European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

Clethodim (ISO); (5*RS*)-2-{(1*EZ*)-1-[(2*E*)-3-chloroallyloxyimino] propyl}-5-[(2*RS*)-2-ethylthio)propyl]-3 hydroxycyclohex-2-en-1-one

Volume 1

Rapporteur Member State: Sweden
Co-Rapporteur Member State: Lithuania

Versions History

When	What
2023/08	Initial RAR

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report includes the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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LEVEL 1

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THE RENEWAL ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the renewal assessment report was prepared

Clethodim is an active substance currently approved until the 31st of May 2023 under Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

This dossier is submitted to support the renewal of the approval of clethodim under Regulation (EC) 1107/2009. The submission is made in accordance with Commission Regulation (EU) No 844/2012 of 18 September 2012, setting out the provisions necessary for the implementation of the renewal procedure for active substances.

This Volume follows the combined RAR/CLH template according to SANCO/12592/2012. rev 1.1, October 2017. Thus, this document also serves as a proposal for classification under Regulation (EC) No 1272/2008.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

Sweden, acting as the rapporteur member state (RMS) evaluated all aspects of the application and the supplementary dossier, in accordance with the procedures specified in Commission Implementing Regulation (EU) No. 844/2012 of 18 September 2012.

Lithuania, acting as the Co-RMS, agreed to review the RAR before the submission to EFSA and the Commission.

1.1.3 EU Regulatory history for use in Plant Protection Products

In the EU-regulatory context, Clethodim was first evaluated within the programme for review of existing active substances provided for in Article 8(2) of EU Council Directive 91/414/EEC. Following the Commission Decision of 5 December 2008 (2008/934/EC) concerning the non-inclusion of clethodim in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance, the applicant Arysta LifeScience S.A.S. made a resubmission application for the inclusion of clethodim in Annex I in accordance with the provisions laid down in Chapter III of Commission Regulation (EC) No. 33/2008. The resubmission dossier included further data in response to the issues identified in the DAR. In accordance with Article 18 of Commission Regulation (EC) No. 33/2008, The Netherlands, being the designated RMS, submitted an

evaluation of the additional data in the format of an Additional Report. The Additional Report was received by the EFSA on 1 December 2009. Addenda were also produced for some of the sections of the DAR in 2010.

To support the discussions that preceded the Annex I inclusion, EFSA was given mandate to perform a peer-review and the authority delivered its final conclusion on the 21st of October 2011 (EFSA Journal 2011;9(10):2417). The Commission then presented a Review Report (SANCO/13456/2010 final). There was a request for confirmatory data on the i) soil and groundwater exposure assessments and ii) the residue definition for risk assessment to be submitted to the Commission by the 31st of May 2013. The Review report was then updated in 2015, after evaluation of confirmatory data.

Clethodim was included in Annex I of EU Council Directive 91/414/EEC on 2 March 2011 and was subsequently approved under Regulation (EC) No. 1107/2009 (repealing Council Directive 91/414/EEC) via Commission Implementing Regulation (EU) No. 540/2011 of 25th May 2011. The current expiry date for this approval is 31/05/2023.

The existing EU MRLs for clethodim are specified in Regulation (EC) No 839/2008. EFSA has published a Reasoned opinion on the review of the existing maximum residue levels (MRLs) for Clethodim according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2019;17(5): 5706). However, the EFSA opinion concluded that a decision on the residue definition for risk assessment could not be made, and new residue definitions and new MRLs have not been established.

1.1.4 Evaluations carried out under other regulatory contexts

The RMS is not aware of any EU-evaluations of Clethodim carried out in the framework of other relevant EU-legislation (e.g. biocides, flavourings, food additives, cosmetics).

Clethodim was included in the Inventory of Evaluations performed by the Joint Meeting on Pesticide Residues (JMPR) http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/lpe/lpe-c/en/

No information has been provided by the applicant on whether Clethodim has been evaluated or registered in any country outside the EU and UK.

1.2 APPLICANT(S) INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

Name: Arysta LifeScience S.A.S.,

Address: Route d'Artix, BP 80, 64150 Noguères, France

Contact: [redacted]

Telephone number: [redacted]

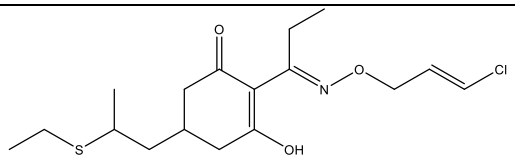
1.2.2 Producer or producers of the active substance

Confidential information provided in Volume 4.

1.2.3 Information relating to the collective provision of dossiers

The RMS received an application for renewal of the approval of Clethodim only from Arysta LifeScience SAS., the main data holder for the dossier supporting the current approval. Besides Arysta LifeScience SAS, there were two other applications submitted for the renewal of approval of clethodim, but they were not followed-up by provision of dossiers.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Clethodim
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	(5*RS*)-2-{(1*EZ*)-1-[(2*E*)-3-chloroallyloxyimino] propyl}-5-[(2*RS*)-2-ethylthio)propyl]-3 hydroxycyclohex-2-en-1-one
CA	2-[1-[[[(2*E*)-3-chloro-2-propen-1-yl]oxy]imino]propyl] -5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one
1.3.3 Producer's development code number	RE 45601 RE-45601
1.3.4 CAS, EEC and CIPAC numbers	
CAS	99129-21-2
EEC	Not assigned[1]
CIPAC	508
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{17}H_{26}ClNO_3S$
Structural formula	
Molecular mass	359.92 g/mol

1.3.6 Method of manufacture (synthesis pathway) of the active substance	Confidential information available in Volume 4.
1.3.7 Specification of purity of the active substance in g/kg	Min 930 g/kg (Commission Implementing Regulation (EU) No 87/2012).
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	Confidential information available in Volume 4.
1.3.8.2 Significant impurities	Confidential information available in Volume 4.
1.3.8.3 Relevant impurities	Toluene max 4 g/kg.
1.3.9 Analytical profile of batches	Confidential information available in Volume 4.

[1]The applicant provided a list number in the dossier that has not been included in the RAR.

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

<table>
<tr><td>1.4.1 Applicant</td><td>Arysta LifeScience S.A.S</td></tr>
<tr><td>1.4.2 Producer of the plant protection product</td><td>Confidential information available in Volume 4.</td></tr>
<tr><td>1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product</td><td>Trade name: Clethodim 120 EC

Code number: H1231bc</td></tr>
<tr><td colspan="2">1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product</td></tr>
<tr><td>1.4.4.1 Composition of the plant protection product</td><td>Pure active substance
<table><tr><th>content</th><th>120 g/L</th><th>13.0 % w/w</th></tr><tr><td>limits</td><td>112.8 – 127.2 g/L</td><td>12.22 – 13.78% w/w</td></tr></table>
Technical active substance
<table><tr><th>content</th><th>125 g/L</th><th>13.5 % w/w</th></tr><tr><td>limits</td><td>117.5 – 132.5 g/L</td><td>12.69 – 14.31 % w/w</td></tr></table>At a minimum purity of the technical active substance of 96 %.</td></tr>
<tr><td>1.4.4.2 Information on the active substances</td><td>ISO common name: Clethodim
CAS: 99129-21-2
EC: Not assigned
CIPAC: 508</td></tr>
<tr><td>1.4.4.3 Information on safeners, synergists and co-formulants</td><td>Confidential information available in Volume 4.</td></tr>
<tr><td>1.4.5 Type and code of the plant protection product</td><td>Emulsifiable Concentrate [Code : EC]</td></tr>
</table>

1.4.6	**Function**	Herbicide
1.4.7	**Field of use envisaged**	Crops (sugar beet, onion, garlic)
1.4.8	**Effects on harmful organisms**	Systemic, selective herbicide (graminicide) for the post-emergence control of annual and perennial grass weeds.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc a.s. g/L (i)	method kind (f-h)	Range of growth stages & season (j)	Number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Sugar beet (*Beta vulgaris* subsp. vulgaris var. altissima) (BEAVA)	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Annuals grasses	EC	120	Spraying / Overall	BBCH 12-33	1	N/A	0.03-0.06	200-400	0.12	BBCH33	
Sugar beet (*Beta vulgaris* subsp. vulgaris var. altissima) (BEAVA)	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Perennial grasses	EC	120	Spraying / Overall	BBCH 12-33	1	N/A	0.075-0.15	200-400	0.3	BBCH33	
Onions (*Allium cepa*) (ALLCE)	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Annuals grasses	EC	120	Spraying / Overall	BBCH 12-19	1	N/A	0.03-0.06	200-400	0.12	BBCH19	
Onions (*Allium cepa*) (ALLCE)	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Perennial grasses	EC	120	Spraying / Overall	BBCH 12-19	1	N/A	0.06-0.12	200-400	0.24	BBCH19	
Garlic (*Allium sativum*) (ALLSA)	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Annuals grasses	EC	120	Spraying / Overall	BBCH 12-19	1	N/A	0.03-0.06	200-400	0.12	BBCH19	
Garlic (*Allium sativum*) (ALLSA)	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Perennial grasses	EC	120	Spraying / Overall	BBCH 12-19	1	N/A	0.06-0.12	200-400	0.24	BBCH19	
Sugar beet (*Beta vulgaris* subsp. vulgaris var. altissima) (BEAVA	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Annuals grasses	EC	120	Spraying / Overall	BBCH 12-33	1 every 3 years	N/A	0.03-0.06	200-400	0.12 (every 3 years)	BBCH33	triennial application (one application every three years)

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc a.s. g/L (i)	method kind (f-h)	Range of growth stages & season (j)	Number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Sugar beet (*Beta vulgaris* subsp. vulgaris var. altissima) (BEAVA	N, C and S EU	Clethodim 120 EC (H1231bc)	F	Perennial grasses	EC	120	Spraying / Overall	BBCH 12-33	1 every 3 years	N/A	0.075-0.15	200-400	0.3 (every 3 years)	BBCH33	triennial application (one application every three years)

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) e.g. biting and sucking insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant-type of equipment used must be indicated

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

1.5.2 Further information on representative uses

Clethodim 120 EC (H1231bc) will be applied in a volume of 200 - 400 L water/ha giving a maximum concentration range of 0.75 – 1.5 g a.s./L in the diluted spray solution when used at the maximum proposed application rate of 2.5 L/ha. The method of application is by field crop sprayer.

The maximum proposed application rate of Clethodim 120 EC (H1231bc) to control annual grass weeds is 1.0 L/ha, equivalent to 120 g a.s./ha, whereas for perennial grass weeds the maximum application rate is 2.5 L/ha, equivalent to 300 g a.s./ha.

Maximum number of applications and their timings:

- One application at BBCH 12-33 for sugar beet.
- One application at BBCH 12-19 for onions and garlic.

Duration of protection afforded by each application: Clethodim 120 EC (H1231bc) is a post-emergence graminicide for control of weeds present at time of application.

No minimum time restriction or special cultivation is necessary before drilling or sowing succeeding or replacement crops.

Instructions for use are provided on the product labels included in Vol 3 CP, section 3.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Not applicable.

1.5.4 Overview on authorisations in EU Member States

Country	Reg. No.	Product tradename	Crops
Austria	-	-	A range of broad-leaved field crops including sugar beet, onions, potatoes, oilseed rape, dry peas, carrot and sunflower
Belgium	9334P/B	Select Prim	
Bulgaria	0348-PPP-4/25.07.2018	Select Super 120 EC	
Croatia	UP/I-320-20/01-01/266	Select Super	
Cyprus	3552	CENTURION 12 EC	
Czech Republic	4903-0	Select Super	
Denmark	-	-	
Estonia	0592/10.02.16	Centurion Plus	
Finland	3231	Select Plus	
France	9900115	Centurion R	
Germany	-	-	
Greece	70276	SELECT 12 EC	
Hungary	04.2/3077-2/2018	Select Super	
Ireland	PCS04948	Centurion Max	
Italy	15868	Centurion Pro	
Latvia	0509	Centurion Plus	
Lithuania	AS2-22H/2015	Centurion Plus	
Luxemburg	L01898-071	Select Prim	
Netherlands	14300	Centurion Plus	
Poland	R-75/2013	Select Super 120 EC	

Country	Reg. No.	Product tradename	Crops
Portugal	00911	Centurion Pro	
Romania	1817	Select Super	
Slovakia	14-11-1419	Centurion Plus	
Slovenia	-	-	
Spain	22.225	Centurion Plus	
Sweden	5293	Select Plus	
UK	MAPP 17911	Centurion Max	

LEVEL 2

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Summary of methodology proposed by the applicant for literature review, for all sections

A literature search was conducted by the applicant in November 2017 and updated in May 2020. The time window for the search was 2010 – 2020. A total of 41 bibliographic databases were searched (i.e., 18 from STN Toxicology Database Cluster and 23 from Dialog, see complete list in Vol 3 CA, B.9.11.1.2.3). Further details on the methodology and outcome of the literature search are presented in the respective parts of Vol 3.

2.1 IDENTITY

2.1.1 Summary of identity

The identity of clethodim is summarized in Level 1, section 1.3 above.

The minimum content of the active substance in technical clethodim is 930 g/kg and remains the same as for the previous approval. There are proposals for revision of the reference specification for impurities, and there is a proposal to consider an impurity as a relevant impurity, which was previously considered a significant impurity. It should be noted that further information is required in order to assess the toxicological, ecotoxicological and environmental relevance of the impurities (please refer to Volume 4 for further information).

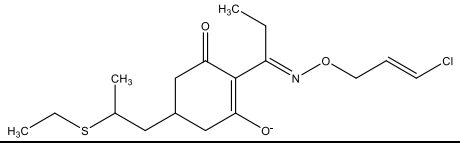
2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Table 1: Summary of physicochemical properties of the active substance.

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid. Amber Munsell Colour Designation: 7.5 YR 6/12 Liquid. Green/yellow Munsell Colour Designation: 2.5 GY 9/2	Ashworth, 1988 Lezberg, 2003a	Visual
Melting/freezing point	- 80 °C	Mak, 2003	Measured
Boiling point	Decomposition starts at 133°C at 100.52 kPa	Butler & O'Connor, 2009	Measured
Vapour pressure	2.68 x 10^{-5} Pa at 20°C 6.71 x 10^{-5} Pa at 25°C	Wöhr, 2022	Extrapolated from measured data
Surface tension	49.9 mN/m at 20.1 °C (90 % saturated solution)	Gould, 2019	Measured
Water solubility	Potassium biphtalate buffer at pH 4: 53.0 mg/L at 20 °C Monopotassium phosphate buffer at pH 7: 5.45 g/L at 20 °C pH adjusted to 10 using 1M NaOH: 30.0 g/L at 20 °C	Li and Baldwin, 2003 Weissenfeld, 2006	Measured Measured
Partition coefficient n-octanol/water	4.21 (estimated by the KOWWIN program – considered supportive by the RMS) Deionized water at pH 6.23 1.87 (at 19.2 °C) (considered supportive) Potassium dihydrogen phosphate buffer at pH 0.394 (at 18.4 °C) (considered supportive) At 20 °C: 3.3 (pH 5) 1.5 (pH 7) 0.908 (pH 9)	Beltran, 2005a Skopec, 2014 Sydney, 2021	Estimated Measured Measured
Partition coefficient n-octanol/water for metabolites	Log P_{OW}	Bendig and Paschke, 2020	Measured

	pH 4	pH 7	pH 9
Clethodim sulfoxide	1.6	0.4	< 0.3
	2.7	1.0	< 0.3
Clethodim sulfone	1.6	0.3	< 0.3

Property	Value				Reference	Comment (e.g. measured or estimated)
				< 0.3		
				< 0.3		
		2.6	0.9	< 0.3		
				< 0.3		
	Clethodim imine	2.8	2.8	2.8		
	Clethodim oxazole sulfoxide	0.5	0.5	0.6		
	Clethodim oxazole sulfone	0.5	0.5	0.5		
		0.6	0.6	0.6		
	Clethodim imine sulfoxide	0.5	0.5	0.4		
	M14R	< 0.3	< 0.3	< 0.3		
				< 0.3		
	M17R	< 0.3	< 0.3	< 0.3		
	M18R	< 0.3	< 0.3	< 0.3		
				< 0.3		
	Clethodim imine ketone	0.4	0.4	0.4		
	CBA ((2-[3-chloroallyloxyimino]-butanoic acid))	2.2	2.1	0.5		
	CAA (trans-3-chloroacrylic acid)	1.0	< 0.3	< 0.3		
				< 0.3		
	trans-3-chloropropenal	1.4	1.4	1.5		
	3-chloroallyl alcohol	0.7	0.6	0.6		
Henry's law constant	1.8 x 10^{-6} Pa m^3 mol^{-1} at 20°C and pH 7				Green, 2022	Calculated
Flash point	108.5 °C				Winkler, 2020	Measured
Flammability	Not applicable (i.e liquid at room temperature).					
Explosive properties	Mechanical Sensitivity: No explosion Thermal Sensitivity: No explosion				Franke, 2005	Measured and visual
Self-ignition temperature	Self-ignition temperature 280 °C				Lezberg, 2003b and Mak, 2004	Measured
Oxidising properties	Not oxidising				Kuchta, 2022b	Measured
Granulometry	Not applicable, clethodim is a liquid.					
Solubility in organic solvents and identity of relevant degradation products	Solubility at 25 °C (g/L), Acetone: >900 Hexane: >900 Ethyl acetate: >900 Dimethyl formamide: >900 Methanol: >100 1,2-Dichloroethane: >100 Xylene: >100 (93 %) At 20 ± 1 °C: Acetone: > 250 g/L Methanol: > 250 g/L 1,2 dichloroethane: > 250 g/L Ethyl acetate: > 250 g/L n-Heptane: > 250 g/L p-Xylene: > 250 g/L				Ashworth, 1988 Baldwin, 2003 Patel, 2019	Measured
Dissociation constant	pKa = 4.47 at 20 °C Species formed following dissociation: 				Ashworth, 1988	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
Viscosity	Dynamic viscosity 689.4 mm^2/s at 23.0 °C 127.9 mm^2/s a 40 °C Kinematic viscosity 768 cP at 21.5 °C	Skopec, 2014	Measured
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	Spectral data consistent with the structure of clethodim.	Möller, 2006 (UV/VIS) Bondarenko, 2010 (UV/VIS) Lezberg and Mahabir 2003 (IR and NMR) Reed, 2003 (MS)	Measured

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives

Table 2: Summary table of studies on explosive properties.

Method	Results	Remarks	Reference
UN Test C.1 (time/pressure test)	No (pressure rise to 2070 kPa not achieved)		Gledhill, 2022 (GLP3016011271R1/2022)
UN Test E.1 (Koenen tube test)	No (no effect in all tests – limiting diameter < 1 mm)	The test was scheduled for a QA audit, which was overseen by an operator. The missed audit does not have any impact on the test result but is a deviation from GLP.	Gledhill, 2022 (GLP3016011271R1/2022)
UN Test A.6 (UN detonation test)	No (average fragmentation length less than 1.5 times the fragmentation time of a reference (water))	Not GLP since the report stated that the test would be conducted in compliance with Commission Directive 2004/10/EC, and following Brexit this directive is no longer applicable in the UK. This has no impact on the test result.	Gledhill, 2022 (GLP3016011271R1/2022)
Structural argument	Clethodim contains nitrogen bonded to oxygen and waiving testing based on the structure is not possible.		
EEC A.2 (DSC Method)	Thermal decomposition onset at ~ 110 °C. Thermal decomposition energy 1078 J/g (measured with DSC)	DSC methodology not performed in accordance with recent WG APCP discussions. Low test material purity (93 %).	Franke, 2006 (20050645.01)
EEC A.14 OECD 113	Mechanical Sensitivity: No explosion Thermal Sensitivity: No explosion Thermal decomposition energy: 1089 J/g (measured with DSC)	DSC methodology not performed in accordance with recent WG APCP discussions (see below). The test material purity is slightly below the specification (92.4 %)	Franke, 2005 (20050374.01)
EEC A.2 (DSC Method)	Thermal decomposition onset at 133±0.5°C at 100.52 kPa. Thermal	DSC methodology not performed in accordance with recent WG APCP discussions.	Butler & O'Connor, 2009 (2699/0001)

Method	Results	Remarks	Reference
	decomposition energy 315 J/g.		
Calculation of oxygen balance	-195.6	Too high for waiving of testing (above -200).	

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

Clethodim was tested for mechanical and thermal explosivity and determined not to be explosive in accordance with test method EEC A.14/OECD 113. No explosion was observed in the mechanical or thermal test; however, it should be noted that there was evolution of smoke without ignition during the mechanical test. This test is however not sufficient for harmonized classification purposes according to the CLP guidance.

Two methodologies based on DSC are available in the dossier that have been used in separate studies to investigate the thermal decomposition (directly or indirectly when testing for boiling point) of clethodim technical (performed in accordance with EEC A.2 – DSC Method). The onset of thermal decomposition was below 500 °C in both cases. In the older studies (20050374.01 and 20050645.01), thermal decomposition onset was found at 110 °C and the thermal decomposition energy per gram was determined to 1089 and 1078 J/g; however, a very wide section of the thermogram was used to determine the integral (~80 - 340°C). Furthermore, the test material in this study was of low purity (93 %), and a heating rate of 3 K/min and closed glass crucibles were used in the study, which is not in alignment of requirements agreed on in recent WG APCP discussions. In the newer study (2699/0001), thermal decomposition onset was found at 133 °C. The average thermal decomposition energy per gram of two DSC runs was 315 J/g. The test material was of higher purity (98.5 %), but the intent of the study was to determine the boiling point and not parameters of the exothermic degradation – perforated aluminium crucibles and a heating rate of 20 K/min was used. Thus, it has not been acceptably demonstrated that the exothermic degradation energy is below 500 J/g. Furthermore, clethodim contains an N-O bond and the oxygen balance is above -200.

UN tests C.1, E.1 and A.6 were performed since performing the test series for self-reactive properties could not be waived based on the SADT (see 2.2.1.1.7) for further information. These tests were all negative, indicating no explosive properties.

2.2.1.1.1.2 Comparison with the CLP criteria

Test method EEC A.14 is not sufficient for classification purposes under the CLP Regulation. Clethodim contains an N-O bond, the oxygen balance is above -200 and it has not been demonstrated that the exothermic decomposition energy is below 500 J/g. In conclusion, none of the available waivers for not performing further testing to determine the explosive hazard in accordance with the CLP Regulation are met, and further testing needs to be carried out for explosive hazards.

UN tests C.1, E.1 and A.6 were submitted for testing the self-reactive properties of clethodim, and match the three tests in test series 2 of the UN MTC that should be used for classification purposes for explosive hazards in accordance with the decision tree in CLP annex I figure 2.1.2. The UN MTC specifies the UN gap test (A.5) and not the detonation test (A.6); however, section 2.8.3 (Relation to other physical hazards) of the CLP guidance states that “The explosive properties do not have to be determined according to the CLP Annex I, Chapter 2.1, because

explosive properties are incorporated in the decision logic for self-reactive substances and mixtures.". The test series/decision logic for self-reactive properties did not indicate any explosive properties for clethodim.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

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

2.2.1.1.2 Flammable gases (including chemically instable gases)

Table 3: Summary table of studies on flammable gases (including chemically unstable gases).

Method	Results	Remarks	Reference
Not applicable, clethodim is not a liquid.			

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not relevant.

2.2.1.1.2.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable.

2.2.1.1.3 Oxidising gases

Table 4: Summary table of studies on oxidising gases.

Method	Results	Remarks	Reference
Not applicable, clethodim is not a gas.			

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant.

2.2.1.1.3.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable.

2.2.1.1.4 Gases under pressure

Table 5: Summary table of studies on gases under pressure.

Method	Results	Remarks	Reference
Not applicable, clethodim is not a gas.			

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant.

2.2.1.1.4.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable.

2.2.1.1.5 Flammable liquids

Table 6: Summary table of studies on flammable liquids.

Method	Results	Remarks	Reference
EC Method A.9	108.5 °C	Flash point > 60 °C and explosive vapour/air mixture not possible.	Winkler, 2020 (PS20190380-1)

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

The flash point of clethodim was determined to be 108.5 °C under atmospheric conditions. The provided information is of relevance for classification purposes.

2.2.1.1.5.2 Comparison with the CLP criteria

The flash point is > 60 °C, which is adequate to conclude on the classification in accordance with the CLP criteria.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids

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

2.2.1.1.6 Flammable solids

Table 7: Summary table of studies on flammable solids.

Method	Results	Remarks	Reference
Hazard not applicable, clethodim is not a solid.			

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

Not relevant.

2.2.1.1.6.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

Hazard class not applicable.

2.2.1.1.7 Self-reactive substances

Table 8: Summary table of studies on self-reactivity.

Method	Results	Remarks	Reference
UN Test H.4	The SADT is ≤ 75°C for a 50 kg package.	The study was considered acceptable.	Arif, 2022 (GLP3016010712R1/2022)
UN Test A.6	Does not propagate a detonation.	Non-GLP	Gledhill, 2022 (GLP3016011271R1/2022)
UN Test C.1	Does not propagate a deflagration		Gledhill, 2022 (GLP3016011271R1/2022)
UN Test C.2	Does not propagate a deflagration		Gledhill, 2022 (GLP3016011271R1/2022)
UN Test E.2	No effect of heating under defined confinement.		Gledhill, 2022 (GLP3016011271R1/2022)
UN Test E.1	No effect of heating under defined confinement.	Non-GLP	Gledhill, 2022 (GLP3016011271R1/2022)
UN Test F.4	Explosive power is none	Non-GLP	Gledhill, 2022 (GLP3016011271R1/2022)
UN Test H.2	SADT for a 50 kg package is 65 °C		Gledhill, 2022 (GLP3016011271R1/2022)

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

The self-accelerating decomposition temperature (SADT) was shown to be equal to or below 75°C, and thus classification as a self-reactive substance cannot be excluded. Therefore, the series of tests (UN tests A.6, C.1, C.2, E.2, E.1, F.4 and H.2) required for self-reactive properties in accordance with the CLP regulation was performed. The test series concluded that clethodim does not detonate in the cavitated state or deflagrates and shows on effect when heated under confinement nor any explosive power and that the SADT is between 60 °C to 75 °C. Clethodim is not mixed with any diluents (see CLP criteria below).

2.2.1.1.7.2 Comparison with the CLP criteria

Annex I: 2.8.2.3 of the CLP regulation states that any self-reactive substance or mixture which, in laboratory testing, neither detonates in the cavitated state nor deflagrates at all and shows no effect when heated under confinement nor any explosive power, provided that it is thermally stable (SADT is 60 °C to 75 °C for a 50 kg package), and, for liquid mixtures, a diluent having a boiling point not less than 150 °C is used for desensitisation shall be defined as self-reactive substance TYPE G.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Harmonized classification proposed (Self-Reactive Type G).

2.2.1.1.8 Pyrophoric liquids

Table 9: Summary table of studies on pyrophoric liquids.

Method	Results	Remarks	Reference
Experience in manufacture and handling shows that clethodim does not ignite spontaneously when coming into contact with air at normal temperatures.			

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

The information is of relevance for classification purposes.

2.2.1.1.8.2 Comparison with the CLP criteria

The provided information is sufficient to conclude on the classification in accordance with Annex I: 2.9.4 of the CLP regulation.

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

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

2.2.1.1.9 Pyrophoric solids

Table 10: Summary table of studies on pyrophoric solids.

Method	Results	Remarks	Reference
Hazard not applicable, clethodim is not a solid.			

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

Not relevant.

2.2.1.1.9.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

Hazard class not applicable.

2.2.1.1.10 Self-heating substances

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

Method	Results	Remarks	Reference
Clethodim is a liquid. In general, the phenomenon of self-heating applies only to solids.			

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

The information is of relevance for classification purposes – no further testing should be required for liquids.

2.2.1.1.10.2 Comparison with the CLP criteria

The Guidance on the Application of the CLP Criteria (ver. 5) states that the phenomenon of self-heating in general only applies to solids since the surface of liquids is not large enough for reaction with air.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

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

2.2.1.1.11 Substances which in contact with water emit flammable gases

Table 12: Summary table of studies on substances which in contact with water emit flammable gases.

Method	Results	Remarks	Reference
Clethodim does contain metals or metalloids, does not react with water and forms a stable mixture with water.			

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

The waiver is of relevance for classification.

2.2.1.1.11.2 Comparison with the CLP criteria

The waiver is acceptable in accordance with Annex I: 2.12.4.1 of the CLP regulation.

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

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

2.2.1.1.12 Oxidising liquids

Table 13: Summary table of studies on oxidising liquids.

Method	Results	Remarks	Reference
EC Method A.21 UN Test O.2	The mean pressure rise time for a clethodim/cellulose mixture was higher than that of the reference mixture.	The study is acceptable and demonstrates that clethodim is not oxidising.	Kuchta, 2022b (CSL-21-1644.01)

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

In the test, the pressure reached 2070 kPa from 670 kPa within 60 seconds in only 2 of the 5 tests and the time taken was significantly longer than for the reference item (35.85 seconds and 16.52 seconds compared to a mean time of 2.27 seconds for the reference item). This study is of relevance for classification purposes and demonstrates that clethodim is not oxidising.

2.2.1.1.12.2 Comparison with the CLP criteria

The used test method in the study by Kuchta (2022b) is the method prescribed by the CLP regulation (UN Test O.2).

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

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

2.2.1.1.13 Oxidising solids

Table 14: Summary table of studies on oxidising solids.

Method	Results	Remarks	Reference
Hazard not applicable, clethodim is not a solid.			

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

Not relevant.

2.2.1.1.13.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

Hazard class not applicable.

2.2.1.1.14 Organic peroxides

Table 15: Summary table of studies on organic peroxides.

Method	Results	Remarks	Reference
Hazard class not applicable – clethodim does not contain the bivalent -O-O- structure and is not an organic peroxide.			

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides

Not relevant.

2.2.1.1.14.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable.

2.2.1.1.15 Corrosive to metals

Table 16: Summary table of studies on the hazard class corrosive to metals.

Method	Results	Remarks	Reference
UN Test C.1	No weight loss > 13.5 % or intrusion > 120 µm in either steel or aluminium plates.	The study is acceptable and demonstrates that clethodim should not be considered corrosive to metals.	Kuchta, 2022c (CSL-21-1644.02)

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

No weight loss of steel or aluminium plates above 13.5% was observed in the test. No localized corrosive resulting in an intrusion greater than 120 µm was observed. The study is of relevance for the classification and demonstrates that clethodim should not be considered corrosive to metals.

2.2.1.1.15.2 Comparison with the CLP criteria

The test method is the prescribed test method for this hazard class in accordance with the Guidance on the Application of the CLP Criteria (ver. 5).

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

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

2.2.2 Summary of physical and chemical properties of the plant protection product

Clethodim 120 EC is an emulsifiable concentrate (EC) formulation containing 120 g/L clethodim. The appearance of the product is that of a clear brown or golden orange homogeneous free flowing liquid of low viscosity. It is not

explosive, has no oxidising properties and is not highly flammable. It has a self-ignition temperature of 275°C. The relative density is 0.9247. A 1% aqueous dilution has a pH of 4.1. The stability data indicate a shelf-life of at least 18 months at ambient temperature. The product has acceptable foaming and emulsion characteristics.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

No data available and not required.

2.3.2 Summary of information on the development of resistance

Resistance among populations of ALOMY, APESV and LOLSS have recently been reported in Europe.

There is known to be cross-resistance between cyclohexanediones (CHDs, including clethodim) and aryloxyphenoxy propionates (APPs), which possess the same mode of action through ACCase enzyme target site, as well as a more general cross-resistance to other modes of action through enhanced metabolism. Clethodim poses the lowest resistance risk of all the ACCase inhibitors due to the small number of target site mutations that confer resistance to this active substance.

The overall risk of resistance when using an Integrated Pest Management strategy is **Low** (0.125-2.25), and therefore acceptable, for all grass weed targets.

If relying on only one herbicide mode of action, there is a moderate to very high risk of resistance arising (3-9), which is unacceptable. Relying only on different modes of action is estimated to result in a low to moderate risk (1.5-4.5), which is not acceptable for the medium to high risk target weeds.

Standardised statements relating to resistance risks and best practice management strategies are included on product labels. More details on the development of resistance are given in Vol 3 CA, Section B.3.7.

2.3.3 Summary of adverse effects on treated crops

No data available.

2.3.4 Summary of observations on other undesirable or unintended side-effects

No data available.

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Ensure good ventilation of the workstation to prevent formation of vapour and avoid breathing dust/vapours/ spray. Avoid contact with skin and eyes. Wear personal protective equipment and do not eat, drink or smoke when using this product.

Keep container closed when not in use, and store in a cool, well-ventilated place away from sources of ignition, including direct sunlight. Transport measures are not regulated.

In case of fire, do not use a heavy water stream; use foam, dry powder, carbon dioxide, water spray or sand. Toxic fumes may be released, complete protective equipment is needed.

2.4.2 Summary of procedures for destruction or decontamination

Dispose of product packaging or contents in a safe manner in accordance with local/national regulations. Soak up spills with inert solids, such as clay or diatomaceous earth as soon as possible. Collect spillage. Store away from other materials.

2.4.3 Summary of emergency measures in case of an accident

Ventilate spillage area. Evacuate unnecessary personnel. Avoid contact with skin and eyes. Avoid breathing dust/fume/gas/mist/vapours/spray.

Prevent entry to sewers and public waters. Notify authorities if liquid enters sewers or public waters. Avoid release into the environment.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

Table 2.5.1-1. Summary of analytical methods for technical active substance.

Matrix	Analyte	Type of method	Validation	References
Technical a.s.	Clethodim	HPLC-UV	The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy and precision in accordance with the requirements of SANCO/3030/99 rev. 5.	Desai, H (2019a, b) and Desai, H (2020a, b) (228-2-12-23783 and 227-2-12-23329)
	Impurities [a)]			

a) Details are reported in Volume 4 confidential part.

Table 2.5.1-2. Summary of analytical methods for formulation analysis.

Matrix	Analyte	Type of method	Validation	References
TM 20015	Clethodim	HPLC-UV	See below	Walker, A F (2015) (TM150171)
H1231bc	Clethodim	HPLC-UV and HPLC-MS/MS	See below	Heermann, A (2017) (S16-07105)
TM-20011	Clethodim	HPLC-UV	See below	Schoop, T. (2007) (A30453)
H1231bc	Toluene	GC-FID	The method has been validated in accordance with SANCO 3030/99 rev. 5. Note that recoveries are only measured at n = 3 per fortification level.	Nikoloska, I. (2020) (GRL-13758)

Table 2.5.1-3. Summary of analytical methods used for data generation in toxicology studies, ecotoxicology studies, e-fate studies, residue studies and phys-chem. studies in Volume 3, B.5 (CA).

Matrix	Analyte	Type of method	Validation	References
A large number of analytical methods were used in toxicology studies, ecotoxicology studies, e-fate studies, residue studies and phys.chem. studies. Please refer to Volume 3 B.5 (CA) for further information.				

Table 2.5.1-4. Summary of analytical methods used for data generation in toxicology studies, ecotoxicology studies, e-fate studies, residue studies and phys-chem. studies in Volume 3, B.5 (CP).

Matrix	Analyte	Type of method	Validation	References
Reconstituted water	Clethodim	HPLC-UV	Acceptable despite minor deviations (see Volume 3 B.5 (CP))	Vinken, R. and Wydra, V. 2006b (30703220)
20X AAP growth medium	Clethodim	HPLC-UV	Acceptable despite minor deviations (see Volume 3 B.5 (CP))	Vinken, R. and Wydra, V. 2006c (30702210)
20X AAP growth medium	Clethodim	HPLC-UV	Acceptable despite minor deviations (see Volume 3 B.5 (CP))	Vinken, R. and Wydra, V. 2007 (35071240)
20X AAP growth medium	Clethodim	HPLC-UV	Acceptable despite minor deviations (see Volume 3 B.5 (CP))	Kuhl R. and Wydra V., 2011 (62161221)

2.5.2 Methods for post control and monitoring purposes

Table 2.5.2-1. Summary of analytical methods covering relevant residue definitions and limits.

Matrix / crop group	Analyte	LOQ	Residue limit
Food of plant origin: High water content commodities	clethodim, clethodim sulfoxide and clethodim sulfone	0.005 mg/kg	Calculated MRL is 0.03 mg/kg for the sum of clethodim, clethodim sulfoxide and clethodim sulfone. Method suitable.
Food of plant origin: High acid content commodities	clethodim, clethodim sulfoxide and clethodim sulfone	0.005 mg/kg	No new MRL proposal Method considered suitable for analytes in the proposed residue definition
Food of plant origin: High starch content commodities	clethodim, clethodim sulfoxide and clethodim sulfone	0.005 mg/kg	Calculated MRL is 0.015* mg/kg for the sum of clethodim, clethodim sulfoxide and clethodim sulfone. Method suitable.
Food of plant origin: Dry commodities	clethodim, clethodim sulfoxide and clethodim sulfone	0.005 mg/kg	No new MRL proposal Method considered suitable for analytes in the proposed residue definition
Food of plant origin: High oil content commodities	clethodim, clethodim sulfoxide and clethodim sulfone	0.005 mg/kg	No new MRL proposal Method considered suitable for analytes in the proposed residue definition
Food of animal origin bovine whole milk, poultry eggs, bovine meat, liver and fat	clethodim, clethodim sulfoxide and clethodim sulfone	0.01 mg/kg	Calculated MRL is 0.03 mg/kg, LOQ for the sum of clethodim, clethodim sulfoxide and clethodim sulfone. Method considered suitable for analytes in the proposed residue definition
Soil	clethodim	0.005 mg/kg	NOEC 47.6 mg a.s./kg soil dw
Drinking water	clethodim	0.1 µg/L	0.1 µg/L EU drinking water limit
Surface water	clethodim	0.1 µg/L	E_rC_{50} = 0.0190 mg a.s./L (twa)
Surface water	clethodim imine and clethodim imine sulfoxide	0.051 µg/L	C. imine: NOEC = 10 mg/L C. imine sulfoxide: E_yC_{50} = 32.1 mg/L
Air	clethodim	1 µg/m^3	60 µg/m^3 *
Body fluids and tissues	clethodim	0.05 mg/L	-

*Calculated from the systemic ADI in accordance with SANCO/825/00 rev. 8.1.

Table 2.5.2-2. Overview of accepted residue analytical methods.

Matrix / crop group	Primary method	Analyte	Confirmatory method	Independent Lab Validation (if appropriate)
Food of plant origin: High water crops (sugarbeet roots and leaves, soybeans and proteagineous peas)	CA 4.2/01Tribolet, R (2005a) LC-MS/MS	clethodim, clethodim sulfoxide and clethodim sulfone	CA 4.2/01 Tribolet, R (2005a) LC-MS/MS	Mende, P. (2006) CA 4.2/02 Holzer, S (2012) CA 4.2/03 Wiesner, F., Breyer, N (2014) CA 4.2/04
Food of plant origin: High oil crops and high water crops (oilseed rape and sugar beet leaves)	CA 4.2/05 Wiesner, F., Breyer, N (2016) LC-MS/MS	clethodim sulfone	CA 4.2/05 Wiesner, F., Breyer, N (2016) LC-MS/MS	
Food of animal origin	CA 4.2/06 Lindner, M. Giesau, A. (2013) LC-MS/MS	clethodim, clethodim sulfoxide and clethodim sulfone	CA 4.2/06 Lindner, M. Giesau, A. (2013) LC-MS/MS	Mewis, A. (2013) CA 4.2/07
Soil	CA 4.2/08 Stahl, F (2019) LC-MS/MS	clethodim	CA 4.2/08 Stahl, F (2019) LC-MS/MS	
Drinking water	(LC-MS/MS)	Clethodim*		Garrigue, P (2020) CA 4.2/10
Surface water	CA 4.2/09 Stahl, F (2019) LC-MS/MS	clethodim	CA 4.2/09 Stahl, F (2019) LC-MS/MS	Garrigue, P (2020) CA 4.2/10

Matrix / crop group	Primary method	Analyte	Confirmatory method	Independent Lab Validation (if appropriate)
Surface water	CA 4.2/11 Stahl, F (2019) LC-MS/MS	clethodim imine and clethodim imine sulfoxide	CA 4.2/11 Stahl, F (2019) LC-MS/MS	
Air	CA 4.2/12 Garrigue, P. (2019) LC-MS/MS	Clethodim	CA 4.2/12 Garrigue, P. (2019) LC-MS/MS	
Body fluids and tissues	CA 4.2/13 Carle, F. (2019) LC-MS/MS	clethodim	CA 4.2/13 Carle, F. (2019) LC-MS/MS	

* The method for groundwater monitoring is only validated for parent, but metabolites clethodim sulfone and clethodim oxazole sulfone are tentatively included in the residue definition for monitoring in groundwater pending submission of further data on the relevance assessment of these metabolites.

2.5.3 Extraction efficiency

The extraction efficiency study S19-00144 (Wiesner, Xu, 2020) is evaluated in Vol.3, B.5.2.1 in accordance with the current guideline SANTE 2017/10632 rev. 3 and was therefore scientifically valid with respect to Commission Regulation (EU) No 283/2013.

The extraction efficiency of the residue analytical methods Holzer, 2012 and Lindner/Giesau, 2012 is considered as being sufficiently proven for high-water content commodities as the residue levels for the sum of all analytes of the residue definitions for monitoring and risk assessment differs by no more than 30% (for residues >0.01 mg/kg) compared to the results obtained with the solvent of the metabolism study. The high-water content commodity group is applicable to the crops under consideration (sugar beet roots and tops with leaves and onion bulb).

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

More detailed results of the studies are presented in Volume 3, section B.6.

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals [equivalent to section 9 of the CLH report template]

Information from two reports on the metabolism of clethodim have been provided to support the application for the renewal of the regulatory approval of clethodim.

Table 17: Summary table of toxicokinetic studies.

Method	Results	Remarks	Reference
In vivo metabolism in rats. Test substance: - clethodim: Lot/Batch:RE-45601-31 Purity: 99% And - [propyl-1-^{14}C]-clethodim Purity >96%. 5 Male and 5 female Sprague-Dawley rats. Single dose oral gavage of 4.4 mg/kg (low dose), 468 mg/kg (high dose) or 4.8 mg/kg repeated dose for 14 days. Conducted under GLP Deviations from OECD TG 417 (2010): - tissue-plasma ratio was not reported - Temperature and humidity were not reported Acceptable	Clethodim appears to be rapidly absorbed with no change in distribution of clethodim or its metabolites between single dose and repeated dose administration or between sexes. Higher tissue concentrations were observed in the 468 mg/kg group in both sexes. Tissue residues were seen primarily in the adrenals, liver and kidneys. A total of ten metabolites were identified of which nine metabolites and the parent were identified in the urine. Urinary metabolites in the repeated dose group which each accounted for more than 5% of the administered dose were clethodim sulfoxide (46-61%), S-methyl sulfoxide (6-11%), imine sulfoxide (5-9%) and 5-OH sulfoxide (2-5%). Clethodim sulfoxide (2-5%) was the only faecal metabolite, which accounted for more than 5% of the administered dose. Clethodim is proposed to either be oxidized to clethodim sulfoxide (dominant process), converted to S-methyl via a sulfonium cation intermediate, cleavage of the oxime N-O bond to generate the imine or hydroxylated at the five position. Clethodim was rapidly excreted with majority of the administered dose (87.2-93.2%) recovered in the urine. There was no difference in excretion pattern between females and males within a treatment group or between treatment groups. A smaller amount (9.3-17.0%) of the administered dose was recovered in the faeces. Expired CO_2, accounted for <1% of the administered dose. The majority of the recovered dose (93.5-98.2%) in all treatment groups was eliminated within 48 h without any signs of accumulation in tissue.	-	[redacted] and [redacted] 1988; Vol.3, B.6.1.1/01 Report No.: MEF-0086 New data for renewal: No
Interspecies comparison of *in vitro* metabolism of [^{14}C]-Clethodim in rat, dog and human hepatocytes. Test substance: - [^{14}C]-clethodim Lot/Batch: 10079RXB001-4 Radiochemical purity: 98.3%, chemical purity: 98.4% - Test material (reference item). Lot/Batch: 4478 Purity: 95.98%	The extent of conversion of [^{14}C]-clethodim was on average 39% in rat hepatocytes, 22% in dog hepatocytes and 66% in human hepatocytes after 120 ± 1 minutes of incubation. The calculated averaged in vitro t1/2 values were >120 min in dog, 99 min in rat and 52 minutes in human hepatocyte incubations. In total five metabolites of [^{14}C]-clethodim were found in the hepatocyte incubations of the three different species. No human specific metabolites were detected. The metabolites representing more than 5% of	There were no human specific metabolites, although M5 may be formed at higher levels in human hepatocytes compared with hepatocytes	Krebbers, S., 2020; Vol.3,.6.1.2/01 Report No. 20182210 New data for renewal: Yes

Method	Results	Remarks	Reference
freshly prepared solution. Incubations in triplicates with 1 and 10 µM for 1, 15, 30, 60, 90 and 120 min. Conducted under GLP. Acceptable	the total radioactivity (M3, M4 and M5) were selected for identification purposes. Metabolic reactions observed included S-oxidation and demethylation (S-Ethyl → S-Methyl).	from rat and dogs.	

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

Two studies are available for this section, one ADME study and one *in vitro* comparable metabolism study. Both studies were conducted in accordance with the OECD Principles of Good Laboratory Practice (1981) and considered acceptable. The *in vitro* comparable metabolism study is new data for the renewal of active substance.

ADME study:

The absorption, distribution, metabolism and excretion of [^{14}C]-clethodim in rat was investigated after a single oral dose of 4.4 and 468 mg/kg bw, and a single oral dose of 4.5 mg/kg bw for 14 daily pre-treatments at the same dose with unlabelled clethodim, and by interspecies comparison of *in vitro* metabolism of [^{14}C]-clethodim in mixed gender rat, dog and human hepatocytes. Both studies were conducted under GLP and acceptable, although it could be noted that the ADME study was restricted since no blood samples were taken for pharmacokinetic analysis.

Clethodim appears to be rapidly absorbed with no change in distribution of clethodim or its metabolites between single dose with repeated dose administration. Higher tissue concentrations were observed in the high dose group relative to the low dose group in both sexes. As a proportion of the dose administered, however, the tissue concentration in the high dose group was similar to or less than the low dose. Highest tissue concentration was observed in the adrenal, followed by the liver and kidneys. No tissue accumulation was observed.

Clethodim was rapidly excreted with majority of the administered dose in the urine (87.2-93.2%). No difference in excretion pattern between females and males within a treatment group or between treatment groups was observed. A smaller amount (< 17%) was recovered in the faeces. Expired CO_2 accounted < 1% of the administered dose. The majority of the recovered dose in all treatment groups was eliminated within 48 h (93.5-98.2%).

