – 145 %
		Photoperiod: 16 hours light: 8 hours darkness (9600 - 11000 lux)

Results:

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.

Under the experimental conditions, the 14-day E_rC_{50} and E_yC_{50} based on shoot length were 6.43 and 3.59 µg test item/L, respectively. For shoot fresh weight, the 14-day E_rC_{50} and E_yC_{50} were calculated to be 6.48 and 2.69 µg test item/L, respectively. The 14-day E_rC_{50} and E_yC_{50} for dry weight were > 100 and 15.4 µg test item/L, respectively. The NOEC for shoot length and fresh weight was determined to be 0.954 µg test item/L and for dry weight the NOEC was 3.05 µg test item/L

The validity criteria of OECD 239 (2014) are met. The study is relevant and acceptable.
Observations of sublethal effects (such as necrosis, chlorosis or damage) are insufficiently reported, but apparently no sublethal effects were observed other than reduced growths.

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

No data available

11.6 Long-term aquatic hazard

Table 66: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results [mg/L]	Remarks	Reference
OECD 204 21d (flow-through)	*Oncorhynchus mykiss* Rainbow trout	Tribenuron-methyl	Length, weight survival, NOEC = 560 (mean measured)		DUP, Hutton, D.G., 1989, HLR 311-89
OECD 210 28d (flow-through)	*Cyprinodon variegatus* Sheepshead	Tribenuron-methyl	Larval length weight, survival, NOEC = 11.9 (mean measured)	Key study	DUP, Rebstock, M., 2012, DuPont-33943

Early lifestage (ELS)	minnow				
OECD 202 21d (static-renewal)	*Daphnia magna* Water flea	Tribenuron-methyl	Adult length, NOEC = 120 Reproduction, EC_{10} = 52 (mean measured)		DUP, Hutton, D.G., 1989 Hoke, R.A. 2013 HLR 164-89, Revision No. 1
OECD 211 21d (static-renewal)	*Daphnia magna* Water flea	Tribenuron-methyl	Adult length, NOEC = 114 (nominal)		DUP, Rebstock, M., 2013, DuPont-35848 Revision No. 1
OECD 211 21d (static-renewal)	*Daphnia magna* Water flea	Tribenuron-methyl	Mortality, reproduction, NOEC = 41 (nominal)	Key study	TTF, Zawadsky, C., 2015 329 TBM
OECD 201 72h (static)	*Pseudokirchneriella subcapitata* Green algae	Tribenuron-methyl	Growth rate, NOEC = 0.004 ErC_{10} = 0.011 (nominal)	Key study	DUP, Sloman, T.L., Leva, S.E., 1998, DuPont-1222
OECD 201 72h (static)	*Anabena flos-aquae* Cyanobacteria	Tribenuron-methyl	Growth rate, NOEC = 10 ErC_{10} = 13.2 (nominal)		TTF: Hermes, H., Sonntag, F. (2016) Ibacon Project 118631218
US EPA guideline 123-2 14d (static)	*Lemna gibba* Duck weed	Tribenuron-methyl	Growth rate, NOErC = 0.001 ErC_{10} = 0.0024 (nominal)		DUP, Kannuck, R.M., Samel, A., 2000, AMR 3070-94, Revision No. 1 Scown, T., et al 2014 DuPont-41634
14d (static)	*Myriophyllum spicatum*	Tribenuron-methyl	Growth rate, NOEC = 94 Necrosis, NOEC = 0.87 ErC_{10} = 3.5 Apical bud damage, NOEC = <0.0022 (mean measured)		DUP, Kirkwood, A., 2015 DuPont-32239, Revision No. 2
OECD 239 14d (static)	*Myriophyllum spicatum*	Tribenuron-methyl	Growth rate (fresh weight), NOEC = 0.00095 ErC_{10} = 0.00081 (nominal)	Key study	TTF, Gonsior , G., 2015, 297 TBM amdt-1
14d (static)	*Elodea canadensis*	Tribenuron-methyl	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

11.6.1 Chronic toxicity to fish

Tribenuron-methyl shows a low chronic toxicity to fish. The NOEC of **11.9 mg/L** determined in the early life stage test with sheepshead minnow is chosen for the chronic toxicity of the substance.