In rat urine a total of nine metabolites and the parent were identified. Urinary metabolites in the repeated dose group which each accounted for more than 5% of the administered dose were clethodim sulfoxide (46-61%), S-methyl sulfoxide (6-11%), imine sulfoxide (5-9%) and 5-OH sulfoxide (2-5%). Clethodim sulfoxide (2-5%) was the only faecal metabolite, which accounted for more than 5% of the administered dose.

Clethodim is proposed to either be oxidized to clethodim sulfoxide (dominant process), converted to S-methyl via a sulfonium cation intermediate, cleavage of the oxime N-O bond to generate the imine or hydroxylated at the five position.

In vitro comparable metabolism study:

The *in vitro* metabolic profile of clethodim was investigated in human, Sprague-Dawley rat and Beagle dog by incubating hepatocytes with 1 µM of [^{14}C]-clethodim, to determine the metabolic stability. The extent of conversion of [^{14}C]-clethodim was on average 39% in rat hepatocytes, 22% in dog hepatocytes and 66% in human hepatocytes after 120 ± 1 minutes of incubation. The calculated averaged in vitro t1/2 values were >120 min in dog, 99 min in rat and 52 min in human hepatocyte incubations.

In hepatocyte incubations a total five metabolites of [^{14}C]-clethodim were found. Metabolites representing more than 5% of the total radioactivity (M3, M4 and M5) were identified and included S-oxidation and demethylation (S-Ethyl → S-Methyl). There were no human specific metabolites, although M5 (unidentified, see proposed structure below) may be formed at higher levels in human hepatocytes compared with hepatocytes from rat and dogs.

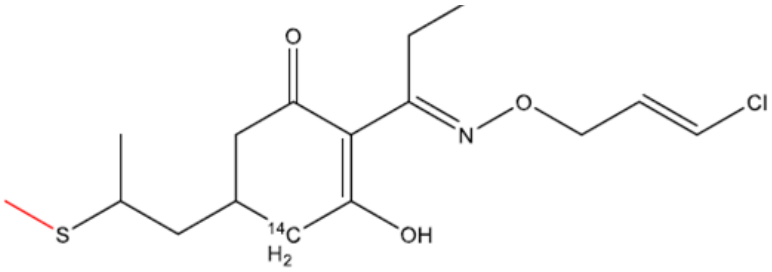


Fig. 2.6.1.1-1: proposed structure for M5

Both studies were conducted under GLP. The rat in vivo study was performed with only basic compliance of OECD TG 417 and no blood samples were taken for pharmacokinetic analysis. The studies are acceptable.

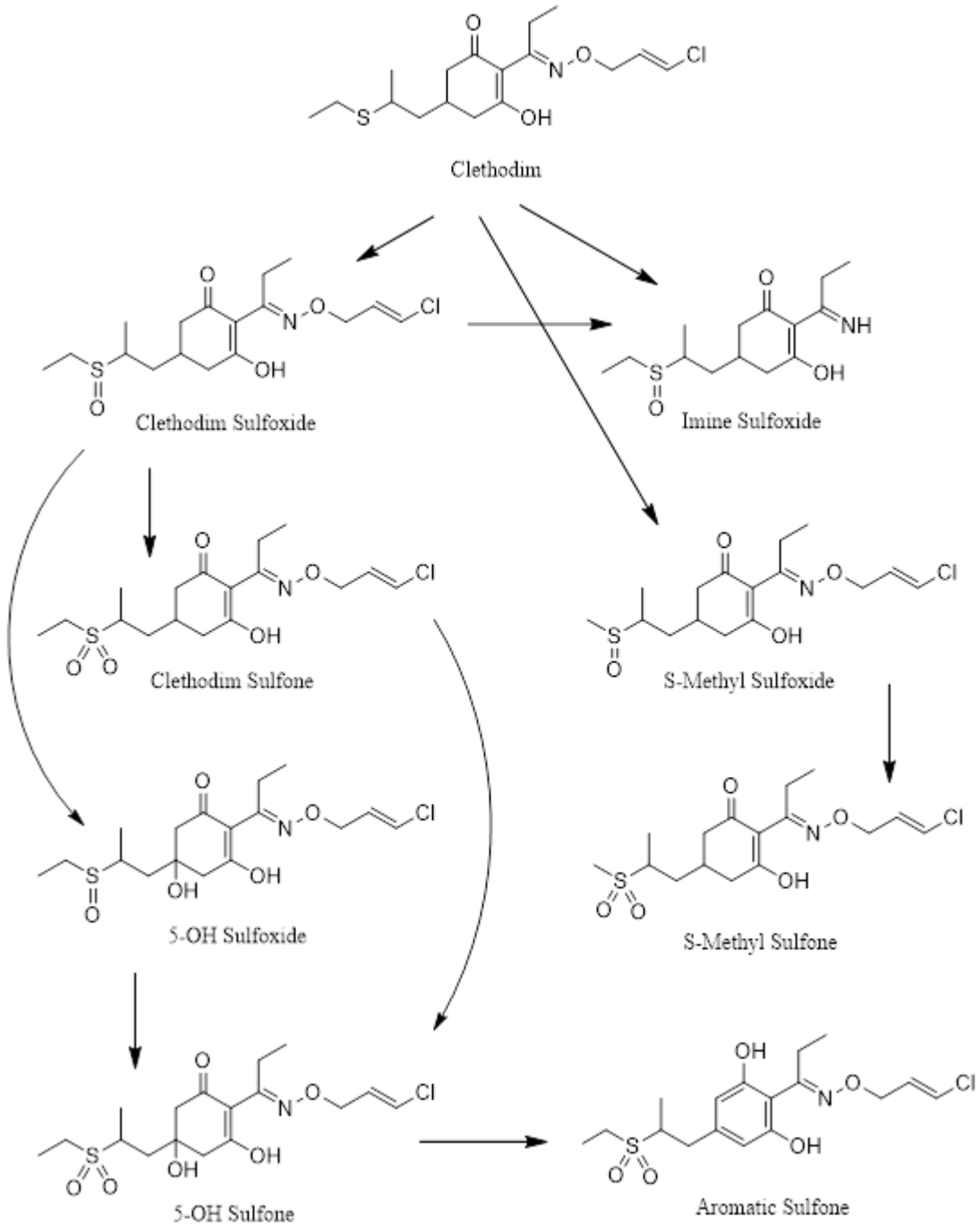


Fig. 2.6.1.1-2: Proposed metabolic pathway of clethodim in rats.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 18: Summary table of animal studies on acute oral toxicity.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD50	Reference
Acute oral toxicity OECD TG 401, No deviations noted. GLP: Yes Acceptable	Rat, Sprague-Dawley, males and females, 5/sex/groups	Chevron RE-45601 Lot/Batch: SX-1688 Purity: 83.3% w/w Vehicle: Suspension in 0.7% CMC (carboxymethylcellulose) and 1.0 % TWEEN 80 in distilled water	Males: 0, 1050, 1450, 1860 or 2500 mg/kg (equal to 0, 875, 1208, 1550 and 2083 mg/kg bw based on correction for purity using a correction factor of 1.2) Females: 0, 800, 1050, 1450 and 2000 mg/kg (equal to 0, 667, 875, 1208 and 1667 mg/kg bw based on correction for purity using a correction factor of 1.2) 14 days observation	Males: 1630 mg/kg bw (confidence limits: 1050-2550 mg/kg) (equal to 1358 mg/kg bw after correction for purity) Females: 1360 mg/kg bw (confidence limits: 820-2230 mg/kg) (equal to 1133 mg/kg bw after correction for purity) Acute tox 4, H302: Harmful if swallowed	[redacted] 1986 Report number: S2498 Vol.3. B.6.2.1/01 New data for renewal: No
Acute oral toxicity study OECD TG 401 No deviations noted GLP: Yes Acceptable	Mouse, CD1, males and females, 5 per sex/group	Chevron RE-45601 Lot/Batch: SX-1688 Purity: 83.3% w/w Vehicle: Suspension in carboxymethyl cellulose sodium salt and TWEEN 80 in distilled water	Males: 0, 1500, 2000, 2500, 3000 mg/kg bw (equal to 1250, 1667, 2083, 2500 mg/kg bw after correction for purity of the test substance using a correction factor of 1.2) Females: 0, 2000, 2500, 3000, and 3500 mg/kg bw (equal to 1667, 2083, 2500, 2917 mg/kg bw after correction for purity of the test substance using a correction factor of 1.2) 14 days observation	Males: 2573 mg/kg bw (confidence limits: 2115-3130 mg/kg) (equal to 1787 mg/kg bw after correction for purity) Females: 2430 (confidence limits: 1956 - 3018 mg/kg bw) (equal to 1688 mg/kg bw after correction for purity) *Acute tox 4, H302: Harmful if swallowed*	[redacted] 1986 Report number: 2107-143 Vol.3. B.6.2.1/02 New data for renewal: No

Table 19: Summary table of human data on acute oral toxicity.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 20: Summary table of other studies relevant for acute oral toxicity.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

There are no new data for this endpoint in this report. Two studies on acute oral toxicity are available, one with Sprague-Dawley rats and one with CD1 mice. Both studies were conducted in accordance with the OECD Principles of Good Laboratory Practice (1981) and according to the OECD 401 (1981). The studies were considered acceptable.

In the DAR (2005) an acute intraperitoneal toxicity study in the rat was evaluated in addition to the studies mentioned above. This study is not included in the dossier by the applicant for the renewal of active substance. However, RMS considers this study as less relevant (intraperitoneal route of administration). Nevertheless, a short study summary is given below (refer to Vol. 3 section B.6.2.1 in DAR (2005) for further details).

Clinical signs in rats included salivation, decreased motor activity, unsteady gait, hyperreactivity, lacrimation, clonic convulsions, red nasal discharge, ocular discharge, and collapse. Clinical signs in mice included hypoactivity, rough coat, hunched appearance, ataxia, tremors, salivation, laboured respiration, and soft faeces and urine stains. Clinical signs of toxicity that subsided after day 6 was observed in all dose groups in both studies. Rats that died during the study had dark red gelatinous material beneath the meninges, reddened meninges, white or black material in the stomach, tan fluid in the stomach and/or small intestine, enlarged adrenals, a dilated renal pelvis, and reddened, darkened, and/or mottled lungs with foam in the trachea (observed in the two highest doses). Two female rats exposed to 1450 mg/kg that survived until termination had trace gliosis in a single spinal nerve in the lower lumbar area. Mice that died during the study had dark-red lungs and compound-like material in the stomach and intestine.

Table 2.6.2.1.1-1. Acute oral toxicity of clethodim technical

Species	Sex	Dose (mg/kg bw)	Number of dead	Total number	LD_{50} (corrected for purity)	Reference
Rat, Sprague Dawley	Female	0	0	5	1133 mg/kg bw	[redacted] 1986 New data for renewal: No
		800	0	5		
		1050	0	5		
		1450	3	5		
		2000	5	5		
	Male	0	0	5	1358 mg/kg bw	
		1050	0	5		
		1450	1	5		
		1860	4	5		
		2500	5	5		
Mouse, CD1	Female	0	0	5	1688 mg/kg bw	[redacted] 1986 New data for renewal: No
		2000	2	5		
		2500	1	5		
		3000	5	5		
		3500	4	5		
	Male	0	0	5	1787 mg/kg bw	
		1500	0	5		
		2000	1	5		
		2500	3	5		
		3000	3	5		

The LD_{50}-values were in the same range for both species, but the lowest value was obtained from female rats (1133 mg a.s./kg bw).

Acute intraperitoneal toxicity study (presented in DAR (2005):

reference	:	[redacted] 1987	exposure	:	Once (5 ml/kg)
type of study	:	Acute intraperitoneal toxicity study	doses	:	0, 700, 1000, 1400, 2000 mg/kg bw (both sexes) [1]
year of execution	:	1986 - 1987	vehicle	:	Tween 80, carboxymethyl cellulose sodium salt high viscosity and distilled water
test substance	:	Chevron RE-45601 technical (Clethodim technical), lot no SX-1688, purity 83.2%	GLP statement	:	Yes
route	:	Intraperitoneal injection	guideline	:	Not applicable
species	:	Rat, Sprague-Dawley, Crl:CD (SD)BR	acceptability	:	Acceptable
group size	:	5/sex/dose	LD_{50}	:	868 mg a.i./kg bw (male) 1001 mg a.i./kg bw (female)

[1] equal to 583, 833, 1167, 1667 mg a.i./L (males and females) after correction for purity of the test substance

Results

Mortality: 5/5 males given 2000 mg/kg, 4/5 males given 1400 mg/kg, 3/5 males given 1000 mg/kg were found dead within 1 d after treatment. 4/5 females given 2000 mg/kg and 1400 mg/kg and 2/5 females given 1000 mg/kg were found dead within 14 d after treatment. No further mortality occurred.

Symptoms of toxicity:

Among the test substance treated animals, clinical signs observed included hypoactivity, rough coat, hunched posture, urine staining of the fur, soft faeces, salivation, ataxia, red stains on nose and eyes and prostration. Most of the clinical signs disappeared by day 4. Pupillary responses were normal for all animals except for 2 animals on day 1 after dosing.

Body weight: No treatment related findings, except for one female animal in the 700 mg/kg dose group, which showed weight loss.

Pathology: A pale liver and bright red lung was observed for animals which died during the study. Pale left lateral lobes and rounded margins of the liver were observed in two 700 mg/kg dose group animals which survived. Compound-like material was found in the abdominal cavity of all animals that died in the 2000 mg/kg dose group. No further treatment-related effects.

Acceptability

The study is considered acceptable.

Conclusions

The acute intraperitoneal LD_{50} of RE-45601 technical was found to be 1041 mg/kg bw for males and 1201 mg/kg bw for females.

After correction for the purity, this is equal to an intraperitoneal LD_{50} of 868 mg a.s./kg bw for males and 1001 mg a.s./kg bw for females.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

The LD_{50} value (female, rat) was 1133 mg a.s./kg bw and thus falls under the criterion for acute oral toxicity category 4 ($300 < ATE \leq 2000$) under regulation (EC) No 1272/2008. This LD50 represents the ATE as it is the lowest LD50 observed in the most sensitive species (females) and is based on results from a well-performed study in rats which is the preferred test species for evaluation of acute toxicity by the oral route.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox. 4. H302: Harmful if swallowed. ATE = 1133 mg/kg bw.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 21: 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 LD50	Reference
Acute dermal toxicity	Rabbit, New Zealand White, 5-10 ind./group	RE-45601 (technical)	Females: 5000 mg/kg	LD_{50}>5000 mg/kg bw (equal to >4167 mg/kg bw	[redacted] 1986

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD50	Reference
No study guideline was reported. Study was conducted in general compliance with guideline OECD 402 (1981). GLP: Yes Acceptable	Males and females	Lot/Batch: SX-1688 Purity: 83.3%w/w	Males: 2000 and 4900 mg/kg 24 h exposure, 14 days observation	based on correction for purity using a correction factor of 1.2)	Report number: CEHB 2510 Vol.3. B.6.2.2/01 New data for renewal: No

Table 22: Summary table of human data on acute dermal toxicity.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 23: Summary table of other studies relevant for acute dermal toxicity.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

There are no new data for this endpoint in this report. One study is available on acute dermal toxicity in which rabbits were exposed for 24 h and observed for 14 days. One animal died during the study (male, 5.0 g/kg bw). Skin irritation occurred in both control and exposed animals, albeit more severe in the latter groups. By day 7, no sign of skin irritation could be observed in the control group while exposed animals displayed both oedema (grade 0-2) and erythema (grade 0-4). Erythema persisted to day 14 in one female dosed with 5.0 g/kg.

Several signs of toxicity were observed. Control animals displayed red, swollen, scabbed, dry/flaky skin. Other signs in the control groups included ocular and nasal discharge, and reduced food intake. Exposed animals showed the same symptoms as the control animals (except nasal discharge and the mouth cut/scab observed in one individual) but usually for a longer period of time. In addition to those symptoms, exposed animals also displayed other dermal effects (abraded, thickened, blackened, crusty, cracked skin) and diarrhoea. The male that was found dead on day 6 displayed reduced food intake, decreased motor activity, decreased body temperature, unkempt appearance, diarrhoea, a lack of faeces, and collapse prior to its death. Body weight was not affected.

The study was conducted in accordance with the OECD Principles of Good Laboratory Practice (1981) and in general compliance with guideline OECD 402 (1981). The study is acceptable.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

LD_{50}>4167 mg/kg bw. This is above the cut-off of 2000 mg/kg bw for acute dermal toxicity classification.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Clethodim is not classified for acute dermal toxicity under Regulation (EC) 1272/2008.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 24: 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 LC50	Reference
Acute inhalation LC_{50} No guideline reported, in general compliance with OECD 403 (2009). Deviation: animals were older than the recommended age of 8-12 weeks, humidity (71-72%) slightly above recommended value of 70% in the guideline GLP: Yes Acceptable	Rat, Sprague-Dawley, both sexes, 5 per sex/group	RE-45601 Lot/Batch: SX-1688 Purity: 83.3% Vehicle: acetone aerosol, MMAD = 2.75 µm	3.9 mg/L, 4 h (maximum attainable concentration) (equal to 3.25 mg/L based on correction for purity using a correction factor of 1.2)	>3.25 mg/L air (4 h, whole body) (value corrected for purity) No mortalities occurred.	[redacted] 1986 Report number: CEHB 2513 Vol.3. B.6.2.3/01 New data for renewal: No

Table 25: Summary table of human data on acute inhalation toxicity.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 26: Summary table of other studies relevant for acute inhalation toxicity.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

There are no new data for this endpoint in this report. One acute inhalation study is available, in which 5 rats of each sex were exposed to an aerosol of diluted test material (3.9 mg clethodim/L) for 240 minutes. It was performed in general compliance with OECD TG 403 and conducted in accordance with the OECD Principles of Good Laboratory Practice. The MMAD was 2.75 µm. Approximately 82% was smaller than 4.5 µm, 23% was smaller than 1.8 µm, and 8% smaller than 1.1 µm. The study is acceptable.

No mortality occurred during the study. During the exposure, salivation was observed in three exposed animals and all animals squinted or had closed eyes. Immediately following the exposure, all exposed animals were salivating, and five of ten animals (four males and one female) had a colourless eye discharge. Additional signs of toxicity observed following exposure included red nasal discharge, abnormal respiratory sounds, decreased faeces, unkempt appearance, and a yellow/red anogenital discharge. All exposed animals appeared normal within 8 days of exposure. In the control group, one male was salivating during the first hour of exposure. Immediately following the exposure and throughout the 14-day observation period, all vehicle control animals appeared normal. No gross pathologic changes that could be attributed to the exposures were seen at necropsy following a 14-day observation period. No exposure-related histologic changes were observed in the lungs or tracheas of exposed animals.

2.6.2.3.2. Comparison with the CLP criteria regarding acute inhalation toxicity

The limit for classification of acute inhalation toxicity under regulation (EC) No 1272/2008 (CLP) is 5.0 mg/L while the concentration tested in the available study was 3.9 mg/L (maximal attainable concentration). The LC_{50} was >3.25 mg/L (value corrected for purity). No classification for acute inhalation toxicity is needed as an LC_{50} equal to or below 5 mg/L has not been demonstrated. There was no mortality at exposure levels relevant to classification.

2.6.2.3.3. Conclusion on classification and labelling for acute inhalation toxicity

Clethodim does not fulfil criteria for classification.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 27: 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
Acute dermal irritation in rabbits OECD TG 404 Deviations: The temperature range was slightly below the recommended (18 ± 3 °C vs 20 ± 3 °C) GLP: Yes Acceptable	Rabbit, New Zealand White, male, 1 (3 min + 1 h) or 3 (4 h) per group	Clethodim technical Lot/Batch: 6F50568000 Purity: 93.4%	0.5 mL undiluted test item, 3 min, 1 h, 4 h	3 minutes (n=1): Very slight erythema (day 2) 1 h (n=1): Very slight erythema (day 2) and dryness of skin (day 5) 4 h (n=3): Very slight to well defined erythema (day 1) Very slight to slight oedema (day 2) Dryness of the skin (day 5) Slight yellow colouration of the skin (day 1) The mean scores were 0.7, 2.0, and 2.0 for erythema and 0.0, 1.7, and 1.3 for oedema. All three animals had recovered completely by day 9.	[redacted] 2005 Report number: 29389 TAL B.6.2.4/01 New data for renewal: No
Acute dermal toxicity	Rabbit, New Zealand White, 5-	RE-45601 (technical) Lot/Batch: SX-1688	Females, 5000 mg/kg,	LD_{50}>4900 mg/kg bw (equal to >4167 mg/kg bw based on correction for purity using a correction factor of 1.2)	[redacted] 1986

No study guideline was reported. Study was conducted in general compliance with guideline OECD TG 402 (1981). GLP: Yes Acceptable	10 ind./group	Purity: 83.3%w/w	Males, 2000 and 4900 mg/kg 24 h exposure, 14 days observation	<u>Symptoms in the control:</u> -red, swollen, scabbed, dry/flaky skin. -ocular and nasal discharge -reduced food intake -erythema (slight to severe) -oedema (non-existing to well-defined) <u>Symptoms in exposed animals:</u> -Red, swollen, scabbed, dry/flaky skin (lasted longer than in the control) -ocular and nasal discharge (lasted longer than in the control) -reduced food intake (lasted longer than in the control) -abraded thickened, blackened, crusty, cracked skin -diarrhoea -death (one male, 5.0 g/kg, on day 6) -erythema (slight to severe) -oedema (moderate to severe) -hyperkeratosis (trace to mild, two 2 g/kg males + one 5/kg female) -dermal necrosis and ulceration (severe, in the female (5 g/kg) with hyperkeratosis in an area where the wrap was secured with tape, unclear if treatment related, all other females had normal skin and all treated males were normal at the 5 g/kg level) Twenty-four h after dosing treated and control animals showed comparable slight to severe erythema; erythema was accompanied by moderate to severe oedema in treated animals and no to well-defined oedema in controls. No erythema or oedema was apparent by day 7 in the controls. Treated animals still had both erythema (no to severe) and oedema (no to well-defined) at this time. Erythema persisted to day 14 in one female dosed with 5.0 g/kg (in an area where the wrap was secured with tape, unclear if treatment related, all other females had normal skin and all treated males were normal at the 5 g/kg level).	Report number: CEHB 2510 Vol.3. B.6.2.2/01 New data for renewal: No
Four-week dermal study in rat OECD TG 410 (1981) Deviations from current guideline: some of the suggested serum measurements were not performed (ornithine decarboxylase, gamma glutamyl transpeptidase, hormone levels, methaemoglobin, cholinesterase activity)	Rat Strain: Sprague-Dawley® Crl:CD® BR, 6/sex/group	RE-45601 (Technical) Lot/Batch: SX-1688 Purity: 83.2% Vehicle: 0.7% carboxymethyl cellulose (CMC) and 1.0% TWEEN 80 in distilled water	0% (vehicle control), 1.0% (low-dose), 10.0% (mid-dose), and 100.0% (high-dose) corresponding to approximately 0, 10, 100, and 1000 mg/kg/day of RE-45601 technical.dose (equal to 0, 8.32, 83.2, and 832 mg/kg bw/day based on correction for purity using a	NOAEL local: <10 mg/kg bw/day (equal to 8.32 mg/kg bw/day after correction for purity of test substance using a correction factor of 1.2) NOAEL systemic: 100 mg/kg bw/day (equiv. 83.2 mg/kg bw/d) (equal to 83.2 mg/kg bw/day after correction for purity of test substance using a correction factor of 1.2) LOAEL local: 10 mg/kg bw/day (equal to 8.32 mg/kg bw/day based on correction for purity using a correction factor of 1.2) LOAEL systemic: 1000 mg/kg bw/day (equal to 832 mg/kg bw/day based on correction for purity using a correction factor of 1.2) <u>Effects at 10 mg/kg bw/day:</u> Skin irritation ↑ triglyceride levels (F: 40%, n.s.)	[redacted] 1987 Report number: S-2848 Vol.3 B.6.3.3/01 New data for renewal: No

GLP: Yes Acceptable			correction factor of 1.2) 21 six-h dermal applications over a 28-day period	<u>Effects at 100 mg/kg bw/day (equal to 83.2 mg/kg bw/day based on correction for purity using a correction factor of 1.2):</u> Skin irritation ↑ triglyceride levels (F: 140%) ↓ BUN/creatinine ratio (M: 22%, F: 9% n.s.) <u>Effects at 1000 mg/kg bw/day (equal to 832 mg/kg bw/day based on correction for purity using a correction factor of 1.2):</u> Skin irritation -clinical signs (anogenital discharge in all males (6 animals) and two females) ↓ food efficiency (M during Weeks 1-2) ↓ body weight gain (M: 35%) ↑ absolute liver weight (F: 20%) ↑ relative liver weight (F: 22%) ↑ liver weight relative to brain weight (F: 24%) ↑ triglyceride levels (F: 160 %) ↓ BUN (M: 22%, F: 20% n.s.) ↓ BUN/creatinine ratio (M: 32%, F: 21% n.s.) ↓ chloride (M: 3%, F: 3%, both within HCD) ↑ relative weight of kidneys (M: 10%) ↑ relative testes weight (M: 13%)	

Table 28: Summary table of human data on skin corrosion/irritation.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 29: Summary table of other studies relevant for skin corrosion/irritation.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

There are no new data for this endpoint in this report.

One skin irritation study is available. It was performed on male rabbits and was conducted in accordance with the OECD Principles of Good Laboratory Practice. The study follows OECD TG 410 except for minor deviations (see Table 27) and is considered acceptable. Exposure to 5.0 mL of undiluted test item for 3 minutes induced a grade 1 erythema on day 2 (n=1). Exposure for 1 h (n=1) resulted in a grade 1 erythema, associated with a dryness of the skin from day 5, that lasted from day 2 up to day 7. Exposure for 4 h (n = 3) resulted in grade 1-2 erythema that appeared on day 1 in all animals and lasted until day 3, 5, and 8. Grade 1-2 oedema appeared in two animals on day 2 and lasted until day 3 and 4. Dryness of the skin was observed from day 5 in one animal and slight yellow colouration of the skin was visible in two individuals on day 1. The mean scores were 0.7, 2.0, and 2.0 for erythema and 0.0, 1.7, and 1.3 for oedema (Report number: 29389 TAL).

Table 2.6.2.4.1-1: Individual and mean skin irritation scores of Clethodim (technical) according to the Draize scheme

Rabbit number	Dermal Irritation	Scores				Mean irritation score (1)	Interpretation (+) (-)
		1h D1	24h D2	48h D3	72h D4		
05	Erythema	1	1	1	0	0.7	(-)
	Oedema	0	0	0	0	0.0	(-)
	Other	*	*	*	*		
35	Erythema	1	2	2	2	2.0	(-)
	Oedema	0	2	2	1	1.7	(-)
	Other	C	*	*	*		
36	Erythema	1	2	2	2	2.0	(-)
	Oedema	0	2	2	0	1.3	(-)
	Other	C	*	*	*		

(1) mean of scores on days 2, 3 and 4
h = hour
D = day
(+) = irritant according to E.E.C. criteria
(-) = non-irritant according to E.E.C. criteria
* = none
C = yellow coloration of the skin

In addition to this study, an acute dermal toxicity study in the rat (refer to section 2.6.2.2), a repeated dose dermal study in the rat (refer to section 2.6.3) and a skin sensitisation study in the Guinea Pig (refer to section 2.6.2.7) are available which also give some information on irritant properties of the active substance.

In the acute dermal toxicity study in which rabbits were exposed for 24 h and observed for 14 days, skin irritation occurred in both control and exposed animals, albeit more severe in the latter group. By day 7, no sign of skin irritation could be observed in the control group while exposed animals displayed both oedema (grade 0-2) and erythema (grade 0-4). Erythema persisted to day 14 in one female dosed with 5.0 g/kg. Other dermal effects reported in exposed animals were red, swollen, scabbed, dry/flaky skin (also observed in the controls for a shorter period of time), and abraded, thickened, blackened, crusty, cracked skin. Histologically, two 2.0 g/kg males and one 5.0 g/kg female had treatment-related trace to mild hyperkeratosis. The 5.0 g/kg female with hyperkeratosis also had severe dermal necrosis and ulceration in an area where the wrap was secured with tape; it is unclear whether or not these lesions are attributable to the test material (all other females had normal skin and all treated males were normal at the 5 g/kg level) (Report number CEHB 2510).

Table 2.6.2.4.1-2. Signs of toxicity in adult rabbits exposed to a single dermal dose of clethodim (SX-1688).

Observed sign of toxicity	Control [1] Females		Females 5.0 g/kg		Control [1] Males		Males 4.9 g/kg		Control [2] Males		Males 2.0 g/kg	
	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]
Death							1	6				
Reduced food intake	1	14	2	4-14			1	2-death	1	1-8	4	1-8
Skin, red	4	1-5	5	1-14	5	1-14	5	1-8	5	1-14	5	1-14
Skin, swollen			5	1-9	3	1	5	1-6	1	1-2	5	1-9
Skin, abraded							1	2-3				
Skin, scabbed	1	8-13	3	3-14	1	5-10	1	3-6			3	7-14
Skin thickened			2	6-7			2	6-7			2	7-9
Skin, dry/flaky	3	5-8	5	6-14	2	3-14	4	6-13			5	7-14
Skin, blackened/darkened											2	4-9
Skin, crusty											1	11-14
Skin, cracked			4	7-9								
Decreased motor activity							1	2-death				
Unkempt appearance							1	4-death				
No faeces							1	5				
Collapse							1	5				

Observed sign of toxicity	Control [1] Females		Females 5.0 g/kg		Control [1] Males		Males 4.9 g/kg		Control [2] Males		Males 2.0 g/kg	
	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]	N[1]	Days[2]
Diarrhoea							1	6			1	14
Decreased body temperature							1	6				
Ocular discharge - colourless									4	1-6	4	5-13
Nasal discharge - yellow									2	1-6		
Mouth cut/scabbed	1	1-5										

[1] Number of animals displaying the sign of toxicity
[2] Observation interval: the first and last day each observation was made

Repeated dermal exposure (exposed for 6 h on 21 out of 28 days) in rats induced skin irritation at all tested doses (8.3, 83, 832 mg/kg). Skin irritation, dry and flaky skin, and scabs were observed as well as erythema and oedema (table below) (Report number S-2848).

Table 2.6.2.4.1-3. Incidence of skin effects in rats exposed to clethodim (technical) for four weeks.

Dose	Day[1]	Irritation	Dry and/or flaky skin	Scab(s)	Mean score for erythema and edema[2]
Males					
0.0%	0	1/6	0/6	0/6	0.2
	2	2/6	1/6	0/6	0.5
	9	3/6	1/6	0/6	0.5
	16	4/6	2/6	0/6	1.2
	23	4/6	3/6	0/6	1.2
	28	6/6	2/6	1/6	2.7
1.0%	0	2/6	0/6	0/6	0.3
	2	3/6	0/6.	0/6	0.5
	9	6/6	0/6	0/6	1.7
	16	6/6	1/6	0/6	2.0
	23	6/6	5/6	1/6	3.0
	28	6/6	4/6	0/6	4.0
10.0%	0	1/6	0/6	0/6	0.2
	2	4/6	0/6	0/6	0.8
	9	6/6	0/6	0/6	1.3
	16	6/6	1/6	0/6	2.7
	23	6/6	0/6	0/6	3.3
	28	6/6	2/6	0/6	2.7
100.0%	0	6/6	0/6	0/6	2.0
	2	6/6	2/6	0/6	3.7
	9	6/6	0/6	0/6	2.7
	16	6/6	3/6	0/6	4.5
	23	6/6	2/6	0/6	5.7
	28	6/6	4/6	0/6	5.0
Females					
0.0%	0	1/6	0/6	0/6	0.2
	2	2/6	0/6	0/6	0.3
	9	6/6	1/6	0/6	1.3
	16	6/6	3/6	0/6	2.2
	23	5/6	2/6	0/6	2.0
	28	6/6	3/6	0/6	3.0
1.0%	0	0/6	0/6	0/6	0.0
	2	2/6	0/6	0/6	0.5
	9	3/6	0/6	0/6	0.8
	16	6/6	4/6	0/6	3.3
	23	6/6	4/6	1/6	3.0
	28	6/6	3/6	0/6	2.2
10.0%	0	2/6	0/6	0/6	0.3
	2	3/6	0/6	0/6	0.8
	9	6/6	0/6	0/6	1.5
	16	6/6	4/6	0/6	4.3
	23	6/6	5/6	1/6	4.5

	28	6/6	5/6	0/6	3.8
100.0%	0	6/6	0/6	0/6	1.7
	2	6/6	0/6	0/6	2.7
	9	6/6	1/6	0/6	3.8
	16	6/6	3/6	0/6	5.5
	23	6/6	2/6	0/6	5.3
	28	6/6	4/6	0/6	4.7

[1] 30 minutes after removal of the test item/vehicle. Day 0 = first application.
[2] Mean of the sum of scores for erythema and oedema; maximum mean score possible: 8.0

In the skin sensitisation study, effects on the skin after the intradermal injection occurred in both control and tested animals (as expected since this is well-known to occur after intradermal injection of FCA). Effects included erythema, oedema, necrotizing dermatitis, encrustation, and exfoliation of encrustation. After the epidermal injection on day 8, no erythematous or oedematous reaction was observed in the animals in control group treated with PEG 300 only but discrete or patchy (grade 1) erythema was observed in five (at 24 h) and six (at 48 h) out of 10 animals after treatment with the test item at 62.5% in PEG 300. After the skin challenge on day 22, no skin reactions were observed in the control group when treated with the test item 50% in PEG 300 or with PEG 300 alone. In the treated animals, discrete or patchy (grade 1) to moderate and confluent (grade 2) erythema were observed in nine (at 24 h) and eight (at 48 h) out of 10 animals after treatment with the test item at 50% in PEG 300. No skin reactions were observed in the animals when treated with PEG 300 only.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

A substance is irritant to skin when it produces reversible damage to the skin following its application for up to 4 h. According to the CLP Guidance Table 3.2.2, the major criterion for the irritation category is as follows:

Category	**Criteria**
Irritation (Category 2)	(1) Mean score 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 h after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
	(2) 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
	(3) 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
[a)] Grading criteria are understood as described in Regulation (EC) No 440/2008	

In the previous RAC opinion (2015) the irritation properties of the test substance were discussed. The following conclusion was done by RAC (text copied from RAC opinion 2015):

"In the rabbit skin irritation study the scores obtained following 4 h (or less) treatment with clethodim did not meet the criteria for classification as Skin Irrit. 2 (mean value of ≥2.3-≥4.0 for erythema or oedema in at least 2/3 animals from gradings at 24, 48 and 72 h after patch removal). There was no evidence of full thickness destruction of the skin. The effects observed were not sufficiently severe to justify classification. Additionally, all effects were found to be reversible within 9 days and there was no evidence of alopecia, hyperkeratosis, hyperplasia or scaling. Therefore, the data from this study indicate that no classification for skin irritation is warranted.

Labelling phrase EUH066 (Repeated exposure may cause skin dryness or cracking) can be applied to substances which may cause concern as a result of skin dryness, flaking or cracking following exposure but which do not meet the criteria for classification.

In the acute dermal toxicity study with clethodim, there were signs of skin irritation noted during the initial 24 h observation period and flaky, dry and/or reddened skin was observed at termination. In the guinea pig skin sensitisation study, discrete or patchy erythema was noted in 60% of animals 48 h after topical induction. Given these results and the fact that this substance is clearly lipophilic (LogP 4.2), it would seem appropriate to apply EUH066 to clethodim.

Therefore, RAC agrees with the DS that clethodim should not be classified for skin irritation but should bear the supplemental labelling phrase, EUH066".

RMS is of the opinion that this conclusion remains for the renewal of active substance.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed.

Labelling phrase EUH066 ("Repeated exposure may cause skin dryness or cracking") is proposed.

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 30: 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
Primary eye irritation – rabbits No guideline reported. In general compliance with OECD TG 405 (1981). The current guideline (OECD TG 405, 2021) recommends: -efforts for the reduction of pain and refers to integrated testing approaches utilizing alternative in vitro guideline studies for hazard classification that were not available at the time of conduct of this study. -The use of a satellite group to assess the influence of washing is not recommended in the current guideline. - The temperature of the water used for rinsing was not reported. - The guideline recommends an initial test using one individual with the possibility	Rabbit, New Zealand white, Male, 9	RE-45601 (technical) Lot/Batch: SX-1688 Purity: 83.3 % w/w	0.1 mL undiluted test material (all individuals) + rinse with water after 30 seconds of exposure (3 individuals). Eyes were examined at 1, 24, 48, and 72 h post treatment	<u>Treated, rinsed eyes:</u> Moderate conjunctival irritation one h after dosing. Slight conjunctival redness 24 h after dosing. All eyes were clear of irritation after 48 h. Mean scores (1, 24, 48, 72h): Cornea: 0, 0, 0, 0 Iris: 0, 0, 0, 0 Redness: 2, 0.7, 0, 0 Chemosis: 1.3, 0, 0, 0 <u>Treated, unrinsed eyes:</u> Moderate-severe conjunctival irritation one h after dosing. Slight-moderate 24 h after dosing. All eyes were clear of irritation 72 h after dosing.	[redacted] 1986 Report number: CEHB 2511 Vol.3. B.6.2.5/01 New data for renewal: No

of extending the test with more animals: in this study, six animals were used from the start. - Humidity (56.2-71.0%) slightly above recommended value of 70% in the guideline GLP: Yes Acceptable				Mean scores (1, 24, 48, 72h): Cornea: 0, 0, 0, 0 Iris: 0, 0, 0, 0 Redness: 2.3, 1.8, 1.0, 0 Chemosis: 1.5, 0.8, 0, 0	

Table 31: Summary table of human data on serious eye damage/eye irritation.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 32: Summary table of other studies relevant for serious eye damage/eye irritation.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

There are no new data for this endpoint in this report.

One study on male New Zealand white rabbits is available. It was conducted in accordance with the principles of Good Laboratory Practice and following the general guidelines of OECD 405 with exception of some deviations (see Table 30) which are not considered to have a major impact on the study outcome. A volume of 0.1 mL undiluted test item was put in the conjunctival sac of male rabbits, three of which were rinsed with distilled water after 30 seconds of exposure and six of which were not. No corneal opacity or iritis was observed during the study. Conjunctival irritation was observed in both groups after 1 h (grade 1-2 in rabbits which eyes were rinsed with water and grade 1-3 in those whose eyes were not rinsed). The irritation gradually cleared out and neither redness nor chemosis were observed after 48 h (rinsed) or 72 h (unrinsed). The study is acceptable.

Table 2.6.2.5.1: Individual animal scores-average (24-72 h)

	Individual animal scores-average (24-72 h)
Cornea/opacity	0-0-0-0-0-0
Iris	0-0-0-0-0-0
Conjunctiva redness	1-0.33-1-1.33-1-1
Conjunctiva chemosis	0.33-0.33-0.33-0-0.33-0.33

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

Eye irritation means the production of changes in the eye, which are fully reversible, occurring after the exposure of the eye to a substance or mixture. A substance should be classified as an eye irritant if it produces in at least 4 of 6 tested animals a positive response of:

(a) corneal opacity ≥ 1; and/or

(b) iritis ≥ 1; and/or

(c) conjunctival redness ≥ 2; and/or

(d) conjunctival oedema (chemosis) ≥ 2

calculated as the mean scores following grading at 24, 48 and 72 h after instillation of the test material, and which fully reverses within an observation period of normally 21 days.

All effects were reversible within 3 days. No effects on the iris or cornea were observed and the effects on conjunctiva redness and chemosis were below 2. Therefore, clethodim does not meet the criteria for classification as an eye irritant.

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

Clethodim does not fulfil criteria for classification.

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

There are no formally recognised and validated animal or *in vitro* tests for respiratory sensitisation.

Table 33: Summary table of animal studies on respiratory sensitisation.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available					

Table 34: Summary table of human data on respiratory sensitisation.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No specific data available				

Table 35: Summary table of other studies relevant for respiratory sensitisation.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

No information was available on respiratory sensitisation. Clethodim was not classified as acutely toxic via the inhalation route (study summarised in section 2.6.2.3). Furthermore, no medical findings have been reported (refer to section 2.6.9).

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

Not relevant as no data are available.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not relevant as no data are available.

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 36: 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
GPMT (Guinea Pig Maximisation Test) OECD TG 406 (1992) Deviations from guideline: -the temperature used was slightly higher than recommended (22±3ºC vs. 20±3ºC) GLP: Yes Acceptable study	Guinea pig, Dunkin/Hartley Albino, Female, 5/group (control) + 10/group (test item)	Clethodim technical Lot/Batch: 6F57523000 Purity: 92.4%	Intradermal induction: 50% dilution of the test item with PEG 300 and an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. Epidermal induction (for 48 h): 62.5% dilution of the test item. Challenge (2 w after epidermal induction): 50% dilution of the test item.	Grade 1 erythema was observed in 5 and 6 animals 24 and 48 h after the epidermal induction of the test item. Grade 1-2 erythema was observed in 9/10 and 8/10 animals 24 and 48 h after the challenge treatment. No reactions were seen in control animals. *Skin Sens. 1 (H317: May cause an allergic skin reaction).*	[redacted] 2006 Report number: A42210 Vol.3. B.6.2.6/01 New data for renewal: No

Table 37: Summary table of human data on skin sensitisation.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 38: Summary table of other studies relevant for skin sensitisation.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

There are no new data for this endpoint in this report.

The skin sensitisation study (old data) on female Guinea pigs was conducted in accordance with the Principles of Good Laboratory Practice and follows OECD TG 406 except for minor deviations (see Table 36). In this study, 80-90 % of the animals in the test group had skin reactions after the challenge with the test item at a concentration of 50%. The study is acceptable.

Table 2.6.2.7.1: Skin response in female Guinea pigs after challenge application of clethodim technical 50% in PEG 300

Treatment	Number of individuals with skin reactions/ total number of individuals	
	24 h	48 h
Control group		
Clethodim technical 50% in PEG 300	0/5	0/5
PEG 300 only	0/5	0/5
Test group		
Clethodim technical 50% in PEG 300	9/10	8/10
PEG 300 only	0/10	0/10

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

For Category 1, when an adjuvant type test method for skin sensitisation is used, a response of at least 30 % of the animals is considered as positive. Clethodim is therefore classified as a skin sensitiser, Category 1, under Regulation (EC) 1272/2008 (CLP) with the hazed statement H317: “May cause an allergic skin reaction”.

Classification in sub-category 1B is appropriate when ≥30% of the animals produce a positive response following an intradermal dose of 1%. However, as clethodim was not tested at an intradermal dose of less than 50% this cannot be assessed adequately. Thus, no sub-categorisation was proposed.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens. 1. H317: May cause an allergic skin reaction.

2.6.2.8 Phototoxicity

Table 39: Summary table of studies on phototoxicity.

Method, guideline, deviations if any	Test substance	Dose levels duration of exposure	Results	Reference
In vitro 3T3 NRU phototoxicity test Balb/c 3T3 fibroblast cells (clone 31, mouse fibroblast cell line) OECD TG 432 (2004) Deviations from guideline: - The proportions of the components in the cell culture medium differed from those recommended in the guideline, the RMS does not consider this	Clethodim technical Lot/Batch: 4478 Purity: 95.98%	0 (vehicle), 0.316, 1.00, 3.16, 10.0, 31.6, 100, 316, and 1000 µg/mL 60 min exposure + 22 min irradiation	IC50 = 73 (irradiated) and 959.8 (not irradiated) µg/mL PIF: >14	Gijsbrechts 2020 Report number: 20182211 Vol.3. B.6.2.7/01 New data for renewal: Yes

to affect the validity of the study. - The irradiation time was 22 minutes as opposed to the ~50 minutes recommended in the guideline. The time was sufficient to cause phototoxicity and thus this deviation does not affect the validity of the study. GLP Acceptable				

Table 40: Summary table of human data on phototoxicity.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 41: Summary table of other studies relevant for phototoxicity.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.8.1 Short summary and overall relevance of the provided information on phototoxicity

For this section a new phototoxicity study is available. In this study, potential phototoxicity of clethodim was assessed using the Neutral red uptake phototoxicity assay in Balb/c 3T3 mouse fibroblasts. The study was conducted in accordance with the OECD Principles of Good Laboratory Practice and follows OECD TG 432 except for some deviations (see Table 39) which are not considered to have a major impact on the study outcome. Cytotoxicity was observed after treatment with clethodim technical after exposure with UV/Visible light irradiation. In the absence of light irradiation, the test item showed no clear cytotoxicity. The test item showed an IC50 value of 73 µg/mL in the presence of irradiation. No IC50 was reached in the absence of irradiation, resulting in a PIF value of >14. Clethodim technical was shown to be phototoxic.

There are currently no classification and labelling criteria for phototoxicity according to the relevant EU regulation (E.C Regulation 1272/2008).

Table 2.6.2.8.1-1: Overview of the results

Irradiation	Value	Concentration of clethodim (µg/mL)								Vehicle Control	IC_{50} (µg/mL)	PIF
		1000	316	100	31.6	10.0	3.16	1.00	0.316			
Not irradiated (-Irr)	OD540	0.87	0.86	0.89	1.00	1.06	1.07	1.07	1.06	1.05	>1000	>14
	SEM	0.02	0.01	0.01	0.03	0.01	0.02	0.02	0.02	0.01		
Irradiated (+Irr)	OD540	0.06	0.11	0.42	0.75	0.94	0.97	1.01	0.99	0.96	73	
	SEM	0.01	0.04	0.03	0.02	0.03	0.03	0.03	0.02	0.02		

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 42: Summary table of evidence for aspiration hazard.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No specific data available				

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

No specific data available.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

According to regulation (EC) No 1272/2008, an active substance is included in the hazard category (Category 1) for aspiration toxicity: (i) based on reliable and good quality human evidence or (ii) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40°C. The second criterion is related only to liquid substances.

No data are available from humans indicating an aspiration hazard. Clethodim has a kinematic viscosity>20.5 mm^2/s.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

No classification for hazard is proposed.

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 43: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure).

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Acute oral toxicity OECD TG 401 (1981) No deviations noted. Rats (Sprague-Dawley), both sexes, 5 individuals/group GLP: Yes Acceptable	Clethodim technical Lot/Batch: SX-1688 Purity: 83.3%w/w Vehicle: Suspension in 0.7% CMC (carboxymethylcellulose) and 1.0 % TWEEN 80 in distilled water Oral gavage 14 days observation period Males: 1050, 1450, 1860, and 2500 mg/kg	NOAEL: - LOAEL: 800 mg/kg bw (equal to 667 mg/kg bw based on correction for purity using a correction factor of 1.2) Clinical signs were observed from 30 minutes after administration in all dose groups during the first days of the study. All surviving animals appeared normal on day 6. Mortality: Group / Sex / Deaths 800 / F / 0/5 1050 / M+F / 0/10 1450 / M+F / 4/10 1860 / M / 4/5 2000 / F / 5/5 2500 / M / 5/5	[redacted] 1986 Report number: S 2498 Vol.3. B.6.2.1/01 New data for renewal: No

	Females: 800, 1050, 1450, and 2000 mg/kg	LD_{50} values (values corrected for purity): Females: 1133 mg a.s./kg bw Males: 1358 mg a.s./kg bw <u>800 mg/kg (F only):</u> Salivation (5/5) ↓ motor activity (5/5) yellow anogenital stains (5/5) unsteady gait (4/5) reduced food consumption (3/5) hyperreactive (1/5) clonic convulsions (1/5) diarrhoea (1/5) red ocular discharge (1/5) <u>1050 mg/kg bw (both sexes):</u> Salivation (10/10) ↓ motor activity (10/10) yellow anogenital discharge and/or stains (8/10) unsteady gait (9/10) hunched or tremoring gait (2/10) lacrimation (3/10) reduced food consumption (9/10) hyperreactive (8/10) red ocular discharge (1/10) red nasal discharge (1/10) <u>1450 mg/kg (both sexes):</u> Salivation (10/10) ↓ motor activity (9/10) Hyperreactive (8/10) Unsteady gait (7/10) Clonic convulsions (3/10) Collapse (4/10) Lacrimation (4/10) Diarrhoea (3/10) yellow anogenital discharge and/or stains (8/10) Hunched and/or tremoring gait (5/10) Reduced food consumption (7/10) Clear ocular discharge (1/10) Red stained fur on the snout (1/10) Brain: dark gelatinous material beneath the meninges (2/10) Trachea: foam/froth (1/10) Stomach: dark black content (/1/10) Trace gliosis in a single spinal nerve in the lower lumbar area (2 individuals), trace focal vacuolar change was associated with one of these lesions. <u>1860 mg/kg (M only):</u> Salivation (5/5) ↓ motor activity (5/5) Unsteady gait (4/5) Clonic convulsions (4/5) Hyperreactive (4/5) Lacrimation (1/5) Collapse (4/5) Hunched and tremoring gait (1/5) Red stained fur on the snout (1/5) Yellow anogenital stain (1/5) Reduced food consumption (1/5) Brain: dark gelatinous material beneath the meninges (3/5)	

		2000 mg/kg (F only): Salivation (5/5) ↓ motor activity (5/5) Clonic convulsions (4/5) Hyperreactive (2/5) Unsteady gait (2/5) Lacrimation (1/5) Collapse (1/5) Yellow anogenital stain (1/5) Brain: dark gelatinous material beneath the meninges (4/5) Brain: reddened meninges (1/5) Lung: reddened/darkened (4/5) Lung: mottled (2/5) Trachea: foam/froth (3/5) Stomach: white fluid/material (1/5) Kidney: dilated pelvis (1/5) 2500 mg/kg (M only): Salivation (5/5) Hyperreactive (4/5) Decreased motor activity (4/5) Unsteady gait (3/5) Clonic convulsions (1/5) Collapse (2/5) Brain: dark gelatinous material beneath the meninges (4/5) Lung: reddened/darkened (4/5) Trachea: foam/froth (3/5) Stomach: white fluid/material (1/5) Stomach: tan fluid (1/5) Small intestine: tan fluid within (1/5)	
Acute oral toxicity OECD TG 401 (1981) No deviations Mice, CD1, both sexes, 5 of each sex/group GLP: Yes Acceptable	Clethodim technical Lot/Batch: SX-1688 Purity: 83.3%w/w Vehicle: Suspension in carboxymethyl cellulose sodium salt and TWEEN 80 in distilled water Vehicle: Suspension in carboxymethyl cellulose sodium salt and TWEEN 80 in distilled water Oral gavage 14-day observation period 1500 (males only), 2000, 2500, 3000 and 3500 (females only) mg/kg bw	NOAEL: - LOAEL: 1500 mg/kg bw (equal to 1250 mg/kg bw based on correction for purity using a correction factor of 1.2) Clinical signs appeared from 1 h post administration in all dose groups (hypoactivity, rough coat, hunched appearance, ataxia, tremors, salivation, laboured respiration, and soft faeces and urine stains) – all visible clinical signs were subsided by day 6. Mortality occurred at doses ≥ 2000 mg/kg. Slightly dark-red lungs were observed in animals that died during the study	[redacted] 1986 Report number: 2107-143 Vol.3. B.6.2.1/02 New data for renewal: No
Acute dermal toxicity No study guideline was reported. Study was conducted in general compliance with guideline OECD TG 402 (1981). Rabbit, New Zealand White, 5-10 ind./group Males and females	Clethodim technical Lot/Batch: SX-1688 Purity: 83.3%w/w Dermal application 24 h exposure, 14 days observation Females: 5000 mg/kg	NOAEL: - LOAEL: 2000 mg/kg (males) (equal to 1667 mg/kg bw based on correction for impurity using a correction factor of 1.2) Control: red, swollen, scabbed, dry/flaky skin. Ocular and nasal discharge, and reduced food intake. Exposed animals:	[redacted] 1986 Report number: CEHB 2510 Vol.3. B.6.2.2/01

GLP: Yes Acceptable	Males: 2000 and 4900 mg/kg	the same symptoms as the control animals (except nasal discharge) but for a longer period of time. In addition to those symptoms, exposed animals also displayed abraded thickened, blackened, crusty, cracked skin, and diarrhoea. Erythema and oedema, trace to mild hyperkeratosis 1 death (male, 4900 mg/kg)	New data for renewal: No
An Oral (Gavage) Acute Neurotoxicity Study of Clethodim in Rats Guidelines followed: OPPTS 870.6200 (1998) OECD TG 424 (1997) Deviations from current guidelines: None Species: Rat Strain: Charles River CD® (Sprague-Dawley) 3 treatment groups and a control group of 12 rats/sex/group GLP: Yes Acceptable	Clethodim TG Lot/Batch: AS 506r Purity: 95.4% Oral gavage, single dose 15 days Dose: 0, 10, 100 and 1000 mg/kg bw	NOAEL neurotox: 1000 mg/kg bw NOAEL systemic: 100 mg/kg bw <u>Effects at 100 mg/kg bw:</u> - reduced foot splay in males (not statistically significant) <u>Effects at 1000 mg/kg bw:</u> - **clinical signs** (↑ soiled fur on day 0 in females (one of these animals also displayed slight salivation) ↓ transient locomotor activity (total and ambulatory counts) in females (stat. sign. in first 10 min interval). - reduced foot splay in males (statistically significant at day 7)	[redacted], (2012a) Report number: WIL-194041 Vol. 3. B.6.7.1.1 New data for renewal: Yes
Acute inhalation toxicity No guideline reported, in general compliance with OECD 403 (2009). Deviation: animals were older than the recommended age of 8-12 weeks, humidity (71-72%) slightly above recommended value of 70% in the guideline Rat, Sprague-Dawley, both sexes, 5 per sex/group GLP: Yes Acceptable study	Clethodim technical Lot/Batch: SX-1688 Purity: 83.3% w/w aerosol, MMAD = 2.75 µm Inhalation of an aerosol of diluted test material (90% v/v in acetone) 240 min exposure + 14 days observation 3.9 mg/L, 4 h (maximum attainable concentration)	NOAEL: - LOAEL: 3.9 mg/L (equal to 3.25 mg/L based on correction for purity using a correction factor of 1.2) <u>Effects observed at 3.9 mg/L:</u> Salivations and colourless eye discharge. Red nasal discharge, abnormal respiratory sounds, decreased faeces, unkempt appearance, and a yellow/red anogenital discharge. All exposed animals appeared normal within 8 days of exposure. No mortalities occurred.	[redacted] 1986 Report number: CEHB 2513 Vol.3. B.6.2.3/01 New data for renewal: No

Table 44: Summary table of human data on STOT SE (specific target organ toxicity-single exposure).

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No information available				

Table 45: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure).

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vivo chromosome aberration assay Acceptable	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: 0.7% Carboxymethylcellulose (CMC) with 1.0% Polyoxyethylene Sorbitan Mono-oleate (Tween-80)	Male and female Sprague-Dawley rats were treated by gavage with 1.5, 0.50 and 0.15 g/kg bw with RE-45601 Technical (SX-1688) which was given as a single administration.	Five of 20 males and 3 of 20 females that received 1.5 g RE-45601 Technical/kg body weight died prior to their scheduled sacrifice. A reduction in the rates of body weight gain as compared to the vehicle control groups was observed in animals treated with 1.5 g/kg; Clinical signs at 1.5 g/kg included prostration, lethargy, hunching, tremors, lacrimation, excessive salivation, crusty eyes and crusty nose; at 0.5 g/kg, lethargy and excessive salivation and at 0.15 g/kg lethargy only.	[redacted] 1987 Report No.: S-2864 Vol. 3. B.6.4.2.1/01 New data for renewal: No
In vivo UDS assay Supportive	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Carboxymethylcellulose (CMC), high viscosity. Polyoxyethylene Sorbitan Mono-oleate (Tween-80).	Male mice (B6C3F1) were given RE-45601 at doses of 0, 100, 1000 and 5000 mg/kg bw 2 or 16 h before sacrifice (single administration).	Three of the five mice treated with 5000 mg/kg (16 hr) were found dead at the time of their scheduled sacrifice; No abnormal clinical signs were reported for the remaining mice.	[redacted] 1986 Report No: S-2762 Vol. 3. B.6.4.2.2/01 New data for renewal: No

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Oral exposure

Two studies on acute oral toxicity are available (refer to section 2.6.2), one with CD1 mice and another with SD rats. Furthermore, an acute neurotoxicity study in the rat is available (refer to section 2.6.7). This latter study is new data for the renewal of active substance.

In the acute oral toxicity study in CD1 mice, animals were exposed to 2000-3500 mg/kg (females) and 1500-3000 mg/kg (males). Clethodim caused clinical signs such as hypoactivity, rough coat, hunched appearance, ataxia, tremors, salivation, laboured respiration, and soft faeces and urine stains. The effects had subsided by day 6. Mice that died during the study had dark-red lungs and compound-like material in the stomach and intestine.

In the acute oral toxicity study in Sprague-Dawley rats, animals were exposed to 800-2000 mg/kg (females) and 1050-2500 mg/kg (males). All five males of the 2500 mg/kg group, 4/5 males of the 1860 mg/kg bw group, 1/5 males of the 1450 mg/kg group, and all five females of the 2000 mg/kg group died within a day of administration. Three out of five females of the 1450 mg/kg group died within 3 days. Clethodim caused clinical signs such as salivation, decreased motor activity, unsteady gait, hyperreactivity, lacrimation, clonic convulsions, red nasal discharge, ocular discharge, and collapse in almost all treated groups. On day 6 all surviving treated animals appeared normal. Dark red gelatinous material beneath the meninges of the brain occurred in two of ten 1450 mg/kg animals, three of five 1860 mg/kg animals, four of five 2000 mg/kg animals, and four of five 2500 mg/kg animals.

Reddened meninges were noted in one of five 2000 mg/kg animals. No brain abnormalities were noted in the animals exposed to ≤ 1050 mg/kg. Abnormalities noted in other tissues included foam or froth in the trachea (three of five animals exposed to 2000 mg/kg, three of five animals exposed to 2500 mg/kg); mottled and/or darkened, reddened lungs (two of ten controls, four of five animals exposed to 2000 mg/kg, four of five animals exposed to 2500 mg/kg); enlarged adrenals (one of ten animals exposed to 1450 mg/kg); dilated kidney pelvis (one of five animals exposed to 2000 mg/kg); and discoloured fluid in the stomach and/or intestine (one of five animals exposed to 2000 g/kg, two of five exposed to 2500 mg/kg). The two female rats exposed to 1450 mg/kg that survived until termination had trace gliosis in a single spinal nerve in the lower lumbar area.

Clethodim was also administered orally to rats in the acute neurotoxicity study but at lower doses (10-1000 mg/kg) compared with the other two acute oral toxicity studies. No mortalities occurred in this study. Some clinical signs were observed; the incidence of hair loss on forelimbs was increased in animals in the high dose group, and a larger number of females in the highest dose group had soiled fur on study day 0 compared to the control group; one female in the highest dose group with soiled fur also had slight salivation. This was not observed at later time points. The effects observed on the brain in the other acute oral toxicity study were not observed in these rats. There were no significant differences in body weight or body weight gain between the control and test substance-treated groups. Hindlimb splayfoot was decreased in males of the high dose (1000 mg/kg bw) (statistically significant at day 7) and middle dose (100 mg/kg bw) (not statistically significant) group. The motor activity was highly variable within the 10 minute-time intervals and differed largely between individuals and groups at times; however, the cumulative values did not indicate any clear trends in affected motor activity. There was a tendency towards lower activity in females on day 0 (both total and ambulatory activity in the 0–10-minute interval was statistically significantly decreased; ↓16%) but no clear trend was observed. The RMS agrees with the applicant that this may be connected to general toxicity. Soiled fur + slight salivation was observed in one animal. There was no apparent effect on habituation patterns in the treated animals. Therefore, the effects noted in this study are not considered adverse. The NOAEL for neurotoxicity was 1000 mg/kg bw, the highest dose tested. NOAEL for systemic toxicity was considered 100 mg/kg bw based on soiled fur in females and salivation in one animal (1000 mg/kg bw). Although salivation was observed in one animal only, this effect was also observed in acute oral toxicity study (Report No.: S- 2498) in the same strain at 800 mg/kg bw/day, and therefore considered reflecting systemic toxicity rather than neurotoxicity.

In the *in vivo* chromosome aberration test, male and female Sprague Dawley rats were treated with RE-45601 Technical (single administration) by gavage at 150, 500 and 1500 mg/kg bw. Five of 20 males and 3 of 20 females that received 1.5 g RE-45601 Technical/kg body weight died prior to their scheduled sacrifice. A reduction in the rates of body weight gain as compared to the vehicle control groups was observed in animals treated with 1.5 g/kg; male animals in this group lost weight from Day 1 to Day 2 and had not net weight gain from Day 0 (pre-treatment) to Day 2. Clinical signs at 1.5 g/kg included prostration, lethargy, hunching, tremors, lacrimation, excessive salivation, crusty eyes and crusty nose: at 0.5 g/kg, lethargy and excessive salivation and at 0.15 g/kg lethargy only.

In the *in vivo* UDS assay, male mice (B6C3F1) were given RE-45601 at doses of 0, 100, 1000 and 5000 mg/kg bw 2 or 16 h before sacrifice (single administration). Three of the five mice treated with 5000 mg/kg (16 hr) were found dead at the time of their scheduled sacrifice. No abnormal clinical signs were reported for the remaining mice.

Dermal exposure

One study was available on acute dermal toxicity (refer to section 2.6.2) in which rabbits were exposed for 24 h (females: 5000 mg/kg males: 2000 and 4900 mg/kg) and observed for 14 days. Skin irritation occurred in both control and exposed animals, albeit more severe in the latter groups. Control animals displayed red, swollen, scabbed, dry/flaky skin. Other signs in the control groups included ocular and nasal discharge, and reduced food intake. Exposed animals showed the same symptoms as the control animals (except nasal discharge and the mouth cut/scab observed in one individual) but usually for a longer period of time. In addition to those symptoms, exposed animals also displayed other dermal effects (abraded, thickened, blackened, crusty, cracked skin) and diarrhoea. One male was found dead on day 6 and it displayed reduced food intake, decreased motor activity, decreased body temperature, unkempt appearance, diarrhoea, a lack of faeces, and collapse prior to its death. Body weight was not affected.

Respiratory exposure

One acute inhalation study was reported (refer to section 2.6.2), in which 5 rats of each sex were exposed to an aerosol of diluted test material (3.9 mg clethodim/L) for 240 minutes. During the exposure, salivation was observed in three exposed animals and all animals squinted or had closed eyes. Immediately following the exposure, all exposed animals were salivating, and five of ten animals (four males and one female) had a colourless eye discharge. Additional signs of toxicity observed following exposure included red nasal discharge, abnormal respiratory sounds, decreased faeces, unkempt appearance, and a yellow/red anogenital discharge. All exposed animals appeared normal within 8 days of exposure. In the control group, one male was salivating during the first h of exposure. Immediately following the exposure and throughout the 14-day observation period, all vehicle control animals appeared normal. No gross pathologic changes that could be attributed to the exposures were seen at necropsy following a 14-day observation period. No exposure-related histologic changes were observed in the lungs or tracheas of exposed animals.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

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 macroscopic 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)..."*

Route of exposure	Units	Category 1	Category 2
Oral	mg/kg bw	$C \leq 300$	$2000 \geq C > 300$
Dermal	mg/kg bw	$C \leq 1000$	$2000 \geq C > 1000$

Inhalation, vapour	mg/l/4h	C ≤ 10	20 ≥ C > 10

Neurotoxic effects were observed in the acute oral studies. Clinical signs that could indicate neurotoxic effects included salivation, decreased motor activity, unsteady gait, hyperactivity, lacrimation, clonic convulsions. The gross necropsy in the acute oral toxicity study in rats revealed effects on the brain, more specifically red gelatinous material beneath the meninges (≥1450 mg/kg) and reddened meninges (only observed in one animal given 2000 mg/kg). Furthermore, upon histopathological examination, trace gliosis in a single spinal nerve in the lower lumbar area was observed in two females of the 1.45 g/kg dose group which survived until necropsy. No effects were however, considered of concern for a classification as STOT-SE, since increased mortality was observed in the dose range (Cat 2: 2000 ≥ C > 300) relevant for classification with STOT-SE. Thus, the effects observed were covered by the acute oral toxicity classification (for acute oral toxicity classification, please see 2.6.2.3).

In the acute dermal toxicity study in the rat, there was no evidence for specific target organ toxicity at 2000 mg/kg bw. Therefore, no classification was needed for dermal STOT-SE.

In an acute inhalation study in rats, no significant toxicity for classification with STOT-SE was observed up to the maximal attainable concentration of 3.25 mg/L. Therefore, no classification was needed for acute inhalation STOT-SE.

There was no evidence of respiratory tract irritation in the available studies; therefore, classification with STOT-SE Category 3 was not proposed.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Clethodim does not meet the criteria for STOT SE under Regulation (EC) 1272/2008.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE)

Table 46: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure).

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
5-week oral study in rat No guideline stated, in general accordance with OECD 407 (1995) Deviations from OECD 407 (2008): - exposure for 5 weeks, not 4	Clethodim technical Lot/Batch: SX-1653 Purity: 83.4% Vehicle: acetone Via the diet for 5 weeks	NOAEL: 200 ppm (12.5 mg/kg/day) LOAEL: 1000 ppm (65.6 mg/kg/day) <u>Effects at 5 ppm:</u> ↓ erythrocyte count (M: 2% n.s., F: 6%) <u>Effects at 200 ppm:</u> ↓ erythrocyte count (M: 3% n.s., F: 4% n.s.) ↑ platelets (M: 30%)	[redacted] 1986 Report number: S-2720 Vol.3 B.6.3.1/01

- weight of epididymis, thymus, spleen and heart was not determined - blood clotting potential was not measured - functional observations were not performed - histopathology on bone marrow was not performed - humidity (72%) slightly above recommended acceptable value of 70% in the guideline Rat (Strain: Sprague-Dawley® Crl:CD® (SD) BR) 10 of each sex/group GLP: Yes Acceptable	0, 5, 200, 1000, 4000, 8000 ppm (Males: 0, 0.26, 12.5, 65.6, 261 and 515 mg/kg bw/day Females: 0, 0.29, 13.9, 70.6, 291 and 554 mg/kg bw/day)	<u>Effects at 1000 ppm:</u> ↓ erythrocyte count (M: 4% n.s., F: 6%) ↓**haemoglobin** (M: 4%, F: 6%) ↑platelets (M: 36%) ↑ **absolute liver weight** (M: 12%) ↑ **liver weight relative to brain weight** (M: 13%, F: 14% n.s.) - **centrilobular hypertrophy** (M) <u>Effects at 4000 ppm:</u> ↓ food consumption, ↓ body weight (F: 8%) ↓ **body weight gain** (M: 11%, F: 25%) ↓ erythrocyte count (M: 5% n.s, F: 4%) ↓ **haemoglobin** (M: 5%, F: 4% n.s.) ↓ **haematocrit** (M: 4%) ↑ platelets (M: 43%), ↑ uric acid (F: 46%) ↑ **absolute liver weight** (M: 13%) ↑ **liver weight relative to brain weight** (M: 16%, F: 12% n.s.) ↑ **liver weight relative to body weight** (M: 19%, F: 18%) - **centrilobular hypertrophy** (M, F) <u>Effects at 8000 ppm:</u> ↓ food consumption, ↓ **body weight** (M: 13%, F: 16%) ↓ **body weight gain** (M: 28%, F: 44%) ↓ **erythrocyte count** (M: 3% n.s., F: 5%) ↓ **haemoglobin** (M: 7%, F: 7%) ↓ **haematocrit** (M: 6%) ↑ platelets (M: 27%) ↑ **cholesterol** (M: 68%) ↑ uric acid (F: 46%) ↑ **absolute liver weight** (M: 15%, F: 13% n.s.) ↑ **liver weight relative to brain weight** (M: 16%, F: 12% n.s.) ↑ **liver weight relative to body weight** (M: 32%, F: 33%) ↑ relative but not absolute, brain, kidneys, and testes weight - **centrilobular hypertrophy** (M, F)	New data for renewal: No
4-week oral study in mouse No guideline stated, in general accordance with OECD 407 (1995) Deviations from OECD 407 (2008): - clinical and functional observations were not performed - blood clotting potential was not determined - thymus, spleen and heart were not weighed - histopathology on bone marrow was not performed. Mouse (strain: CD-1® (ICR-derived)) 10 of each sex/group	Clethodim technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: acetone Exposed via the diet for 4 weeks. 0, 100, 250, 625, 1500 and 4000 ppm (equal to 0, 11.9, 29.7, 74.4, 179 and 476 mg/kg bw per day)	NOAEL: 250 ppm (29.7 mg/kg bw/day) LOAEL: 625 ppm (74.4 mg/kg bw/day) <u>Effects at 625 ppm:</u> ↓ **haemoglobin** (M: 4%) <u>Effects at 1500 ppm:</u> ↓ **erythrocyte count** (M: 4%) ↓ **haemoglobin** (M: 4%, F: 6%) ↑ **absolute liver weight incl. gallbladder** (M: 13%) ↑ **relative liver weight incl. gallbladder** (M: 14%) <u>Effects at 4000 ppm:</u> ↓ **erythrocyte count** (M: 9%) ↓ **haemoglobin** (M: 8%, F: 6% n.s.) ↓ **haematocrit** (M: 8%) ↑ **absolute liver weight incl. gallbladder** (M 42%, F: 16%) ↑ **relative liver weight incl. gallbladder** (M: 42%, F: 22%)	[redacted] 1986 Report number: S-2733 Vol.3 B.6.3.1/02 New data for renewal: No

GLP: Yes Acceptable		**- hepatic centrilobular hypertrophy** (all males: minimal to moderate, eight females: minimal to slight)	
13-weeks oral study in rat In general accordance with OECD 408 (1998) Deviations from OECD 408 (2018): Parameters/endpoints not examined in this study include: - blood measurements of thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH); - plasma/serum measurements of low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and other hormones (on a case-by-case basis) - weights of prostate and seminal vesicles with coagulating glands as a whole, pituitary and thyroid gland - determination of oestrus cycle stage of all females at necropsy - enumeration of cauda epididymis sperm reserves, sperm morphology or sperm motility (optional) - histopathology of coagulation glands and male mammary glands - sensory reactivity and functional observations were not performed. -the weight of the epididymides, thymus, spleen, heart and uterus - blood clotting potential - histopathology on bone marrow - humidity (78%) above recommended acceptable value of 70% Species: Rat (Strain: Sprague-Dawley® Crl:CD® (SD)BR) Groups: 12 rats/sex/group GLP: Yes Acceptable	RE-45601 (Technical) Lot/Batch: SX-1688 Purity: 84% Vehicle: Acetone Exposure via the diet for 13 weeks + 6-week recovery period <u>Doses:</u> 0, 50, 500, 2500, 5000 ppm/diet (0, 2.3, 25, 134 and 279 mg/kg bw/day for males; 0, 2.8, 30, 159 and 341 mg/kg bw/day for females)	NOAEL: 500 ppm (25 mg/kg bw/day) LOAEL: 2500 ppm (134 mg/kg bw/day) <u>Effects at 2500 ppm, 13 weeks:</u> ↓ food consumption (M, sporadic) ↓ body weight (M: 7%) ↓**bodyweight gain** (M: 10%) ↑ **relative liver weight** (M: 12%, F: 12%) - **hepatic centrilobular hypertrophy** (M: 8/12, F: 2/12) <u>Effects at 5000 ppm, 13 weeks:</u> ↓ food consumption (M, F) ↓ **body weight** (M: 11%, F: 11%) ↓**body weight gain** (M: 18%, F: 24%) ↑ **serum cholesterol** (M: 31%) ↑ **total protein** (M: 5%) ↑ **globulin levels** (M: 9%), ↑ **absolute liver weight** (M: 9% n.s., F: 14%) ↑ **relative liver weight** (M: 26%, F: 28%) ↑ **relative brain weight** (M: 16%, F: 13%) ↑ relative kidney weight (M: 10%, F: 14%) - **hepatic centrilobular hypertrophy** (M 10/12, F: 7/12) <u>Recovery period:</u> Food consumption and body weight gain was reduced during the exposure period but was increased during the recovery period. Final body weight (week 19) was similar between groups except for females of the high dose group (↓7%). The only organ weight that was significantly different after the 6-week recovery period was relative liver weight in females of mid-dose (↑11%) and high (↑13%) dose groups. There were no treatment-related changes present among males and females at the recovery sacrifice. Including no liver hypertrophy.	[redacted] 1986 Report number: S-2765 Vol.3 B.6.3.2/01 New data for renewal: No
90-day oral study in dog In general accordance with OECD 409 (1998) Deviations from OECD 409 (1998): - the weight of the epididymides, thymus, spleen	RE-45601 (Technical) Lot/Batch: SX-1688 Purity: 83.3% Oral in gelatine capsules for 13 weeks	NOAEL: 25 mg/kg bw per day (equal to 21 mg/kg bw/day based on correction for purity) LOAEL: 75 mg/kg bw per day (equal to 62 mg/kg bw/day based after correction for purity) <u>Effects at 75 mg/kg bw/day:</u> ↑ **absolute liver weight** (M: 16% n.s., F: 15% n.s.)	[redacted] 1987 Report number: S-2759 Vol.3 B.6.3.2/02

and uterus were not determined. - histopathology on the bone marrow was not performed. Species: Dogs Strain: Beagle Group: 4/sex/group GLP: Yes Acceptable study	Doses: 0, 1, 25, 75, and 125 mg/kg bw/day (0, 0.83, 21, 62 and 104 mg/kg bw/day when corrected for purity)	↑ **relative liver weight** (M: 12% n.s., F: 6% n.s.) ↑ **cholesterol** (F) (Day 91: ↑32% n.s., Day 55: ↑39%, Day 35: ↑42%) Effects at 125 mg/kg bw/day: ↑ **alkaline phosphatase** (increasing over time, M: 67% n.s., F:88%) ↑ **cholesterol** (F) (Day 91: ↑57% n.s., Day 55: ↑40%, Day 35: ↑58%) ↑ **globulin** (M: 22%) ↓ **albumin/globulin** (M: 21%) ↑ **absolute liver weight** (M: 34%, F: 30%) ↑ **relative liver weight** (M: 27% n.s., F: 19% n.s.) - **increased severity of centrilobular vesicles/vacuoles** (M, F)	New data for renewal: No
One-year oral study in dog U.S. Environmental Protection Agency (1982). Pesticide Assessment Guidelines – Subdivision F- Hazard Evaluation: Human and Domestic Animals. In general accordance with OECD 452 (1998). Deviations from OECD TG 452 (2018): - no histopathologic evaluation of the harderian gland and lacrimal gland - ornithine decarboxylase was not determined. - the temperature and humidity varied greatly and were outside of the recommended range Species: Dogs Strain: Beagle Group: 6/sex/group GLP: Yes Acceptable	RE-45601 (Technical) Lot/Batch: SX-1688 Purity: 83.3% Oral in gelatine capsules for 52 weeks Doses: 0, 1, 75 and 300 mg/kg bw per day (equal to 0, 0.83, 62 and 250 mg/kg bw per day after correction for purity of test substance using a correction factor of 1.2) Note - the high dose group received 200 mg/kg/day the first 7 weeks and 300 mg/kg bw/day the remainder of the exposure period	NOAEL: 1 mg/kg bw/day (equal to 0.83 mg/kg bw/day after correction for purity) LOAEL: 75 mg/kg bw/day (equal to 62 mg/kg bw/day after correction for purity) Effects at 1 mg/kg bw/day: ↑ absolute thyroid/parathyroid weight (M: 22% n.s.) ↑ relative thyroid/parathyroid weight (M: 33% n.s.) Effects at 75 mg/kg bw/day: ↑ **absolute liver weight** (M: 27% n.s., F: 34%) ↑ **relative liver weight** (M: 16%, F: 25%) ↑ absolute thyroid/parathyroid weight (M: 45% n.s.) ↑ relative thyroid/parathyroid weight (M: 33% n.s.) ↑**WBC** (Day 360: F: 41% n.s., Day 180: 22% n.s., Day 90: 27%) ↑ **platelet count** (M: 20% n.s., F: 39%) ↓ A/G Ratio (9% n.s.) ↓ **glucose** (M: 8% n.s., F: 9%) -**histopathological changes in the sternal bone marrow** (hyperplasia (males: 1/6, females: 1/6)) Effects at 300 mg/kg bw/day: ↑ **absolute liver weight** (M: 56%, F: 70%) ↑ **relative liver weight** (M: 60%, F: 75%) ↑ **absolute thyroid/parathyroid weight** (M: 91%) ↑ **relative thyroid/parathyroid weight** (M: 100%) ↑ **platelet count** (M: 69%, F: 104%) ↓ **erythrocytes** (M: 9%, F: 18%) ↓ **haemoglobin** (M: 8% n.s., F: 14%) ↓ **haematocrit** (M: 8%, F: 14%) ↑ **reticulocytes** (F: Day 360: 233% n.s., Day 180: 350% on day 180) ↑ **WBC** (Day 360: M: 35% n.s., F: 60%; Day 180: M: 28%, F: 28%; Day 90: M: 23% n.s., F42%) ↓ **A/G Ratio** (M: 14% n.s., F: 26%) ↓ glucose (M: 12% n.s., F: 13%) ↑ **ALK** (increasing over time, M: 273%, F: 341%)	[redacted] 1988 Report number: S-2964 Vol.3 B.6.3.2/03 New data for renewal: No

		↑ **cholesterol** (M: 32%, F: 61%) ↑ **triglycerides** (M: 65%, F:84%) ↑ **ALT** (M: 167%, F: 144%) **-macroscopical changes in the liver** (enlarged liver (2M, 2F) and dark liver (4M, 4F) **-histopathological changes in the liver** (hepatocyte hypertrophy (males: 5/6, females: 4/6), hepatocyte pigment (males: 6/6, females 6/6)) **-histopathological changes in the sternal bone marrow** (hyperplasia (males: 6/6, females: 6/6))	
Four-week dermal study in rat OECD TG 410 (1981) Deviations from current guideline: - some of the suggested serum measurements were not performed (ornithine decarboxylase, gamma glutamyl transpeptidase, hormone levels, methaemoglobin, cholinesterase activity) - 2 days acclimation period instead of 5 Species: Rat Strain: Sprague-Dawley® Crl:CD® BR Group: 6/sex/group GLP: yes Acceptable	RE-45601 (Technical) Lot/Batch: SX-1688 Purity: 83.2% Vehicle: 0.7% carboxymethyl cellulose (CMC) and 1.0% TWEEN 80 in distilled water 21 six-h dermal applications over a 28-day period <u>Doses:</u> 0, 10, 100 and 1000 mg/kg bw/day (equal to 0, 8.32, 83.2, and 832 mg/kg bw/day after correction for purity of test substance using a correction factor of 1.2)	NOAEL for local effects: <10 mg/kg bw/day (equal to <8.32 mg/kg bw/day after correction for purity of test substance using a correction factor of 1.2) LOAEL for local effects: 10 mg/kg bw/day (equal to 8.32 mg/kg bw/day after correction for purity of test substance using a correction factor of 1.2) NOAEL for systemic toxicity: 100 mg/kg bw/day (equal to 83.2 mg/kg bw/day after correction for purity of test substance using a correction factor of 1.2) LOAEL systemic: 1000 mg/kg bw/day (equal to 832 mg/kg bw/day based on correction for purity using a correction factor of 1.2) <u>Effects at 10 mg/kg bw/day:</u> Skin irritation ↑ triglyceride levels (F: 40%, n.s.) <u>Effects at 100 mg/kg bw/day (equal to 83.3 mg/kg bw/day after correction for purity pf test substance using a correction factor of 1.2):</u> Skin irritation ↑ triglyceride levels (F: 140%) ↓ BUN/creatinine ratio (M: 22%, F: 9% n.s.) <u>Effects at 1000 mg/kg bw/day (equal to 833 mg/kg bw/day after correction for purity of test substance using a correction factor of 1.2):</u> Skin irritation -clinical signs (anogenital discharge in all males (6 animals) and two females) ↓ food efficiency (M during Weeks 1-2) ↓ **body weight gain** (M: 35%) ↑ **absolute liver weight** (F: 20%) ↑ relative liver weight (F: 22%) ↑ **liver weight relative to brain weight** (F: 24%) ↑ **triglyceride levels** (F: 160 %) ↓ **BUN** (M: 22%, F: 20% n.s.) ↓ **BUN/creatinine ratio** (M: 32%, F: 21% n.s.) ↓ chloride (M: 3%, F: 3%, both within HCD) ↑ relative weight of kidneys (M: 10%) ↑ relative testes weight (M: 13%)	[redacted] J.H. 1987 Report number: S-2848 Vol.3 B.6.3.3/01 New data for renewal: No

Combined Chronic Oral Toxicity/ Oncogenicity Study in Rats OECD TG 453 (1981) Deviations from current OECD 453 (2018): - prothrombin time and activated partial thromboplastin time were not measured - weight of thyroid, epididymis, heart, spleen, and uterus were not measured - coagulating gland, vagina, and lacrimal gland were not fixed and/or examined - the humidity varied a lot and was outside of the recommended range Species: Rat Strain: Sprague-Dawley® Crl:CD® BR Group: 65/sex/group 10 animals/sex/group were sacrificed at interim sacrifice (1 year) GLP: Yes Acceptable	RE-45601 Technical Lot/Batch: SX-1688 Purity: ~83% Vehicle: Acetone <u>Doses:</u> 0, 5, 20, 500, 2500 ppm (equivalent to 0, 0.15, 0.57, 16 and 86 mg/kg bw/day (♂) and 0, 0.2, 0.72, 21 and 113 mg/kg bw/day (♀)) Oral exposure via the diet Duration of exposure: 104 weeks	NOAEL: 500 ppm (16 mg/kg bw/day) LOAEL: 2500 ppm (86 mg/kg bw/day) <u>Effects at 500 ppm:</u> ↑ relative liver weight after 1 y (F: 18% n.s.) and after 2 y (F: 12% n.s.) ↑ liver weight relative to brain weight after 1 y (F: 24%) <u>Effects at 2500 ppm:</u> ↓ body weight (At Day 91: M: 7%, F: 6%; At Day 360: M: 7%, F: 8%; At Day 724: M: 8% n.s., F:13% n.s) ↓ **bodyweight gain** calculated for the first 3 months (M:11%, F: 12%) ↓ food consumption at intervals during the study (M, F) ↓ food efficiency during the first three months (M) ↑ **absolute liver weight** after 1 y (M: 15% n.s., F: 24%) but not 2 y ↑ **relative liver weight** after 1 y (M: 22%, F: 18% n.s.) and after 2 y (F: 21%) ↑ **liver weight relative to brain weight** after 1 y (M: 16% n.s., F: 23%) but not 2 y - **hypertrophy in hepatocytes** (after 1 year: 1 M and 3 F, none in the control; after 2 years: 1 M and 2 F in this dose group vs 1 F in the control) - **binucleated cells in the liver** after 1 y (6 F vs 1 in the control) but not after 2 y - ↑**chronic pancreatitis** (F: 15 animals compared to 4 animals in the control group) (unclear relevance)	[redacted] 1988a Report number: S-2766 Vol. 3. B.6.5/02 New data for renewal: No
Chronic Oral Oncogenicity Study in Mice Guidelines followed: OECD 451 (1981) Deviations from OECD 451 (2018) Organs not harvested/assessed: coagulating gland, lacrimal gland, mammary glands from males (note that this is only required if visibly dissectible, no information on this) Species: Mouse Strain: CD-1 60 animals per sex and dose level GLP: Yes Acceptable	Chevron RE-45601 Technical Purity: 83.3% Vehicle: Acetone <u>Doses:</u> 0, 20, 200, 1000, 2000/3000* ppm (equal to 0, 2.4, 24, 119 and 238/357 mg/kg bw/day after correction for purity of test substance) *Mice in the highest dose group received 2000 ppm the first 15 weeks. Thereafter 3000 ppm Oral exposure (via the diet) Duration of exposure: 52 weeks (10 mice/group) or 78 weeks	NOAEL: 200 ppm (24 mg/kg bw/day) LOAEL: 1000 ppm (119 mg/kg bw/day) <u>Effects at 1000 ppm:</u> ↑ **absolute liver weight** at week 53 (M: 12% n.s.) ↑ **relative liver weight** (Week 53: M: 17%) ↑ liver weight relative to brain weight (Week 53: M: 15%) - **histopathological changes in the liver** (centrilobular hypertrophy (M, F), increased pigment (F), and bile duct hyperplasia (M)) - **histopatohological changes in the lung** (foci of amphophilic alveolar macrophages (M, F) <u>Effects at 2000/3000 ppm:</u> ↑ **mortality** (M: 68% vs 42% in the control, F: 52% vs 33% in the control) ↑ **absolute liver weight** at week 53 (M: 16%, F: 16% n.s.) and at week 79 (M: 12% n.s., F: 12% n.s.) ↑ **relative liver weight** at Week 53 (M: 27%, F: 28%) and at week 79 (M: 13% n.s., F: 16%) ↑ **liver weight relative to brain weight** at Week 53 (M: 21%, F: 18%) and at week 79 (M: 15% n.s., F: 20%) - macroscopical changes in the kidney (pale, in animals dying or sacrificed due to moribund status)	[redacted] (1988) Report number: S-2867 Vol. 3. B.6.5/01 New data for renewal: No

		- **histopathological changes in the liver** (centrilobular hypertrophy (M, F), increased pigment (M), and bile duct hyperplasia (M)) - **histopathological changes in the lung** (foci of amphophilic alveolar macrophages in the lung (M, F)) ↓ **erythrocytes** (Week 27: M: 8%, F:5%; Week 53: M:19% n.s., F: 8% n.s.; Week 79: M: 14%) ↓ **haematocrit** (Week 79: M: 12% n.s.; Week 27: M: 8%) ↓ **haemoglobin** (Week 79: M: 12% n.s,; Week 27: M:7%) ↑ **incidence of systemic amyloidosis** in animals that died/was sacrificed due to a moribund state (M: 42% vs 28% in the control, F: 36% vs 22% in the control) There was an increased incidence of lung adenomas and carcinomas in the treated males relative to control males. The incidence of these tumours for unscheduled deaths and terminally sacrificed animals was 8, 16, 20, 22 and 22% for males in groups treated with 0, 20, 200, 1000, and 2000/3000 ppm, respectively. The incidence was also higher in control females (16%) compared with control males. These values were all within the historical control range: the means in the historical control mice were 14.9% (range: 5.5-26.5%) and 10.2% (range: 4.0-18.4%) in males and females, respectively.	
Pilot Teratology Study in Rats with Chevron RE-45601 Technical Guidelines followed: Not a guideline study Major deviations from a full OECD TG 414 (2018): - ten dams/group, TG recommends 20 to achieve at least 16 animals with implantation sites. - the exposure period ended at day 15 instead of the day prior to termination (day 19). - anogenital distance in foetuses not investigated, thyroid weight, thyroid histopathology, and blood thyroid hormone levels (T4, T3 and TSH) in the maternal animals not investigated. - it is noted that there were indications of SDA viral infections in some dams at gestation day 20. This was noted in 1, 2, 2, 3, and 2 females in the 0, 50, 150, 300, and 500 mg/kg bw/day group, respectively. Species: Rat Strain: CD® Sprague-Dawley	RE-45601 Technical Purity: 83.3% Exposure: Oral gavage, single daily dose on gestational days 6-15 <u>Doses:</u> 0, 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw per day, after correction for purity)	No NOAEL was set in study* <u>Effects at 300 mg/kg bw/day (250 mg/kg bw/day after correction for purity of test substance):</u> - **clinical signs** (excessive salivation, 4 of 10 dams) ↓ **pup weight** (7%, not statistically significant) <u>Effects at 500 mg/kg bw/day (417 mg/kg bw/day after correction for purity of test substance):</u> - **clinical signs** (excessive salivation, 8 of 10 dams) ↓**body weight** (Day 20: ↓10%, n.s.) ↓ **bodyweight gain** (Day 15-20: ↓38.8%; Day 6-20: ↓62.5%) ↓ number of implantation sites (87 versus 126 in control, n.s.) ↑ pre-implantation loss ratio (0.289 versus 0.082 in control, n.s.) ↓ total number of viable foetuses (86 versus 122 in control, within historical controls) ↓ **foetal weight** of viable foetuses (↓11%) This study was used to determine dose levels in Schroeder 1987 It was noted that there were indications of SDA viral infections in some dams at gestation day 20 which restricts the reliability of the study. This was noted in 1, 2, 2, 3 and 2 females in the 0, 50, 150, 300 and 500 mg/kg bw/day groups, respectively	[redacted] (1986) Report number: S-2807 Vol. 3. B.6.6.2.1/01 New data for renewal: Yes

10 mated females per group GLP Supportive data			
Teratology Study in Rats Guidelines followed: EPA/FIFRA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation (October 1982) Deviations from current OECD 414 (2018): The following endpoints were not assessed: - anogenital distance in foetuses - thyroid weight, thyroid histopathology, and blood thyroid hormone levels (T4, T3 and TSH) in the maternal animals. The exposure period ended at day 15 instead of the day prior to termination (shorter exposure period). Species: Rat Strain: Crl:CD® (COBS) 4 treatment groups consisting of 25 rats each Evaluated and accepted in the DAR (2005) GLP Acceptable study	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Carboxymethyl cellulose, Tween 80 aqueous suspension Exposure: Oral gavage, single daily dose on gestational days 6-15 <u>Doses:</u> 0, 10, 100, 350 and 700 mg/kg bw per day (equal to 0, 8.3, 83.3, 292 and 583 mg/kg bw per day, after correction for purity)	NOAEL maternal and developmental toxicity: 100 mg/kg bw/day (83.3 mg/kg bw/day after correction for purity of test substance) LOAEL maternal and developmental toxicity: 350 mg/kg bw/day (292 mg/kg bw/day after correction for purity of test substance) <u>Effects at 350 mg/kg bw/day (292 mg/kg bw/day after correction for purity of test substance):</u> - **clinical signs** (excessive salivation, poor condition, red nasal discharge, alopecia, staining ano-genital area) ↓ body weight (GD 20: 7%; GD20 corrected value: 6%) ↓ **bodyweight gain** (GD 6-15: 15% n.s., GD 15-20: 17%, GD 0-20 corrected value: 77%) ↓ absolute uterine weight (10% n.s.) ↓ foetal weight (11%) ↑ **skeletal variations** (incomplete or unossified vertebrae, unossified 5th and/or 6th sternebrae) (foetal:88.8% compared to 72.6% in control) <u>Effects at 700 mg/kg bw/day (583 mg/kg bw/day after correction for purity of test substance):</u> - **mortality** (5 females died at GD 11-16) - **clinical signs** (excessive salivation, excessive lacrimation, red/mucoid nasal discharge, alopecia, staining ano-genital area, chromodacryorrhea) ↓ body weight (GD 20: 6-8%; GD 20 corrected value: 13%) ↓ **bodyweight gain** (GD 6-15: 40%, GD 15-20: 17%, GD 0-20 corrected value: 11%) ↓ food consumption at GD 7, 8, 9, 10 (24-31%) ↓ absolute uterine weight (27%) ↑ resorptions (1.9 n.s. vs 0.8 in control) ↑ resorptions per implant (0.13 n.s. vs 0.05 in control) ↓ number of litters with viable foetuses (18 vs 25 in control within HCD) ↑ **external malformations** (foetal: 4% compared to 0% in control; litter: 33.3% compared to 0% in control) ↑ **skeletal variations** (incomplete or unossified sacral and caudal vertebrae and unossified 5th and/or 6th sternebrae) (96.4% compared to 72.6% in control) ↑ **skeletal malformations** (foetal: 6.4% n.s. compared to 5.4% in control; litter: 22.2% n.s. compared to 16% in control) (observations generally restricted to foetuses noted externally with tail defects, 7 foetuses) ↑**visceral malformations** (foetal: 3.4% compared to 0% in control; litter: 16.7% compared to 0% in control). Distortion of the	[redacted] (1987) Report number: S-2808 Vol. 3. B.6.6.2.2/01 New data for renewal: No

		cerebral hemisphere and an opening in the cranium were seen in one foetus with exencephaly, dissimilar aortic arch defects were observed in two foetuses, one with short tail, absence of the kidney and ureter, bladder and a defect of the large intestine were observed in one foetus that was tailless, oedematous and had an imperforate anus. ↓ **foetal weight** (25%) *STOT-RE 2: H373 ("May cause damage to organs through prolonged or repeated exposure").* Classification with STOT-RE 2 proposed due to mortalities seen in dams.	
Teratology Study in Rabbits (dose range finding study) Guidelines followed: 40 CFR 158.135, Pesticide Assessment Guideline No.83-3 Deviations from current OECD TG 414: Major deviations from a full OECD 414 (2018): - eight dams/group, TG recommends 20 to achieve at least 16 animals with implantation sites. - the exposure period ended at day 19 instead of the day prior to termination (day 28). Species: Rabbit Strain: New Zealand White SPF 4 groups of 8 rabbits each GLP Supportive	Chevron RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Aqueous 0.7% carboxy-methyl cellulose (w/v) and 0.5% Tween 80 (w/v) solution Exposure: Gavage. Single daily dose on gestational day 7-19 <u>Doses:</u> 0, 50, 150, 300 or 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw/day, after correction for purity of technical substance using a correction factor of 1.2)	No NOAEL was set in study* <u>Effects at 50 mg/kg bw/day (equal to 41.7 mg/kg bw/day after correction for purity of test substance):</u> Tendencies of ↓ food consumption during the later stage of the dosage period, and dried faeces (one animal) – the effects were not statistically significant. Considered treatment related but not adverse. <u>Effects at 150 mg/kg bw/day (equal to 125 mg/kg bw/day after correction for purity of test substance):</u> - clinical signs (increased incidence of dried faeces, n.s.) ↓ **body weight gain** day 7-20 (+0.02 kg vs +0.2 kg in the control) ↓ food consumption during the later stage of the dosage period (day 13-20) (n.s.) <u>Effects at 300 mg/kg bw/day (equal to 250 mg/kg bw/day after correction for purity of test substance):</u> - **mortality** (2/7) - clinical signs (increased incidence of dried faeces) ↓ **body weight** (Day 20: 11%) ↓ **body weight gain** (Day 7-20: -0.31 kg vs +0.2 kg in the control, n.s.) ↓ food consumption during the dosage period and some days after (day 7-24) followed by an increase compared with the control (n.s) ↑ absolute liver weight (19% n.s.) ↑ relative liver weight (23% n.s.) ↑ **resorptions** (1.4 vs 0.3 in the control, i.e. 2/5 vs 1/7 in the control) -hairball in stomach (observed in 2 rabbits that died) ↓ **foetal body weight/litter** (13%) <u>Effects at 500 mg/kg bw/day (equal to 417 mg/kg bw/day after correction for purity of test substance):</u> - **mortality** (2/7) - clinical signs (increased incidence of dried faeces) ↓ **body weight** (Day 16:15%, Day 20: 22%)	[redacted] G.E., (1986) Report number: S-2734 Vol. 3. B.6.6.2.3/01 New data for renewal: Yes