Reference:	**DUP: Hutton, 1989a** Flow-through 21-day LC50 and NOEC of DPX-L5300-20 to rainbow trout (*Salmo gairdneri*)
Company Report No.:	HLR 311-89
Guideline:	OECD Guideline 204 (1984)
GLP:	Yes
Material and methods:	
Test material:	Tribenuron methyl technical

Lot/Batch #:	L5300-20
Purity:	95.8%
Test system/test conditions:	The chronic effect of tribenuron-methyl on rainbow trout was examined in a 21-day study under flow-through conditions. The mean length of the fish was 2.7 cm and the mean wet weight was 0.41 g. pH of the stock solution was elevated by NaOH to promote the dissolution of the test substance. Ten fish per test concentration (two replicates of 5 fish each) were exposed to the following nominal test concentrations: 31, 62, 125, 250 and 500 mg/l, along with a dilution water control. The water temperature during the test was 10 - 14°C, the pH was 6.3-7.8, and the O_2-content was 9.8-10.8 mg/l. Hardness and alkalinity was 76 and 84 mg/l, respectively. The flow velocity was six aquaria turnovers (7 litres) per 24 hours.

Results: Recovery was in the range of 96 to 112 % of nominal concentrations. No mortality occurred during the course of the study. A few fish darkened in colour, but recovered before study termination. All dose groups had a significantly shorter body length than the control. There was no dose-trend in the effects on body length, and the finding was probably due to the fact that the fish in one of the control groups by chance were relatively large compared to the other fish. The 21 days EC_{50} and LC_{50} were above 560 mg/l, and NOEC was set to 560 mg/l. The values are based on measured concentrations.

The study was conducted under guideline OECD 204 (1984). The validity criteria of OECD 204 (1984) are fulfilled. A review of this study indicates that it partially meets the current guideline; deviations include that the oxygen concentration dropped below 60% in all of the test solutions and the control at some point during the study. However, reconducting the study is unlikely to yield a significantly different result. The study is considered valid
The temperature is lower and variation exceeds the recommendation in the guideline (test: 10.1 – 13.8 °C; Guideline: 13-17°C with maximum ± 2°C variation). However, it is not likely that this deviation substantially influenced the results. All dose groups had a significantly shorter body length than the control. However, there was no dose-trend in the effects on body length, and the finding was most likely due to the fact that the fish **in only one of the 2** control groups by chance were relatively large compared to the other fish. Therefore, the observed decrease in length was likely not a result of the exposure.

Reference:	**DUP: Rebstock 2012** Tribenuron-methyl (DPX-L5300) technical: Early life-stage toxicity test with the sheepshead minnow, *Cyprinodon variegatus*, under flow-through conditions
Company Report No.:	DuPont-33943
Guideline:	OECD 210 (1992), U.S. EPA 850.1400 (1996)
GLP:	Yes

Material and methods:

Test material:	Tribenuron-methyl technical
Lot/Batch #:	L5300-281
Purity:	98.2%
Control:	Dilution (laboratory blended water) water
Solvent control:	None
Test vehicle:	None
Toxic reference:	None
Test organism:	Sheepshead Minnow
Species:	*Cyprinodon variegatus*

Age at dosing:	<24 hours
Initial population:	20 embryos per test chamber
Source:	In-house culture
Diet:	Brine shrimp nauplii and/or salmon starter at least twice daily except 24 hours prior to termination
Test chamber:	Glass aquaria measuring approximately 15 cm wide by 22 cm long by 24 cm high with a test solution depth of 14 cm
Experimental treatments	- 28d post-hatch - The fish were fed during treatment. - unaerated, - flow-through, - nominal concentrations of 0 (control), 0.63, 1.3, 2.5, 5.0, and 10 mg a.s./L. 20 embryos were used per replicate with four replicates per test concentration and control
Environmental conditions (in-life period)	
Temperature:	21.1 to 24.9°C for fry
Photoperiod:	16 hr photoperiod (646 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
Endpoints and observations	egg hatchability, post-hatch survival, standard length, and blotted wet weight
Statistics	The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration for egg hatchability, fish survival (28-day post-hatch), standard length, and blotted wet weight, were estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test, with the alternate hypothesis being the mean for the parameter was reduced in comparison to the control mean. Prior to the Dunnett's test, a Shapiro Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. The results from the Shapiro-Wilk's and Levene's tests indicated non-normality and heterogeneity of variance for percent hatch and post-hatch survival. Therefore, these parameters were analysed with non-parametric analyses on the ranks of the values. The results from the Shapiro-Wilk's and Levene's tests indicated normality and homogeneity of variance for standard length and blotted wet weight. Therefore, these parameters were analysed with a parametric ANOVA and Dunnett's test on the non-transformed data.