		↓ **body weight gain** day 7-20 (-0.72 kg vs +0.2 kg in the control) ↓ food consumption during the dosage period (day 7-24) with a post dosage increase compared with the control ↑ absolute liver weight (20% n.s.) ↑ relative liver weight (19% n.s.) - **gastric ulceration** (observed in 3 of 4 rabbit that aborted and/or died) -hairball in stomach (observed in 2 or 4 rabbits that aborted and/or died) - abortions (4 vs 0 in the control) - **premature delivery** (1 individual) ↓ **foetal body weight/litter** (32%)	
Developmental toxicity study in rabbits Guidelines followed: Teratogenicity 40 CFR 158.135, Pesticide 1Asessment Guideline 83-3 Deviations from OECD 414 (2001; the 2018 update is not applicable to rabbits): the exposure period ended at day 19 instead of the day prior to termination (shorter exposure period). Species: Rabbit Strain: New Zealand White SPF 19-20 animals/group GLP Acceptable	Chevron RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Exposure: Gavage. Single daily dose on gestational day 7-19 <u>Doses:</u> 0, 25, 100 and 300 mg/kg bw per day (equal to 0, 20.8, 83.3 and 250 mg/kg bw/day after correction for purity)	NOAEL maternal: 25 mg/kg bw/day (20.8 mg/kg bw/day, corrected for purity) NOAEL developmental: 100 mg/kg bw per day (83.3 mg/kg bw/day, corrected for purity) LOAEL maternal: 100 mg/kg bw per day (83.3 mg/kg bw/day, corrected for purity) LOAEL developmental: 300 mg/kg bw per day (250 mg/kg bw/day, corrected for purity) <u>Effects observed at 100 mg/kg bw per day (83.3 mg/kg bw/day, corrected for purity):</u> - clinical signs (dried faeces, red substance in pan) ↓ **body weight gain** during the dosage period, day 7-20 (+0.05 kg vs +0.18 kg in the control, n.s.) ↓ food consumption during both the dosage period,day 7-20 (15% n.s.) and during the post-dosage period (10% n.s.) <u>Effects observed at 300 mg/kg bw per day (250 mg/kg bw/day, corrected for purity):</u> - clinical signs (dried faeces, red substance in pan) ↓ **body weight gain** during the dosage period, day 7-20 (-0.10 kg vs +0.18 kg in the control), followed by a ↑ in the post-dosage period, day 20-29 (+0.24 kg vs +0.09 kg in the control) ↓ food consumption during the dosing period, day 7-20 (28%) followed by an ↑ in the post-dosage period, day 20-29 (11%) ↓ absolute uterine weight (10% n.s.) ↑ **foetal incidence of angulated hyoid alae** (6.3% vs 1.4% in the control), **misaligned sutures** (fontanelle; 3.6 % vs 0% in the control), and **nasal irregular ossification** (6.3% vs 2.2% in the control)	[redacted] G.E., (1987) Report number: S-2869 Vol. 3. B.6.6.2.4/01 New data for renewal: No
Rat Reproduction Study (dose range finding study) Guidelines followed: 40 CFR 158.135, Pesticide Assessment Guideline No.83-4 Species: Rat Strain: Albino Crl: CD Sprague-Dawley	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Acetone	No NOAEL was set in study* <u>Parental effects:</u> <u>2000 ppm:</u> No treatment related effects <u>5000 ppm:</u> ↓ food consumption during the first week (pre-mating) (M: 15%)	[redacted]., (1986) Report number: S-2758 Vol. 3. B.6.6.1/01

P generation: 8 males and 8 females per group Major deviations from OECD 416 (2001): • treatment initiated one week before mating rather than 10 weeks before mating • only one generation, F0 dams and F1 pups terminated on lactation day 7 • low number of females (8), GL recommends use of sufficient number of animals to yield preferably not less than 20 pregnant females at or near parturition. • oestrous cycle length and normality not investigated • testis and epididymis weight not investigated • sperm motility and sperm morphology not analysed • total number of homogenisation-resistant testicular spermatids and cauda epididymal sperm not enumerated • physical development of the offspring not investigated • haematological and clinical parameters not investigated, organ weights not recorded, histopathological investigations not made • less number of observation points GLP: Yes Supportive	Dietary exposure from 1 week before mating until day 7 of lactation. <u>Doses:</u> 0, 500, 2000, and 5000 ppm (equal to 0, 25, 100 and 250 mg/kg bw/day using a default value of 0.05 for chronic rat studies as recommended by EFSA guidance on selected default values (EFSA Journal 2012;10(3):2579)) Values corrected for purity of test substance using a correction factor of 1.2): 0, 20.8, 83.3, 208.3 mg/kg bw/day	↓ **body weights** during week 0-2 of the study (M: 2%)), or gestational day 20 (F: 13%), lactational day 0 (F: 14%), and lactational day 7 (F: 16%) ↓ **bodyweight gain** during week 0-3 (M: 18%) or week 0-1 (F: 63%) <u>Offspring effects:</u> <u>500 ppm</u>: ↓**combined pup weight** on day 7 (9%) ↓ **combined pup weight gain** between day 0 and 7 (13%) <u>2000 ppm:</u> ↓**combined pup weight** on day 7 (9%) ↓ **combined pup weight gain** between day 0 and 7 (14%) <u>5000 ppm:</u> ↓**combined pup weight** on day 7 (11%) ↓ **combined pup weight gain** between day 0 and 7 (16%) <u>Comment:</u> The reduced food consumption (observed in both sexes but only significant in males) could be a result of reduced palatability of the food containing the test item. The observed parental effects, which mainly included reduced body weights, could at least in part be attributable to the reduced food intake.	New data for renewal: Yes
Two Generation (One Litter) Reproduction Study in Rats Guidelines followed: Reproductive and Fertility Effects 40 CFR 158.135, Pesticide Assessment Guideline 83-4 Deviations from OECD 416 (2001): - no analysis of sperm parameters - developmental and functional observations of pups were not performed - weighing of adrenals, brain, liver, pituitary gland, spleen, thyroids were not performed - histopathology of the vagina was not performed - dosing before mating period seems to be 9 weeks (the guideline recommends dosing to be continued for at least 10	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Acetone 10 ml acetone/kg food Exposure: The F0 males and females received the test material via the diet throughout pre-mating, mating, gestation, and lactation F1a indirect exposure in utero and through nursing, and direct exposure from weaning to pre-mating, mating, gestation, and lactation. F2 indirect exposure in utero and through nursing <u>Doses:</u> 0, 5, 20, 500 and 2500 ppm (equal to 0, 0.5, 1.2, 32.2 and 163 mg/kg bw/day for	NOAEL parental toxicity: 500 ppm (32.2 mg/kg bw/day) NOAEL offspring toxicity: 500 ppm (32.2 mg/kg bw/day) NOAEL reproductive toxicity: 2500 ppm (163 mg/kg bw/day) LOAEL parental toxicity: 2500 ppm (163 mg/kg bw/day) LOAEL offspring toxicity: 2500 ppm (163 mg/kg bw/day) LOAEL reproductive toxicity: Not determined. <u>Effects at 2500 ppm:</u> <u>F0 adults</u> ↓ food intake (during a few days) ↓ body weight (M: 4-9%) ↑ relative testis weight (10%)	[redacted] (1987) Report number: S-2778 Vol. 3. B.6.6.1/02 New data for renewal: No

weeks before the mating period) Species: Rat Strain: Albino Crl: COBS/ CD Sprague-Dawley F0 generation: 30 males and 30 females per group F1 generation: 30 males and 30 females per group GLP: Yes Acceptable	males; 0, 0.5, 1.5, 37.4 and 181 mg/kg bw/day for females in the pre-mating period after correction for purity as calculated by the applicant)	 <u>F1 adults</u> ↓ food intake ↓ **body weight** (M: 10-19%, F: 6-10%) ↓ absolute prostate and seminal vesicles weight (25 and 11%, respectively), unclear relevance ↑ relative weight of the left epididymis (18%) <u>F1 pups:</u> slightly increased number of stillborn pups (unclear relevance) <u>F2 pups:</u> no effects	
A 28-Day Dietary Dose Range-Finding Neurotoxicity Study of Clethodim in Rats Guidelines followed: None (dose range finding study) Deviations from 424 (1997): fewer animals, no histopathological examination, FOB performed only during week 3, haematology and clinical biochemistry parameters were not assessed. Species: Rat Strain: Crl:CD(SD) (Sprague-Dawley) 3 treatment groups and a control group of 5 rats/sex/group GLP Supportive	Clethodim TG Purity: 95.4% Exposure via the diet Dose: 0, 500, 1500 or 5000 ppm (equal to 0, 45, 132, and 441 mg/kg/day for ♂, 0, 51, 155, and 475 mg/kg bw per day for ♀)	No NOAEL was set in study* <u>Effects at 5000 ppm (441 mg/kg bw/d ♂ and 475 mg/kg bw/d ♀):</u> ↓ mean body weights (M: 15%, F: 5%) ↓ mean body weight gain, day 0-28 (M: 30%, F: 21% n.s) ↓ absolute brain weight (M: 4%) No treatment related notes were made during the gross necropsy which is the only endpoint assessed that is relevant for STOT-RE.	[redacted] (2012b) Report number: WIL-194039 Vol. 3. B.6.7.1.2 New data for renewal: Yes
A 90-Day Oral Dietary Neurotoxicity Study of Clethodim in Rats Guidelines followed: OPPTS 870.6200 (1998) Deviations from OECD 424 (1997): None Species: Rat Strain: Crl:CD(SD) (Sprague-Dawley) 3 treatment groups and a control group of 12 rats/sex/group GLP Acceptable study	Clethodim TG Lot/batch: AS 506r Purity: 95.4% Exposure via the diet Dose: 0, 500, 1500 and 5000 ppm (equal to 0, 31, 94 and 331 mg/kg bw per day for ♂, 0, 38, 115 and 380 mg/kg bw per day for ♀)	NOAELsys: 1500 ppm (94 mg/kg bw/d ♂, 115 mg/kg bw/d ♀) LOAELsys: 5000 ppm (331 mg/kg bw/d ♂ and 380 mg/kg bw/d ♀) NOAELneuro: 5000 ppm (331 mg/kg bw/d ♂, 380 mg/kg bw/d ♀) LOAELneuro: None <u>Effects at 5000 ppm (331 mg/kg bw/d ♂ and 380 mg/kg bw/d ♀:</u> M: ↓ **mean final body weight** (10%) and ↓ body weight gain between day 0-42 (resulting in a 16% reduced bw gain over the entire period, day 0-91) F: ↓ mean final body weight (8%, n.s.) and ↓ body weight gain until day 35 (resulting in a 19% **reduced bw gain** over the entire period, day 0-91)	[redacted] (2012d) Report number: WIL-194040 Vol. 3. B.6.7.1.3 New data for renewal: Yes

		No neurotoxic effects	
A 28-Day Oral (Dietary) Dose Range-Finding Immunotoxicity Study of Clethodim in Female B6C3F1 Mice (GLP) Guidelines followed: OPPTS 870.7800 (1998) Deviations from current guidelines: No positive control Species: Mice Strain: B6C3F1 Female 8 mice/group GLP Supportive	Clethodim TG, Batch: AS 506r Purity: 95.4% <u>Doses:</u> 400, 2000 and 4000 ppm (equal to 101, 551 and 958 mg/kg bw/day) Clethodim was offered ad libitum in the diet for 28 consecutive days	NOAELsystemic: 400 ppm (101 mg/kg bw/day) LOAELsystemic: 2000 ppm (551 mg/kg bw/day) NOAELimmunotoxicity: 4000 mg/kg bw (958 mg/kg bw/day) LOAELimmunotoxicity: - <u>Effects observed at 2000 ppm (551 mg/kg bw/day):</u> ↑ **absolute and relative liver weight** (16%) <u>Effects observed at 4000 ppm (958 mg/kg bw/day):</u> ↑ **absolute and relative liver weight** (41 and 39 %, respectively) ↓ food consumption No evidence of immunotoxicity.	[redacted] (2012a) Report number: WIL-194037 Vol. 3, B.6.8.2/01 New data for the Annex I renewal: Yes
A 28-Day Oral (Dietary) Immunotoxicity Study of Clethodim in Female B6C3F1 Mice Guidelines followed: OPPTS 870.7800 (1998) Deviations from OPPTS 870.7800 (1998): None Mice Strain: B6C3F1 Female 10 mice/group GLP Acceptable	Clethodim TG Purity: 95.4% Dose: 0, 400, 2000 and 4000 ppm (equal to 0, 136, 603 and 1312 mg/kg bw per day) Clethodim was offered ad libitum in the diet for 28 consecutive days	NOAELsystemic: 400 ppm (136 mg/kg bw/day) LOAELsystemic: 2000 ppm (603 mg/kg bw/day) NOAELimmunotoxicity: 4000 ppm (1312 mg/kg bw/day): LOAELimmunotoxicity: - <u>Effects observed at 2000 ppm (603 mg/kg bw/day):</u> ↑ **absolute and relative liver weight** (17 and 13 %, respectively) ↓ food consumption day 0-7 <u>Effects observed at 4000 ppm (1312 mg/kg bw/day):</u> ↑ **absolute and relative liver weight** (45 and 42 %, respectively) ↓ food consumption day 0-7 No evidence of immunotoxicity.	[redacted] (2012b) Report number: WIL-194038 Vol. 3, B.6.8.2/02 New data for the Annex I renewal: Yes
Five-Week Subchronic Feeding Study of High Purity RE-45601 (SX-1718) and RE-45601 Process Neutrals (SX-1717) in Rats No guideline followed. Sprague-Dawley® Crl:CD® (SD) BR 10 rats/sex/group GLP	High Purity RE-4560, Purity: 96.2% Dose: 6800 ppm (equal 597 mg/kg bw/day for males and 667 mg/kg bw/day for females) Process Neutrals of RE-45601 <u>Dose:</u>	<u>Effects observed rats treated with 6800 ppm clethodim (597 mg/kg bw/day for males and 667 mg/kg bw/day for females):</u> ↓**body weight** (F: 9-15%) ↓ **body weight gain** (M: 33%, F: 42%) **- mild anaemia** (5-7% reductions in erythrocyte, haemoglobin and haematocrit values) ↑ **liver weight** (M: abs.:12%, rel.: 34%, F: rel. 24%) accompanied by centrilobular hypertrophy. ↓ **adrenal weight** (M: 26%, F: 17%) Males were more severely affected.	[redacted] 1987 Report no. S-2763 Vol. 3. B.6.8.2/03 New data for the Annex I renewal: No

Supportive	1200 ppm (equal 4.87 mg clethodim/kg bw/day for males and 5.78 mg clethodim/kg bw/day for females) The test items were offered ad libitum in the diet for 5 consecutive weeks	<u>Effects observed rats treated with 1200 ppm process neutrals (148 and 175 mg Process Neutrals/kg body weight/day containing 4.87 and 5.78 mg clethodim/kg bw/day for males and females, respectively):</u> ↓ body weight (Day 35: M: 6%) ↓ **body weight gain** (M: 12%) ↑ **liver weight** (F: abs and rel: 10%) **-hepatic centrilobular hypertrophy** ↓ testis weight (abs 5% n.s, rel 6%) In general, animals exposed to clethodim were more severely affected than those exposed to process neutrals. <u>Conclusion:</u> Clethodim alters several health parameters in rats but impurities may contribute partially to some of these results.	
Cytochrome P-450 concentration following 21-day oral administration in male rats. No guideline followed. Rat. Sprague-Dawley Crl:CD® BR 8 males/group GLP Supplementary	RE-45601 Technical (batch SX-1688) Purity: 83.3% 250 mg/kg/day (208 mg/kg/day, corrected for purity)	<u>Effects observed at 208 mg/kg bw/day:</u> ↑ **liver weight** (M: abs: 21%, rel: 23%) No difference in CYP450 concentration was observed	[redacted] 1989 Report no. S-3055 Vol. 3. B.6.8.2/04 New data for the Annex I renewal: Yes

*Study not suitable for NOAEL setting (low number of animals used and limited parameters investigated)

Table 47: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure).

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 48: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure).

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

For this section there are a number of studies which are new for the renewal of active substance: two pilot developmental toxicity studies (one in rat and one in rabbit), one reproductive toxicity dose range finding study, one acute neurotoxicity study, one 90-day neurotoxicity study, one dose range findings immunotoxicity study, one 28-

day immunotoxicity study, and one 21-day oral toxicity study. The latter one was considered supplementary. All other studies (old and new data) were considered acceptable or supportive.

Table 49: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days.

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
5-week oral toxicity study in the rat Report No. S-2720	200 ppm (12.5 mg/kg bw/day) (changes in haematological parameters indicating mild anaemia (↓Hb <10%), effects on the liver (increased cholesterol, increased liver weights, centrilobular hypertrophy)	5 weeks	24<C≤240 mg/kg bw/day (Cat 2) C≤24 mg/kg bw/day (Cat 1)	-
5-week feeding study of high purity RE-45601 and RE-45601 process Neutrals in rats Report No.: S-2763	Clethodim: 8000 ppm (597 mg/kg bw/day) (effects on the liver (changes in haematological parameters indicating mild anaemia, increased weight, centrilobular hypertrophy)	5 weeks	24<C≤240 mg/kg bw/day (Cat 2) C≤24 mg/kg bw/day (Cat 1)	-
28-day oral dose range finding neurotoxicity study in the rat Report No.: WIL-194039	5000 ppm (441 mg/kg bw/day) (slightly reduced absolute brain weight	28 days	30<C≤300 mg/kg bw/day (Cat 2) C≤ 30 mg/kg bw/day (Cat 1)	-
28-day oral toxicity study in mice Report No.: S-2733	625 ppm (74.4 mg/kg bw/day) (changes in haematological parameters indicating mild anaemia (↓haemoglobin 4-8%) 1500 ppm (179 mg/kg bw/day (changes in haematological parameters indicating mild anaemia, increased liver weights) 4000 ppm (476 mg/kg bw/day) (changes in haematological parameters indicating mild anaemia, hepatic centribolular hypertrophy)	28 days	30<C≤300 mg/kg bw/day (Cat 2) C≤ 30 mg/kg bw/day (Cat 1)	-
28-day oral immunotoxicity study in mice Report No.: WIL-194038	2000 ppm (603 mg/kg bw/day) (increased liver weight)	28 days	30<C≤300 mg/kg bw/day (Cat 2) C≤ 30 mg/kg bw/day (Cat 1)	-
4-week dermal toxicity study in the rat Report number: S-2848	100 mg/kg bw/day (changes in biochemical parameters indicating liver toxicity) 1000 mg/kg bw/day (clinical signs (anogenital discharge), increased liver weight	28 days	60<C≤600 mg/kg bw/day (Cat 2) C≤60 mg/kg bw/day (Cat 1)	-
1-year oral (gavage) study in dogs Report No.: S-2964	75 mg/kg bw/day (histopathological changes in sternal bone marrow (hyperplasia)) 300 mg/kg bw/day (increased cholesterol, enlarged liver, increased, hepatocyte hypertrophy, pigment, increased thyroid/parathyroid weight, changes in haematological parameters)	1 year	2.5<C≤25 mg/kg bw/day (Cat 2) C≤ 2.5 mg/kg bw/day (Cat 1)	-
2-year feeding study in the rat	2500 ppm (86 mg/kg bw/day) (increased liver weight,	2 years	1.25<C≤12.5 mg/kg bw/day (Cat 2)	-

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Report number: S-2766	histopathological changes in the liver (hypertrophy (observed after 1 y and 2 y), binucleated cells (observer after 1y))		C≤1.25 mg/kg bw/day (Cat 1)	
78-week feeding study in mice Report No.: S-2867	1000 ppm (119 mg/kg bw/day (increased liver weight and histopathological changes in liver (centrilobular hypertrophy, increased pigemt, bile duct hyperplasia) 2000/3000 ppm (238/357 mg/kg bw/day) (histopathological changes in lungs (foci of amphophilic alveolar macrophages), increased incidence of amyloidosis and mortalities	78 weeks	1.5<C≤15 mg/kg bw/day (Cat 2) C≤1.5 mg/kg bw/day (Cat 1)	-

Rat:

5-week oral toxicity study in rats (refer to Vol. 3, B.6.3.1/01)

In this pilot study, Clethodim Technical (purity: 83.4%) was administered to Sprague-Dawley rats (10/sex/group) via the diet at concentrations of 0, 5, 200, 1000, 4000, 8000 ppm (equal to 0, 0.26, 12.5, 65.6, 261 and 515 mg/kg bw/day for males, and 0, 0.29, 13.9, 70.6, 291 and 554 mg/kg bw/day for females) for 5 weeks. Vehicle used in study was acetone. Treatment was associated with reduced body weight noted in females at 291 mg/kg bw/day (↓8%) and 554 mg/kg bw/day (↓16%) and in males at 515 mg/kg bw/day (↓13%), reduced bodyweight gain (>10%) noted in both sexes at ≥261/291 (M/F) mg/kg bw/day, changes in haematological parameters noted in males at ≥12.5 mg/kg bw/day and in females at ≥70.6 mg/kg bw/day, changes in biochemical parameters (increased cholesterol) noted in males at 515 mg/kg bw/day (↑68%), changes in urinalysis (increased uric acid) noted in females at ≥291 mg/kg bw/day, increased liver weights noted in both sexes at ≥65.6/70.6 (M/F) mg/kg bw/day, and histopathological findings in the liver (centrilobular hypertrophy) noted in males at ≥70.6 mg/kg bw/day and in females at ≥291 mg/kg bw/day. Haematological changes (indicating mild anaemia) included: reduced erythrocyte counts (females at ≥70.6 mg/kg bw/day, up to 7% reduction), reduced haemoglobin levels (males at ≥65.6 mg/kg bw/day, 4-7%, females at ≥70.6 mg/kg bw/day, 4-7%), and reduced haematocrits (males at ≥261 mg/kg bw/day, 4-6%). In addition, increased platelets were noted in males at ≥13.9 mg/kg bw/day (30-43%).

The NOAEL in this study is 200 ppm (12.5 mg/kg bw/day) based on reduced body weight grow observed in both sexes at ≥261/291 (M/F) mg/kg bw/day, changes in haematological parameters (indicating mild anaemia) observed in both sexes at ≥65.6/70.6 (M/F) mg/kg bw/day, changes in biochemical parameters indicating liver toxicity (increased cholesterol) noted at 515 mg/kg bw/day (males only), increased liver weights noted in both sexes at ≥65.5/70.6 (M/F) mg/kg bw/day, and histopathological changes in the liver (centrilobular hypertrophy) observed in males at ≥65.6 mg/kg bw/day and in females at ≥261 mg/kg bw/day.

The study was performed in accordance with Good Laboratory Practice. The deviations from the current OECD TG 407 are presented in Table 46. These deviations concern endpoints that were not studied which limit the interpretations of the results but do not affect the validity of the study. The study is considered acceptable.

Table 2.6.3.1.1-01: Histopathology findings in the liver- males

Diagnosis	Group 1A, 0 ppm	Group 4D, 1000 ppm	Group 5E, 4000 ppm	Group 6F, 8000 ppm
Total examined	**10**	**10**	**10**	**10**
Hematopoiesis, extramedullary	10	9	6	5
trace	8	9	6	5
mild	2	0	0	0
Hepatite, chronic	0	0	0	1
trace	0	0	0	1
Hypertrophy, centrilobular	0	9	9	9
trace	0	6	3	1
mild	0	3	6	8

Table 2.6.3.1.1-02: Histopathology findings in the liver- females

Diagnosis	Group 1A, 0 ppm	Group 4D, 1000 ppm	Group 5E, 4000 ppm	Group 6F, 8000 ppm
Total examined	**10**	**10**	**10**	**10**
Hematopoiesis, extramedullary	5	6	5	6
trace	5	5	4	6
mild	0	1	1	0
Hepatite, chronic	2	0	0	1
trace	2	0	0	1
Hypertrophy, centrilobular	0	0	9	9
trace	0	0	8	5
mild	0	0	1	4
Within normal limits	3	4	1	0

13-week oral study in rats (refer to Vol. 3, B.6.3.2/01)

In this study RE-45601 Technical (purity: 84%) was administered to Sprague-Dawley rats (12/sex/group) via the diet at concentrations of 0, 50, 500, 2500 and 5000 ppm (equal to 0, 2.3, 25, 134 and 279 mg/kg bw/day for males; 0, 2.8, 30, 159 and 341 mg/kg bw/day for females) for 13 weeks. Vehicle used in study was acetone. Following this treatment phase, 12 rats/sex/group were sacrificed. The remaining animals (12 rats/sex/group) in the control and two high dose groups were fed untreated basal diet for an additional six weeks and were sacrificed at the end of this recovery phase. Treatment was associated with reduced body weight noted in males at 134 mg/kg bw/day (7%) and in both sexes at 279/341 (M/F) mg/kg bw/day (>10%), reduced bodyweight gain noted in males at 134 mg/kg bw/day (10%) and in both sexes at 279/341 (M/F) mg/kg bw/day (>10%), reduced food consumption noted in both sexes at 279/341 (M/F) mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 279/341 (M/F) mg/kg bw/day, increased liver weight noted in both sexes at ≥134/159 (M/F) mg/kg bw/day, increased relative kidney weight noted in both sexes at 279/341 (M/F) mg/kg bw/day (M:10%, F: 14%), and histopathological findings in the liver (hepatic centrilobular hypertrophy) noted in both sexes at ≥134/159 (M/F) mg/kg bw/day. Changes in biochemical parameters indicating liver toxicity at 279/341 (M/F) mg/kg bw/day included: increased serum cholesterol (↑M: 31%), total protein (M: ↑5%), globulin (M: ↑9%).

Food consumption and body weight gain was reduced during the exposure period but was increased during the recovery period. Final body weight (week 19) was similar between groups except for females of the high dose group (↓7%). The only organ weight that was significantly different after the 6-week recovery period was relative liver weight in females of mid-dose (↑11%) and high (↑13%) dose groups. There were no treatment-related changes present among males and females at the recovery sacrifice.

The NOAEL in study was set at 500 ppm (equal to 25 mg/kg bw /day) based on reduced bodyweight noted at 279/341 (M/F) mg/kg bw/day (>10%), reduced bodyweight gain noted at ≥134/159 (M/F) mg/kg bw/day (≥10%), changes in biochemical parameters (indicating liver toxicity) noted in males at 279 mg/kg bw/day (increased serum cholesterol, total protein and globulin levels), increased liver weight noted at ≥134/159 (M/F) mg/kg bw/day, and histopathological changes in the liver (hypertrophy) observed at ≥134/159 (M/F) mg/kg bw/day.

The study was conducted in accordance with good laboratory practice and according to OECD TG 408 (1998). There were a number of endocrine sensitive endpoint (included in the current version of the guideline) that were not analysed. This limits the usefulness of the study, but it does not affect the reliability of the obtained results. The study is considered acceptable.

Table 2.6.3.1.1-03: Selected histopathological results of rats administered clethodim (RE-45601 Technical) in the diet for 13 weeks (mean ± SD)

Sex	**Males**					**Females**				
Dose (ppm)	0	50	500	2500	5000	0	50	500	2500	5000
Dose (mg/kg bw/day)	0	2.3	25	134	279	0	2.8	30	159	341
Liver: centrilobular hypertrophy	0/12	0/12	0/12	8/12	10/12	0/12	0/12	0/12	2/12	7/12

4-week dermal study in rats (refer to Vol. 3, B.6.3.3/01)

In this study Sprague Dawley rats (6/sex/group) were exposed to repeated dermal doses of RE-45601 Technical (purity: 83.2%) during a 28-day period (21 six-h dermal applications) at doses of 0 (control), 10, 100 or 1000 mg/kg bw/day. The vehicle used in study was 0.7% carboxymethyl cellulose (CMC) and 1% TWEEN 80 in distilled water. Treatment was associated with local effects of skin irritation observed at all dose levels. Furthermore treatment was associated with clinical signs (anogenital discharge) noted in both sexes at 1000 mg/kg bw/day, reduced bodyweight gains observed in males at 1000 mg/kg bw/day, changes in biochemical parameters (increased triglyceride levels (F: ↑140-160%); reduced BUN/creatinine ratio (M: 22%)) noted at ≥100 mg/kg bw/day, lower food efficiency values noted in males at 1000 mg/kg bw/day, increased liver weights (about 20%) noted in females at 1000 mg/kg bw/day, increased relative kidney weight (10%) noted in males at 1000 mg/kg bw/day, increased relative testes weight (13%) noted at 1000 mg/kg bw/day.

No NOAEL for local effects could be set in this study since skin irritation was observed from 10 mg/kg bw/day. LOAEL for local effects is 10 mg/kg bw/day (equal to 8.3 mg/kg bw/day after correction for purity using a correction factor of 1.2). The systemic NOAEL was 100 mg/kg bw per day (equal to 83.3 mg/kg bw/day after correction for purity using a correction factor of 1.2) based on reduced bodyweight gain noted in males at 1000 mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 1000 mg/kg bw/day (increased triglyceride levels (F), reduced BUN (M), reduced BUN/creatinine ratio (M)), and increased liver weights noted in both sexes at 1000 mg/kg bw/day.

The study was performed in accordance with OECD TG 410 and conducted to GLP. Some serum measurements mentioned in OECD TG 410 were not performed (ornithine decarboxylase, gamma glutamyl transpeptidase, hormone levels, methaemoglobin, cholinesterase activity); however, it is noted that these are not required but suggested. The study is acceptable.

Combined chronic oral toxicity/oncogenicity study in rats (refer to Vol. 3, B.6.5/02)

In this study Sprague Dawley rats were exposed to RE-45601 Technical (purity: 83%) in the diet for 2 years at doses of 0 (control), 5, 20, 500, 2500 ppm (equivalent to 0, 0.15, 0.57, 16 and 86 mg/kg bw/day (males) and 0, 0.2, 0.72, 21 and 113 mg/kg bw/day (females)). The vehicle used in study was Acetone 10 mL/kg diet. Treatment was associated with reduced body weight noted in both sexes at 86/113 (M/F) mg/kg bw/day (At Day 91: M: 7%, F: 6%; At Day 360: M: 7%, F: 8%; At Day 724: M: 8% n.s., F:13% n.s), reduced bodyweight gain noted at 86/113 (M/F) mg/kg bw/day (M:11%, F: 12%, calculated for the first 3 months), reduced food consumption noted at 86/113 (M/F) mg/kg bw/day (noted at intervals during the study), reduced food efficiency noted in males at 86 mg/kg bw/day (during the first three months), increased liver weights noted in females at 21 mg/kg bw/day (rel weight: after 1 year: ↑18% n.s.; after 2 y: 12%, n.s) and in both sexes at 86/113 (M/F) mg/kg bw/day (abs weight after 1 y: M: 15% n.s., F: 24%; rel weight after 1y: M: 22%, F: 18% n.s.; rel weight after 2 y: F: 21%), and histopathological findings in the liver noted in both sexes at 86/113 (M/F) mg/kg bw/day. The histopathological findings consisted of hypertrophy in hepatocytes (observed in both sexes after 1 y and 2 y) and binucleated cells in the liver observed in females after 1 y but not after 2 y. No treatment-related increase in the incidence of neoplasms or other microscopic lesions was found in any of the groups.

The NOAEL in study is 500 ppm (equal to 16 mg/kg bw/day) based on reduced bodyweight gain noted in both sexes at 86/113 (M/F) mg/kg bw/day, increased liver weights noted in both sexes at 86/113 (M/F) mg/kg bw/day, and histopathological findings in the liver noted at 86/113 (M/F) mg/kg bw/day (hypertrophy (both sexes), binucleated cells (F)).

The study was performed in accordance with OECD 453 and with EPA, FIFRA and TSCA Good Laboratory Practice (GLP) Standards. The deviations from the current guideline (OECD 453, 2018) includes the ones listed in Table 46. These deviations are not considered to have a major impact on the study outcome. The study is considered acceptable.

Table 2.6.3.1.1-04: Selected pathology parameters of rats administered RE-45601 Technical in the diet for 104 weeks (mean±SD)

	Males					**Females**				
Dose (ppm)	**0**	**5**	**20**	**500**	**2500**	**0**	**5**	**20**	**500**	**2500**
mg/kg bw/day	**0**	**0.15**	**0.57**	**16**	**86**	**0**	**0.20**	**0.72**	**21**	**113**
Pathology										
Non-neoplastic lesions										
Interim sacrifice#										
Centrilobular hypertrophy	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	3/10
All study animals										
Binucleated cells	2/65	0/65	0/65	0/65	1/65	1/65	0/65	0/65	1/65	6/65

No. of animals with lesion/ No. of animals in group.

Two generation (one litter) reproduction study in rats (refer to Vol. 3, B.6.6.1/02)

In this two generation study, RE-45601 Technical (purity: 83.3%) was administered in the diet to groups of 30 males and females per generation Treatment was associated with reduced body weight noted in F0 generation males at 163 mg/kg bw/day (↓4-9%) and in F1 generation males (↓10-19%) and females (↓6-10%), and minor reductions in food consumption in both generations at 163/181 (M/F) mg/kg bw/day, organ weight changes noted at 163 mg/kg bw/day (F0 generation: increased relative testis weight (10%); F1 generation: increased relative epididymis weight (18%),

reduced absolute prostate (25%) and seminal vesicles weights (11%)). Relative weights of prostate and seminal vesicles were comparable to control. Furthermore, slightly increased number of still born pups was observed in F1 generation at 163/181 (M/F) mg/kg bw/day. The relevance of this latter finding was unclear. The lack of similar effect in the F2 pups and the four-fold higher value in the F2 controls indicates that the effect may be incidental.

The NOAEL for parental toxicity in study is 500 ppm (32.2 mg/kg bw/day) based on reduced body weights noted in both generations at 163/181 (M/F) mg/kg bw/day covering also reduced absolute prostate and seminal vesicles weights of unclear relevance noted in F1 adults at 163 mg/kg bw/day. NOAEL for reproductive toxicity is 2500 ppm (163 mg/kg bw/day, highest dose tested). The NOAEL for offspring toxicity is 500 ppm (32.2 mg/kg bw/day) based on slightly increased number of stillborn noted in F1 pups at 2500 ppm (163/181 (M/F) mg/kg bw/day) (although unclear relevance).

The study was performed in general accordance with OECD 416 and with EPA, FIFRA and TSCA Good Laboratory Practice (GLP) Standards. There were some deviations from the current version of the guideline, see Table 46. The deviations include parameters that were not measured, and while these limits the scope of the study, they do not affect the reliability. The study is considered acceptable.

(F0 and F1) at levels of 0, (control), 5, 20, 500, and 2500 ppm (equal to 0, 0.5, 1.2, 32.2 and 163 mg/kg bw/day for males; 0, 0.5, 1.5, 37.4 and 181 mg/kg bw/day for females in the pre-mating period after correction for purity as calculated by the applicant). The vehicle used in study for preparation of diet was Acetone.

Pilot rat reproduction study in rats (refer to Vol. 3, B.6.6.2.1/01)

In this pilot study, groups of 8 male and 8 female Sprague-Dawley Crl:CD strain rats were fed diet containing 0, 500, 2000 or 5000 ppm RE-45601 Technical (purity: 83.3%) for 1 week before mating. The doses equal to 0, 20.8, 83.3, 208.3 mg/kg bw/day when corrected for purity of active substance. The vehicle used in study for preparation of diet was Acetone. Females received the diet continuously throughout mating and gestation, and until Day 7 of lactation when they were necropsied. The offspring were exposed to the test material in utero and while nursing until they were sacrificed and necropsied on Day 7 of lactation. Effects on adults and offspring were observed at the maximum dose level of 5000 ppm (208.3 mg/kg bw/day).

Treatment was associated with reduced bodyweight noted in adults at 208.3 mg/kg bw/day (Males: week 0-2: 2%; Females: GD 20 13%, LD 0 F: 14%, LD: 7 (16%)), reduced bodyweight gain noted in adults at 5000 ppm (M: 18%, F: 63%) and reduced food consumption noted in adult males during the first week (pre-mating) (15%). In the offspring reduced combined pup weights were noted at all dose levels (On day 7: 9%, 9%, and 11% in the low, middle, and high dose, respectively; Day 0-7: 13%, 14%, and 16% in the low, middle, and high dose, respectively). There were no effects on reproduction indices for males or females, or on pup litter size, survival, and sex ration.

The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated). The study is considered as supportive data (dose range finding study).

Teratology study in rats (refer to Vol. 3, B.6.6.2.2/01)

In this study, pregnant dams (Crl:CD rats) (25/dose) were administered clethodim (purity: 83.3%) by gavage during gestational days 6-15 at doses of 0, 10, 100, 300 and 700 mg/kg bw per day (equal to 0, 8.3, 83.3, 292 and 583

mg/kg bw/day after correction for purity of test substance). Maternal toxicity was observed in the top two doses, with increasing severity with dose. Manifestations of maternal toxicity included mortality (5 of 25 animals) noted at 700 mg/kg bw/day (583 mg/kg bw/day after correction for purity of test substance), clinical signs (excessive salivation, excessive lacrimation, poor condition, red/mucoid nasal discharge, alopecia, staining of the ano-genital area, chromodocryorrhea (top dose only)) noted at ≥350 mg/kg bw/day, reduced maternal body weight noted at 350 mg/kg bw/day (GD 20: 7%; GD 20 corrected value: 6%) and 700 mg/kg bw/day (GD 20: 8%; GD 20 corrected value: 13%), reduced bodyweight gain noted at 350 mg/kg bw/day (GD 6-15: 15% n.s.; GD 15-20: 17% GD 20 corrected value: 77%) and 700 mg/kg bw/day (GD 6-15: 40%; GD 15-20: 17%; GD 20 corrected value: 11%). Furthermore, food consumption was reduced in the highest dose group during the exposure period (except for the last day). Uterine weight was reduced in a dose dependent manner: 7% reduction in the 100 mg/kg bw/day group, 10 % in the 350 mg/kg bw/day group, and 27% in the 700 mg/kg bw/day group (only the top dose was statistically significant). The mean number of resorptions and resorptions per implant was increased in the top dose group (not statistically significant). There were fewer litters with viable foetuses in the highest dose group. Foetal body weight was reduced at 350 mg/kg bw/day (11%) and 700 mg/kg bw/day (25%). Furthermore, the incidence of skeletal variations (retarded ossification processes) was increased in the top two doses. There was also a higher incidence of external and visceral malformations among the top dose foetuses. Seven out of the 8 foetuses with external malformations had (among other things) deformed tails, an effect that is associated with maternal toxicity. Because the fetotoxic effects only were observed in the presence of maternal toxicity, the distinction between direct and indirect effects on the foetus is unclear.

NOAEL for maternal toxicity is 100 mg/kg bw/day (equal to 83.3 mg/kg bw per day after correction for purity of test substance) based on mortalities noted at 700 mg/kg bw/day, clinical signs noted at ≥350 mg/kg bw/day, reduced body weight noted at 700 mg/kg bw/day and reduced bodyweight gain noted at ≥350 mg/kg bw. NOAEL for developmental toxicity is 100 mg/kg bw/day (equal to 83.3 mg/kg bw per day after correction for purity of test substance) based on decreased foetal weight noted at ≥350 mg/kg bw/day, increased incidence of skeletal variations noted at ≥350 mg/kg bw/day, and increased incidence of external and visceral malformations at 700 mg/kg bw/day.

The study was performed in general accordance with OECD 414 and with FIFRA Good Laboratory Practice (GLP) Standards. The deviations from the current guideline include endpoints that would have been valuable for the endocrine disruption assessment (see Table 46); however, the lack of such information does not invalidate the study. The exposure period in the study is shorter than described in the current version of the guideline. The exposure period used (day 5-15) covers the main part of organogenesis. The study is considered acceptable.

Pilot teratology study in rats (refer to Vol. 3, B.6.6.2.1/01)

In this dose range finding study, RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 6-15 to groups of 10 female rats at doses of 0, (control), 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw per day, after correction for purity of test substance).

At the top dose of 500 mg/kg bw/day (417 mg/kg bw/day when corrected for purity of test substance), observed effects included increased salivation (8/10 dams), reduced body weight (Day 20: ↓10% n.s.), reduced bodyweight gain (Day 15-20: ↓38.8%; Day 6-20: ↓62.5%), reduced number of implantation sites (87 versus 126 in control, n.s.),

and increased pre-implantation loss ratio (0.289 versus 0.082 in control, n.s.), reduced number of viable foetuses (86 versus 122 in control, within historical control values), and reduced foetal weight of viable foetuses (↓11%) .

In the second highest dose of 300 mg/kg bw/day (250 mg/kg bw/day when corrected for purity), observed effects included increased salivation in the dams (8/10 dams) and reduced pup weight (7%, not statistically significant).

The study was performed in accordance FIFRA Good Laboratory Practice (GLP) Standards. The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated) (pilot study). It is also noted that there were indications of SDA viral infections in some dams at gestation day 20 which restricts the reliability of the study. This was noted in 1, 2, 2, 3, and 2 females in the 0, 50, 150, 300, and 500 mg/kg bw/day group, respectively. The study is considered as supportive data.

90-day oral dietary neurotoxicity study in rats (refer to Vol. 3, B.6.7.1.3)

In this study, RE-45601 (purity: 95.4%) was administered in the diet to groups of 12 males and females at levels of 0 (control), 500, 1500 and 5000 ppm (equal to 0, 31, 94 and 331 mg/kg bw/day for males, and 0, 38, 115 and 380 mg/kg bw/day for females) for 28 days. No mortality occurred and no clear treatment related clinical signs were observed. Body weights of both sexes were lower in the highest dose group than those of the control group throughout the study (7-11% in males and 7-9% in females). Body weights of the low and middle dose were comparable to control weights or higher (body weight of female of the 500-ppm group). Body weight gain was reduced in both sexes (16% in males and 19% in females) over the entire study period, in general due to lower gains during the first month. Food consumption in males was reduced during the first week, potentially indicating palatability issues, but the food consumption per animal was slightly lower also after that, the consumption per kg bw was similar or slightly higher the rest of the study. In females, the food consumption per animal was lower/slightly lower throughout the study while the consumption per kg bw was similar to the control group overall. The functional battery revealed no treatment related effect on home cage, handling, open field, sensory, or neuromuscular observations. Physiological observations included lower body weight in both sexes. No clear treatment related trends were observed in the treated animals. Total and ambulatory motor activity counts for the 5000 ppm (380 mg/kg bw/day) group females at the study week 7 evaluation was lower than that of the control. The value was also lower than the HCD and the control value was higher than the HCD. No effects on habituation were observed. There were no effects on liver weight, brain weight, or brain length or width but it is noted that relative weights were not reported. No treatment related changes were noted during necropsy.

NOAEL for systemic toxicity was set at 1500 ppm (94 mg/kg/day for males and 115 mg/kg/day for females) based on reduced body weight observed in males and reduced bodyweight gain observed in both sexes at 5000 ppm. NOAEL for neurotoxicity was set at 5000 ppm (331 mg/kg/day for males and 380 mg/kg/day for females) (highest dose level). The study was performed in accordance with good laboratory practice and follows OECD TG 424 and is considered acceptable.

Table 2.6.3.1.1-05: Body weight (g) of rats administered clethodim in the diet for 13 weeks (mean ± SD, n=12) and assessed in the functional observational battery

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**500**	**1500**	**5000**	**0**	**500**	**1500**	**5000**
Week 3	374.8 ± 25.70	377.8 ± 30.54	374.9 ± 31.77	341.5 ± 19.39* (↓9%)	207.6 ± 15.71	229.6 ± 21.57** (↑11%)	211.7 ± 17.22	190.5 ± 11.94* (↓8%)

Sex	Male				Female			
Dose (ppm)	**0**	**500**	**1500**	**5000**	**0**	**500**	**1500**	**5000**
Week 7	493.3 ± 32.78	495.3 ± 41.26	487.7 ± 50.14	441.1 ± 33.55** (↓11%)	246.3 ± 18.91	273.1 ± 26.74** (↑11%)	256.7 ± 18.40	223.8 ± 17.75* (↓9%)
Week 12	572.9 ± 35.95	574.2 ± 49.59	568.9 ± 58.25	516.4 ± 46.28* (↓10%)	268.9 ± 21.72	295.5 ± 30.51* (↑8%)	282.4 ± 23.91	244.5 ± 21.83

Table 2.6.3.1.1-06: Motor activity counts for female rats at selected time points during study week 7

Study week	Time point	Statistic	Dose (ppm)				
			0	**500**	**1500**	**5000**	**HCD**
			Total motor activity counts				
7	21-30 mins	Mean ± SD	573 ± 350.2	436 ± 237.7	408 ± 223.8	288 ± 265.1	357 – 622
		Linear Trend p-value#		NT	0.142	0.013* (↓50%)	NT
		Overall (Statistical model: LinTrt*Time p-value#) 0.026*					
			Ambulatory motor activity counts				
7	21-30 mins	Mean ± SD	142 ± 120.0	79 ± 63.9	86 ± 62.8	60 ± 75.0	50 – 123
		Linear Trend p-value#		NT	0.131	0.042* (↓102%)	NT
		Overall (Statistical model: LinTrt*Time p-value#) 0.034*					

Table 2.6.3.1.1-07: Motor activity counts for male rats administered clethodim in the diet

Week	Time interval (min)	Mean values				% difference to control				% of 0-10 minute interval (within the same group)			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Total													
-1	0-10	1316	1227	1236	1309	n.a.	-7	-6	-1	100	100	100	100
	11-20	722	664	628	670	n.a.	-8	-13	-7	55	54	51	51
	21-30	563	467	348	507	n.a.	-17	-38	-10	43	38	28	39
	31-40	104	464	150	326	n.a.	346	44	213	8	38	12	25
	41-50	135	217	104	205	n.a.	61	-23	52	10	18	8	16
	51-60	148	98	147	189	n.a.	-34	-1	28	11	8	12	14
	Cumulative	2989	3138	2613	3206	n.a.	5	-13	7	-	-	-	-
3	0-10	1272	1260	1179	1209	n.a.	-1	-7	-5	100	100	100	100
	11-20	932	832	731	833	n.a.	-11	-22	-11	73	66	62	69
	21-30	684	735	563	595	n.a.	7	-18	-13	54	58	48	49
	31-40	552	585	343	372	n.a.	6	-38	-33	43	46	29	31
	41-50	311	470	278	312	n.a.	51	-11	0	24	37	24	26
	51-60	230	392	186	316	n.a.	70	-19	37	18	31	16	26
	Cumulative	3981	4274	3281	3637	n.a.	7	-18	-9	-	-	-	-
7	0-10	1249	1114	1142	1129	n.a.	-11	-9	-10	100	100	100	100
	11-20	807	694	705	736	n.a.	-14	-13	-9	65	62	62	65
	21-30	573	500	495	508	n.a.	-13	-14	-11	46	45	43	45
	31-40	463	355	363	477	n.a.	-23	-22	3	37	32	32	42
	41-50	317	297	261	293	n.a.	-6	-18	-8	25	27	23	26
	51-60	84	172	139	165	n.a.	105	65	96	7	15	12	15
	Cumulative	3493	3132	3105	3307	n.a.	-10	-11	-5	-	-	-	-
12	0-10	1183	1135	1098	1216	n.a.	-4	-7	3	100	100	100	100
	11-20	658	559	565	713	n.a.	-15	-14	8	56	49	51	59
	21-30	395	461	335	434	n.a.	17	-15	10	33	41	31	36
	31-40	360	337	334	239	n.a.	-6	-7	-34	30	30	30	20
	41-50	249	251	196	230	n.a.	1	-21	-8	21	22	18	19
	51-60	192	158	129	231	n.a.	-18	-33	20	16	14	12	19
	Cumulative	3037	2899	2657	3063	n.a.	-5	-13	1	-	-	-	-
Ambulatory													
-1	0-10	436	401	379	425	n.a.	-8	-13	-3	100	100	100	100
	11-20	178	144	133	147	n.a.	-19	-25	-17	41	36	35	35
	21-30	112	85	50	94	n.a.	-24	-55	-16	26	21	13	22
	31-40	8	88	12	49	n.a.	1000	50	513	2	22	3	12
	41-50	6	29	16	19	n.a.	383	167	217	1	7	4	4
	51-60	16	3	15	11	n.a.	-81	-6	-31	4	1	4	3
	Cumulative	757	749	604	745	n.a.	-1	-20	-2	-	-	-	-
3	0-10	316	348	268	326	n.a.	10	-15	3	100	100	100	100
	11-20	196	183	137	180	n.a.	-7	-30	-8	62	53	51	55
	21-30	142	162	98	107	n.a.	14	-31	-25	45	47	37	33
	31-40	105	119	53	56	n.a.	13	-50	-47	33	34	20	17
	41-50	52	84	56	54	n.a.	62	8	4	16	24	21	17
	51-60	38	71	29	58	n.a.	87	-24	53	12	20	11	18
	Cumulative	848	966	641	780	n.a.	14	-24	-8	-	-	-	-

Week	Time interval (min)	Mean values				% difference to control				% of 0-10 minute interval (within the same group)			
		1	2	3	4	1	2	3	4	1	2	3	4
7	0-10	287	239	244	263	n.a.	-17	-15	-8	100	100	100	100
	11-20	145	108	99	144	n.a.	-26	-32	-1	51	45	41	55
	21-30	100	86	82	95	n.a.	-14	-18	-5	35	36	34	36
	31-40	75	59	50	90	n.a.	-21	-33	20	26	25	20	34
	41-50	60	41	39	52	n.a.	-32	-35	-13	21	17	16	20
	51-60	15	29	24	28	n.a.	93	60	87	5	12	10	11
	Cumulative	682	563	537	672	n.a.	-17	-21	-1	-	-	-	-
12	0-10	238	228	209	269	n.a.	-4	-12	13	100	100	100	100
	11-20	100	85	81	115	n.a.	-15	-19	15	42	37	39	43
	21-30	64	64	45	68	n.a.	0	-30	6	27	28	22	25
	31-40	58	54	45	38	n.a.	-7	-22	-34	24	24	22	14
	41-50	34	34	21	44	n.a.	0	-38	29	14	15	10	16
	51-60	32	22	13	35	n.a.	-31	-59	9	13	10	6	13
	Cumulative	527	486	413	569	n.a.	-8	-22	8	-	-	-	-

Groups: 1 = vehicle control, 2 = 500 ppm, 3 = 1500 ppm, 4 = 5000 ppm

Table 2.6.3.1.1-08: Motor activity counts for female rats administered clethodim in the diet

Week	Time interval (min)	Mean values				% difference to control				% of 0-10 minute interval (within the same group)			
		1	2	3	4	1	2	3	4	1	2	3	4
Total													
-1	0-10	1211	1183	1381	1259	n.a.	-2	14	4	100	100	100	100
	11-20	348	535	446	389	n.a.	54	28	12	29	45	32	31
	21-30	319	300	508	197	n.a.	-6	59	-38	26	25	37	16
	31-40	186	178	360	312	n.a.	-4	94	68	15	15	26	25
	41-50	269	235	241	253	n.a.	-13	-10	-6	22	20	17	20
	51-60	340	221	222	189	n.a.	-35	-35	-44	28	19	16	15
	Cumulative	2673	2652	3157	2599	n.a.	-1	18	-3	-	-	-	-
3	0-10	1630	1469	1503	1469	n.a.	-10	-8	-10	100	100	100	100
	11-20	792	787	803	832	n.a.	-1	1	5	49	54	53	57
	21-30	486	620	492	582	n.a.	28	1	20	30	42	33	40
	31-40	516	512	593	276	n.a.	-1	15	-47	32	35	39	19
	41-50	508	346	375	283	n.a.	-32	-26	-44	31	24	25	19
	51-60	375	327	428	357	n.a.	-13	14	-5	23	22	28	24
	Cumulative	4306	4062	4193	3798	n.a.	-6	-3	-12	-	-	-	-
7	0-10	1293	1321	1363	1288	n.a.	2	5	0	100	100	100	100
	11-20	751	742	665	603	n.a.	-1	-11	-20	58	56	49	47
	21-30	573	436	408	288	n.a.	-24	-29	-50	44	33	30	22
	31-40	436	390	352	306	n.a.	-11	-19	-30	34	30	26	24
	41-50	448	305	309	222	n.a.	-32	-31	-50	35	23	23	17
	51-60	251	186	249	350	n.a.	-26	-1	39	19	14	18	27
	Cumulative	3752	3380	3346	3057	n.a.	-10	-11	-19	-	-	-	-
12	0-10	1362	1273	1370	1309	n.a.	-7	1	-4	100	100	100	100
	11-20	650	560	708	508	n.a.	-14	9	-22	48	44	52	39
	21-30	338	385	585	246	n.a.	14	73	-27	25	30	43	19
	31-40	429	316	433	295	n.a.	-26	1	-31	31	25	32	23
	41-50	414	288	380	327	n.a.	-30	-8	-21	30	23	28	25
	51-60	374	243	351	207	n.a.	-35	-6	-45	27	19	26	16
	Cumulative	3568	3065	3827	2893	n.a.	-14	7	-19	-	-	-	-
Ambulatory													
-1	0-10	439	398	504	428	n.a.	-9	15	-3	100	100	100	100
	11-20	91	107	94	87	n.a.	18	3	-4	21	27	19	20
	21-30	80	53	154	39	n.a.	-34	93	-51	18	13	31	9
	31-40	31	33	88	60	n.a.	6	184	94	7	8	17	14
	41-50	48	35	51	45	n.a.	-27	6	-6	11	9	10	11
	51-60	60	12	41	23	n.a.	-80	-32	-62	14	3	8	5
	Cumulative	749	638	932	682	n.a.	-15	24	-9	-	-	-	-
3	0-10	540	447	476	473	n.a.	-17	-12	-12	100	100	100	100
	11-20	204	179	193	221	n.a.	-12	-5	8	38	40	41	47
	21-30	135	143	116	140	n.a.	6	-14	4	25	32	24	30
	31-40	135	121	153	60	n.a.	-10	13	-56	25	27	32	13
	41-50	127	70	90	63	n.a.	-45	-29	-50	24	16	19	13
	51-60	89	68	119	84	n.a.	-24	34	-6	16	15	25	18

Week	Time interval (min)	Mean values				% difference to control				% of 0-10 minute interval (within the same group)			
		1	2	3	4	1	2	3	4	1	2	3	4
	Cumulative	1230	1027	1146	1041	n.a.	-17	-7	-15	-	-	-	-
7	0-10	378	356	407	375	n.a.	-6	8	-1	100	100	100	100
	11-20	182	166	150	148	n.a.	-9	-18	-19	48	47	37	39
	21-30	142	79	86	60	n.a.	-44	-39	-58	38	22	21	16
	31-40	103	76	84	63	n.a.	-26	-18	-39	27	21	21	17
	41-50	106	56	58	40	n.a.	-47	-45	-62	28	16	14	11
	51-60	63	37	51	101	n.a.	-41	-19	60	17	10	13	27
	Cumulative	974	770	836	787	n.a.	-21	-14	-19	-	-	-	-
12	0-10	403	326	395	374	n.a.	-19	-2	-7	100	100	100	100
	11-20	158	111	159	94	n.a.	-30	1	-41	39	34	40	25
	21-30	80	77	123	61	n.a.	-4	54	-24	20	24	31	16
	31-40	110	63	103	67	n.a.	-43	-6	-39	27	19	26	18
	41-50	91	60	82	74	n.a.	-34	-10	-19	23	18	21	20
	51-60	94	46	78	35	n.a.	-51	-17	-63	23	14	20	9
	Cumulative	936	683	940	705	n.a.	-27	0	-25	-	-	-	-

Groups: 1 = vehicle control, 2 = 500 ppm, 3 = 1500 ppm, 4 = 5000 ppm

28-day dietary dose range finding study neurotoxicity study (refer to Vol. 3, B.6.7.1.2)

In this dose range finding study, RE-45601 (purity: 95.4%) was administered in the diet to groups of 5 males and females at levels of 0 (control), 500, 1500 and 5000 ppm for 28 days. The mean test substance consumption in the 500, 1500, and 5000 ppm groups was 45, 132, and 441 mg/kg/day, respectively, for males and 51, 155, and 475 mg/kg/day, respectively, for females over the entire study (study days 0-28). It is not clear whether the results from the chemical analysis was used to calculate these values or if only feed consumption was used.

No deaths occurred and no clinical signs stood out in the exposed groups. Body weight and/or body weight gain were affected in all dose groups at one interval or more. The final weight at day 28 was 15 % lower in males of the high dose (441 mg/kg bw/day) group, and body weight gain was reduced by 16, 14, and 30 % in the low, middle, and high dose males, respectively. A similar trend was observed in females. The absolute weight was not significantly affected in females (a 5% reduction at the top dose) and the overall body weight gain was reduced by 21% in this group (475 mg/kg bw/day), mainly due to a significant decrease of body weight change during the first week (48%). No clear effects on food consumption were observed, but body weight gained as percent of feed consumed was lower in the 5000 ppm (441 mg/kg bw/day) males. No treatment related effects on home cage, handling, sensory or neuromuscular observations, or motor activity were observed. Absolute brain weight, but not liver weight in males, was slightly reduced in the top and lowest dose group (↓5%) in the low dose and ↓ 4% in the top dose. No effect on brain weight was observed in middle dose group, thus no dose-response.

The study was performed in accordance with good laboratory practice. It was performed as a dose range finding study and did not follow any specific guideline. It was generally performed in line with OECD TG 424 (1997) with some exceptions which are listed in Table 46. The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated). The study is considered as supportive data.

Five-week sub-chronic feeding study of high purity RE-45601 (SX-1718) and RE-45601 process Neutrals (SX-1717) in rats (refer to Vol. 3, B.6.8.2/03)

This study was designed to investigate whether the observed toxicity in the studies performed with low purity RE-45601 (84.3% purity) could be ascribed to the impurities or process Neutrals.

RE-45601 Technical and RE-45601 Process Neutrals (containing 3.3% RE-45601) were administered to rats (Sprague-Dawley) (10/sex/group) via the diet for 5 weeks. Dose levels were: Clethodim: 8000 ppm (equivalent to 597 and 667 mg/kg bw/day for males and females); Process Neutrals: 1200 ppm (4.87/5.78 mg clethodim/kg body weight/day (males/females). Control animals received the vehicle (10 mL/kg feed) only.

In summation, exposure to 597/667 mg clethodim/kg body weight/day via the diet (males/females) resulted in reduced body weight (F: 9-15%) and bodyweight gain (M: 33%, F: 42%), mild anaemia (5-7% reductions in erythrocyte, haemoglobin and haematocrit values), increased liver weight (M: abs.:12%, rel.: 34%, F: rel. 24%), liver centrilobular hypertrophy, and altered serum chemistry values (albeit within historical control values) (males only). In addition, adrenal weight was reduced (M: 26%, F: 17%) but no histopathological lesions were observed. Males were more severely affected than females. Animals exposed to 148/175 mg process neutrals/kg body weight/day containing 4.87/5.78 mg clethodim/kg body weight/day (males/females) were also affected, but not as severely. This exposure led to reduced body weight gain (males), reduced alkaline phosphatase values (within historical controls), centrilobular hypertrophy, increased liver weight (females), and reduced testes weight. In general, the animals exposed to clethodim was more severely affected, and increased albumin and total protein levels, and anaemia was observed in these animals only. However, the process neutrals also affected the animals.

The incidence of centrilobular hypertrophy was slightly higher for the RE-45601 treatment groups (RE-45601 treatment group: 10 of 10 males and 8 of 10 females; Process Neutrals treatment group: 6 of 10 males and 3 of 10 females).

The study was performed with GLP compliance. It was not conducted according to a specific OECD test guideline. The study is considered supportive.

Cytochrome P-450 concentration following 21-day oral administration in male rats (refer to Vol. 3, B.6.8.2/04)

This study was designed to investigate the potential of RE-45601 technical to induce cytochrome P-450 following 21-days of oral administration in male Sprague-Dawley rats. Male rats were administered 208 mg clethodim/kg bw/day for 21 days via oral gavage. This exposure resulted in increased liver weights (abs weight ↑21%, rel. weight ↑23%) but no other signs of overt toxicity. The mean CYP450 concentration, determined in liver samples from the exposed rats, did not statistically differ from that of the control. The study was considered as supplementary data.

Mouse:

4-week oral study in mice (refer to Vol. 3, B.6.3.1/02)

In this study, RE-45601 Technical (purity: 83.3%) was administered to mice (CD-1) (10/sex/group) via the diet at concentrations of 0, 100, 250, 625, 1500 and 4000 ppm (equivalent to 0, 11.9, 29.7, 74.4, 179 and 476 mg/kg bw per day as calculated by applicant) for 28 days. Vehicle used in study was acetone. Treatment was associated with changes in haematological parameters noted in males at ≥74.4 mg/kg bw/day and in females at ≥179 mg/kg bw/day, increased liver weights noted in males at ≥179 mg/kg bw/day and in females at 476 mg/kg bw/day, and histopathological findings in the liver (hepatic centrilobular hypertrophy) noted in both sexes at 476 mg/kg bw/day. Haematological changes included: reduced haemoglobin noted in males at ≥74.4 mg/kg bw/day (4-8%) and in females at ≥179 mg/kg bw/day (6%), reduced haematocrit noted in males at 476 mg/kg bw/day (8%), and reduced erythrocyte count noted in males at ≥179 mg/kg bw/day (4-9%).

The NOAEL for clethodim in this study was 250 ppm (equivalent to 29.7 mg/kg bw/day) based on haematological changes noted in males at ≥74.4 mg/kg bw/day (reduced haemoglobin at ≥74.4 mg/kg bw/day, reduced haematocrit at 476 mg/kg bw/day, reduced erythrocyte count at ≥179 mg/kg bw/day), increased liver weights noted in males at ≥179 mg/kg bw/day and in females at 476 mg/kg bw/day, and histopathological findings in the liver (hepatic centrilobular hypertrophy) noted in males at 476 mg/kg bw/day.

The study was performed in accordance with Good Laboratory Practice. The deviations from the current OECD TG 407 are presented in Table 46. These deviations concern endpoints that were not studied which limit the interpretations of the results but do not affect the validity of the study. The study is considered acceptable.

Table 2.6.3.1.1-09: Hypertrophy of centrilobular hepatocytes

	Male Groups						Female Groups					
Number Examined	1/10	2/10	3/10	4/10	5/10	6/10	1/10	2/10	3/10	4/10	5/10	6/10
Unremarkable	6	10	7	8	6	0	10	10	10	10	9	2
Minimal	4	0	3	2	3	1	0	0	0	0	1	4
Slight	0	0	0	0	1	3	0	0	0	0	0	4
Moderate	0	0	0	0	0	6	0	0	0	0	0	0
Total Affected	4	0	3	2	4	10	0	0	0	0	1	8

Chronic oral oncogenicity study in mice (refer to Vol. 3, B.6.5/01)

In this study mice (CD-1) (60/sex/group) were orally exposed to Chevron RE-45601 Technical (83.3%) for 78 weeks at doses of 0 (control), 20, 200, 1000, 2000/3000 ppm (equal to 0, 2.4, 24, 119 and 238/357 mg/kg bw/day after correction for purity of test substance) for 78 weeks. The vehicle used in study was Acetone 1.5 mL/kg of feed. Treatment was associated with increased mortalities noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day) (due to amyloidosis), changes in haematological parameters noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day), increased liver weights noted in males at ≥1000 ppm (119 mg/kg bw/day) and in females at 2000/3000 ppm (238/357 mg/kg bw/day), macroscopical changes in the kidney (pale kidney in animals dying or sacrificed due to moribund status) noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day), and histopathological findings noted in the liver (males and females at ≥1000 ppm (119 mg/kg bw/day)) and the lung (males at ≥1000 ppm (119 mg/kg bw/day)) and findings of increased systemic amyloidosis noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day). Changes in haematological parameters noted at 2000/3000 ppm (238/357 mg/kg bw/day) consisted of reduced erythrocytes (Week 79: M: ↓14%; Week 53: M: ↓19%, F: ↓8% n.s., Week 27: M: ↓8%, F: ↓5%), haematocrit (M: ↓12% n.s.) and haemoglobin (M: ↓12% n.s.). Histopathological findings in the liver at ≥1000 ppm (119 mg/kg bw/day) consisted of centrilobular hypertrophy (both sexes), increased pigment (F) and bile duct hyperplasia (M). Treatment-related microscopic findings in the lungs consisted of foci of amphophilic alveolar macrophages (at ≥1000 ppm (119 mg/kg bw/day), both sexes). An additional treatment-related microscopic finding for unscheduled deaths included an increased incidence in systemic amyloidosis for the 3000 ppm (357 mg/kg bw/day) animals. Although amyloidosis is frequently noted in mice of this age and strain, the increased incidence in the high-dose group suggests a treatment-related exacerbation of this finding. The study did not show carcinogenic potential of clethodim technical.

The NOAEL of this study is 200 ppm (equal to 24 mg/kg bw/day, value corrected for purity of test substance) based on increased mortalities noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day), changes in haematological parameters (reduced cell mass) noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day), increased liver weights noted in males at ≥1000 ppm (119 mg/kg bw/day) and in females at 2000/3000 ppm (238/357 mg/kg bw/day), and microscopical finding in the liver noted at ≥1000 ppm (119 mg/kg bw/day) (centrilobular hypertrophy (both sexes), increased pigment (females), bile duct hyperplasia (males)) and in the lung noted at ≥1000 ppm (119 mg/kg bw/day) (foci of amphophilic alveolar macrophages, both sexes) and increased incidence of systemic amyloidosis noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day).

The study was performed in accordance with OECD 451 and FIFRA Good Laboratory Practice. There were some organs that were not harvested/assessed that are listed in the current guideline (OECD 451, 2018), specifically coagulating gland, lacrimal gland, and mammary glands from males (note that this is only required if the glands are visibly dissectible, no information on this). This does not invalidate the outcome of the study. The study is considered acceptable.

Table 2.6.3.1.1-10: Selected histopathology parameters of mice administered RE-45601 Technical in the diet.

	Males					**Females**				
Dose (ppm)	**0**	**20**	**200**	**1000**	**2000 3000**	**0**	**20**	**200**	**1000**	**2000 3000**
Main groups (mg/kg bw/day)	**0**	**2.4**	**24**	**119**	**238/ 257**	**0**	**2.4**	**24**	**119**	**238/ 357**
Non-neoplastic lesions[1]										
Interim sacrifice (week 53)										
Liver: centrilobular hypertrophy	0/10	1/10	1/10	**8/10**	**10/10**	1/10	2/10	2/10	**8/10**	**9/10**
Liver: increased pigment	0/10	0/10	0/10	0/10	**5/10**	0/10	0/10	0/10	0/10	0/10
Terminal sacrifice										
Liver: centrilobular hypertrophy	1/28	1/31	1/30	**10/24**	**16/16**	0/32	0/41	0/39	**4/29**	**10/22**
Liver: hyperplasia bile duct	0/28	0/31	1/30	**4/24**	**5/16**	1/32	0/41	0/39	0/29	2/22
Liver: increased pigment	0/28	0/31	0/30	**7/24**	**11/16**	2/32	1/41	**4/39**	**5/29**	**8/22**
Lung: foci of amphophilic alveolar macrophages	0/28	0/31	**1/30**	**5/24**	**8/16**	0/32	0/41	0/39	**3/29**	**13/22**

28-day oral (dietary) immunotoxicity studies (refer to Vol. 3, B.6.8.2/01 and B.6.8.2/02)

Two immunotoxicity studies were performed, one dose range finding study (Vol. 3, B.6.8.2/01) and one main study (Vol. 3, B.6.8.2/02). Both were performed according to OPPTS 870.7800 (1998) with no deviations except that the dose range finding study did not include a positive control. In these studies, Clethodim TG (purity: 95.4%) was administered in the diet to groups of 10 female mice (main study) or 8 female mice (range finding study) at levels of 0, 400, 2000 and 4000 ppm (corresponding to 101, 551 and 958 mg/kg bw/day in the dose range finding study and 0, 136, 603 and 1312 mg/kg bw per day in the main study). No signs of toxicity except for increased liver weights and lower food consumption were observed. The absolute liver weights in the dose range finding study were 16 and 41% higher than that of the control group in the middle (603 mg/kg bw/day) and high (1312 mg/kg bw/day) dose, respectively. The corresponding relative liver weight values were 16 and 39% higher. In the main study, the

absolute and relative liver weights were 17 and 13 % higher, respectively, in the middle dose, and 45 and 42 %, respectively, in the high dose when compared to control.

No immunosuppressant effect was observed in the dose range finding study. There was a statistically significantly higher mean AFC response in the 2000 ppm (551 mg/kg/bw/day) group (↑54%). There was a similar tendency in the 4000 ppm (958 mg/kg bw/day) group, the mean value was 36% higher than that of the control group (not statistically significant) but the value was lower than that of the 2000 ppm (551 mg/kg/bw/day) group. In the main study, there was a 19-15% reduction in AFC response in the top two doses but there was no dose response, the differences were not statistically significant, and there was an increase in this endpoint in the dose-range finding study. There was also a statistically significant decreasing trend in relative spleen weight (Jonckheere's Test); however, the differences between the exposed groups and the control were not statistically significant and the mean value of the highest dose group was only 8% lower than that of the control (0.36 vs 0.39). Overall, clethodim does not appear to be immunotoxic at these dose levels. NOAEL for systemic toxicity was set at 400 ppm (136 mg/kg bw/day in the main study) based on increased liver weights observed at 2000 ppm (603 mg/kg bw/day in the main study). NOAEL for immunotoxicity was set at 4000 ppm (1312 mg/kg bw/day in the main study) (highest dose level). The studies were considered acceptable (main study) or supportive (dose-range finding study).

Table 2.6.3.1.1-11: Spleen antibody-forming cell response to T-dependent antigen sheep erythrocytes in female mice exposed to clethodim for 28 days (mean ± standard error, n=10)

Dose	0 ppm	400 ppm	2000 ppm	4000 ppm	50 mg/kg CPS	Trend analysis [a]
Body weight (g)	22.9 ± 0.3	23.7 ± 0.3	23.6 ± 0.2	23.2 ± 0.3	22.7 ± 0.2	n.s.
Spleen weight (mg)	90.5 ± 3.4	90.7 ± 3.1	82.7 ± 4.5	84.1 ± 3.9	42.0 ± 2.3** (↓54%)	n.s.
Relative spleen weight	0.39 ± 0.01	0.38 ± 0.01	0.35 ± 0.02	0.36 ± 0.02	0.19 ± 0.01** (↓51%)	$p \leq 0.05$
Spleen cells (x 10^7)	12.32 ± 0.53	12.89 ± 0.44	11.46 ± 0.42	11.38 ± 0.64	3.91 ± 0.21** (↓68%)	n.s.
IgM AFC (10^6 spleen cells)	2515 ± 292	2450 ± 274	2025 ± 275 (↓19%)	2119 ± 245 (↓15%)	0 ± 0** (↓100%)	n.s.
IgM AFC/Spleen (x 10^3)	308 ± 41	321 ± 42	237 ± 37	249 ± 39	0 ± 0** (↓100%)	n.s.

Dog:

<u>90-day oral study in dogs (refer to Vol. 3, B.6.3.2/02)</u>

In this study, RE-45601 Technical (purity: 83.3%) was administered to Beagle dogs (4/sex/group) orally via gelatine capsules at doses of 0 (control), 1, 25, 75 and 125 mg/kg bw/day (equal to 0, 0.83, 21, 62 and 104 mg/kg bw/day when corrected for purity) for 13 weeks. Treatment was associated with changes in biochemical parameters noted in females at ≥75 mg/kg bw/day and in males at 125 mg/kg bw/day, increased liver weights noted in both sexes at ≥75 mg/kg bw/day, and histopathological changes in the liver (increased severity of centrilobular vesicles/vacuoles) noted in both sexes at 125 mg/kg bw/day. Changes in biochemical parameters noted at 125 mg/kg bw/day included: increased cholesterol (F: 40-58%), increased alkaline phosphatase (M: 67% n.s., F: 88%), increased globulin (M: 22%) and reduced albumin/globulin (M: 21%).

The NOAEL is 25 mg/kg bw/day (equal to 21 mg/kg bw/day after correction for purity) based on increased liver weights noted in both sexes at ≥75 mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity)

noted in females at ≥75 mg/kg bw/day and in males at 125 mg/kg bw/day, and histopathological findings in the liver (increased severity of centrilobular vesicles/vacuoles) noted in both sexes at 125 mg/kg bw/day.

The study was performed in line with OECD guideline 409 with some deviations (see Table 46). The deviations did not affect the reliability of the study. No GLP-certificate was included however, the study is well reported and appear well performed. The study is considered acceptable.

Table 2.6.3.1.1-12: Hepatic histopathological findings in dogs administered clethodim (RE-45601 Technical) for 13 weeks (mean ± SD)

Sex	**Males**					**Females**				
Dose (mg clethodim/kg bw/day)	**0**	**1**	**25**	**75**	**125**	**0**	**1**	**25**	**75**	**125**
Pathology - microscopic										
Liver: Centrilobular vesicles/vacuoles	4/4	4/4	4/4	4/4	4/4	3/4	4/4	4/4	4/4	4/4
Severity[1]	1.75	2.00	2.25	2.25	3.25	1.67	2.25	2.25	1.75	3.00

One-year oral study in dogs (refer to Vol.3, B.6.3.2/03)

In this study Chevron RE-45601 Technical (purity: 83.3%) was administered to Beagle dogs (6/sex/group) orally via gelatine capsules at doses of 0 (control), 1, 75, and 300 mg/kg bw/day for 52 weeks. Treatment was associated with changes in haematological and biochemical parameters noted at ≥75 mg/kg bw/day, organ weight changes (increased liver weights noted in both sexes at ≥75 mg/kg bw/day; increased thyroid/parathyroid weights noted in males of all treated groups but only statistically significant at 300 mg/kg bw/day), macroscopical findings in the liver noted in both sexes at 300 mg/kg bw/day (enlarged, dark liver), and histopathological changes noted in the sternal bone marrow at ≥75 mg/kg/kg bw/day (hyperplasia, both sexes) and in the liver at 300 mg/kg bw/day (hepatocyte hypertrophy, pigment (both sexes)). Treatment-related findings in clinical pathology parameters for the 75 mg/kg group included increases in mean platelet counts (F), leukocyte counts (F), corrected leukocyte counts (F) and decreased glucose values (F). In addition to changes in these parameters, clinical pathological changes for the 300 mg/kg group included decreases in erythrocyte counts (M:9%, F: 18%), haemoglobin concentration (M: 8% n.s, F: 14%), haematocrit levels (M: 8%, F: 14%) and glucose levels (M: 12% n.s., F: 13%); and increase in total cholesterol (M; 32%, F: 61%), alanine aminotransferase (M: 167%, F: 144%), alkaline phosphatase (M: 273%, F: 341%), and triglycerides (M: 65%, F: 84%).

The NOAEL in study is set at 1 mg/kg bw/day (equal to 0.83 mg/kg bw/day after correction for purity for test substance) based on haematological changes (indicating anaemia) noted in both sexes at 300 mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 300 mg/kg bw/day, changes in organ weights noted at 75 mg/kg bw/day (increased liver weights (both sexes) and 300 mg/kg bw/day (increased liver weights (both sexes) and thyroid/parathyroid weights (M)), gross pathological findings in the liver (dark and enlarged) noted at 300 mg/kg bw/day (both sexes), and microscopical findings in the sternal bone marrow (hyperplasia) noted at ≥75 mg/kg bw/day (both sexes) and in the liver (hepatocyte hypertrophy, pigment) noted at 300 mg/kg bw/day (both sexes).

The study was performed in accordance with Good Laboratory Practice. The deviations from the current OECD TG 407 are presented in Table 46. These deviations concern endpoints that were not studied which limit the interpretations of the results but do not affect the validity of the study. The study is considered acceptable.

Table 2.6.3.1.1-13: Gross pathology and histopathological findings in dogs administered clethodim (RE-45601 Technical) for 52 weeks.

	Males				**Females**			
Dose (mg clethodim/kg bw/day)	**0**	**1**	**75**	**300**	**0**	**1**	**75**	**300**
Macroscopy[1]								
Liver, dark	0/6	0/6	0/6	4/6	0/6	0/6	0/6	4/6
Liver, enlarged	0/6	0/6	0/6	2/6	0/6	0/6	0/6	2/6
Thyroid: enlarged	0/6	0/6	1/6	1/6	0/6	1/6	0/6	0/6
Thymus: dark	0/6	0/6	1/6	1/6	0/6	0/6	0/6	0/6
Uterus: thickened wall	-	-	-	-	0/6	2/6	2/6	1/6
Uterus: thickened H-wall	-	-	-	-	0/6	0/6	0/6	1/6
Uterus: thickened cervix wall	-	-	-	-	0/6	1/6	2/6	1/6
Vagina: wall thickened	-	-	-	-	0/6	1/6	1/6	2/6
Microscopic findings[1]								
Hepatocyte hypertrophy	0/6	0/6	0/6	5/6	0/6	0/6	0/6	4/6
Hepatocyte pigment	0/6	0/6	1/6	6/6	0/6	0/6	0/6	6/6
Hyperplasia of marrow (sternum)	0/6	0/6	1/6	6/6	0/6	0/6	1/6	6/6
Testis: hypospermia	0/6	0/6	0/6	1/6	-	-	-	-
Testis: abnormal sperm	0/6	0/6	0/6	1/6	-	-	-	-
Testis: chronic active orchitis	0/6	0/6	0/6	1/6	-	-	-	-
Epididymis: abnormal sperm	0/6	0/6	0/6	1/6	-	-	-	-

[1] Number of animals with lesion/number of animals in group

Rabbit:

Developmental toxicity study in rabbits (refer to Vol. 3, B.6.6.2.4/01)

In this study, Chevron RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 7-19 to groups of 19-20 female rabbits at doses of 0, (control), 25, 100 and 300 mg/kg bw/day (equal to 0, 20.8, 83.3 and 250 mg/kg bw/day, after correction for purity of test substance). Treatment related effects were associated with clinical signs (dried faeces, red substance in pan) observed in dams at ≥100 mg/kg bw/day (≥83.3 mg/kg bw/day after correction for purity of test substance), reduced bodyweight gain observed in dams at ≥100 mg/kg bw/day (≥83.3 mg/kg bw/day after correction for purity of test substance), and reduced food consumption observed in dams at ≥100 mg/kg bw/day (≥83.3 mg/kg bw/day after correction for purity of test substance). Since neither of the high dosage group does with red substance in the cage pans aborted and each had viable foetuses at scheduled Caesarean-sectioning, the red substance in the cage pans may reflect rectal irritation and bleeding of these does according to study author. At the high dose level of 300 mg/kg bw/day (250 mg/kg bw/day after correction for purity of test substance) the following developmental effects were observed: increased foetal incidence of angulated hyoid alae, misaligned sutures and nasal irregular ossification. NOAEL for maternal toxicity was set at 25 mg/kg bw/day (20.8 mg/kg bw/day after correction for purity of test substance) based on reduced bodyweight gain observed at ≥100 mg/kg bw/day (≥83.3 mg/kg bw/day after correction for purity of test substance). NOAEL for developmental toxicity was set at 100 mg/kg bw/day based on increased foetal incidence of angulated hyoid alae, misaligned sutures and nasal irregular ossification observed at 300 mg/kg bw/day (250 mg/kg bw/day after correction for purity of test substance).

The study was performed in general accordance with OECD 414 and with FIFRA Good Laboratory Practice (GLP) Standards. The current guideline applicable to rabbits is the version from 2001 since the updates published in 2018 includes rat-specific requirements. The exposure period in the study is shorter than described in the latest relevant

version of the guideline. The exposure period used (day 7-19) covers the main part of organogenesis but organs are still under development later than that. The study is considered acceptable.

Pilot teratology study in rabbits (refer to Vol. 3, B.6.6.2.3/01)

In this dose range finding study, Chevron RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 7-19 to groups of 8 female rabbits at doses of 0, (control), 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw/day, after correction for purity of technical substance using a correction factor of 1.2). Treatment related effects were associated with mortality (≥300 mg/kg bw/day), clinical signs of dried faeces (≥50 mg/kg bw/day, statistical significant at ≥300 mg/kg bw/day), reduced body weight (≥300 mg/kg bw/day), reduced bodyweight gain (≥150 mg/kg/day), reduced feed consumption during the dosage period (≥50 mg/kg/day, statistically significant at 500 mg/kg bw/day) with a post dosage increase in food consumption compared with the control (≥150 mg/kg/day), increased maternal liver weight and liver/body weight ratio (≥300 mg/kg/day, not statistically significant but ~20% increase), gross pathological findings observed in animals that aborted and/or died (hairball in stomach at ≥300 mg/kg bw/day, gastric ulceration at 500 mg/kg bw/day), abortion (500 mg/kg/day), and premature delivery (one animals at 500 mg/kg/day). There was also a possible increase in resorptions: the number of resorptions was 1.4 in the 300 mg/kg bw/day group compared with the 0.3 in the control. There was none in the highest dose group but only one female was available for assessment in that group. In addition, the foetal body weight was 13% and 32% lower in the 300 and 500 mg/kg bw/day dosage groups, respectively, compared with the control. The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated).

The study was not compared to any guideline since it is a pilot study, however the major deviations from a full OECD TG 414 study include the use of less animals per group and a shorter exposure duration. It was performed in accordance EPA, FIFRA, and TSCA Good Laboratory Practice (GLP) Standards. The study is considered as supportive data.

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

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 3.9.2), a substance should be classified in Category 1 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:

Route of Exposure	**Units**	**Guidance Values Ranges: (dose/concentration)**
Oral (rat)	mg/kg bw/day	C≤10
Dermal (rat or rabbit)	mg/kg bw/day	C≤20
Inhalation (rat) gas	ppm V/6h/day	C≤50
Inhalation (rat) vapour	mg/litre/6h/day	C≤0.2
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	C≤0.2

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:

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 tables above is increased by a factor of three for a 28-day study.

The CLP Guidance also states the following for STOT RE (in 3.9.1):

"Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs."

Rat:

<u>5-week oral toxicity study in rats (RAR Vol. 3, B.6.3.1/01)</u>

In this study Clethodim Technical (purity: 83.4%) was administered to Sprague-Dawley rats (10/sex/group) via the diet at concentrations of 0, 5, 200, 1000, 4000, 8000 ppm (equal to 0, 0.26, 12.5, 65.6, 261 and 515 mg/kg bw/day for males, and 0, 0.29, 13.9, 70.6, 291 and 554 mg/kg bw/day for females) for 5 weeks. Vehicle used in study was acetone. Treatment was associated with reduced body weight noted in females at 291 mg/kg bw/day (↓8%) and 554 mg/kg bw/day (↓16%) and in males at 515 mg/kg bw/day (↓13%), reduced bodyweight gain (>10%) noted in both sexes at ≥261/291 (M/F) mg/kg bw/day, changes in haematological parameters noted in males at ≥12.5 mg/kg bw/day and in females at ≥70.6 mg/kg bw/day, changes in biochemical parameters (increased cholesterol) noted in males at 515 mg/kg bw/day (↑68%), changes in urinalysis (increased uric acid) noted in females at ≥291 mg/kg

bw/day, increased liver weights noted in both sexes at ≥65.6/70.6 (M/F) mg/kg bw/day, and histopathological findings in the liver (centrilobular hypertrophy) noted in males at ≥70.6 mg/kg bw/day and in females at ≥291 mg/kg bw/day. Haematological changes (indicating mild anaemia) included: reduced erythrocyte counts (females at ≥70.6 mg/kg bw/day, up to 7% reduction), reduced haemoglobin levels (males at ≥65.6 mg/kg bw/day, 4-7%, females at ≥70.6 mg/kg bw/day, 4-7%), and reduced haematocrits (males at ≥261 mg/kg bw/day, 4-6%). In addition, increased platelets were noted in males at ≥13.9 mg/kg bw/day (30-43%).

The changes in haematological parameters indicating mild anaemia (a reduction of Hb less than 10%) and urinalysis (increased uric acid) were not considered severe enough for classification with STOT-RE. Also, effects on the liver (increased cholesterol, increased weight and centrilobular hypertrophy) were not considered severe enough for STOT-RE classification.

13-week oral study in rats (Vol. 3, B.6.3.2/01)

In this study RE-45601 Technical (purity: 84%) was administered to Sprague-Dawley rats (12/sex/group) via the diet at concentrations of 0, 50, 500, 2500 and 5000 ppm (equal to 0, 2.3, 25, 134 and 279 mg/kg bw/day for males; 0, 2.8, 30, 159 and 341 mg/kg bw/day for females) for 13 weeks. Vehicle used in study was acetone. Following this treatment phase, 12 rats/sex/group were sacrificed. The remaining animals (12 rats/sex/group) in the control and two high dose groups were fed untreated basal diet for an additional six weeks and were sacrificed at the end of this recovery phase. Treatment was associated with reduced body weight noted in males at 134 mg/kg bw/day (7%) and in both sexes at 279/341 (M/F) mg/kg bw/day (>10%), reduced bodyweight gain noted in males at 134 mg/kg bw/day (10%) and in both sexes at 279/341 (M/F) mg/kg bw/day (>10%), reduced food consumption noted in both sexes at 279/341 (M/F) mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 279/341 (M/F) mg/kg bw/day, increased liver weight noted in both sexes at ≥134/159 (M/F) mg/kg bw/day, increased relative kidney weight noted in both sexes at 279/341 (M/F) mg/kg bw/day (M:10%, F: 14%), and histopathological findings in the liver (hepatic centrilobular hypertrophy) noted in both sexes at ≥134/159 (M/F) mg/kg bw/day. Changes in biochemical parameters indicating liver toxicity at 279/341 (M/F) mg/kg bw/day included: increased serum cholesterol (↑M: 31%), total protein (M: ↑5%), globulin (M: ↑9%). Food consumption and body weight gain was reduced during the exposure period but was increased during the recovery period. Final body weight (week 19) was similar between groups except for females of the high dose group (↓7%). The only organ weight that was significantly different after the 6-week recovery period was relative liver weight in females of mid-dose (↑11%) and high (↑13%) dose groups. There were no treatment-related changes present among males and females at the recovery sacrifice.

The effects on the liver (changes in biochemical parameters, increased weight, and hypertrophy) were not considered severe enough for classification with STOT-RE. It could also be noted that no effects were observed within the critical range of doses for Cat 2 classification (i.e. 10<C≤100 mg/kg bw/day).

4-week dermal study in rats (Vol. 3, B.6.3.3/01)

In this study Sprague Dawley rats (6/sex/group) were exposed to repeated dermal doses of RE-45601 Technical (purity: 83.2%) during a 28-day period (21 six-h dermal applications) at doses of 0 (control), 10, 100 or 1000 mg/kg bw/day. The vehicle used in study was 0.7% carboxymethyl cellulose (CMC) and 1% TWEEN 80 in distilled water.

Treatment was associated with local effects of skin irritation observed at all dose levels. Furthermore treatment was associated with clinical signs (anogenital discharge) noted in both sexes at 1000 mg/kg bw/day, reduced bodyweight gains observed in males at 1000 mg/kg bw/day, changes in biochemical parameters (increased triglyceride levels (F: ↑140-160%); reduced BUN/creatinine ratio (M: 22%)) noted at ≥100 mg/kg bw/day, lower food efficiency values noted in males at 1000 mg/kg bw/day, increased liver weights (about 20%) noted in females at 1000 mg/kg bw/day, increased relative kidney weight (10%) noted in males at 1000 mg/kg bw/day, increased relative testes weight (13%) noted at 1000 mg/kg bw/day.

The effects on the liver (changes in biochemical parameters and increased weight) were not considered severe enough for classification with STOT-RE. It could also be noted that the findings in the liver did not occur within the critical range of doses for Cat 2 classification (i.e. 60<C≤600 mg/kg bw/day) (Haber´s rule considered for exposure duration of 28 days). Increased relative kidney weight noted in males at 1000 mg/kg bw/day might reflect the reduced bodyweight gains observed in males at this dose level and is not considered relevant for STOT-RE classification. Furthermore, this effect did not occur within the critical range of doses for Cat 2 classification.

Combined chronic oral toxicity/oncogenicity study in rats (Vol. 3, B.6.5/02)

In this study Sprague Dawley rats were exposed to RE-45601 Technical (purity: 83%) in the diet for 2 years at doses of 0 (control), 5, 20, 500, 2500 ppm (equivalent to 0, 0.15, 0.57, 16 and 86 mg/kg bw/day (males) and 0, 0.2, 0.72, 21 and 113 mg/kg bw/day (females)). The vehicle used in study was Acetone 10 mL/kg diet. Treatment was associated with reduced body weight noted in both sexes at 86/113 (M/F) mg/kg bw/day (At Day 91: M: 7%, F: 6%; At Day 360: M: 7%, F: 8%; At Day 724: M: 8% n.s., F:13% n.s.), reduced bodyweight gain noted at 86/113 (M/F) mg/kg bw/day (M:11%, F: 12%, calculated for the first 3 months), reduced food consumption noted at 86/113 (M/F) mg/kg bw/day (noted at intervals during the study), reduced food efficiency noted in males at 86 mg/kg bw/day (during the first three months), increased liver weights noted in females at 21 mg/kg bw/day (rel. weight: after 1 year: ↑18% n.s.; after 2 y: 12%, n.s.) and in both sexes at 86/113 (M/F) mg/kg bw/day (abs weight after 1 y: M: 15% n.s., F: 24%; rel weight after 1y: M: 22%, F: 18% n.s.; rel weight after 2 y: F: 21%), and histopathological findings in the liver noted in both sexes at 86/113 (M/F) mg/kg bw/day. The histopathological findings consisted of hypertrophy in hepatocytes (observed in both sexes after 1 y and 2 y) and binucleated cells in the liver observed in females after 1 y but not after 2 y. No treatment-related increase in the incidence of neoplasms or other microscopic lesions was found in any of the groups.

The effects on the liver (increased weight and histopathological findings of hypertrophy in hepatocytes and binucleated cells) were not considered severe enough for classification with STOT-RE. It could also be noted that the findings in the liver did not occur within the critical range of doses for Cat 2 classification (i.e. 2.5<C≤12.5 mg/kg bw/day) (Haber´s rule considered for exposure durations of 104 weeks).

Two generation (one litter) reproduction study in rats (Vol. 3, B.6.6.1/02)

In this two generation study, RE-45601 Technical (purity: 83.3%) was administered in the diet to groups of 30 males and females per generation (F0 and F1) at levels of 0, (control), 5, 20, 500, and 2500 ppm (equal to 0, 0.5, 1.2, 32.2 and 163 mg/kg bw/day for males; 0, 0.5, 1.5, 37.4 and 181 mg/kg bw/day for females in the pre-mating period after correction for purity as calculated by the applicant). The vehicle used in study for preparation of diet was Acetone.