Results:

The mean measured concentrations of tribenuron-methyl in the control and test substance treatments during the study were <LOD (control), 0.754, 1.46, 2.99, 6.03, and 11.9 mg a.s./L, which represented 112 to 121% of the nominal concentrations. No residues of tribenuron-methyl were detected in the control above the LOD of 0.036 mg a.s./L. All test acceptability criteria were met with the exception of the 25 ± 2°C temperature range. The temperature was below the protocol-specified range in the control and test treatment replicates at initiation, but remained within specifications for the remainder of the test. This deviation did not impact the overall quality and results of the study.

Based on mean measured concentrations of tribenuron-methyl , the NOEC values for egg hatchability, post-hatch survival, standard length, and blotted wet weight was 11.9 mg a.s./L, the highest mean measured concentration tested.

11.6.2 Chronic toxicity to aquatic invertebrates

Tribenuron-methyl shows a low chronic toxicity to aquatic invertebrates. The lowest value is the NOEC of **41 mg/L**.

Reference:	**DUP: Hutton 1989b** Chronic toxicity of IN-L5300-20 to *Daphnia magna* **DUP: Hoke 2013** Chronic toxicity of IN L5300-20 to *Daphnia magna*
Company Report No.:	164-89, Revision No. 1
Guideline:	OECD 202 (1984)
GLP:	Yes
Material and methods:	
Test material:	Tribenuron-methyl
Lot/Batch No:	IN-L5300-20 (15, 527)
Purity:	95.8% (w/w)
Test system/test conditions:	The chronic toxicity of tribenuron-methyl (based on immobilisation, growth and reproduction) to *Daphnia magna* was evaluated in a static renewal test over an exposure period of 21 days. Forty daphnids (ten replicates of 4 daphnids each) per test concentration were exposed to nominal concentrations of 31, 62, 125, 250, 500, and 1000 mg/l, in addition to a dilution water control. The water temperature during the test was 20°C, the pH was 7.1-8.8, and the O2-content was 4.7-8.7 mg/l. Hardness and alkalinity was 75±2 and 82±2 mg/l, respectively.

Results:

Measured concentrations remained between 92 and 116 % of nominal. Results are presented based on average measured values.

Only in the highest dose group the survival was significantly lower than in the control. Hence, the NOEC for survival was 480 mg/l, and the LOEC was 940 mg/l. The 21-day EC_{50} for immobilisation was > 940 mg/l. Growth was the most sensitive parameter, as concentration levels of 250 mg/l and higher produced significantly shorter average adult lengths than the control group. Thus, the NOEC for growth was 120 mg/l and the LOEC was 250 mg/l. All results were based on measured concentrations.

The RMS requested a closer look at the reproduction data. The results are:

ECx/NOEC	Endpoint (mg/L)	95% Confidence interval (mg/L)
EC_{10} =	51.6	(33.0 - 70.3)
EC_{20} =	109.4	(70 - 148.8)
EC_{50} =	339.8	(217.3 - 462.3)
NOEC=	250	-

The study fulfils the validity criteria stated in OECD 211 (2012).

The oxygen concentration is below 60% saturation at some points in all test solutions including control, but not dose related. However, the requirement of OECD 211 (Daphnia reproduction) is met, i.e. the oxygen concentration is always above 3 mg/L.