Treatment was associated with reduced body weight noted in F0 generation males at 163 mg/kg bw/day (↓4-9%) and in F1 generation males (↓10-19%) and females (↓6-10%), and minor reductions in food consumption in both generations at 163/181 (M/F) mg/kg bw/day, organ weight changes noted at 163 mg/kg bw/day (F0 generation: increased relative testis weight (10%); F1 generation: increased relative epididymis weight (18%), reduced absolute prostate (25%) and seminal vesicles weights (11%)). Relative weights of prostate and seminal vesicles were comparable to control. Furthermore, slightly increased number of still born pups was observed in F1 generation at 163/181 (M/F) mg/kg bw/day. The relevance of this latter finding was unclear. The lack of similar effect in the F2 pups and the four-fold higher value in the F2 controls indicates that the effect may be incidental.

No adverse effects relevant for STOT-RE classification were observed in this study. Effect on organ weight changes observed in this study were not considered severe enough for classification and did not occur within the critical range of doses for STOT-RE Cat 2 classification (i.e. 10<C≤100 mg/kg bw/day) (Haber´s rule considered for exposure durations of 90 days). Effects on offspring (slightly increased number of stillborn) were not considered relevant for STOT-RE classification but were further discussed in section 2.6.6.1.

Pilot rat reproduction study in rats (Vol. 3, B.6.6.1/01)

In this pilot study, groups of 8 male and 8 female Sprague-Dawley Crl:CD strain rats were fed diet containing 0, 500, 2000 or 5000 ppm RE-45601 Technical (purity: 83.3%) for 1 week before mating. The doses equal to 0, 20.8, 83.3, 208.3 mg/kg bw/day when corrected for purity of active substance. The vehicle used in study for preparation of diet was Acetone. Females received the diet continuously throughout mating and gestation, and until Day 7 of lactation when they were necropsied. The offspring were exposed to the test material in utero and while nursing until they were sacrificed and necropsied on Day 7 of lactation. Effects on adults and offspring were observed at the maximum dose level of 5000 ppm (208.3 mg/kg bw/day).

Treatment was associated with reduced bodyweight noted in adults at 208.3 mg/kg bw/day (Males: week 0-2: 2%; Females: GD 20 13%, LD 0 F: 14%, LD: 7 (16%)), reduced bodyweight gain noted in adults at 5000 ppm (M: 18%, F: 63%) and reduced food consumption noted in adult males during the first week (pre-mating) (15%). In the offspring reduced combined pup weights were noted at all dose levels (On day 7: 9%, 9%, and 11% in the low, middle and high dose, respectively; Day 0-7: 13%, 14%, and 16% in the low, middle and high dose, respectively). There were no effects on reproduction indices for males or females, or on pup litter size, survival, and sex ration.

The systemic effects observed in the dams (reduced bodyweight gain and food consumption) were not considered severe enough or relevant for STOT-RE classification. Effects on offspring (reduced pup weight) were further discussed in section 2.6.6.1.

Teratology study in rats (Vol. 3, B.6.6.2.2/01)

In this study, pregnant dams (Crl:CD rats) (25/dose) were administered clethodim (purity: 83.3%) by gavage during gestational days 6-15 at doses of 0, 10, 100, 300 and 700 mg/kg bw per day (equal to 0, 8.3, 83.3, 292 and 583 mg/kg bw/day after correction for purity of test substance). Maternal toxicity was observed in the top two doses, with increasing severity with dose. Manifestations of maternal toxicity included mortality (5 of 25 animals) noted at 700 mg/kg bw/day (583 mg/kg bw/day after correction for purity of test substance), clinical signs (excessive salivation, excessive lacrimation, poor condition, red/mucoid nasal discharge, alopecia, staining of the ano-genital

area, chromodocryorrhea (top dose only)) noted at ≥350 mg/kg bw/day, reduced maternal body weight noted at 350 mg/kg bw/day (GD 20: 7%; GD 20 corrected value: 6%) and 700 mg/kg bw/day (GD 20: 8%; GD 20 corrected value: 13%), reduced bodyweight gain noted at 350 mg/kg bw/day (GD 6-15: 15% n.s.; GD 15-20: 17%; GD 0-20 corrected value: 77%) and 700 mg/kg bw/day (GD 6-15: 40%; GD 15-20: 17%; GD 0-20 corrected value: 11%). Furthermore, food consumption was reduced in the highest dose group during the exposure period (except for the last day). Uterine weight was reduced in a dose dependent manner: 7% reduction in the 100 mg/kg bw/day group, 10 % in the 350 mg/kg bw/day group, and 27% in the 700 mg/kg bw/day group (only the top dose was statistically significant). The mean number of resorptions and resorptions per implant was increased in the top dose group (not statistically significant). There were fewer litters with viable foetuses in the highest dose group. Foetal body weight was reduced at 350 mg/kg bw/day (11%) and 700 mg/kg bw/day (25%). Furthermore, the incidence of skeletal variations (retarded ossification processes) was increased in the top two doses. There was also a higher incidence of external and visceral malformations among the top dose foetuses. Seven out of the 8 foetuses with external malformations had (among other things) deformed tails, an effect that is associated with maternal toxicity. Because the fetotoxic effects only were observed in the presence of maternal toxicity, the distinction between direct and indirect effects on the foetus is unclear.

Mortality was observed in dams (5 of 25 dams) at 700 mg/kg bw/day (583 mg/kg bw/day after correction for purity of test substance). This effect was considered severe and relevant for human health and noted within the critical range of doses for STOT-RE Category 2 classification (i.e. C≤1000 mg/kg bw/day) (Haber´s rule considered for exposure duration of 9 days). Several animals in the 700 mg/kg bw/day dose group began to show signs of toxicity (salivation, red nasal discharge, poor condition, staining of the fur in the ano-genital area) at Day 10 of gestation which appeared to be treatment-related, and five females died during the Day 11-16 gestation interval after five to 10 days of treatment. Mortality was not observed in the pilot study (Report No. S-2807, Vol. 3, B.6.6.2.1/01) or in other repeated dose toxicity studies conducted with the rat. However, it could be noted that dose levels used in these studies were lower compared to this study. The developmental effects observed in this study were not considered relevant for STOT-RE classification but have been further discussed in section 2.6.6.2.

Pilot teratology study in rats (Vol. 3, B.6.6.2.1/01)

In this dose range finding study, RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 6-15 to groups of 10 females at doses of 0, (control), 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw per day, after correction for purity of test substance).

At the top dose of 500 mg/kg bw/day (417 mg/kg bw/day when corrected for purity of test substance), observed effects included increased salivation (8/10 dams), reduced body weight (Day 20: ↓10% n.s.), reduced bodyweight gain (Day 15-20: ↓38.8%; Day 6-20: ↓62.5%), reduced number of implantation sites (87 versus 126 in control, n.s.), and increased pre-implantation loss ratio (0.289 versus 0.082 in control, n.s.), reduced number of viable foetuses (86 versus 122 in control, within historical control values), and reduced foetal weight of viable foetuses (↓11%) .

In the second highest dose of 300 mg/kg bw/day (250 mg/kg bw/day when corrected for purity), observed effects included increased salivation in the dams (8/10 dams) and reduced pup weight (7%, not statistically significant).

The systemic effects observed in the dams (salivation, reduced body weight/bodyweight gain) were not considered severe enough or relevant for STOT-RE classification. The developmental effects observed in this study were further discussed in section 2.6.6.2.

90-day oral dietary neurotoxicity study in rats (Vol. 3, B.6.7.1.3)

In this study, RE-45601 (purity: 95.4%) was administered in the diet to groups of 12 males and females at levels of 0 (control), 500, 1500 and 5000 ppm (equal to 0, 31, 94 and 331 mg/kg bw/day for males, and 0, 38, 115 and 380 mg/kg bw/day for females) for 28 days. No mortality occurred and no clear treatment related clinical signs were observed. Body weights of both sexes were lower in the highest dose group than those of the control group throughout the study (7-11% in males and 7-9% in females). Body weights of the low and middle dose were comparable to control weights or higher (body weight of female of the 500-ppm group). Body weight gain was reduced in both sexes (16% in males and 19% in females) over the entire study period, in general due to lower gains during the first month. Food consumption in males was reduced during the first week, potentially indicating palatability issues, but the food consumption per animal was slightly lower also after that, the consumption per kg bw was similar or slightly higher the rest of the study. In females, the food consumption per animal was lower/slightly lower throughout the study while the consumption per kg bw was similar to the control group overall. The functional battery revealed no treatment related effect on home cage, handling, open field, sensory, or neuromuscular observations. Physiological observations included lower body weight in both sexes. No clear treatment related trends were observed in the treated animals. Total and ambulatory motor activity counts for the 5000 ppm (380 mg/kg bw/day) group females at the study week 7 evaluation was lower than that of the control. The value was also lower than the HCD and the control value was higher than the HCD. No effects on habituation were observed. There were no effects on liver weight, brain weight, or brain length or width but it is noted that relative weights were not reported. No treatment related changes were noted during necropsy.

The effects noted in this study (reduced body weight/bodyweight gain) were not considered relevant for STOT-RE Category 2 classification.

28-day dietary dose range finding study neurotoxicity study (Vol. 3, B.6.7.1.2)

In this dose range finding study, RE-45601 (purity: 95.4%) was administered in the diet to groups of 5 males and females at levels of 0 (control), 500, 1500 and 5000 ppm for 28 days. The mean test substance consumption in the 500, 1500, and 5000 ppm groups was 45, 132, and 441 mg/kg/day, respectively, for males and 51, 155, and 475 mg/kg/day, respectively, for females over the entire study (study days 0-28). It is not clear whether the results from the chemical analysis was used to calculate these values or if only feed consumption was used.

No deaths occurred and no clinical signs stood out in the exposed groups. Body weight and/or body weight gain were affected in all dose groups at one interval or more. The final weight at day 28 was 15 % lower in males of the high dose (441 mg/kg bw/day) group, and body weight gain was reduced by 16, 14, and 30 % in the low, middle, and high dose males, respectively. A similar trend was observed in females. The absolute weight was not significantly affected in females (a 5% reduction at the top dose) and the overall body weight gain was reduced by 21% in this group (475 mg/kg bw/day), mainly due to a significant decrease of body weight change during the first week (48%). No clear effects on food consumption were observed, but body weight gained as percent of feed consumed was lower in the 5000 ppm (441 mg/kg bw/day) males. No treatment related effects on home cage,

handling, sensory or neuromuscular observations, or motor activity were observed. Absolute brain weight, but not liver weight in males, was slightly reduced in the top and lowest dose group (↓5%) in the low dose and ↓ 4% in the top dose. No effect on brain weight was observed in middle dose group, thus no dose-response.

The effects noted in this study (reduced body weight/bodyweight gain, slightly reduced brain weight) were not considered of concern for a STOT-RE Category 2 classification. It could be noted that no effects occurred within the critical range of doses for Cat 2 classification (i.e. 30<C≤300 mg/kg bw/day) (Haber´s rule considered for exposure duration of 28 days).

Five-week sub-chronic feeding study of high purity RE-45601 (SX-1718) and RE-45601 process Neutrals (SX-1717) in rats (B.6.8.2/03)

This study was designed to investigate whether the observed toxicity in the studies performed with low purity RE-45601 (84.3% purity) could be ascribed to the impurities or process Neutrals.

RE-45601 Technical and RE-45601 Process Neutrals (containing 3.3% RE-45601) were administered to rats (Sprague-Dawley) (10/sex/group) via the diet for 5 weeks. Dose levels were: Clethodim: 8000 ppm (equivalent to 597 and 667 mg/kg bw/day for males and females); Process Neutrals: 1200 ppm (4.87/5.78 mg clethodim/kg body weight/day (males/females). Control animals received the vehicle (10 mL/kg feed) only.

In summation, exposure to 597/667 mg clethodim/kg body weight/day via the diet (males/females) resulted in reduced body weight (F: 9-15%) and bodyweight gain (M: 33%, F: 42%), mild anaemia (5-7% reductions in erythrocyte, haemoglobin and haematocrit values), increased liver weight (M: abs.:12%, rel.: 34%, F: rel. 24%), liver centrilobular hypertrophy, and altered serum chemistry values (albeit within historical control values) (males only). In addition, adrenal weight was reduced (M: 26%, F: 17%) but no histopathological lesions were observed. Males were more severely affected than females. Animals exposed to 148/175 mg process neutrals/kg body weight/day containing 4.87/5.78 mg clethodim/kg body weight/day (males/females) were also affected, but not as severely. This exposure led to reduced body weight gain (males), reduced alkaline phosphatase values (within historical controls), centrilobular hypertrophy, increased liver weight (females), and reduced testes weight. In general, the animals exposed to clethodim was more severely affected, and increased albumin and total protein levels, and anaemia was observed in these animals only. However, the process neutrals also affected the animals.

The incidence of centrilobular hypertrophy was slightly higher for the RE-45601 treatment groups (RE-45601 treatment group: 10 of 10 males and 8 of 10 females; Process Neutrals treatment group: 6 of 10 males and 3 of 10 females).

Effects observed in the liver (increased weight and centrilobular hypertrophy) were not considered severe enough for STOT-RE classification and did not occur within the critical range of doses for Cat 2 classification (i.e. 30<C≤300 mg/kg bw/day) (Haber´s rule considered for exposure duration of 28 days).

The study was performed with GLP compliance. It was not conducted according to a specific OECD test guideline.

Cytochrome P-450 concentration following 21-day oral administration in male rats (Vol. 3, B.6.8.2/04)

This study was designed to investigate the potential of RE-45601 technical to induce cytochrome P-450 following 21-days of oral administration in male Sprague-Dawley rats. Male rats were administered 208 mg clethodim/kg

bw/day for 21 days via oral gavage. This exposure resulted in increased liver weights (abs weight ↑21%, rel. weight ↑23%) but no other signs of overt toxicity. The mean CYP450 concentration, determined in liver samples from the exposed rats, did not statistically differ from that of the control.

Effects observed in the study (increased weight) were not considered severe enough for STOT-RE classification.

Mouse:

4-week oral study in mice (RAR Vol. 3, B.6.3.1/02)

In this study, RE-45601 Technical (purity: 83.3%) was administered to mice (CD-1) (10/sex/group) via the diet at concentrations of 0, 100, 250, 625, 1500 and 4000 ppm (equivalent to 0, 11.9, 29.7, 74.4, 179 and 476 mg/kg bw per day as calculated by applicant) for 28 days. Vehicle used in study was acetone. Treatment was associated with changes in haematological parameters noted in males at ≥74.4 mg/kg bw/day and in females at ≥179 mg/kg bw/day, increased liver weights noted in males at ≥179 mg/kg bw/day and in females at 476 mg/kg bw/day, and histopathological findings in the liver (hepatic centrilobular hypertrophy) noted in both sexes at 476 mg/kg bw/day. Haematological changes included: reduced haemoglobin noted in males at ≥74.4 mg/kg bw/day (4-8%) and in females at ≥179 mg/kg bw/day (6%), reduced haematocrit noted in males at 476 mg/kg bw/day (8%), and reduced erythrocyte count noted in males at ≥179 mg/kg bw/day (4-9%).

The changes in haematological parameters indicating mild anaemia were not considered severe enough for classification with STOT-RE (a reduction of Hb less than 10%). Nor were the findings in the liver (increased weight and hepatic centrilobular hypertrophy) considered severe enough for a classification as STOT-RE. It could also be noted that the histopathological findings in the liver did not occur within the critical range of doses for Cat 2 classification (i.e. 30<C≤300 mg/kg bw/day) (Haber´s rule considered for exposure duration of 28 days).

Chronic oral oncogenicity study in mice (Vol. 3, B.6.5/01)

In this study mice (CD-1) (60/sex/group) were orally exposed to Chevron RE-45601 Technical (83.3%) for 78 weeks at doses of 0 (control), 20, 200, 1000, 2000/3000 ppm (equal to 0, 2.4, 24, 119 and 238/357 mg/kg bw/day after correction for purity of test substance) for 78 weeks. The vehicle used in study was Acetone 1.5 mL/kg of feed. Treatment was associated with increased mortalities noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day) (due to amyloidosis), changes in haematological parameters noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day), increased liver weights noted in males at ≥1000 ppm (119 mg/kg bw/day) and in females at 2000/3000 ppm (238/357 mg/kg bw/day), macroscopical changes in the kidney (pale kidney in animals dying or sacrificed due to moribund status) noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day), and histopathological findings noted in the liver (males and females at ≥1000 ppm (119 mg/kg bw/day)) and the lung (males at ≥1000 ppm (119 mg/kg bw/day)) and findings of increased systemic amyloidosis noted in both sexes at 2000/3000 ppm (238/357 mg/kg bw/day). Changes in haematological parameters noted at 2000/3000 ppm (238/357 mg/kg bw/day) consisted of reduced erythrocytes (Week 79: M: ↓14%; Week 53: M: ↓19%, F: ↓8% n.s., Week 27: M: ↓8%, F: ↓5%), haematocrit (M: ↓12% n.s.) and haemoglobin (M: ↓12% n.s.). Histopathological findings in the liver at ≥1000 ppm (119 mg/kg bw/day) consisted of centrilobular hypertrophy (both sexes), increased pigment (F) and bile duct hyperplasia (M). Treatment-related microscopic findings in the lungs consisted of foci of amphophilic alveolar macrophages (at ≥1000 ppm (119 mg/kg bw/day), both sexes). An additional treatment-related microscopic finding

for unscheduled deaths included an increased incidence in systemic amyloidosis for the 3000 ppm (357 mg/kg bw/day) animals. Although amyloidosis is frequently noted in mice of this age and strain, the increased incidence in the high-dose group suggests a treatment-related exacerbation of this finding. The study did not show carcinogenic potential of clethodim technical.

The effects observed in the study were not considered of concern for a STOT-RE classification since the effects were not severe enough and did not occur within the critical range of doses for Cat 2 classification (i.e. $1.5 < C \leq 15$ mg/kg bw/day) (Haber´s rule considered for exposure duration of 78 weeks).

28-day oral (dietary) immunotoxicity studies (Vol.3, B.6.8.2/01-02)

Two immunotoxicity studies were performed, one dose range finding study (Vol. 3, B.6.8.2/01) and one main study (Vol. 3, B.6.8.2/02). Both were performed according to OPPTS 870.7800 (1998) with no deviations except that the dose range finding study did not include a positive control. In these studies, Clethodim TG (purity: 95.4%) was administered in the diet to groups of 10 female mice (main study) or 8 female mice (range finding study) at levels of 0, 400, 2000 and 4000 ppm (corresponding to 101, 551 and 958 mg/kg bw/day in the dose range finding study and 0, 136, 603 and 1312 mg/kg bw per day in the main study). No signs of toxicity except for increased liver weights and lower food consumption were observed. The absolute liver weights in the dose range finding study were 16 and 41% higher in the middle (603 mg/kg bw/day) and high (1312 mg/kg bw/day) dose, respectively when compared to the control. The corresponding relative liver weight values were 16 and 39% higher in the middle and high dose, respectively when compared to the control. In the main study, the absolute and relative liver weights were 17 and 13 % higher in the middle and high dose, respectively, when compared to the control group, and the corresponding relative liver weights were 45 and 42 % higher in the middle and high dose, respectively when compared to the control.

No immunosuppressant effect was observed in the dose range finding study. There was a statistically significantly higher mean AFC response in the 2000 ppm (551 mg/kg/bw/day) group (↑54%). There was a similar tendency in the 4000 ppm (958 mg/kg bw/day) group, the mean value was 36% higher than that of the control group (not statistically significant) but the value was lower than that of the 2000 ppm (551 mg/kg/bw/day) group. In the main study, there was a 19-15% reduction in AFC response in the top two doses but there was no dose response, the differences were not statistically significant, and there was an increase in this endpoint in the dose-range finding study. There was also a statistically significant decreasing trend in relative spleen weight (Jonckheere's Test); however, the differences between the exposed groups and the control were not statistically significant and the mean value of the highest dose group was only 8% lower than that of the control (0.36 vs 0.39). Overall, clethodim does not appear to be immunotoxic at these dose levels.

The effects on the liver (increased weights) were not considered severe enough for classification with STOT-RE and did not occur within the critical range of doses for Cat 2 classification (i.e. $30<C\leq300$ mg/kg bw/day) (Haber´s rule considered for exposure duration of 28 days).

Dog:

90-day oral study in dogs (Vol. 3, B.6.3.2/02)

In this study, RE-45601 Technical (purity: 83.3%) was administered to Beagle dogs (4/sex/group) orally via gelatine capsules at doses of 0 (control), 1, 25, 75 and 125 mg/kg bw/day (equal to 0, 0.83, 21, 62 and 104 mg/kg bw/day when corrected for purity) for 13 weeks. Treatment was associated with changes in biochemical parameters noted in females at ≥75 mg/kg bw/day and in males at 125 mg/kg bw/day, increased liver weights noted in both sexes at ≥75 mg/kg bw/day, and histopathological changes in the liver (increased severity of centrilobular vesicles/vacuoles) noted in both sexes at 125 mg/kg bw/day. Changes in biochemical parameters noted at 125 mg/kg bw/day included: increased cholesterol (F: 40-58%), increased alkaline phosphatase (M: 67% n.s., F: 88%), increased globulin (M: 22%) and reduced albumin/globulin (M: 21%).

The effects on the liver (changes in biochemical parameters, increased weight and increased severity of centrilobular vesicles/vacuoles) were not considered severe enough for classification with STOT-RE. It could also be noted that the histopathological findings in the liver did not occur within the critical range of doses for Cat 2 classification (i.e. 10<C≤100 mg/kg bw/day).

One-year oral study in dogs (Vol.3, B.6.3.2/03)

In this study Chevron RE-45601 Technical (purity: 83.3%) was administered to Beagle dogs (6/sex/group) orally via gelatine capsules at doses of 0 (control), 1, 75, and 300 mg/kg bw/day for 52 weeks. Treatment was associated with changes in haematological and biochemical parameters noted at ≥75 mg/kg bw/day, organ weight changes (increased liver weights noted in both sexes at ≥75 mg/kg bw/day; increased thyroid/parathyroid weights noted in males of all treated groups but only statistically significant at 300 mg/kg bw/day), macroscopical findings in the liver noted in both sexes at 300 mg/kg bw/day (enlarged, dark liver), and histopathological changes noted in the sternal bone marrow at ≥75 mg/kg/kg bw/day (hyperplasia, both sexes) and in the liver at 300 mg/kg bw/day (hepatocyte hypertrophy, pigment (both sexes)). Treatment-related findings in clinical pathology parameters for the 75 mg/kg group included increases in mean platelet counts (F), leukocyte counts (F), corrected leukocyte counts (F) and decreased glucose values (F). In addition to changes in these parameters, clinical pathological changes for the 300 mg/kg group included decreases in erythrocyte counts (M:9%, F: 18%), haemoglobin concentration (M: 8% n.s, F: 14%), haematocrit levels (M: 8%, F: 14%) and glucose levels (M: 12% n.s., F: 13%); and increase in total cholesterol (M; 32%, F: 61%), alanine aminotransferase (M: 167%, F: 144%), alkaline phosphatase (M: 273%, F: 341%), and triglycerides (M: 65%, F: 84%).

The effects observed in the study were not considered of concern for a classification as STOT-RE. No adverse effects occurred within the critical range of doses for Cat 2 classification (i.e. 2.5 < C ≤ 25 mg/kg bw/day) (Haber´s rule considered for exposure duration of 52 weeks).

Rabbit:

Developmental toxicity study in rabbits (Vol. 3, B.6.6.2.4/01)

In this study, Chevron RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 7-19 to groups of 19-20 female rabbits at doses of 0, (control), 25, 100 and 300 mg/kg bw/day (equal to 0, 20.8, 83.3 and

250 mg/kg bw/day, after correction for purity of test substance). Treatment related effects were associated with clinical signs (dried faeces, red substance in pan) observed in dams at ≥100 mg/kg bw/day (≥83.3 mg/kg bw/day after correction for purity of test substance), reduced bodyweight gain observed in dams at ≥100 mg/kg bw/day (≥83.3 mg/kg bw/day after correction for purity of test substance), and reduced food consumption observed in dams at ≥100 mg/kg bw/day (≥83.3 mg/kg bw/day after correction for purity of test substance). Since neither of the high dosage group does with red substance in the cage pans aborted and each had viable foetuses at scheduled Caesarean-sectioning, the red substance in the cage pans may reflect rectal irritation and bleeding of these does according to study author. At the high dose level of 300 mg/kg bw/day (250 mg/kg bw/day after correction for purity of test substance) the following developmental effects were observed: increased foetal incidence of angulated hyoid alae, misaligned sutures and nasal irregular ossification.

The effects noted in dams (clinical signs of dried faeces and red substance in pan, and reduced bodyweight gain) were not considered severe enough for a STOT-RE Category 2 classification. The developmental effects observed in this study were not considered relevant for STOT-RE classification but have been further discussed in section 2.6.6.2.

Pilot teratology study in rabbits (Vol. 3, B.6.6.2.3/01)

In this dose range finding study, Chevron RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 7-19 to groups of 8 female rabbits at doses of 0, (control), 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw/day, after correction for purity of technical substance using a correction factor of 1.2). Treatment related effects were associated with mortality (≥300 mg/kg bw/day), clinical signs of dried faeces (≥50 mg/kg bw/day, statistical significant at ≥300 mg/kg bw/day), reduced body weight (≥300 mg/kg bw/day), reduced bodyweight gain (≥150 mg/kg/day), reduced feed consumption during the dosage period (≥50 mg/kg/day, statistically significant at 500 mg/kg bw/day) with a post dosage increase in food consumption compared with the control (≥150 mg/kg/day), increased maternal liver weight and liver/body weight ratio (≥300 mg/kg/day, not statistically significant but ~20% increase), gross pathological findings observed in animals that aborted and/or died (hairball in stomach at ≥300 mg/kg bw/day, gastric ulceration at 500 mg/kg bw/day), abortion (500 mg/kg/day), and premature delivery (one animals at 500 mg/kg/day). There was also a possible increase in resorptions: the number of resorptions was 1.4 in the 300 mg/kg bw/day group compared with the 0.3 in the control. There was none In the highest dose group but only one female was available for assessment in that group. In addition, the foetal body weight was 13% and 32% lower in the 300 and 500 mg/kg bw/day dosage groups, respectively, compared with the control.

The mortalities observed in dams at 300 mg/kg bw/day (250 mg/kg bw/day after correction for purity of test substance) and 500 mg/kg bw/day (417 mg/kg bw/day after correction for purity of test substance) were considered as a severe and relevant effect for human health and noted within the critical range of doses for STOT-RE Category 2 classification (i.e. 75<C≤750 mg/kg bw/day) (Haber´s rule considered for exposure duration of 12 days). One dam in the 300 mg/kg bw/day group was found dead on day 26 of gestation. It had persistent weight loss occur from day 10 of gestation (body weight: 3.79 kg) until its death on day 25 (body weight: 2.94 kg). Its feed consumption was inhibited throughout the dosage period, as compared with the pre-dosage period, and from day 1 until its death. Clinical signs interrelated with the observed decrease in feed consumption were dried faeces (days 17 to 23 of gestation) and no faeces (days 24 and 25 of gestation). At necropsy, the rabbit had a small hairball present in the

stomach and paraovarian cysts. Another dam at 300 mg/kg bw/day was found dead on day 21 of gestation. This rabbit had a general pattern of weight loss occur between days 7 (body weight: 4.15 kg) and 20 (body weight: 3.74 kg) of gestation. Daily feed consumption for this doe was remarkably inhibited beginning on day 14 of gestation; on days 17 through 19 of gestation. Soft or liquid faeces were observed for the doe on days 19 and 20 of gestation. Necropsy revealed a hairball in the stomach and dilated, blood-filled intestinal blood vessels. One rabbit in the 500 mg/kg bw/day group aborted and was found dead on day 20 of gestation; red substance, assumed to be blood and a sign of abortion, was observed in the cage pan on the day death occurred. This rabbit had persistent body weight loss occur from the initiation of dosage (day 7 body weight: 4.25 kg; day 19 body weight: 3.40 kg). Its daily feed consumption was first remarkably decreased on day 8 of gestation; from day 12 of gestation until its death. Dried faeces were observed for the doe on days 13 through 19 of gestation. Necropsy of this doe revealed paraovarian cysts, numerous ulcerations in the gastric pylorus and a hairball present in the stomach. Another dam at 500 mg/kg bw/day was found dead on day 8 of gestation. This death may have been interrelated with a possible intubation accident during administration of the second dosage. This rabbit had clonic convulsions occur within approximately seven minutes after intubation, the rabbit died within 15 minutes of intubation and at necropsy had haemorrhagic lungs. The haemorrhagic lungs may have resulted from convulsive activity; test substance was present in the stomach and not apparent in the lungs. In addition to the lung changes, necropsy revealed paraovarian cysts.

The developmental effects observed in this study were not considered relevant for STOT-RE classification but have been further discussed in section 2.6.6.2.

Overall conclusion- findings relevant for STOT-RE:

Mortality was observed in the developmental toxicity study in rats (Vol. 3, B.6.6.2.2). Five of 25 dams died at 700 mg/kg bw/day (583 mg/kg bw/day, value corrected for purity of test substance). This effect was considered relevant for STOT-RE classification since the effect is severe and occurred within the critical range of doses for STOT-RE Category 2 classification (i.e. C≤1000 mg/kg bw/day) (Haber´s rule considered for exposure duration of 9 days). Several animals in the 700 mg/kg bw/day dose group began to show signs of toxicity (salivation, red nasal discharge, poor condition, staining of the fur in the ano-genital area) at Day 10 of gestation which appeared to be treatment-related, and five females died during the Day 11-16 gestation interval after five to 10 days of treatment. Mortality was not observed in the pilot study (Vol. 3, B.6.6.2.1/01) or in other repeated dose toxicity studies conducted with the rat. However, it could be noted that dose levels used in these studies were lower compared to this study.

Furthermore, mortality was observed in the pilot developmental toxicity study in rabbits (Vol. 3. B.6.6.2.3/01). Two of seven pregnant at 300 mg/kg bw/day (250 mg/kg bw/day, value corrected for purity of test substance) dosage group rabbits died, and one of seven pregnant 500 mg/kg bw/day (417 mg/kg bw/day, value corrected for purity of test substance) dosage group rabbits aborted and died. The death of a second 500 mg/kg bw/day dosage group doe was probable related to the test substance according to study author, although this event may be the result of a possible intubation accident. All deaths appeared to be interrelated with decreased feed consumption, weight loss, gastrointestinal lesions (hairball and/or ulceration) and/or abortion. There were no deaths, abortion or premature delivery observed in the main study at the highest dose level of 300 mg/kg bw/day (250 mg/kg bw/day, value corrected for purity of test substance). However, maternal toxicity (clinical signs of dried faeces and red substance in pan, inhibited maternal body weight gain and feed consumption) was produced by the 100 and 300 mg/kg bw/day dosages of the test substance indicating toxicity. The mortalities were noted at a dose level within the critical range

of doses for STOT-RE Category 2 classification (i.e C≤750 mg/kg bw/day) (Haber´s rule considered for exposure duration of 12 days). The mortalities observed in the pilot study could support a STOT-RE classification.

As a conclusion STOT-RE Cat 2 is proposed based on mortalities observed in the rat developmental toxicity supported by mortalities observed in the pilot teratogenicity study in rabbits.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Classification as STOT-RE 2. H373 ("May cause damage through prolonged or repeated exposure") is proposed based on mortalities observed in rats (developmental study, Vol. 3, B.6.6.2.2/01) and rabbits (pilot developmental study, Vol. 3, B.6.6.2.3/01).

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 50: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro.*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial gene mutation OECD Guideline 471. Genetic Toxicology: Bacterial Reverse Mutation Test. (Adopted July 21, 1997). Deviations from current guidelines: None GLP: Yes Acceptable	Clethodim Technical Lot/Batch: 4478 Purity: 95.98 ± 0.04% w/w Vehicle: Dimethylsulphoxide (DMSO)	<u>Plate incorporation method and the pre-incubation method</u> With/without S9 Strains: TA1535, TA1537, TA98, TA100 (*S. typhimurium*) and WP2*uvr*A (*E. coli*) <u>Doses:</u> 52, 164, 512, 1600 and 5000 µg/plate <u>Plate incorporation method</u> With/without S9 Strains: TA1535, TA1537, TA98, TA100 (*S. typhimurium*) <u>Doses:</u> 0, 100, 300, 1000, 3300, and 10000 µg/plate	Negative. Not mutagenic in *Salmonella typhimurium* and *Escherichia coli* under the specified experimental conditions. Cytotoxicity was observed in tester strains TA1535, TA1537, TA98 and TA100 at the highest tested concentration both with and without metabolic activation.	Groot 2020 Report No.: 20182212 Vol.3. B.6.4.1/01 New data for renewal: Yes
Bacterial gene mutation 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2 (1983) Deviations from OECD 471: - *Escherichia coli* WP2*uvr*A was not included, and no	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Dimethylsulphoxide (DMSO)	Plate incorporation method With/without S9 Strains: TA1535, TA1537, TA98, TA100 (*S. typhimurium*) <u>Doses:</u> 0, 100, 300, 1000, 3300, and 10000 µg/plate	Negative Clethodim was not shown to be mutagenic under these conditions in this *Salmonella typhimurium*. The test item was not completely miscible with the top agar at ≥ 3.3 mg/plate. The test item was slightly cytotoxic to TA100 and	Machado 1986a Report No.: S-2760 Vol. 3. B.6.4.1/02 New data for

independent repeat test was performed GLP: Yes Acceptable			TA1535 at 3300 g/plate with metabolic activation and at 10 mg/plate without metabolic activation.	renewal: No
Bacterial gene mutation 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2 (1983) Deviations from OECD 471: - laboratory historical control data was not reported. GLP: Yes Acceptable with limitations (uncertainties regarding statistical analysis)	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Dimethylsulphoxide (DMSO)	Plate incorporation method With/without S9 Strains: TA1535, TA1537, TA98, TA100 (*S. typhimurium*) and WP2*uvr*A (*E. coli*) <u>Doses:</u> 0, 100, 333, 1000, 3333, and 10000 µg/plate	Equivocal results Negative for the strains TA1535, TA1537, TA100 and WP2*uvr*A both with and without metabolic activation. In TA98, the mean number of revertant colonies was 1.9 times higher than the control value (both +S9 and -S9) in the first experiment, and 1.8 (+S9) and 2.1 (-S9) times higher than the control value in the second experiment. Slight cytotoxicity was observed only in TA100 with S-9 at 10 mg/plate.	Machado 1986b Report No.: S-2859 Vol. 3. B.6.4.1/03 New data for renewal: No
Mammalian cell gene mutation assay (CHO-HGPRT assay in vitro) Guidelines followed: None mentioned, study performed in general accordance with OECD 476 Deviations from TG 476 (2016): - shorter concentration intervals GLP: Yes Acceptable	Clethodim (Select) Lot/Batch: 10195-36 Purity: 92.7% Vehicle: Dimethylsulphoxide (DMSO)	Chinese Hamster Ovarian CHO cells With/without S9 <u>Doses:</u> 100, 200, 300, 400, 450, 500 µg/mL	Negative both with and without metabolic activation Cytotoxicity was observed at higher concentrations (≥450 µg/mL) (without metabolic activation) Precipitation occurred in the highest dose group.	Lehn 1990 Report No.: T6033343 Vol. 3. B.6.4.1/04 New data for renewal: No
Chromosome aberration assay Guidelines followed: 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2 (1983) Deviations from OECD 473 (2016): - laboratory historical control data was not reported. - the evaluation criteria are inconsistent with recommendations. - fewer cells than recommended were scored.	Chevron RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3%	Chinese Hamster Ovarian (CHO) cells With/without S9 <u>Doses:</u> <u>First study:</u> 0.03, 0.1, 0.3, 1.0 µL/mL <u>Second study:</u> 0.6, 0.8, 1.0, 1.2 µL/mL	Negative with metabolic activation Positive without metabolic activation at the top two concentrations	Putman 1986a Report No.: S-2761 Vol. 3. B.6.4.1/05 New data for renewal: No

- the exposure time was shorter than recommended. GLP: Yes				
Chromosome aberration assay Guidelines followed: 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2 (1983) Deviations from OECD 473 (2016): - laboratory historical control data was not reported. - the evaluation criteria are inconsistent with recommendations. - fewer cells than recommended were scored - the exposure time was shorter than recommended. GLP: Yes Acceptable with limitations	Purified Chevron RE-45601 Lot/Batch: SX-1718 Purity: 96.1%	Chinese Hamster Ovarian (CHO) cells With/without S9 <u>Doses:</u> <u>First study:</u> 0.03, 0.1, 0.3, 1.0 µL/mL <u>Second study:</u> 0.6, 0.8, 1.0, 1.2 µL/mL	Negative both with and without metabolic activation	Putman 1986b Report No.: S-2865 Vol. 3. B.6.4.1/06 New data for renewal: No
In vitro Micronucleus Assay in Cultured Peripheral Human Lymphocytes Guidelines followed: OECD 487 (2016) Deviations from guideline: none GLP: Yes Acceptable	Clethodim Technical Lot/Batch: 4478 Vehicle: Dimethyl sulfoxide (DMSO) Purity: 95.98%	Concentration levels Assay 1A (3 h exposure time, 27 h harvest time): 50, 600 and 900 µg/mL culture medium (without S9). 50, 600 and 1000 µg/mL medium (with S9) Assay 2 (24h exposure, 24 h harvest time): 10, 100, and 250 µg/mL culture medium (without S9) Concentration selection was based on the presence/absence of precipitation and cytotoxicity.	Negative	De Jong 2021 Report No.: 2020-33038 Vol. 3. B.6.4.1/07 New data for renewal: Yes

Table 51: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells in vivo.

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
In vivo mammalian bone marrow chromosomal aberration assay Guideline followed: 40 CFR 158.135, Pesticide Assessment Guideline No.84-2 of 1983 The study was checked for deviations from	Clethodim Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: 0.7% Carboxymethylcellulose (CMC) with 1.0%	Rats (Sprague Dawley) exposed to clethodim by oral gavage. <u>First dose range finding study:</u> not considered reliable due to poor homogeneity of the suspension and variable analytical results. <u>Second dose range finding study (5/sex):</u> 5.0, 2.5, 1.8, 1.2 and 0.6 g	Negative No measurements of plasma/blood concentration of the test substance were performed and there was no depression of mitotic index. However, TK data is available showing bone marrow exposure (see	[redacted] 1987 Report No.: S-2864 Vol. 3, B.6.4.2/01

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
OECD TG 475 (2016). The following deviations were observed: -laboratory historical control data have not been reported -no linear trend assessments has been performed - only 50 metaphases are analysed instead of the prescribed 200 metaphases - no blood samples were taken GLP: Yes Acceptable	Polyoxyethylene Sorbitan Mono-oleate (Tween-80)	test article in 0.7% CMC with 1% Tween-80/kg bw <u>Cytogenetics study (15/sex):</u> Test substance: 0.15, 0.5, 1.5 g/kg bw Positive control: 0.1 mg/mL thriethylenemelamine (TEM) in distilled water: 0.5 mg/kg bw <u>Study duration:</u> Five animals per sex and dose group were sacrificed 12, 24, and 48 h after the exposure was initiated. The positive control animals were sacrificed 24 h after exposure initiation.	Vol. 3, B.6.1, Report No.: MEF-0086).	New data for renewal: No
In vivo-in vitro unscheduled DNA (UDS) synthesis test in hepatocytes of male mice Guideline followed in study: 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2 (1983) The study was checked for deviations from OECD TG 486 (1997). The following deviations were observed: -no control animals for the 2 h timepoint - only two analysable animals in the control (due to technical error) GLP: Yes Supportive	Clethodim Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: 0.7% Carboxymethylcellulose (CMC) high viscosity. Polyoxyethylene Sorbitan Mono-oleate (Tween-80).	Male mouse (B6C3F1) exposed to clethodim by oral gavage. Exposure duration: 16 h <u>Main study:</u> Vehicle control (3 animals sacrificed at 16 h): 0.7% CMC and 0.5% Tween-80 Test substance (3 animals/group sacrificed at 2 h, 3-5 animals/group sacrificed at 16 h): 100, 1000, 5000 mg/kg bw Positive control: 10 mg DMB/kg bw (1.0% tween-80 was used in the middle and high dose group)	Negative	[redacted] 1986 Report No.: S-2762 Vol. 3, B.6.4.2/02 New data for renewal: No

Table 52: Summary table of human data relevant for genotoxicity / germ cell mutagenicity.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

The potential mutagenicity of clethodim has been studied *in vitro* in both bacteria and mammalian cells, and *in vivo* in a rat bone marrow chromosomal aberration test and a mouse liver UDS assay. All these studies were conducted in accordance with the OECD Principles of Good Laboratory Practice (1981). The studies were considered acceptable, acceptable with limitations, or supportive. One Ames test and one *in vitro* micronucleus assay are new data for the renewal of active substance.

Three bacterial mutagenicity studies (Ames tests) are available. Two of these studies are old data (presented in DAR, 2005) and one is new data for the renewal of active substance. One previous study investigating *S. typhimurium* as tester strain, gave negative results, both in the presence and absence of metabolic activation. In this study, the test item was slightly cytotoxic to TA100 and TA1535 at 3300 g/plate with metabolic activation and at 10 mg/plate without metabolic activation (Report No.: S-2760). Another previous study showed increased number of revertant colonies of the strain TA98 in the highest dose group (both with and without metabolic activation). In this study *S-typhimurium* and *E.coli* as tester strains were investigated at concentrations up to 10000 µg/plate. Slight cytotoxicity was observed to TA100 only at the highest concentration. RMS considers the result of this study as equivocal. There are limitations in this study since the statistical analysis was not included in the tabulations and due to lack of historical control data (Report No.: S-2859). A new Ames test is submitted for the renewal procedure which gave negative results, both in the presence and absence of metabolic activation (Report No.: 20182212). In this study *S-typhimurium* and *E.coli* as tester strains, were tested at concentrations up to 5000 µg/plate. At this concentration cytotoxicity as evidenced by a decrease in the number of revertants, reduction of the bacterial background lawn and the increase in the size of the microcolonies were present. In the Mammalian cell gene mutation assay in Chinese hamster ovary cells, clethodim produced negative results (Report No.: T6033343). In all these studies mentioned above, the top dose tested was limited by the toxicity of the test substance.

In both *in vitro* chromosome aberration tests in Chinese Hamster ovary cells, clethodim technical was not clastogenic in the presence of metabolic activation. In the initial *in vitro* chromosome aberration test, without metabolic activation, clethodim was found to be positive at the two highest doses tested (0.91 and 1.1 mg/mL) (Report No.: S-2761). However, the second *in vitro* chromosome aberration test performed with a purified clethodim (96.1%) demonstrated negative result without metabolic activation (Report No.: S-2865). Further, the clastogenic responses observed in the chromosome aberration assay in the absence of a metabolic activation system were not confirmed in the *in vivo* studies.

A micronucleus study on peripheral human lymphocytes was submitted (new data for the renewal of active substance) in which no increase in the number of mononucleated and binucleated cells with micronuclei was observed in exposed groups (i.e. the study did not indicate clastogenic or aneugenic potential) (Report No: 2020-33038).

The *in vivo* chromosome aberration test in rats was negative using clethodim technical. Limitations of the study included that no historical control data was provided and fewer cells than specified in OECD 475 (2016) were assessed. In addition, no measurements of plasma/blood concentration of the test substance were performed and there was no depression of mitotic index. However, TK data is available (Report No.: MEF-0086) showing bone marrow exposure (Report No.: S-2864). Also negative was the Unscheduled DNA Synthesis test in hepatocytes of male mice. However, this latter study is considered as supportive data only (Report No.: S-2762).

Considering the results in the genotoxicity studies as presented above, clethodim technical is considered non-genotoxic. The available *in vivo* chromosome aberration test did not confirm the positive/equivocal results from some of the *in vitro* studies.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

The criteria for classification for germ cell mutagenicity under Regulation 1272/2008 (CLP) is as followed:

Category 1: Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1A is based on positive evidence from human epidemiological studies. The classification in Category 1B is based on positive results from in vivo heritable germ cell mutagenicity tests in mammals or positive results from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence has potential to cause mutations to germ cells or positive results from tests showing mutagenic effect in the germ cells of humans, without demonstration of transmission to progeny.

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 tests which are supported by positive results from *in vitro* assays.

The available *in vivo* chromosome aberration test did not confirm the positive/equivocal results from some of the *in vitro* studies.