Reference:	**DUP: Rebstock 2013** Tribenuron-methyl (DPX-L5300) technical: Chronic toxicity to the cladoceran, *Daphnia magna*, determined under statistical renewal test conditions
Company Report No.:	DuPont-35848, Revision No. 1

Guideline:	OECD 211 (2008),
GLP:	Yes

Material and methods:

Test material:	Tribenuron-methyl technical
Lot/Batch #:	L5300-281
Purity:	98.2%
Control:	Dilution water (laboratory blended fresh water)
Solvent control:	NA
Test vehicle:	Dilution water (laboratory blended fresh water)
Toxic reference:	None
Test organism:	Cladoceran
Species:	*Daphnia magna*
Age at dosing:	Neonates (<24 hours old)
Initial population:	10 daphnids per treatment
Source:	In-house culture
Diet:	Test period: algal suspension (Pseudokirchneriella subcapitata) plus a prepared invertebrate food (YCT)
Test chamber:	Glass jars measuring approximately 6.5 cm high with a diameter of 5.0 cm and a solution depth of 3.5 cm
Experimental treatments	The chronic toxicity of tribenuron-methyl to *Daphnia magna* was determined in a static-renewal, 21 day test. Treatments consisted of a dilution water control and five nominal concentrations of 7.5, 15, 30, 60, and 120 mg a.s./L. Ten daphnids were used per treatment.
Environmental conditions (in-life period)	
Temperature:	19.4 to 20.3°C (of replicate test chambers)
Photoperiod:	16 hr photoperiod (588 lux) and 8 hr darkness which included 30 min transitional light preceding and following the 16-hr light interval
Dissolved oxygen:	6.4 to 8.9 mg/L
pH:	7.7 to 8.6
Endpoints and observations	number of surviving adult daphnids, occurrence of abnormalities, and production of neonates
Statistics	The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) for mortality/immobility data were determined by using a one-tailed Dunnett's test and a Fisher's exact test with the alternate hypothesis being that the mean for the parameter was reduced in comparison to the control mean. A Hochberg adjustment was used to control the experiment-wise error rate for the Fisher's test at the same level ($p = 0.05$). Reproduction, length and weight data were analysed with a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test with the alternate hypothesis being that the mean for the parameter was reduced in comparison to the control mean. Prior to the Dunnett's test, a Shapiro Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. The results from the Shapiro-Wilk's and Levene's tests indicated the assumptions of normality and homogeneity of variance were not met for the survival and time to first brood data. Therefore, these parameters were analysed with a non parametric ANOVA and Dunnett's test on the ranks of the values. Assumptions of normality and homogeneity of variance were met for reproduction and length data. These parameters were analysed with a parametric ANOVA and Dunnett's test on the untransformed data. Estimates of the EC50 concentration values and their 95% confidence limits for immobilisation data were calculated using the probit method and Trimmed Spearman-Karber method. When the p value for Goodness of Fit was >0.05 and there was no other evidence of questionable convergence, the probit method was selected for reporting. When this criterion was not achieved, the Trimmed Spearman-Karber method was selected for reporting.

Results:

The mean, measured concentrations of tribenuron-methyl during the 21-day exposure were 7.94, 15.4, 29.7, 57.3, and 114 mg a.s./L or 95 to 106% of nominal. No residues of tribenuron-methyl were detected in the dilution water control solution above the LOD of 0.0386 mg a.s./L, with the exception of the Day 21 sample in which a single replicate of the 7.5 mg a.s./L treatment was erroneously composited with the control replicates. The relevant test acceptability criteria were met for this study. The water-quality characteristics remained within the tolerance limits set forth in the protocol. Survival of the dilution water control daphnids was 80%. The average number of live young per surviving first generation daphnid was 181 and no ephippia were produced by control animals.

Conclusion:

Based on survival and mean measured concentrations, the 21-day NOEC and LOEC were 114 and >114 mg a.s./L, respectively. MATC values could not be calculated. The 7-, 14-, and 21-day EC_{50} value, based on immobilisation of the first generation daphnids, was >114 mg a.s./L, the highest concentration tested.

Based on days to first brood, number of young produced, and mean measured concentrations, the NOEC and LOEC values were 114 and >114 mg a.s./L, respectively.

Based on adult length and mean measured concentrations, the NOEC and LOEC values were 114 and >114 mg a.s./L, respectively.

The study fulfils the validity criteria stated in OECD 211 (2012).

Reference:	**TTF: Zawadsky, C., 2015** Tribenuron-Methyl TC: Toxicity to the water Flea *Daphnia magna* Straus under Laboratory Conditions (Reproduction Test)
Company Report No.:	329 TBM
Guideline:	OECD 211 (2012),
GLP:	Yes

Material and methods:	
Test material:	Tribenuron-methyl TC
Batch no.:	63B3011159
Purity:	98.23 % w/w
Description:	Light brown solid
Test organism:	*Daphnia magna* STRAUS, Clone V
Age:	< 24 hours, at test initiation
Source:	In-house culture, originally purchased from the Federal Environmental Agency in Berlin, Germany
Diet:	Single cell green algae (*Desmodesmus subspicatus*), provided daily
Treatment:	0, 1.05, 2.63, 6.56, 16.4 and 41.0 mg test item/L, semi-static conditions, with renewal of test solutions every Monday, Wednesday and Friday.
Test medium:	Elendt M4
pH:	7.74 – 8.01
Dissolved oxygen:	8.4 – 8.8 mg/L
Hardness:	232 – 250 mg CaCO3/L
Test vessels:	100 mL Glass beakers, containing 50 mL test medium
Environmental conditions:	
Temperature:	19.1 – 21.8 °C
pH:	7.35 – 8.46
Dissolved oxygen:	6.9 – 10.8 mg/L
Photoperiod:	16 hours light: 8 hours darkness
Light intensity:	1250 lux

Observations: Mortality, presence of eggs in the brood pouch, males or winter eggs, obvious differences in condition and size of the adult daphnids.
Number of offspring, body length of the adult daphnids.

Statistics: For determination of the LOEC and NOEC the data was first tested for normality and homogeneity of variance using Shapiro-Wilk test and Bartlett's test, respectively. Thereafter, the Dunnett's t-test was performed to compare the treatment groups with the control. All data was analysed using SAS Proprietary Software Version 9.3.

Results:

In the fresh medium the measured concentrations were between 100 and 111 % of the nominal test concentrations. The aged medium showed measured concentrations from 97 to 121 % of nominal test concentrations. Therefore, all results were based on nominal concentrations.

During the test no unusual observations were made for the control or any of the test item concentrations. In the control group and in the group exposed to the highest test item concentration of 41.0 mg/L two daphnids died. In the other test groups, one dead daphnid was observed.

The mean number of live offspring produced per adult in the control group was 107.4. There was no statistically significant difference in the number of offspring per adult between any of the test item exposed groups and the control

The 21-day NOEC for tribenuron-methyl was 41.0 mg/L and the LOEC was >41.0 mg/L.

The study fulfils the validity criteria stated in OECD 211 (2012).

11.6.3 Chronic toxicity to algae or other aquatic plants

Several studies are available. Both algae and aquatic plants are sensitive to tribenuron-methyl, which is expected for a herbicide. Higher plants are more sensitive than algae. The lowest EC_{10} is **0.00081 mg/L** derived with *Myriophyllum spicatum*.

The study summaries are found in chapter 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

No data available

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Adequate acute toxicity data are available for all taxonomic levels (fish, crustacea and algae or other aquatic plants).

The 14d ErC_{50} for *Lemna gibba* of 0.0047 mg/L is lower than the classification criterion for Category Acute 1: ≤ 1 mg/l. The appropriate M-factor is 100, since the toxicity is within the range $0.001 < L(E)C_{50} \leq 0.01$.

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

Adequate chronic toxicity data are available for all taxonomic levels (fish, crustacea and algae or other aquatic plants).

Tribenuron-methyl is not rapidly degradable in the aquatic environment.

The 14d EC_{10} for *Myriophyllum spicatum* of 0.00081 mg/L is lower than the classification criterion for Category Chronic 1: ≤ 0.1 mg/l. The appropriate M-factor is 100, since the toxicity is within the range of $0.0001 < EC_{10} \leq 0.001$.

Tribenuron-methyl has a low potential for bioaccumulation (log Kow≤4).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Tribenuron-methyl shall be classified as

(a) Acute (short-term) aquatic hazard

Category Acute 1. M-factor = 100

(b) Long-term aquatic hazard

Category Chronic 1. M-factor = 100

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 EC10 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.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No data available

13 ADDITIONAL LABELLING

Not relevant

14 REFERENCES

Reference lists are included in Annex I – IV.

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.

15 ANNEXES

Further details, including study summaries of the referred data are provided in Annex I – IV to this CLH-report:

Annex I Physical and Chemical properties

Annex II Toxicology and metabolism

Annex III Environmental Fate and Behaviour

Annex IV Ecotoxicology