RMS agrees with the previous conclusion by RAC (2015) that no Cat. 2 classification of clethodim for germ cell mutagenicity is warranted.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

No classification of clethodim for germ cell mutagenicity is warranted.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 53: Summary table of animal studies on long-term toxicity and carcinogenicity.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
Chronic Oral Oncogenicity Study in Mice Guidelines followed: OECD 451 (1981) Deviations from OECD 451 (2018) Organs not harvested/assessed: coagulating gland, lacrimal gland, mammary glands from males (note that this is only required if visibly dissectible, no information on this) Species: Mouse	Chevron RE-45601 Technical Purity: 83.3% Vehicle: Acetone <u>Doses:</u> 0, 20, 200, 1000, 2000/3000* ppm (equal to 0, 2.4, 24, 119 and 238/357 mg/kg bw/day after correction for purity of test substance)	NOAEL: 200 ppm (24 mg/kg bw/day) LOAEL: 1000 ppm (119 mg/kg bw/day) <u>Effects at 1000 ppm:</u> ↑ **absolute liver weight** at week 53 (M: 12% n.s.) ↑ **relative liver weight** (Week 53: M: 17%) ↑ liver weight relative to brain weight (Week 53: M: 15%) - **histopathological changes in the liver** (centrilobular hypertrophy (M, F), increased pigment (F), and bile duct hyperplasia (M)) - **histopathological changes in the lung** (foci of amphophilic alveolar macrophages (M, F)	[redacted] (1988) Report number: S-2867 Vol. 3. B.6.5/01 New data for renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
Strain: CD-1 60 animals per sex and dose level GLP: Yes Acceptable	*Mice in the highest dose group received 2000 ppm the first 15 weeks. Thereafter 3000 ppm Oral exposure (via the diet) Duration of exposure: 52 weeks (10 mice/group) or 78 weeks	<u>Effects at 2000/3000 ppm:</u> ↑ **mortality** (M: 68% vs 42% in the control, F: 52% vs 33% in the control) ↑ **absolute liver weight** at week 53 (M: 16%, F: 16% n.s.) and at week 79 (M: 12% n.s., F: 12% n.s.) ↑ **relative liver weight** at Week 53 (M: 27%, F: 28%) and at week 79 (M: 13% n.s., F: 16%) ↑ **liver weight relative to brain weight** at Week 53 (M: 21%, F: 18%) and at week 79 (M: 15% n.s., F: 20%) - macroscopical changes in the kidney (pale, in animals dying or sacrificed due to moribund status) - **histopathological changes in the liver** (centrilobular hypertrophy (M, F), increased pigment (M), and bile duct hyperplasia (M)) - **histopathological changes in the lung** (foci of amphophilic alveolar macrophages in the lung (M, F)) ↓ **erythrocytes** (Week 27: M: 8%, F:5%; Week 53: M:19% n.s., F: 8% n.s.; Week 79: M: 14%) ↓ **haematocrit** (Week 79: M: 12% n.s.; Week 27: M: 8%) ↓ **haemoglobin** (Week 79: M: 12% n.s,; Week 27: M:7%) ↑ **incidence of systemic amyloidosis** in animals that died/was sacrificed due to a moribund state (M: 42% vs 28% in the control, F: 36% vs 22% in the control) There was an increased incidence of lung adenomas and carcinomas in the treated males relative to control males. The incidence of these tumours for unscheduled deaths and terminally sacrificed animals was 8, 16, 20, 22 and 22% for males in groups treated with 0, 20, 200, 1000, and 2000/3000 ppm, respectively. The incidence was also higher in control females (16%) compared with control males. These values were all within the historical control range: the means in the historical control mice were 14.9% (range: 5.5-26.5%) and 10.2% (range: 4.0-18.4%) in males and females, respectively.	
Combined Chronic Oral Toxicity/ Oncogenicity Study in Rats Guidelines followed: OECD 453 (1981) Deviations from OECD 453 (2018): - prothrombin time and activated partial thromboplastin time were not measured - weight of thyroid, epididymis, heart, spleen, and uterus were not measured - coagulating gland, vagina, and lacrimal gland were not fixed and/or examined	RE-45601 Technical Lot/Batch: SX-1688 Purity: ~83% Vehicle: Acetone Doses: 0, 5, 20, 500, 2500 ppm (equivalent to 0, 0.15, 0.57, 16 and 86 mg/kg bw/day (♂) and 0, 0.2, 0.72, 21 and 113 mg/kg bw/day (♀)) Oral exposure via the diet Duration of exposure:	NOAEL: 500 ppm (16 mg/kg bw/day) LOAEL: 2500 ppm (86 mg/kg bw/day) <u>Effects at 500 ppm:</u> ↑ relative liver weight after 1 y (F: 18% n.s.) and after 2 y (F: 12% n.s.) ↑ liver weight relative to brain weight after 1 y (F: 24%) <u>Effects at 2500 ppm:</u> ↓ body weight (At Day 91: M: 7%, F: 6%; At Day 360: M: 7%, F: 8%; At Day 724: M: 8% n.s., F:13% n.s) ↓ **bodyweight gain** calculated for the first 3 months (M:11%, F: 12%) ↓ food consumption at intervals during the study (M, F) ↓ food efficiency during the first three months (M)	[redacted] (1988a) Report number: S-2766 Vol.3. B.6.5/02 New data for renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL Bold text=adverse effect	Reference
- the humidity varied a lot and was outside the recommended range Species: Rat Strain: Sprague-Dawley® Crl:CD® BR 65 animals/sex/ group 10 animals/sex/ group were sacrificed at interim sacrifice (1 year) GLP: Yes Acceptable	104 weeks	↑ **absolute liver weight** after 1 y (M: 15% n.s., F: 24%) but not 2 y ↑ **relative liver weight** after 1 y (M: 22%, F: 18% n.s.) and after 2 y (F: 21%) ↑ **liver weight relative to brain** weight after 1 y (M: 16% n.s., F: 23%) but not 2 y - **hypertrophy in hepatocytes** (after 1 year: 1 M and 3 F, none in the control; after 2 years: 1 M and 2 F in this dose group vs 1 F in the control) - **binucleated cells in the liver** after 1 y (6 F vs 1 in the control) but not after 2 y - ↑chronic pancreatitis (F: 15 animals compared to 4 animals in the control group) (unclear relevance)	

Table 54: Summary table of human data on long-term toxicity and carcinogenicity.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 55: Summary table of other studies relevant for long-term toxicity and carcinogenicity.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

The dossier includes one long-term toxicity/carcinogenicity study in the rat and one long-term toxicity/ carcinogenicity study in the mouse (Table 53). Both studies were included in the previous EU evaluation (DAR 2005). There are no new data for this endpoint in this report. The submitted studies are shortly summarised in text (below):

Combined Chronic Oral Toxicity/Oncogenicity Study in Rats (Report No. S-2766)

In this study, sixty-five male and female Sprague-Dawley rats per group were fed diets containing 0 (control), 5, 20, 500 and 2500 ppm RE-45601 Technical for two years. The concentrations in feed equal to 0, 0.15, 0.57, 16 and 86 mg/kg bw per day for males, and 0, 0.20, 0.72, 21 and 113 mg/kg bw per day for females. The vehicle used in study was Acetone 10 mL/kg diet. Ten animals/sex/group were sacrificed at one year. Survivors were sacrificed after 731-739 days on study. There were no significant differences in mortality rates after one year or at the end of the study. Mortality after two years (excluding accidental deaths) was 53, 51, 54, 50 and 67% for males and 45, 42, 49, 60 and 51% for females in the 0, 5, 20, 500 and 2500 ppm groups, respectively. Treatment was associated with reduced body weights noted in both sexes at 2500 ppm (86 mg/kg bw/day) (At Day 91: M: 7%, F: 6%; At Day 360: M: 7%, F: 8%; At Day 724: M: 8% n.s., F:13% n.s). After three months of feeding, the bodyweight gains of males and females in this group were 89 and 88% respectively, of controls. Decreased food consumption was observed for animals in the 2500 ppm group during the first year of the study (noted at intervals); males in this group also had decreased food efficiency during the first three months. No treatment-related clinical signs, ophthalmic

abnormalities, clinical pathology changes, or differences in brain, kidney, adrenal, or gonad weights were observed during the study. The main target organ was the liver as presented by effects on liver weights and histopathological changes in the liver. Increased absolute and/or relative liver weights and trace to mild centrilobular hypertrophy in a few animals were seen in both sexes in the 2500 ppm group at the end of one year. Females treated at 2500 ppm also showed increased (9%) incidence of binucleated cells in the liver compared to control (2%) but the effect was of uncertain toxicological significance. Relative liver weights were also increased in 500 ppm females, but no hypertrophy was observed. At the end of the study, the liver/body weight ratio was increased in females in the 2500 ppm group; this reflected the decreased body weight in these animals. There were no absolute or relative liver weight changes in males and no treatment-related centrilobular hypertrophy in either sex. It is noted that the intake of the active substance was lower during the second year of the study in all dose groups. This could contribute to the more pronounced effects on the liver at 1 year compared with 2 years. There was no evidence of carcinogenicity in this study.

The NOAEL in study is 500 ppm (equal to 16 mg/kg bw/day) based on reduced bodyweight gain noted at 2500 ppm (both sexes), increased liver weights noted at 2500 ppm (both sexes), and histopathological findings in the liver noted at 2500 ppm (hypertrophy (both sexes) and nucleated cells (females)). The NOAEL set in previous evaluation DAR (2005) remains. The study was performed in accordance with OECD 453 and with EPA, FIFRA and TSCA Good Laboratory Practice (GLP) Standards. The deviations from the current guideline (OECD 453, 2018) includes the ones listed in table 53. The deviations are not considered to have a major impact on the study outcome. The study is considered acceptable.

Table 2.6.5.1-1: Selected pathology parameters of rats administered RE-45601 Technical in the diet for 104 weeks (mean±SD)

	Males					**Females**				
Dose (ppm)	**0**	**5**	**20**	**500**	**2500**	**0**	**5**	**20**	**500**	**2500**
mg/kg bw/day	**0**	**0.15**	**0.57**	**16**	**86**	**0**	**0.20**	**0.72**	**21**	**113**
Pathology										
Non-neoplastic lesions										
Interim sacrifice#										
Centrilobular hypertrophy	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	3/10
All study animals										
Binucleated cells	2/65	0/65	0/65	0/65	1/65	1/65	0/65	0/65	1/65	6/65

No. of animals with lesion/ No. of animals in group.

Combined Chronic Oral Toxicity/Oncogenicity Study in mice (Report number: S-2867)

In this study mice (CD-1) (60/sex/group) were exposed to Chevron RE-45601 Technical for 78 weeks at doses of 0 (control), 20, 200, 1000, 2000/3000 ppm (equal to 0, 2.4, 24, 119 and 238/357 mg/kg bw/day after correction for purity of test substance). The vehicle used in study was Acetone 1.5 mL/kg of feed. Ten mice per sex per group were randomly selected for sacrifice after 52 weeks of treatment. Animals remaining on study following 78 weeks of treatment were sacrificed. No consistent signs of toxicity were apparent in the gross clinical signs noted during Weeks 7-78. At 78 weeks, there was a significant treatment related increase in mortality. Survival incidence was 58, 66, 60, 52 and 32% for the 0, 20, 200, 1000 and 2000/3000 ppm group males, respectively, and 67, 84, 80, 59 and 48% for the 0, 20, 200, 1000 and 2000/3000 ppm group females, respectively. The predominant cause of death was an increased incidence and severity of systemic amyloidosis. There was no statistically significant effect on body weight, bodyweight gain or food consumption. Evaluation of the haematologic values revealed a slight decrease in

red cell mass, i.e., decreased erythrocyte counts, haemoglobin (males only), and haematocrit (males only) values in the 2000/3000 ppm (238/357 mg/kg bw/day) group mice at Weeks 27 and 53 of treatment. Statistically significant differences were noted for some of these values at Week 27 only. The only treatment-related change in red cell mass at Week 79 was a significant decrease in mean erythrocyte count in Group 2000/3000 ppm males. An increased incidence of pale kidneys was noted for the 2000/3000 ppm mice dying or sacrificed due to moribund status which probably correlated with the increased amyloidosis noted microscopically. Mean values of absolute and/or relative, i.e., to terminal body weight and brain weight, liver weights were increased in the 1000 ppm (119 mg/kg bw/day) group males and the 2000/3000 ppm group males and females at Week 53, and in the 2000/3000 ppm group females at Week 79. Microscopic changes consisted of increased incidence of systemic amyloidosis as a cause of death in the high-dose group. This finding was noted in 42% of the high-dose males versus 28% of the control males, and in 36% of the high-dose females versus 22% of the control females Treatment-related microscopic findings were also observed in the liver and in the lung. Centrilobular hypertrophy was noted in the 1000 ppm group and 2000/3000 ppm group males and the 2000/3000 ppm group females following 52 weeks of treatment. After 78 weeks increased pigment and bile duct hyperplasia in the liver of 1000 ppm and 2000/3000 ppm group animals were observed in addition. Increased pigment was also observed in 2000/3000 ppm group males after 53 weeks. After 78 weeks of the test material administration, foci of amphophilic alveolar macrophages were observed in the lung of 1000 ppm and 2000/3000 ppm group animals.

There was an increased incidence of lung adenomas and carcinomas in control females and treated males and females relative to control males. However, these tumours are not believed to be related to treatment with Chevron RE-45601 Technical due to the absence of a dose response, a similar incidence in historical data, the apparent late onset of the tumour, and the lack of statistical support. The incidence of these tumours for unscheduled deaths and terminally sacrificed animals was 8, 16, 20, 22, and 22 % for males in groups 0, 20, 200, 1000, and 2000/3000 ppm, respectively, and 16, 26, 20, 22, and 18% for females in groups 0, 20, 200, 1000, and 2000/3000 ppm, respectively. The range of incidences of these types of tumours in the historical control mice were 5.5-26.5 % and 4.0-18.4 % in males and females, respectively.

The NOAEL of this study is 200 ppm (equal to 24 mg/kg bw/day, value corrected for purity of test substance) based on increased mortalities noted in both sexes at 2000/3000 ppm, changes in haematological parameters (reduced cell mass) noted in both sexes at 2000/3000 ppm, increased liver weights noted in males at ≥1000 ppm and in females at 2000/3000 ppm, and microscopical finding in the liver noted at ≥1000 ppm (centrilobular hypertrophy (both sexes), increased pigment (females), bile duct hyperplasia (males)) and in the lungs noted at ≥1000 ppm (foci of amphophilic alveolar macrophages, both sexes) and increased incidence of systemic amyloidosis noted in both sexes at 2000/3000 ppm. The NOAEL set in previous evaluation DAR (2005) remains. The study was performed In accordance with OECD 451 and FIFRA Good Laboratory Practice. There were some organs that were not harvested/assessed that are listed in the current guideline (OECD 451, 2018), specifically coagulating gland, lacrimal gland, and mammary glands from males (note that this is only required if the glands are visibly dissectible, no information on this). This does not invalidate the study. The study is considered acceptable.

Table 2.6.5.1-2: Selected histopathology parameters of mice administered RE-45601 Technical in the diet

	Males					**Females**				
Dose (ppm)	**0**	**20**	**200**	**1000**	**2000 3000**	**0**	**20**	**200**	**1000**	**2000 3000**

Main groups (mg/kg bw/day)	**0**	**2.4**	**24**	**119**	**238/ 257**	**0**	**2.4**	**24**	**119**	**238/ 357**
Non-neoplastic lesions[1]										
Interim sacrifice (week 53)										
Liver: centrilobular hypertrophy	0/10	1/10	1/10	8/10	10/10	1/10	2/10	2/10	8/10	9/10
Liver: increased pigment	0/10	0/10	0/10	0/10	5/10	0/10	0/10	0/10	0/10	0/10
Terminal sacrifice										
Liver: centrilobular hypertrophy	1/28	1/31	1/30	10/24	16/16	0/32	0/41	0/39	4/29	10/22
Liver: hyperplasia bile duct	0/28	0/31	1/30	4/24	5/16	1/32	0/41	0/39	0/29	2/22
Liver: increased pigment	0/28	0/31	0/30	7/24	11/16	2/32	1/41	4/39	5/29	8/22
Lung: foci of amphophilic alveolar macrophages	0/28	0/31	1/30	5/24	8/16	0/32	0/41	0/39	3/29	13/22

[1] No. of animals with lesion/ No. of animals in group.

Table 2.6.5.1-3: Summary of neoplastic findings in lungs of mice administered RE-45601 Technical in the diet

		Males					**Females**				
Dose (ppm)		**0**	**20**	**200**	**1000**	**2000/3000**	**0**	**20**	**200**	**1000**	**2000/3000**
Main groups (mg/kg bw/day)		0	2.4	24	119	238/ 257	0	2.4	24	119	238/ 357
All deaths (unscheduled, week 53, and terminal sacrifice)											
	Number examined	60	60	60	60	60	60	60	60	60	60
	B – alveolar/bronchiolar adenoma	5	10	12	11	10	9	10	11	10	8
	B – multiple alveolar/bronchiolar adenoma	0	0	0	0	2	0	2	0	1	2
	M – alveolar/bronchiolar carcinoma	0	0	2	2	0	1	3	1	0	0
	N – carcinoma, undifferentiated	0	0	0	0	1	0	0	0	0	0
	N – hepatocellular carcinoma	0	0	0	0	0	1	0	0	0	0
	N – mammary carcinoma	0	0	0	0	0	0	0	2	0	1

B = Primary, Benign Neoplasm; M = Primary, Malignant Neoplasm; N = Metastatic Neoplasm

Table 2.6.5.1-4: Statistical results from the analysis of lung tumour incidence in male mice exposed to clethodim

Tumour type	**Comparison**	**Probability**		
		Prevalence	**Unadjusted[1]**	**Gross adjusted[2]**
Lung adenoma and carcinoma	Trend	0.2624	0.2023	0.1921
	1 vs 2	0.1528	0.1347	0.1588
	1 vs 3	0.0203*	0.0217*	0.0259*
	1 vs 4	0.0750	0.0572	0.0660
	1 vs 5	0.0625	0.0572	0.0611

* Not significant using Bonferroni correction (critical value for p = 0.0125)

[1] All animals included.

[2] Animals which died prior to the first occurrence of any tumour of interest were excluded.

Table 2.6.5.1-5: Historical control data on lung alveolar/bronchiolar tumours in CD-1 mice.

				Terminal		**Total**	
Study	**Year**	**Weeks**	**Finding**	**M**	**F**	**M**	**F**
A	1984	78	Adenoma	3/41	3/35	4/50	3/51
			Adenoma, Multiple	0/41	0/35	0/50	0/51
			Carcinoma	1/41	1/35	2/50	1/51
			Combined	4/41	4/35	6/50	4/51

B	1984	78	Adenoma Adenoma, Multiple Carcinoma Combined	2/37 2/37 3/37 7/37	0/37 1/37 0/37 1/37	2/50 2/50 4/50 8/50	0/50 1/50 1/50 2/50
C	1985	78	Adenoma Adenoma, Multiple Carcinoma Combined	1/38 1/38 2/38 4/38	3/43 0/43 1/43 4/43	1/50 1/50 2/50 4/50	3/50 0/50 1/50 4/50
D	. 1985	78	Adenoma Adenoma, Multiple Carcinoma Combined	8/41 0/41 0/41 8/41	6/50 0/50 0/50 6/50	10/69 0/69 0/69 10/69	7/69 0/69 0/69 7/69
E	1986	78	Adenoma Adenoma, Multiple Carcinoma Combined	2/40 0/40 1/40 3/40	4/46 0/46 1/46 5/46	2/55 0/55 1/55 3/55	4/55 0/55 1/55 5/55
F	1987	78	Adenoma Adenoma, Multiple Carcinoma Combined	13/44 0/44 0/44 13/44	6/40 0/40 3/40 9/40	13/49 0/49 0/49 13/49	6/49 0/49 3/49 9/49
G	1987	78	Adenoma Adenoma, Multiple Carcinoma Combined	7/32 0/32 1/32 8/32	4/32 0/32 1/32 5/32	10/50 0/50 1/50 11/50	6/50 0/50 1/50 7/50

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The placing of a substance in Category 2 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. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

There are no human studies available. The two combined long term/carcinogenicity studies with clethodim technical did not demonstrate treatment-related increases in tumours in rats or mice. Therefore, clethodim should not be classified for carcinogenicity.

Table 56: Compilation of factors to be taken into consideration in the hazard assessment.

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Refer to assessment above at section 2.6.5.1. There were no treatment-related increases in tumours in rats or mice.								

2.6.5.3 Conclusion on classification and labelling for carcinogenicity.

Clethodim does not meet the criteria for carcinogenicity under Regulation (EC) 1272/2008. No classification was proposed for carcinogenicity.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies

Table 57: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
Rat Reproduction Study (dose range finding study) Guidelines followed: 40 CFR 158.135, Pesticide Assessment Guideline No.83-4 Species: Rat Strain: Albino Crl: CD Sprague-Dawley P generation: 8 males and 8 females per group Major deviations from OECD 416 (2001): • treatment initiated one week before mating rather than 10 weeks before mating • only one generation, F0 dams and F1 pups terminated on lactation day 7 • low number of females (8), GL recommends use of sufficient number of animals to yield preferably not less than 20 pregnant females at or near parturition. • oestrous cycle length and normality not investigated • testis and epididymis weight not investigated • sperm motility and sperm morphology not analysed • total number of homogenisation-resistant testicular spermatids and cauda epididymal sperm not enumerated • physical development of the offspring not investigated • haematological and clinical parameters not investigated, organ weights not recorded, histopathological investigations not made • less number of observation points GLP: Yes	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Acetone Dietary exposure from 1 week before mating until day 7 of lactation Doses: 0, 500, 2000, and 5000 ppm (equal to 0, 25, 100 and 250 mg/kg bw/day using a default value of 0.05 for chronic rat studies as recommended by EFSA guidance on selected default values (EFSA Journal 2012;10(3):2579)) Values corrected for purity of test substance using a correction factor of 1.2): 0, 20.8, 83.3, 208.3 mg/kg bw/day	No NOAEL was set in study* <u>Parental effects:</u> <u>2000 ppm:</u> No treatment related effects <u>5000 ppm:</u> ↓ food consumption during the first week (pre-mating) (M: 15%) ↓ body weights during week 0-2 of the study (M: 2%), or gestational day 20 (F: 13%), lactational day 0 (F: 14%), and lactational day 7 (F: 16%) ↓ **bodyweight gain during** week 0-3 (M: 18%) or week 0-1 (F: 63%) <u>Offspring effects:</u> <u>500 ppm:</u> ↓**combined pup weight** on day 7 (9%) ↓ **combined pup weight gain** between day 0 and 7 (13%) <u>2000 ppm:</u> ↓**combined pup weight** on day 7 (9%) ↓ **combined pup weight gain** between day 0 and 7 (14%) <u>5000 ppm:</u> ↓**combined pup weight** on day 7 (11%) ↓ **combined pup weight gain** between day 0 and 7 (16%) <u>Comment:</u> The reduced food consumption (observed in both sexes but only significant in males) could be a result of reduced palatability of the food containing the test item. The observed parental effects, which mainly included reduced body weights, could at least in part be attributable to the reduced food intake	[redacted] (1986) Report number: S-2758 Vol. 3. B.6.6.1./01 New data for renewal: Yes

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL Bold text=adverse effect	Reference
Supportive			
Two Generation (One Litter) Reproduction Study in Rats Guidelines followed: Reproductive and Fertility Effects 40 CFR 158.135, Pesticide Assessment Guideline 83-4 Deviations from OECD 416 (2001): - no analysis of sperm parameters - developmental and functional observations of pups were not performed - weighing of adrenals, brain, liver, pituitary gland, spleen, thyroids were not performed - histopathology of the vagina was not performed - dosing before mating period seems to be 9 weeks (the guideline recommends dosing to be continued for at least 10 weeks before the mating period) Species: Rat Strain: Albino Crl: COBS/ CD Sprague-Dawley F0 generation: 30 males and 30 females per group F1 generation: 30 males and 30 females per group GLP: Yes Acceptable	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Acetone 10 ml acetone/kg food Exposure: The F0 males and females received the test material via the diet throughout pre-mating, mating, gestation, and lactation F1a indirect exposure in utero and through nursing, and direct exposure from weaning to pre-mating, mating, gestation, and lactation. F2 indirect exposure in utero and through nursing <u>Doses:</u> 0, 5, 20, 500 and 2500 ppm (equal to 0, 0.5, 1.2, 32.2 and 163 mg/kg bw/day for males; 0, 0.5, 1.5, 37.4 and 181 mg/kg bw/day for females in the pre-mating period after correction for purity as calculated by the applicant)	NOAEL parental toxicity: 500 ppm (32.2 mg/kg bw/day) NOAEL offspring toxicity: 500 mg/kg bw/day (32.2 mg/kg bw/day) NOAEL reproductive toxicity: 2500 ppm (163 mg/kg bw/day) LOAEL parental toxicity: 2500 ppm (163 mg/kg bw/day) LOAEL offspring toxicity: 2500 ppm (163 mg/kg bw/day) LOAEL reproductive toxicity: Not determined. <u>Effects at 2500 ppm:</u> <u>F0 adults</u> ↓ food intake (during a few days) ↓ body weight (M: 4-9%) ↑ relative testis weight (10%) <u>F1 adults</u> ↓ food intake ↓ **body weight** (M: 10-19%, F: 6-10%) ↓ absolute prostate and seminal vesicles weight (25 and 11%, respectively), unclear relevance ↑ relative weight of the left epididymis (18%) <u>F1 pups:</u> **- slightly increased number of stillborn pups** (unclear relevance) - decreased bodyweight gain (5% n.s.) <u>F2 pups:</u> - decreased bodyweight (6% n.s.)	[redacted] (1987) Report number: S-2778 Vol. 3. B.6.6.1/02 New data for renewal: No

*Study not suitable for NOAEL setting (low number of animals used and limited parameters investigated)

Table 58: Summary table of human data on adverse effects on sexual function and fertility.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 59: Summary table of other studies relevant for toxicity on sexual function and fertility.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

The potential of clethodim to cause effects on sexual function and fertility was examined in one two-generational (one litter) study in the rat. This study was submitted and evaluated in previous EU evaluation (DAR 2005). For the renewal of active substance, the pilot study for the main study has been submitted in addition.

The submitted studies (Table 57) are shortly summarised in text (below):

A two-generation (one litter) reproduction study in rats (Report No: S-2778)

A two-generation reproductive toxicity study was performed in which rats (30 males and females/generation (F0 and F1)) were given clethodim at a dietary concentration of 0, 5, 20, 500, and 2500 ppm (equal to 0, 0.5, 1.2, 32.2 and 163 mg/kg bw/day for males; 0, 0.5, 1.5, 37.4 and 181 mg/kg bw/day for females in the pre-mating period after correction for purity as calculated by the applicant)).

No test material-related changes in clinical observations or mortality were observed in either generation. Mean body weights were significantly reduced for both F0 and F1a adult males exposed to 2500 ppm of RE-45601. Body weights for F1a adult females were significantly reduced during the pre-mating (7-10%) and gestation periods (6-9%) up through day-7 (6%) of lactation. While body weights were reduced for F1a females, body weight gain during gestation was not affected by treatment but corrected maternal body weight during pregnancy calculated as a change in maternal body weight gain compared to controls using data point GD0 and LD1 shows a reduced bodyweight gain of 14% in F1 females at 2500 ppm. In F1a males of the high dose group, food consumption was reduced during some time periods in the pre-mating period, during the whole mating period when food consumption was measured, and at times after mating. Mean food consumption values were significantly reduced on days 0-2, 2-5, and 9-12 of gestation of the F1 females However, in all cases, effects were mild (<18%). There was no effect on body weight or food consumption in the F0 females. No effect on the reproductive ability of F0 and F1a adults were observed. Mating indices, pregnancy rates, male fertility, and the oestrous cycle were not affected by treatment.

An increase in the number of stillborn F1 pups was observed (14 pups which corresponds to 3.8% of the delivered pups in the highest treatment group compared with 2 pups, i.e. 0.7%, in the control group). The number of F0 females with at least one stillborn pup was 7 (25%) in the 2500 ppm group and 2 (9.1%) in the control group. The number of stillborn F2 pups in the control group was 7 (2.7%), indicating that the control value in the F1 generation may be in the lower range of the spectrum. No increase in stillborn F2 pups was observed. According to the applicant, historical control data was not available from the lab, but a reference was made to a historical value of 9 stillborn pups from 6 litters cited from one control group in a different 2-generation study performed earlier by the same laboratory. The effect increases with dose, and it is noted that a decrease in the number of litters with viable foetuses was also observed in the high dose group in the developmental toxicity study performed in the same strain although the incidence was yet within historical control range. Overall, the lack of effect in the F2 pups indicates that this may be an incidental finding.

Dilation of the renal pelvis was observed in five F1 pups (1.8%) in 4 litters (16.7%) in the high dose group. No incidence of this was observed in the control group. According to the applicant, no further details is available with respect to the historical control data. Considering that the kidney has not been identified as target organ in other

studies and that there were no indications of renal toxicity in the developmental study performed in the same strain, this finding is not considered to demonstrate teratogenic effect.

In the F1 males, the absolute weights of the prostate and seminal vesicles were 25 and 11% lower than the controls, respectively. The relative weights were similar to the controls. It is noted that while reduced prostate and seminal vesicles weights were observed, no histological lesions were increased in this group. The terminal body weights of both F0 and F1 males were lower in the high dose group. An increase in relative, but not absolute, testis weight was observed in the F0 generation. The absolute, but not relative, weight of the left epididymis was increased in the F1 generation. These increases in organ weights are likely a result of the reduction in body weight. No differences in terminal body weight or organ weights were observed in F0 or F1 females.

The NOAEL for parental toxicity in study is 500 ppm (32.2 mg/kg bw/day) based on reduced body weights noted at 2500 ppm covering also reduced absolute prostate and seminal vesicles weights of unclear relevance noted in F1 adults at 2500 ppm. NOAEL for reproductive toxicity is 2500 ppm (163 mg/kg bw/day, highest dose tested). The toxicological relevance of the slightly increased number of stillborn noted in F1 pups at 2500 ppm is unclear. However, since it is not considered safe to fully exclude an effect of treatment, it is considered appropriate to take a prudent approach and set the NOAEL for offspring toxicity at the same level as the parent NOAEL, i.e. 500 ppm. This NOAEL would also cover for the reduced bodyweight observed in high dose pups on day 21 after culling (6%, not statistically significant) that are seen at all dose levels in the dose-range findings study on day 7 (13-16%, statistically significant). This proposed NOAEL is a change from the previous assessment (DAR 2005) in which the NOAEL for offspring toxicity was set at 2500 ppm. The NOAELs for parental and reproductive toxicity set in previous evaluation remains.

The study was performed in general accordance with OECD 416 and with EPA, FIFRA and TSCA Good Laboratory Practice (GLP) Standards. There were some deviations from the current version of the guideline. Endpoints required in OECD 416 (2001) that was not assessed/measured in the study included analysis of sperm parameters, developmental and functional observations of pups, weight of adrenals, brain, liver, pituitary gland, spleen, and thyroid, and histopathology of the vagina. While these limits the scope of the study, they do not affect the acceptability. The study is considered acceptable.

Table 2.6.6.1.1-1: Body weights (g) of F0 and F1a males (mean ± SD)

Day	Dietary concentration of RE-45601 Technical (mg/kg)				
	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
F0 generation premating males					
0	215 ± 10.1	210 ± 8.7	213 ± 9.9	211 ± 9.1	210 ± 10.8
7	269 ± 14.5	269 ± 11.2	265 ± 13.5	263 ± 13.3	258 ± 15.1** (↓4%)
14	315 ± 17.3	312 ± 15.8	310 ± 20.2	306 ± 27.6	301 ± 16.5** (↓4%)
21	355 ± 20.6	352 ± 19.3	353 ± 22.9	346 ± 20.1	336 ± 19.5** (↓5%)
28	388 ± 23.7	386 ± 22.9	386 ± 27.5	375 ± 26.0	367 ± 25.0** (↓5%)
35	417 ± 25.9	413 ± 23.7	415 ± 29.8	404 ± 25.2	395 ± 26.0** (↓5%)
42	440 ± 28.5	434 ± 23.9	440 ± 31.9	424 ± 28.0	414 ± 28.6** (↓6%)
49	659 ± 31.7	454 ± 25.7	460 ± 34.0	443 ± 32.8	427 ± 32.7** (↓4%)
56	477 ± 33.5	474 ± 28.9	480 ± 36.5	463 ± 34.6	447 ± 31.9** (↓6%)

Day	Dietary concentration of RE-45601 Technical (mg/kg)				
	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
63	495 ± 34.9	490 ± 29.9	495 ± 37.6	474 ± 43.3	460 ± 34.7** (↓7%)
F0 generation mated males					
70	502 ± 35.8	493 ± 30.5	500 ± 33.4	494 ± 40.8	466 ± 35.0** (↓7%)
77	517 ± 38.4	508 ± 27.5	515 ± 40.8	496 ± 40.5	480 ± 36.2** (↓7%)
84	525 ± 41.9	516 ± 29.8	524 ± 42.4	506 ± 40.6	490 ± 38.8** (↓7%)
91	533 ± 44.1	524 ± 32.3	533 ± 46.4	514 ± 43.8	494 ± 39.6** (↓7%)
98	537 ± 47.6	527 ± 33.6	538 ± 47.4	521 ± 43.8	498 ± 40.8** (↓7%)
105	546 ± 48.9	536 ± 33.2	549 ± 49.7	533 ± 46.6	508 ± 43.0** (↓7%)
112	556 ± 48.9	543 ± 34.5	557 ± 53.2	541 ± 47.3	516 ± 45.4** (↓7%)
119	562 ± 50.6	548 ± 37.7	563 ± 58.6	548 ± 49.9	522 ± 46.6** (↓7%)
126	568 ± 50.1	553 ± 37.1	570 ± 59.4	552 ± 52.2	527 ± 47.9** (↓7%)
133	577 ± 57.7	558 = 43.7	573 ± 59.0	548 ± 51.0	523 ± 55.2** (↓9%)
F1a generation premating males					
119	77 ± 10.2	74 ± 8.8	75 ± 10.4	74 ± 8.4	67 ± 11.1** (↓13%)
126	124 ± 16.1	123 ± 23.0	123 ± 17.4	121 ± 12.0	108 ± 19.9** (↓13%)
133	183 ± 21.8	180 ± 24.7	179 ± 27.8	179 ± 14.8	149 ± 38.5** (↓19%)
140	243 ± 24.5	240 ± 27.8	238 ± 29.3	238 ± 18.1	203 ± 43.3** (↓16%)
147	297 ± 27.4	296 ± 31.0	293 ± 29.6	294 ± 22.6	255 ± 45.9** (↓14%)
154	348 ± 31.1	347 ± 34.3	341 ± 29.0	344 ± 25.9	303 ± 45.4** (↓13%)
161	383 ± 34.3	384 ± 36.7	376 ± 28.4	378 ± 29.2	335 ± 43.6** (↓13%)
168	411 ± 38.6	415 ± 39.7	408 ± 29.5	409 ± 34.8	363 ± 43.1** (↓12%)
175	440 ± 62.1	440 ± 45.1	437 ± 30.3	436 ± 37.8	390 ± 46.3** (↓11%)
182	462 ± 45.3	461 ± 46.3	456 ± 33.2	458 ± 39.1	411 ± 48.1** (↓11%)
189	478 ± 47.8	477 ± 49.6	473 ± 34.8	476 ± 43.3	427 ± 49.5** (↓11%)
196	492 ± 51.9	491 ± 51.8	487 ± 36.3	489 ± 43.6	438 ± 50.8** (↓11%)
F1a generation mated males					
203	499 ± 52.7	499 ± 52.8	492 ± 38.0	493 ± 46.4	444 ± 50.9** (↓11%)
210	513 ± 55.8	509 ± 54.1	509 ± 38.0	512 ± 49.6	462 ± 53.9** (↓10%)
217	527 ± 58.4	524 ± 56.4	524 ± 41.0	525 ± 51.5	478 ± 56.4** (↓9%)
224	539 ± 62.4	536 ± 60.5	534 ± 43.8	536 ± 53.3	485 ± 57.3** (↓10%)
231	551 ± 63.3	544 ± 63.1	543 ± 47.5	543 ± 54.1	492 ± 57.0* (↓11%)
238	563 ± 65.0	555 ± 63.8	552 ± 48.5	552 ± 55/7	501 ± 59.0* (↓11%)
245	572 ± 66.9	566 ± 65.3	563 ± 50.2	566 ± 58.8	511 ± 62.1* (↓11%)

Day	Dietary concentration of RE-45601 Technical (mg/kg)				
	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
252	583 ± 69.3	575 ± 68.7	573 ± 50.7	580 ± 61.7	520 ± 63.3* (↓11%)
259	591 ± 71.5	581 ± 74.4	581 ± 52.7	586 ± 63.9	527 ± 64.1* (↓11%)
266	606 ± 74.0	577 ± 76.6	590 ± 53.6	596 ± 52.1	531 ± 56.6* (↓12%)

* p<0.05 different from control; ** p<0.01 different from control

Table 2.6.6.1.1-2: Body weights (g) of F0 and F1a females (mean ± SD)

Day	Dietary concentration of RE-45601 Technical (mg/kg)				
	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
F0 generation premating females					
0	152 ± 10.4	154 ± 9.8	152 ± 8.0	153 ± 8.0	156 ± 11.1
7	173 ± 23.0	177 ± 11.7	173 ± 10.4	177 ± 12.9	175 ± 15.3
14	193 ± 16.2	196 ± 15.8	192 ± 10.2	197 ± 13.2	194 ± 16.5
21	208 ± 18.8	211 ± 17.3	207 ± 13.0	216 ± 16.5	211 ± 18.8
28	221 ± 20.9	225 ± 19.1	221 ± 13.3	228 ± 17.5	223 ± 22.4
35	232 ± 22.7	237 ± 19.3	230 ± 14.8	239 ± 10.5	234 ± 19.3
42	242 ± 24.0	246 ± 19.8	245 ± 16.6	246 ± 18.9	242 ± 21.8
49	231 ± 26.0	252 ± 22.8	253 ± 16.0	256 ± 19.5	250 ± 22.7
56	257 ± 27.6	262 ± 22.6	259 =18.6	263 ± 22.3	256 ± 24.0
63	265 ± 28.6	270 ± 24.1	265 ± 16.5	270 ± 22.6	262 ± 24.6
F0 generation mated females					
70	273 ± 28.3	270 ± 22.5	269 ± 17.9	274 ± 20.7	264 ± 26.3
771	309 ± 17.4	288 ± 22.5	280 ± 7.8	291 ± 11.1	243 ± 25.6
841	300 ± 0.0	314 ± 34.8	288 ± 13.8	308 ± 0.0	237 ± 19.3
911	-	-	-	293 ± 0.0	245 ± 0.0
981	325 ± 41.3	297 ± 42.9	285 ± 21.6	267 ± 26.0	279 ± 35.9
1051	312 ± 35.9	294 ± 36.3	286 ± 14.9	269 ± 24.4	286 ± 28.2
1121	317 ± 31.5	302 ± 28.1	302 ± 16.5	278 ± 34.6	290 ± 36.7
1191	329 ± 0.0	-	313 ± 24.1	296 ± 43.8	325 ± 0.0
F0 maternal body weights during gestation					
0	264 ± 29.4	267 ± 25.4	263 ± 19.8	275 ± 21.3	263 ± 26.7
7	296 ± 32.9	297 ± 25.6	296 ± 18.8	307 ± 23.1	293 ± 26.9
14	326 ± 32.7	327 ± 27.5	322 ± 21.1	331 ± 24.9	320 ± 27.7
21	396 ± 33.0	387 ± 42.6	389 ± 21.3	393 ± 38.8	385 ± 34.7
F0 maternal body weights during lactation					
0	301 ± 20.1	298 ± 28.2	297 ± 16.9	304 ± 26.4	292 ± 29.2
7	312 ± 27.5	312 ± 26.0	315 ± 16.0	318 ± 21.1	308 ± 24.8
14	332 ± 24.1	333 ± 21.0	334 ± 16.9	334 ± 19.0	331 ± 26.7
21	319 ± 24.6	311 ± 18.7	312 ± 15.0	317 ± 21.9	312 ± 27.1
F1a generation premating females					
119	70 ± 8.3	69 ± 8.3	70 ± 8.4	70 ± 7.1	65 ± 8.1* (↓7%)
126	108 ± 12.0	108 ± 11.4	106 ± 14.7	109 ± 8.9	100 ± 13.6 (↓10%)
133	143 ± 14.2	137 ± 21.8	135 ± 23.9	145 ± 10.1	128 ± 26.1* (↓8%)
140	171 ± 17.6	168 ± 20.0	163 ± 22.4	173 ± 11.4	157 ± 23.3
147	194 ± 18.9	191 ± 20.4	188 ± 21.3	196 ± 13.9	178 ± 21.8** (↓8%)
154	218 ± 22.0	215 ± 21.4	212 ± 24.0	218 ± 17.0	199 ± 21.9** (↓9%)
161	233 ± 24.6	226 ± 23.8	225 ± 25.3	231 ± 17.5	211 ± 21.4** (↓9%)
168	245 ± 25.4	239 ± 25.0	237 ± 24.0	244 ± 20.6	222 ± 18.5** (↓9%)
175	258 ± 29.9	249 ± 25.4	249 ± 24.3	258 ± 20.5	236 ± 19.1** (↓9%)

Day	Dietary concentration of RE-45601 Technical (mg/kg)				
	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
182	267 ± 29.4	258 ± 24.5	257 ± 27.3	268 ± 22.7	246 ± 20.0** (↓8%)
189	274 ± 30.9	263 ± 26.3	264 ± 27.4	274 ± 22.8	251 ± 21.3** (↓8%)
196	279 ± 29.9	269 ± 27.2	269 ± 26.0	279 ± 25.1	256 ± 22.7** (↓8%)
F1a generation mated females					
203	282 ± 36.0	275 ± 31.8	270 ± 25.7	279 ± 23.6	258 ± 26.6
210	329 ± 0.0	288 ± 23.5	-	294 ± 25.5	254 ± 0.0
217	342 ± 0.0	305 ± 32.6	-	333 ± 0.0	257 ± 0.0
224	344 ± 0.0	-	-	341 ± 0.0	-
231	317 ± 39.4	293 ± 39.5	271 ± 0.0	320 ± 11.9	289 ± 25.3
238	316 ± 39.2	300 ± 34.8	285 ± 0.0	333 ± 21.7	294 ± 27.5
245	356 ± 0.0	-	-	322 ± 0.0	-
252	361 ± 0.0	-	-	330 ± 0.0	-
F1a maternal body weights during gestation					
0	273 ± 25.3	267 ± 26.9	272 ± 25.0	277 ± 22.6	254 ± 24.3* (↓7%)
7	304 ± 25.9	294 ± 31.2	300 ± 23.5	304 ± 25.4	279 ± 22.8** (↓8%)
14	334 ± 29.6	317 ± 29.2	327 ± 25.2	333 ± 27.6	304 ± 21.3** (↓9%)
21	402 ± 38.7	379 ± 28.1	397 ± 30.7	398 ± 35.7	378 ± 22.4* (↓6%)
F1a maternal body weight during lactation					
0	310 ± 36.3	297 ± 31.8	305 ± 26.7	306 ± 28.7	286 ± 18.8* (↓8%)
7	317 ± 30.6	309 ± 24.5	316 ± 26.0	312 ± 23.4	298 ± 17.4* (↓6%)
14	330 ± 31.9	326 ± 24.6	337 ± 24.4	325 ± 22.8	310 ± 17.5
21	316 ± 32.4	312 ± 26.1	321 ± 23.4	320 ± 22.3	314 ± 16.3

* p<0.05 different from control; ** p<0.01 different from control

[1] Not including pregnant females (their weights are reported under "maternal weight"). Statistical analysis not reported due to small sample size (n=1-5).

Table 2.6.6.1.1-3: Reproductive data, F0→F1a.

Parameter	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
Number of mated females/ treated females	28/28	30/30	30/30	29/30	29/30
Pregnant females (% of mated)	78.6	83.3	76.7	82.8	96.6
Number of mated males/ treated males	29/30	29/30	26/30	28/30	25/29
Males that impregnated at least one female (% mated males)	78.6	82.8	88.5	85.7	96.0
Duration of gestation (days)	21.7	21.9	22.0	22.0*	22.0*
Dams with at least one stillborn pup	2 (9.1%)	5 (20%)	4 (17.4%)	4 (16.7%)	7 (25%)
Dams with only stillborn pups	0	0	0	0	0
Surviving dams with no surviving pups 21 days postpartum	0	1 (4%)	0	0	0
Number pups born	298	308	286	285	367
Number pups born alive	296	303	281	278	353
Number of stillborn	2 (0.7%)	5 (1.6%)	5 (1.7%)	7 (2.5%)	14* (3.8%)
% born stillborn	180	200	186	180	226
Liveborn, not culled	9	13	6	1	7
Number of pups dying 0-21 days	13.5	12.1	12.2	11.6	12.6
Live pups per litter, day 0 (before culling)	7.9	7.8	7.8	7.5	7.8
Live pups per litter, day 4 (after culling)	7.9	7.8	7.8	7.5	7.8
Live pups per litter, day 21	6.0	6.0	6.1	6.3	6.1

Parameter	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
Mean pup weight (g), day 0	9.3	9.6	9.9	10.0	9.4
Mean pup weight (g), day 4 (after culling)	50.0	49.5	51.6	50.5	47.7
Mean pup weight (g), day 21 (after culling)	28/28	30/30	30/30	29/30	29/30

* p<0.05 different from control; ** p<0.01 different from control

Table 2.6.6.1.1-4: Reproductive data, F1a→F2.

Parameter	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
Number of mated females/ treated females	29/30	29/29	30/30	29/30	30/30
Pregnant females (% of mated)	72.4	79.3	96.7*	93.1	83.3
Number of mated males/ treated males	29/30	26/30	30/30	29/30	29/30
Males that impregnated at least one female (% mated males)	72.4	88.5	96.7	93.1	86.2
Duration of gestation (days)	22	21.9	21.9	21.9	21.9
Dams with at least one stillborn pup	5 (23.8%)	5 (21.7%)	6 (20.7%)	8 (29.6%)	6 (24.0%)
Dams with only stillborn pups	0	0	0	0	0
Surviving dams with no surviving pups 21 days postpartum	2 (9.5%)	0	1 (3.4%)	1 (3.7%)	1 (4.0%)
Number pups born	264	254	371	332	321
Number pups born alive	257	247	363	332	311
Number of stillborn	7	6	7	11	10
% born stillborn	2.7%	2.4%	1.9%	3.2%	3.1%
Liveborn, not culled	172	182	234	219	216
Number of pups dying 0-21 days	25	8	16	12	30
Live pups per litter, day 0 (before culling)	12.2	10.7	12.5	12.3	12.4
Live pups per litter, day 4 (after culling)	7.7	7.6	7.9	8.0	7.8
Live pups per litter, day 21	7.7	7.6	7.8	8.0	7.8
Mean pup weight (g), day 0	5.9	6.1	6.1	6.1	5.9
Mean pup weight (g), day 4 (after culling)	9.0	9.2	9.1	9.2	9.6
Mean pup weight (g), day 21 (after culling)	47.1	47.6	49.5	47.9	44.3

Table 2.6.6.1.1-5: Terminal body weight and selected organ weights

Organ	0 ppm	5 ppm	20 ppm	500 ppm	2500 ppm
F0 Males					
Terminal body weight (g)	572.59	552.99	579.76	556.06	526.63* (↓8%)
Testis, left (g)	1.75	1.79	1.79	1.78	1.76
Testis, right (g)	1.76	1.78	1.79	1.80	1.77
Testis, left (g/100 g BW)	0.31	0.32	0.31	0.32	0.34* (↑10%)
Testis, right (g/100 g BW)	0.31	0.33	0.31	0.33	0.34* (↑10%)
F1a males					
Terminal body weight (g)	602.67	589.97	587.79	598.46	536.67** (↓11%)
Epididymis, left (g)	0.68	0.63	0.68	0.71	0.69
Prostate (g)	0.84	0.66	0.78	0.71	0.63* (↓25%)
Seminal vesicles (g)	2.09	1.82** (↓13%)	1.94	1.92	1.86* (↓11%)
Epididymis, left (g/100 g BW)	0.11	0.11	0.12	0.12	0.13* (↑18%)
Prostate (g/100 g BW)	0.14	0.11	0.14	0.12	0.12
Seminal vesicles (g/100 g BW)	0.35	0.31	0.33	0.33	0.35

* p<0.05 different from control; ** p<0.01 different from control

Reproduction toxicity study (dose range finding study) (Report S-2758):

In addition to the two-generation study, a pilot study was performed with groups of 8 male and 8 female Sprague-Dawley Crl:CD strain rats were fed diet containing 0, 500, 2000 or 5000 ppm RE-45601 Technical (purity: 83.3%) for 1 week before mating. The doses equal to 0, 20.8, 83.3, 208.3 mg/kg bw/day when corrected for purity of active substance. The vehicle used in study for preparation of diet was Acetone. Females received the diet continuously throughout mating and gestation, and until Day 7 of lactation when they were necropsied. The offspring were exposed to the test material in utero and while nursing until they were sacrificed and necropsied on Day 7 of lactation. Effects on adults and offspring were observed at the maximum dose level of 5000 ppm.

Treatment was associated with reduced bodyweight noted in adults at 5000 ppm (Males: week 0-2: 2%; Females: GD 20 13%, LD 0 F: 14%, LD: 7 (16%)), reduced bodyweight gain noted in adults at 5000 ppm (M: 18%, F: 63%) and reduced food consumption noted in adult males during the first week (pre-mating) (15%). In the offspring reduced combined pup weights were noted at all dose levels (On day 7: 9%, 9%, and 11% in the groups 500, 2000 and 5000 ppm, respectively). Pup weight gain (day 0-7) was also reduced in all dose groups (↓13%, ↓14%, and ↓16% in the groups 500, 2000, and 5000 ppm, respectively). There were no effects on reproduction indices for males or females, or on pup litter size, survival, and sex ration. The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated). The study was not compared to any guideline since it is a pilot study. It was performed in accordance FIFRA Good Laboratory Practice (GLP) Standards. The study is considered as supportive data (dose range finding study)

Table 2.6.6.1.1-6: Summary of litter data

	RE-45601 (ppm)			
	0	500	2,000	5,000
Number of females on test at time of mating	8	8	8	8
Number (%) of females mated[a]	8 (100)	8 (100)	8 (100)	8 (100)
Number (%) of females pregnant[b]	8 (100)	8 (100)	7 (88)	8 (100)
Number (%) of mated females				
With viable litter[c]	8 (100)	8 (100)	7 (100)	8 (100)
With nonviable litter	0 (0)	0 (0)	0 (0)	0 (0)
Number (%) of males mated	8 (100)	8 (100)	8 (100)	8 (100)
Number (%) males siring litters[d]	8 (100)	8 (100)	7 (88)	8 (100)
Days to mate				
Mean	3	2	3	2
S.D.	1.1	0.8	2.1	1.2
Length of gestation (days)				
Mean	22	22	22	23
S.D.	0.4	0.4	0.4	0.5
Day 0				
Number of litters	8	8	7	8
Total number (%) of pups born alive	118 (98)	120 (99)	111 (100)	107 (99)
Total number (%) of pups found dead	3 (2)	1 (1)	0 (0)	1 (1)
Total number of live males	64	63	63	58
Total number of live females	54	57	48	49
Total number of dead males	0	0	0	1
Total number of dead females	3	1	0	0
Number of pups (male & female) born alive				
Mean	15	15	16	13
S.D.	2.05	1.8	1.2	1.7
Number of male pups born alive				
Mean	8	8	9	7
S.D.	2.6	2.4	2.7	2.5
Number of female pups born alive				
Mean	7	7	7	6
S.D.	2.2	2.6	3.4	1.9

a Also defined as the "mating index."
b Also defined as the female "fertility index."
c Also defined as the "gestation index."
d Also defined as the male "fertility index."

	RE-45601 (ppm)			
	0	500	2,000	5,000
Day 0 (Continued)				
Number of pups (male & female) found dead				
Mean	0	0	0	0
S.D.	0.7	0.4	0.0	0.4
Number of male pups found dead				
Mean	0	0	0	0
S.D.	0.0	0.0	0.0	0.4
Number of female pups found dead				
Mean	0	0	0	0
S.D.	0.7	0.4	0.0	0.0
Combined pup weight (g)				
Mean	6.5	6.3	6.3	6.3
S.D.	0.51	0.46	0.50	0.48
Male pup weight (g)				
Mean	6.7	6.5	6.5	6.4
S.D.	0.51	0.41	0.45	0.46
Female pup weight (g)				
Mean	6.3	6.2	6.2	6.1
S.D.	0.48	0.46	0.55	0.47
Day 4				
Number of litters	8	8	7	8
Total number of live pups	118	120	110	105
Number of pups (male & female)				
Mean	15	15	16	13
S.D.	2.0	1.8	1.1	1.8
Number of male pups				
Mean	8	8	9	7
S.D.	2.6	2.4	2.7	2.7
Number of female pups				
Mean	7	7	7	6
S.D.	2.2	2.6	3.2	1.7
Combined pup weight (g)				
Mean	10.8	10.1	10.0	9.9
S.D.	1.21	0.82	1.05	1.04
Male pup weight (g)				
Mean	11.2	10.3	10.2	10.2
S.D.	1.27	0.91	0.90	0.98
Female pup weight (g)				
Mean	10.5	10.0	9.9	9.7
S.D.	1.15	0.75	1.23	1.13

	RE-45601 (ppm)			
	0	500	2,000	5,000
Day 7				
Number of litters	8	8	7	8
Total number of live pups	118	118	109	102
Total number of live males	64	62	62	56
Total number of live females	54	56	47	46
Number of pups (male and female)				
Mean	15	15	16	13
S.D.	2.0	1.9	1.1	2.0
Number of male pups				
Mean	8	8	9	7
S.D.	2.6	2.2	2.9	2.8
Number of female pups				
Mean	7	7	7	6
S.D.	2.2	2.6	3.2	1.5
Percent of survivors (Days 0-7)[e]				
Mean	100	98	98	95
S.D.	0.0	3.5	4.5	7.4
Combined pup weight (g)				
Mean	15.9	14.5*	14.4*	14.2*
S.D.	1.87	1.13	1.86	1.25
Male pup weight (g)				
Mean	16.4	14.9	14.8	14.5
S.D.	1.97	0.96	1.77	1.12
Female pup weight (g)				
Mean	15.4	14.1	14.1	13.8
S.D.	1.75	1.21	2.00	1.34
Combined pup weight gain (g) (Days 0-7)				
Mean	9.4	8.2*	8.1*	7.9*
S.D.	1.50	0.95	1.55	0.94
Male pup weight gain (g) (Days 0-7)				
Mean	9.7	8.4	8.4	8.1
S.D.	1.62	0.86	1.53	0.83
Female pup weight gain (g) (Days 0-7)				
Mean	9.1	8.0	7.9	7.7
S.D.	1.40	1.04	1.65	1.06
Sex ratio (M/M+F)x100				
Mean	54	53	57	53
S.D.	15.4	14.5	19.7	15.1

e Also defined as the "viability index."

Table 2.6.6.1.1-7: Combined pup weight and pup weight gain

Day	0 ppm	500 ppm	2000 ppm	5000 ppm
0	6.5	6.3	6.3	6.3
4	10.8	10.1	10.0	9.9
7	15.9	14.5* (↓9%)	14.4* (↓9%)	14.2* (↓11%)
0-7	9.4	8.2* (↓13%)	8.1* (↓14%)	7.9* (↓16%)

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

In regulation 1272/2008 (CLP), adverse effects on sexual function and fertility are defined as “Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature

reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."

Table 2.6.6.1.2-1. Hazard categories for reproductive toxicants (corresponding to table 3.7.1(a) in regulation 1272/2008)

Category	Description
1	Known or presumed human reproductive toxicant. Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
1A	Known human reproductive toxicant. The classification of a substance in Category 1A is largely based on evidence from humans.
1B	Presumed human reproductive toxicant. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility 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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
2	Suspected human reproductive toxicant. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed 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 the other toxic effects.

As clethodim is not a known human reproductive toxicant and there is no human data available providing clear evidence of an adverse effect on sexual function, the criteria for category 1A is not fulfilled.

Effects of clethodim on sexual function and fertility were investigated in rats in one two-generational (one litter) study considered of acceptable quality (Report No: S-2778). In addition, a range-finding one-generation toxicity study (Report S-2758) is available, but the study is limited and considered as supportive only.

The effects noted in the studies mentioned above, that are considered potentially relevant for classification are as follows: changes in weights of male sexual organs in adult (prostate, seminal vesicles, testis, epididymis) and an increase in the number of stillborn F1 pups.

Changes in weights of male sexual organs (prostate, seminal vesicles, testis and epididymis):

In the main study (Report S-2758), the absolute weights of the prostate and seminal vesicles were 25 and 11% lower in F1 males at 2500 ppm (151.2 mg/kg bw/day) compared to controls. However, no histological lesions were increased in this group. The terminal body weights of both F0 and F1 males were lower in the high dose group. An increase in relative, but not absolute, testis weight was observed in the F0 generation. The absolute, but not relative, weight of the left epididymis was increased in the F1 generation. These relative increased in organ weights are likely a result of the reduction in body weight. Sperm parameters were not investigated in the studies. However, the results for clethodim technical obtained in the steroidogenesis assay (new data for renewal) were concluded to be negative.

Overall, effects on male sexual organ weights were observed, but findings were confined to a dose level with presence of general toxicity (reduced body weight >10%) and no histopathological findings were observed. Thus, data no not provide convincing evidence for a classification of the substance in Cat. 2.

Increase in the number of stillborn F1 pups:

In the main study (Report S-2758), an increase in the number of stillborn F1 pups was observed. This type of effect is considered to reflect developmental toxicity rather than fertility and is thus discussed in section 2.6.6.2.

Overall conclusion, available data did not provide convincing evidence for a classification with regards to sexual function and fertility. Therefore, no classification for sexual function and fertility is considered warranted.

Weight of the prostate and seminal vesicles in males of the F1 generation were lower in the high dose group in the 2-generation study provided. It is not clear whether this was caused by the reduced food consumption or the treatment, and no effect on the fertility index of these males was observed. This does not warrant for classification for adverse effects on sexual function and fertility.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 60: Summary table of animal studies on adverse effects on development.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
Rat Reproduction Study (dose range finding study) Guidelines followed: 40 CFR 158.135, Pesticide Assessment Guideline No.83-4 Species: Rat Strain: Albino Crl: CD Sprague-Dawley P generation: 8 males and 8 females per group Major deviations from OECD 416 (2001): - treatment initiated one week before mating rather than 10 weeks before mating - only one generation, F0 dams and F1 pups terminated on lactation day 7 - low number of females (8), GL recommends use of sufficient number of animals to yield preferably not less than 20 pregnant females at or near parturition. - oestrous cycle length and normality not investigated - testis and epididymis weight not investigated - sperm motility and sperm morphology not analysed	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Acetone Dietary exposure from 1 week before mating until day 7 of lactation. Doses: 0, 500, 2000, and 5000 ppm (equal to 0, 25, 100 and 250 mg/kg bw/day using a default value of 0.05 for chronic rat studies as recommended by EFSA guidance on selected default values (EFSA Journal 2012;10(3):2579)) Values corrected for purity of test substance using a correction factor of 1.2): 0, 20.8, 83.3, 208.3 mg/kg bw/day	No NOAEL was set in study* <u>Parental effects:</u> <u>2000 ppm:</u> No treatment related effects <u>5000 ppm:</u> ↓ food consumption during the first week (pre-mating) (M: 15%) ↓ **body weights** during week 0-2 of the study (M: 2%), or gestational day 20 (F: 13%), lactational day 0 (F: 14%), and lactational day 7 (F: 16%) ↓ **bodyweight gain** during week 0-3 (M: 18%) or week 0-1 (F: 63%) <u>Offspring effects:</u> <u>500 ppm:</u> ↓**combined pup weight** on day 7 (9%) ↓ **combined pup weight gain** between day 0 and 7 (13%) <u>2000 ppm:</u> ↓**combined pup weight** on day 7 (9%) ↓ **combined pup weight gain** between day 0 and 7 (14%) <u>5000 ppm:</u> ↓**combined pup weight** on day 7 (11%) ↓ **combined pup weight gain** between day 0 and 7 (16%) <u>Comment:</u>	[redacted] (1986) Report number: S-2758 Vol. 3. B.6.6.1/01 New data for renewal: Yes

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
- total number of homogenisation-resistant testicular spermatids and cauda epididymal sperm not enumerated - physical development of the offspring not investigated - haematological and clinical parameters not investigated, organ weights not recorded, histopathological investigations not made - less number of observation points GLP: Yes Supportive		The reduced food consumption (observed in both sexes but only significant in males) could be a result of reduced palatability of the food containing the test item. The observed parental effects, which mainly included reduced body weights, could at least in part be attributable to the reduced food intake	
Two Generation (One Litter) Reproduction Study in Rats Guidelines followed: Reproductive and Fertility Effects 40 CFR 158.135, Pesticide Assessment Guideline 83-4 Deviations from OECD 416 (2001): - no analysis of sperm parameters - developmental and functional observations of pups were not performed - weighing of adrenals, brain, liver, pituitary gland, spleen, thyroids were not performed - histopathology of the vagina was not performed - dosing before mating period seems to be 9 weeks (the guideline recommends dosing to be continued for at least 10 weeks before the mating period) Species: Rat Strain: Albino Crl: COBS/ CD Sprague-Dawley F0 generation: 30 males and 30 females per group F1 generation: 30 males and 30 females per group GLP: Yes Acceptable	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Acetone 10 ml acetone/kg food Exposure: The F0 males and females received the test material via the diet throughout pre-mating, mating, gestation, and lactation F1a indirect exposure in utero and through nursing, and direct exposure from weaning to pre-mating, mating, gestation, and lactation. F2 indirect exposure in utero and through nursing <u>Doses:</u> 0, 5, 20, 500 and 2500 ppm (equal to 0, 0.5, 1.2, 32.2 and 163 mg/kg bw/day for males; 0, 0.5, 1.5, 37.4 and 181 mg/kg bw/day for females in the pre-mating period after correction for purity as calculated by the applicant)	NOAEL parental toxicity: 500 ppm (32.2 mg/kg bw/day) NOAEL offspring toxicity: 500 mg/kg bw/day (163 mg/kg bw/day) NOAEL reproductive toxicity: 2500 ppm 163 mg/kg bw/day) LOAEL parental toxicity: 2500 ppm (163 mg/kg bw/day) LOAEL offspring toxicity: - LOAEL reproductive toxicity: - <u>Effects at 2500 ppm:</u> <u>F0 adults</u> ↓ food intake (during a few days) ↓ body weight (M: 4-9%) <u>F1 adults</u> ↓ food intake ↓ **body weight** (M: 10-19%, F: 6-10%) <u>F1 pups:</u> - **slightly increased number of stillborn pups** (unclear relevance) - decreased bodyweight gain (5% n.s.) <u>F2 pups:</u> - decreased bodyweight (6% n.s.)	[redacted] (1987) Report number: S-2778 Vol. 3. B.6.6.1/02 New data for renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
Teratology Study in Rats (dose range finding study) Guidelines followed: Not a guideline study Major deviations from a full OECD 414 (2018): - ten dams/group, TG recommends 20 to achieve at least 16 animals with implantation sites. - the exposure period ended at day 15 instead of the day prior to termination (day 19). - anogenital distance in foetuses not investigated, thyroid weight, thyroid histopathology, and blood thyroid hormone levels (T4, T3 and TSH) in the maternal animals not investigated. - it is noted that there were indications of SDA viral infections in some dams at gestation day 20. This was noted in 1, 2, 2, 3, and 2 females in the 0, 50, 150, 300, and 500 mg/kg bw/day group, respectively. Species: Rat Strain: CD® Sprague-Dawley 10 mated females per group GLP: Yes Supportive	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Carboxymethyl cellulose, Tween 80 aqueous suspension Exposure: Oral gavage, single daily dose on gestational days 6-15 <u>Doses:</u> 0, 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw per day, after correction for purity)	No NOAEL was set in study* <u>Effects at 300 mg/kg bw/day (250 mg/kg bw/day after correction for purity of test substance):</u> - **clinical signs** (excessive salivation, 4 of 10 dams) ↓ **pup weight** (7%, not statistically significant) <u>Effects at 500 mg/kg bw/day (417 mg/kg bw/day after correction for purity of test substance):</u> - **clinical signs** (excessive salivation, 8/10 dams) ↓**body weight** (Day 20: ↓10%, n.s.) ↓ bodyweight gain (Day 15-20: ↓38.8%; Day 6-20: ↓62.5%) ↓ number of implantation sites (87 versus 126 in control, n.s.) ↑ pre-implantation loss ratio (0.289 versus 0.082 in control, n.s.) ↓ total number of viable foetuses (86 versus 122 in control, within historical controls) ↓ **foetal weight** of viable foetuses (↓11%) This study was used to determine dose levels in Schroeder 1987 It was noted that there were indications of SDA viral infections in some dams at gestation day 20 which restricts the reliability of the study. This was noted in 1, 2, 2, 3 and 2 females in the 0, 50, 150, 300 and 500 mg/kg bw/day groups, respectively	[redacted] (1986) Report number: S-2807 Vol. 3. B.6.6.2.1/01 New data for renewal: Yes
Teratology Study in Rats Guidelines followed: EPA/FIFRA Pesticide Assessment Guidelines Subdivision F, Hazard Evaluation (October 1982) Deviations from current OECD TG 414 (2018): The following endpoints were not assessed: - anogenital distance in foetuses - thyroid weight, thyroid histopathology, and blood thyroid hormone levels (T4, T3 and TSH) in the maternal animals	RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Vehicle: Carboxymethyl cellulose, Tween 80 aqueous suspension Exposure: Oral gavage, single daily dose on gestational days 6-15 Doses: 0, 10, 100, 350 and 700 mg/kg bw per day (equal to 0, 8.3, 83.3, 292 and 583 mg/kg bw per day, after correction for purity)	NOAEL maternal and developmental toxicity: 100 mg/kg bw/day (83.3 mg/kg bw/day after correction for purity of test substance) LOAEL maternal and developmental toxicity: 350 mg/kg bw/day (292 mg/kg bw/day after correction for purity of test substance) <u>Effects at 350 mg/kg bw/day (292 mg/kg bw/day after correction for purity of test substance):</u> - **clinical signs** (excessive salivation, poor condition, red nasal discharge, alopecia, staining ano-genital area) ↓ body weight (GD 20: 7%; GD 20 corrected value: 6%) ↓ **bodyweight gain** (GD 6-15: 15% n.s., GD 15-20: 17%; GD 0-20 corrected value: 77%) ↓ absolute uterine weight (10% n.s.)	[redacted] (1987) Report number: S-2808 Vol. 3. B.6.6.2.2/01 New data for renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
The exposure period ended at day 15 instead of the day prior to termination (shorter exposure period). Species: Rat Strain: Crl:CD® (COBS) 4 treatment groups consisting of 25 rats each. GLP: Yes Acceptable		↓ **foetal weight** (11%) ↑ **skeletal variations** (incomplete or unossified vertebrae, unossified 5th and/or 6th sternebrae) (foetal:88.8% compared to 72.6% in control) <u>Effects at 700 mg/kg bw/day (583 mg/kg bw/day after correction for purity of test substance):</u> - **mortality** (5 females died at GD 11-16) - **clinical signs** (excessive salivation, excessive lacrimation, red/mucoid nasal discharge, alopecia, staining ano-genital area, chromodacryorrhea) ↓ body weight (GD 20: 6-8%; GD 20 corrected value: 13%) ↓ **bodyweight gain** (GD 6-15: 40%, GD 15-20: 17%; GD 0-20 corrected value: 11%) ↓ food consumption at GD 7, 8, 9, 10 (24-31%) ↓ absolute uterine weight (27%) ↑ resorptions (1.9 n.s. vs 0.8 in control) ↑ resorptions per implant (0.13 n.s. vs 0.05 in control) ↓ number of litters with viable foetuses (18 vs 25 in control within HCD) ↑ **external malformations** (foetal: 4% compared to 0% in control; litter: 33.3% compared to 0% in control) ↑ **skeletal variations** (incomplete or unossified sacral and caudal vertebrae and unossified 5th and/or 6th sternebrae) (96.4% compared to 72.6% in control) ↑ **skeletal malformations** (foetal: 6.4% n.s. compared to 5.4% in control; litter: 22.2% n.s. compared to 16% in control) (observations generally restricted to foetuses noted externally with tail defects, 7 foetuses) ↑**visceral malformations** (foetal: 3.4% compared to 0% in control; litter: 16.7% compared to 0% in control). Distortion of the cerebral hemisphere and an opening in the cranium were seen in one foetus with exencephaly, dissimilar aortic arch defects were observed in two foetuses, one with short tail, absence of the kidney and ureter, bladder and a defect of the large intestine were observed in one foetus that was tailless, oedematous and had an imperforate anus. ↓ **foetal weight** (25%)	
Teratology Study in Rabbits (dose range finding study) Guidelines followed: 40 CFR 158.135, Pesticide Assessment Guideline No.83-3 Deviations from current OECD TG 414:	Chevron RE-45601 Technical Lot/Batch: SX-1688 Purity: 83.3% Aqueous 0.7% carboxy-methyl cellulose (w/v) and 0.5% Tween 80 (w/v) solution	No NOAEL was set in study* <u>Effects at 50 mg/kg bw/day (equal to 41.7 mg/kg bw/day after correction for purity of test substance):</u> Tendencies of ↓ food consumption during the later stage of the dosage period, and dried faeces (one animal) – the effects were not	[redacted] G.E. (1986) Report number: S-2734 Vol. 3. B.6.6.2.3/01

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
Major deviations from a full OECD 414 (2018): - eight dams/group, TG recommends 20 to achieve at least 16 animals with implantation sites. - the exposure period ended at day 19 instead of the day prior to termination (day 28). Species: Rabbit Strain: New Zealand White SPF 4 groups of 8 rabbits each GLP Supportive	Exposure: Gavage. Single daily dose on gestational day 7-19 Doses: 0, 50, 150, 300 or 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw/day, after correction for purity of technical substance using a correction factor of 1.2)	statistically significant. Considered treatment related but not adverse. <u>Effects at 150 mg/kg bw/day (equal to 125 mg/kg bw/day after correction for purity of test substance):</u> - clinical signs (increased incidence of dried faeces, n.s.) ↓ **body weight gain** day 7-20 (+0.02 kg vs +0.2 kg in the control) ↓ food consumption during the later stage of the dosage period (day 13-20) (n.s.) <u>Effects at 300 mg/kg bw/day (equal to 250 mg/kg bw/day after correction for purity of test substance):</u> - **mortality** (2/7) - clinical signs (increased incidence of dried faeces) ↓ **body weight** (Day 20: 11%) ↓ **body weight gain** (Day 7-20: -0.31 kg vs +0.2 kg in the control, n.s.) ↓ food consumption during the dosage period and some days after (day 7-24) followed by an increase compared with the control (n.s) ↑ absolute liver weight (19% n.s.) ↑ relative liver weight (23% n.s.) ↑ **resorptions** (1.4 vs 0.3 in the control, i.e. 2/5 vs 1/7 in the control) -hairball in stomach (observed in 2 rabbits that died) ↓ **foetal body weight/litter** (13%) <u>Effects at 500 mg/kg bw/day (equal to 417 mg/kg bw/day after correction for purity of test substance</u>): - **mortality** (2/7) - clinical signs (increased incidence of dried faeces) ↓ **body weight** (Day 16:15%, Day 20: 22%) ↓ **body weight gain** day 7-20 (-0.72 kg vs +0.2 kg in the control) ↓ food consumption during the dosage period (day 7-24) with a post dosage increase compared with the control. ↑ absolute liver weight (20% n.s.) ↑ relative liver weight (19% n.s.) - **gastric ulceration** (observed in 3 of 4 rabbit that aborted and/or died) -hairball in stomach (observed in 2 or 4 rabbits that aborted and/or died) - abortions (4 vs 0 in the control) - **premature delivery** (1 individual) ↓ **foetal body weight/litter** (32%)	New data for renewal: Yes
Developmental toxicity study in rabbits	Chevron RE-45601 Technical Lot/Batch: SX-1688	NOAEL maternal: 25 mg/kg bw/day (20.8 mg/kg bw/day, corrected for purity)	[redacted] [redacted] (1987)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL** Bold text=adverse effect	**Reference**
Guidelines followed: Teratogenicity 40 CFR 158.135, Pesticide 1Asessment Guideline 83-3 Deviations from OECD 414 (2001; the 2018 update is not applicable to rabbits): the exposure period ended at day 19 instead of the day prior to termination (shorter exposure period). Species: Rabbit Strain: New Zealand White SPF 19-20 animals/group GLP Acceptable	Purity: 83.3% Exposure: Gavage. Single daily dose on gestational day 7-19 Doses: 0, 25, 100 and 300 mg/kg bw per day (equal to 0, 20.8, 83.3 and 250 mg/kg bw/day after correction for purity)	NOAEL developmental: 100 mg/kg bw per day (83.3 mg/kg bw/day, corrected for purity) LOAEL maternal: 100 mg/kg bw per day (83.3 mg/kg bw/day, corrected for purity) LOAEL developmental: 300 mg/kg bw per day (250 mg/kg bw/day, corrected for purity) <u>Effects observed at 100 mg/kg bw per day (83.3 mg/kg bw/day, corrected for purity):</u> - clinical signs (dried faeces, red substance in pan) ↓ **body weight gain** during the dosage period, day 7-20 (+0.05 kg vs +0.18 kg in the control, n.s.) ↓ food consumption during both the dosage period, day 7-20 (15% n.s.) and during the post-dosage period (10% n.s.) <u>Effects observed at 300 mg/kg bw per day (250 mg/kg bw/day, corrected for purity):</u> - clinical signs (dried faeces, red substance in pan) ↓ **body weight gain** during the dosage period, day 7-20 (-0.10 kg vs +0.18 kg in the control), followed by a ↑ in the post-dosage period, day 20-29 (+0.24 kg vs +0.09 kg in the control) ↓ food consumption during the dosing period, day 7-20 (28%) followed by an ↑ in the post-dosage period, day 20-29 (11%) ↓ absolute uterine weight (10% n.s.) ↑ **foetal incidence of angulated hyoid alae (**6.3% vs 1.4% in the control), **misaligned sutures** (fontanelle; 3.6 % vs 0% in the control), and **nasal irregular ossification** (6.3% vs 2.2% in the control)	Report number: S-2869 Vol. 3. B.6.6.2.4/01 New data for renewal: No

*study not suitable for NOAEL setting (low number of animals used and limited parameters investigated)

Table 61: Summary table of human data on adverse effects on development.

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data available				

Table 62: Summary table of other studies relevant for developmental toxicity.

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data available				

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

The potential of clethodim to cause adverse effects on developmental was examined in two developmental toxicity studies, one in the rat and the other one in the rabbit. These studies were submitted and evaluated in previous EU evaluation (DAR 2005). For the renewal of active substance, the pilot studies for the main studies have been submitted in addition.

The submitted studies (Table 60) are shortly summarised in text (below):

Rat

Pilot developmental toxicity study in rats (Report No.: S-2807):

In this dose range finding study, RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 6-15 to groups of 10 females at doses of 0, (control), 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw per day, after correction for purity). At the top dose of 500 mg/kg bw/day (417 mg/kg bw/day), observed effects included increased salivation (8/10 dams), reduced body weight (Day 20: ↓10% n.s.), reduced bodyweight gain (Day 15-20: ↓38.8%; Day 6-20: ↓62.5%), reduced number of implantation sites (87 versus 126 in control, n.s.), and increased pre-implantation loss ratio (0.289 versus 0.082 in control, n.s.), reduced number of viable foetuses (86 versus 122 in control, within historical control values), and reduced foetal weight of viable foetuses (↓11%) . In the second highest dose of 300 mg/kg bw/day (250 mg/kg bw/day when corrected for purity), observed effects included increased salivation in the dams (8/10 dams) and reduced pup weight (7%, not statistically significant).

The study was performed in accordance FIFRA Good Laboratory Practice (GLP) Standards. The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated). It is also noted that there were indications of SDA viral infections in some dams at gestation day 20, which restricts the reliability of the study. This was noted in 1, 2, 2, 3, and 2 females in the 0, 50, 150, 300, and 500 mg/kg bw/day group, respectively. The study is considered as supportive data.

Table 2.6.6.2.1-1: Maternal body weights during gestation (g) (mean values)

	0 (mg/kg bw/day)	**50 (mg/kg bw/day)**	**150 (mg/kg bw/day)**	**300 (mg/kg bw/day)**	**500 (mg/kg bw/day)**
Day 20	338±46	338±30	349±18	335±24	305±45 (10%)

* p<0.05 different from control
**p<0.01 different from control

Table 2.6.6.2.1-2: Maternal bodyweight change (g)

	0 (mg/kg bw/day)	**50 (mg/kg bw/day)**	**150 (mg/kg bw/day)**	**300 (mg/kg bw/day)**	**500 (mg/kg bw/day)**
Day 6-20	30.4±7.4	32.6±10	34.2±9.8	28.5±9.9	11.4±18.7 ** (62.5%)
Day 15-20	67±11	66±14	74±5	62±12	41±25* (38.8%)

* p<0.05 different from control
**p<0.01 different from control

Table 2.6.6.2.1-3: Selected foetal data.

Effect	Exposure group				
	Vehicle control	**50 mg/kg bw/day**	**150 mg/kg bw/day**	**300 mg/kg bw/day**	**500 mg/kg bw/day**
Total number of viable foetuses	122	131	149	138	86
Body weight of viable foetuses (g)	3.48	3.50	3.43	3.24 (↓7%)	3.11** (↓11%)
Body weight of viable male foetuses	3.57	3.61	3.49	3.33 (↓7%)	3.17** (↓11%)
Body weight of viable female foetuses	3.39	3.37	3.37	3.15 (↓7%)	3.12 (↓8%)
External malformations	0	1	1	0	1

Developmental toxicity study in rats (Report No.: S-2808)

A developmental rat study was performed in which pregnant dams (Crl:CD rats) (25/dose) were administered clethodim by gavage during gestational days 6-15 at doses of 0, 10, 100, 300 and 700 mg/kg bw per day (equal to 0, 8.3, 83.3, 292 and 583 mg/kg bw/day after correction for purity). Maternal toxicity occurred at the two highest doses (of increasing severity with increasing doses). Manifestations of maternal toxicity included mortality noted at 700 mg/kg bw/day (5 of 25 animals), clinical signs (excessive salivation, excessive lacrimation, poor condition, red/mucoid nasal discharge, alopecia, staining of the ano-genital area, chromodocryorrhea (top dose only)) noted at ≥350 mg/kg bw/day, reduced maternal body weight noted at 350 mg/kg bw/day (GD 20: 7%, GD 20 corrected value: 6%) and 700 mg/kg bw/day (GD 10-20: 6-8%, GD 20 corrected value: 13%), reduced bodyweight gain noted at 350 mg/kg bw/day (GD 6-15: 15% n.s.; GD 15-20: 17%; GD 0-20 corrected value: 77%) and 700 mg/kg bw/day (GD 6-15: 40%; GD 15-20: 17%; GD 0-20 corrected value: 11%). Furthermore, food consumption was reduced in the highest dose group during the exposure period (except for the last day). Uterine weight was reduced in a dose dependent manner: 7% reduction in the 100 mg/kg bw/day group, 10 % in the 350 mg/kg bw/day group, and 27% in the 700 mg/kg bw/day group (only the top dose was statistically significant). The mean number of resorptions and resorptions per implant (not statistically significant) was increased in the top dose group.

There was a statistically significant reduction of litters with viable foetuses in the highest dose group (18 versus the 25 in the control group) and it is noted that the non-statistically significant increase in resorption sites (1.9) was slightly above the range of historical control data (mean 0.7 (0.2-1.8)). Nevertheless, there was no statistically significance difference in litter size compared to concurrent controls and no difference from historical control data (mean 12.3 in high dose compared to a mean of 12.9 (10.5-14.8) in historical controls. Foetal body weight was reduced at 350 mg/kg bw/day (11%) and 700 mg/kg bw/day (25%). Furthermore, the incidence of skeletal variations (retarded ossification processes) was increased in the top two doses. There was also a higher incidence of external and visceral malformations among the top dose foetuses. Seven out of the 8 foetuses with external malformations had (among other things) deformed tails, an effect that is associated with maternal toxicity. Because the fetotoxic effects only were observed in the presence of maternal toxicity, the distinction between direct and indirect effects on the foetus is unclear.

NOAEL for maternal toxicity is 100 mg/kg bw/day (equal to 83.3 mg/kg bw per day after correction for purity of test substance) based on mortalities noted at 700 mg/kg bw/day, clinical signs noted at ≥350 mg/kg bw/day, reduced body weight noted at 700 mg/kg bw/day and reduced bodyweight gain noted at ≥350 mg/kg bw

NOAEL for developmental toxicity is 100 mg/kg bw/day (equal to 83.3 mg/kg bw per day after correction for purity of test substance) based on decreased foetal weight noted at ≥350 mg/kg bw/day, increased incidence of skeletal variations noted at ≥350 mg/kg bw/day, and increased incidence of external and visceral malformations at 700 mg/kg bw/day. The NOAELs for maternal and developmental toxicity set in previous evaluation (DAR 2005) remains.

The study was performed in general accordance with OECD 414 and with FIFRA Good Laboratory Practice (GLP) Standards. The deviations from the current guideline (OECD 414, 208) included a shorter exposure period and that some endpoints (anogenital distance in foetuses, thyroid weight, thyroid histopathology, and blood T4, T3 and TSH concentrations in the dams) were not assessed. The deviations from the current guideline include endpoints that would have been valuable for the endocrine disruption assessment; however, the lack of such information does not invalidate the study. The study is considered acceptable.

Table 2.6.6.2.1-4: Maternal body weight during gestation in rats dosed with Clethodim Technical during gestational day 6-15

Day 0	**Day 3**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**	**Day 15**	**Day 20**	**Day 20corr1**
A-	**A-**	**A-**	**A-**	**A-**	**A-**	**AL**	**A+L+**	**AL**	**AL**	**A+L+**	**A+L+**	**K+J+**	**A+L+**
Control – 0 mg/kg													
215± 17	233± 18	246± 19	248± 20	232± 26	257± 22	263± 20	268± 20	272± 20	278± 20	284± 20	293± 20	362± 26	281± 20
10 mg/kg													
212± 17	232± 19	245± 21	241± 19	251± 21	257± 21	263± 21	270± 20	274± 20	279± 20	284± 19	292± 20	362± 26	282± 19
100 mg/kg													
211± 16	230± 16	244± 18	244± 19	249± 20	252± 18	260± 18	264± 17	270± 18	275± 17	280± 17	288± 18	357± 19	281± 17
350 mg/kg													
249± 15	225± 16	239± 14	239± 15	242± 17	246± 19	251± 18	258± 18	262± 18	268± 19	273± 18	279± 22	337± 36* (↓7%)	264± 28* (↓6%)
700 mg/kg													
213± 12	233± 13	245± 15	246± 15	245± 15	248± 17	248± 16* (↓6%)	250± 13** (↓7%)	258± 12 (↓5%)	263± 15 (↓5%)	266± 16* (↓6%)	271± 18** (↓9%)	332± 18** (↓8%)	272± 14 (↓3%)

A-: No statistical differences among the means (parametric ANOVA).
A($p<0.05$) / A+ ($p<0.01$): The means differ significantly (parametric ANOVA).
L($p<0.05$) / L+ ($p<0.01$): The response is linearly related to the dose levels.
Parametric: *($p<0.05$) / ** ($p<0.01$): Significantly different from control (Dunnett's).
K-: No statistical differences among the means (Kruskal-Wallis, nonparametric).
K($p<0.05$) / K+ ($p<0.01$): The means differ significantly (Kruskal-Wallis, nonparametric).
J($p<0.05$) / J+ ($p<0.01$): There is an ordered response to dosage.
Nonparametric: *($p<0.05$) / ** ($p<0.01$): Significantly different from control (Dunn's Rank Sum).
1 Day 20 gestation weight minus the weight of the gravid uterus

Table 2.6.6.2.1-5: Maternal body weight change during gestation in rats dosed with Clethodim Technical during gestational day 6-15

Days													
0-3	**3-6**	**0-6**	**6-7**	**7- 8**	**8- 9**	**9-10**	**10-11**	**11-12**	**12-13**	**13-14**	**14-15**	**6-15**	**15-20**
A-	**A-**	**A-**	**A-**	**A+L+**	**A-**	**AL**	**K-**	**A-**	**A-**	**K-**	**K-**	**A+L+**	**K+L+**
							Control – 0 mg/kg						
17±6	14±6	31±19	2±4	5±4	5±6	6±6	5±4	4±6	6±3	6±4	8±4	47±8	70±10
							10 mg/kg						
19±5	15±5	33±8	2±6	4±4	6±5	6±4	7±5	5±4	5±4	4±4	9±4	47±9	69±11
							100 mg/kg						
18±8	16±8	33±8	0±6	5±5	3±5	8±6	5±5	6±5	4±5	6±4	8±4	45±10	69±8
							350 mg/kg						
16±7	15±7	30±7	0±5	3±6	4±6	5±4	7±4	4±3	6±4	5±6	6±7	40±14 (↓15%)	58±20 * (↓17%)
							700 mg/kg						
19±5	13±6	32±6	1±7	-1±7**	3±8	0±12*	2±11	6±4	5±6	3±12	5±17	28±15 ** (↓40%)	58±13 ** (↓17%)

A-: No statistical differences among the means (parametric ANOVA).
A($p<0.05$) / A+ ($p<0.01$): The means differ significantly (parametric ANOVA).
L($p<0.05$) / L+ ($p<0.01$): The response is linearly related to the dose levels.
Parametric: *($p<0.05$) / ** ($p<0.01$): Significantly different from control (Dunnett's).
K-: No statistical differences among the means (Kruskal-Wallis, nonparametric).
K($p<0.05$) / K+ ($p<0.01$): The means differ significantly (Kruskal-Wallis, nonparametric).
J($p<0.05$) / J+ ($p<0.01$): There is an ordered response to dosage.
Nonparametric: *($p<0.05$) / ** ($p<0.01$): Significantly different from control (Dunn's Rank Sum).

Table 2.6.6.2.1-6: Food consumption during gestation in rats dosed with Clethodim Technical during gestational day 6-15

Days											
0-3	**3-6**	**6-7**	**7- 8**	**8- 9**	**9-10**	**10-11**	**11-12**	**12-13**	**13-14**	**14-15**	**15-20**
K-	**A-**	**K+J+**	**K+J+**	**K+J+**	**K+J+**	**K+J+**	**K-**	**K-**	**K-**	**K-**	**K-**
Control – 0 mg/kg											
120±37	108±10	101±11	100±13	99±10	102±14	102±11	101±22	98±10	97±12	93±8	104±7
10 mg/kg											
113±12	110±10	103±15	100±9	99±9	103±10	105±13	100±11	96±13	95±12	95±8	103±7
100 mg/kg											
115±16	111±9	96±16	96±13	95±10	101±13	99±12	100±10	95±10	94±13	95±13	102±8
350 mg/kg											
119±28	107±10	92±14	86±16**	100±40	97±24	99±16	95±8	101±22	95±19	105±42	107±26
700 mg/kg											
109±9	107±7	137±181* (↑36%)	76±19** (↓24%)	75±21** (↓24%)	70±27** (↓31%)	71±35** (↓30%)	82±35 (↓19%)	89±23 (↓9%)	84±27 (↓13%)	92±22 (↓1%)	106±7 (↑2%)

K($p<0.05$) / K+ ($p<0.01$): The means differ significantly (Kruskal-Wallis, nonparametric).
J($p<0.05$) / J+ ($p<0.01$): There is an ordered response to dosage.
Nonparametric: *($p<0.05$) / ** ($p<0.01$): Significantly different from control (Dunn's Rank Sum).

Table 2.6.6.2.1-7: Selected reproductive data in dams exposed to Clethodim technical at gestational days 6-15

Effect	Exposure group					Historical control	
	Vehicle control	10 mg/kg bw/day	100 mg/kg bw/day	350 mg/kg bw/day	700 mg/kg bw/day	Mean value (range)	Number of control groups
Pregnancy rates	25/25 (100%)	25/25 (100%)	24/25 (96%)	25/25 (100%)	24/25 (96%)2	94% (68-100%)	38
Mean number of corpora lutea	16.3 ± 3.3	16.3 ± 2.3	15.5 ± 2.3	16.5 ± 2.3	15.5 ± 1.3	15.3 (13.5-18.3)	36
Mean number of implantation sites	14.9 ± 2.2	14.7 ± 1.5	14.3 ± 1.6	14.6 ± 2.0	14.2 ± 1.6	13.7 (11.3-15.5)	38
Mean pre-implantation loss ratio	0.074 ± 0.089	0.091 ± 0.093	0.092 ± 0.095	0.111 ± 0.124	0.085 ± 0.083	-	-
Uterine weight (g)	82 ± 11	80 ±10	76 ± 9 (↓7%)	74 ± 11 (↓10%)	60 ± 15** (↓27%)	-	-
Number of litters with viable foetuses	25	25	24	25	18*	-	-
Mean litter size	14.1 ± 1.9	14.0 ± 1.6	13.7 ± 1.5	14.0 ± 2.0	12.3 ± 3.5	-	-
Mean number of resorptions	0.8 ± 0.8	0.96 ± 0.9	0.5 ± 0.7	0.6 ± 0.6	1.9 ± 3.5	0.7 (0.2-1.8)	38
Mean number resorptions/implant	0.052 ± 0.047	0.043 ± 0.057	0.036 ± 0.046	0.038 ± 0.040	0.128 ± 0.222	-	-

Table 2.6.6.2.1-8: Summary of selected reproduction data – foetus mean body weights (mean ±SD)

Mean Body Weight (g)	0 mg/kg	10 mg/kg	100 mg/kg	350 mg/kg	700 mg/kg	Stat. Symbol
Viable Foetuses	3.65±0.24	3.61±0.17	3.48±0.25	3.26±0.51** (11%)	2.75±0.37** (25%)	K+J+
Male Foetuses	3.82±0.24	3.71±0.17	3.58±0.26	3.33±0.52** (13%)	2.79±0.36** (27%)	K+J+
Female Foetuses	3.57±0.25	3.51±0.19	3.38±0.26	3.18±0.49** (11%)	2.77±0.36** (22%)	K+J+

K($p<0.05$) / K+ ($p<0.01$): The means differ significantly (Kruskal-Wallis, nonparametric).
J($p<0.05$) / J+ ($p<0.01$): There is an ordered response to dosage.
Nonparametric: *($p<0.05$) / ** ($p<0.01$): Significantly different from control (Dunn's Rank Sum).

Table 2.6.6.2.1-9: Incidence- external malformations (number of affected foetuses)

	0 (mg/kg bw/day)	10 (mg/kg bw/day)	100 (mg/kg bw/day)	350 (mg/kg bw/day)	700 (mg/kg bw/day)
Number of foetuses examined	353	351	329	350	221
Agnathia (shown to be micrognathia during skeletal examination)	0	0	1	0	0
Small foetus with glassy appearance of skin	0	0	1	0	0
Exencephaly	0	0	0	0	1
Tail malformations (absent, filamentous, or short tail)	0	0	0	0	7
Oedematous	0	0	0	0	1
Imperforated anus	0	0	0	0	2

Table 2.6.6.2.1-10: Incidence – Visceral malformations (number of affected foetuses)

	0 (mg/kg bw/day)	**10 (mg/kg bw/day)**	**100 (mg/kg bw/day)**	**350 (mg/kg bw/day)**	**700 (mg/kg bw/day)**
Number of foetuses examined	185	181	172	181	118
Distended lateral ventricles of the brain	0	1	1	0	0
Distortion of the cerebral hemisphere and opening in the cranium (seen in one foetus with exencephaly)	0	0	0	0	1
Dissimilar aortic arch defects	0	0	0	0	2
Bilateral absence of the kidney and ureter, absence of a bladder and a defect of the large intestine	0	0	0	0	1

Table 2.6.6.2.1-11: Incidence – skeletal malformations (number of affected foetuses)

	0 (mg/kg bw/day)	**10 (mg/kg bw/day)**	**100 (mg/kg bw/day)**	**350 (mg/kg bw/day)**	**700 (mg/kg bw/day)**
Number of foetuses examined	185	181	172	181	118
Tail defects; short, absent	0	0	0	0	5
Tail; filamentous	0	0	0	0	2
Misshapen mandible and malformation of several cranial bones (tympanic, basisphenoid)	0	0	1	0	0
Wavy ribs	7	0	0	3	0
5 lumbar vertebrae	1	0	4	1	2
Fused sternebra	1	0	0	0	0

Table 2.6.6.2.1-12: Selected developmental data.

Effect	**Exposure group**					**Historical control1**	
	Vehicle control	**10 mg/kg bw/day**	**100 mg/kg bw/day**	**350 mg/kg bw/day**	**700 mg/kg bw/day**	**Mean value (range)**	**Number of control groups**
Number of litters	25	25	24	25	24	-	-
Number of litters with viable foetuses	25	25	24	25	18* (28%)	-	-
Mean litter size	14.1 ± 1.9	14.0 ± 1.6	13.7 ± 1.5	14.0 ± 2.0	12.3 ± 3.5 (13%)	-	-
Mean number of resorptions	0.8 ± 0.8	0.96 ± 0.9	0.5 ± 0.7	0.6 ± 0.6	1.9 ± 3.5 (138%)	0.7 (0.2-1.8)	38
Mean number resorptions/implant	0.052 ± 0.047	0.043 ± 0.057	0.036 ± 0.046	0.038 ± 0.040	0.128 ± 0.222 (146%)	-	-
Body weight of viable foetuses (g)	3.65±0.24	3.61±0.17	3.48±0.25 (5%)	3.26±0.51** (11%)	2.75±0.37** (25%)	3.28-3.69	10
Body weight of viable male foetuses	3.82±0.24	3.71±0.17	3.58±0.26 (6%)	3.33±0.52** (13%)	2.79±0.36** (27%)	3.23-3.99	28
Body weight of viable female foetuses	3.57±0.25	3.51±0.19	3.38±0.26 (5%)	3.18±0.49** (11%)	2.77±0.36** (22%)	3.07-3.78	28
Sex ratio of viable foetused (Males: females)	1.1	1.0	1.0	1.0	0.8	-	-
Incidence of foetal external malformations	0/353	0/351	1/329	0/350	8/221 ** (3.6%)	-	-
Litter incidence of foetal external malformations	0/25	0/25	1/24	0/25	6/18 * (33.3%)	-	-

Effect	Exposure group					Historical control1	
	Vehicle control	10 mg/kg bw/day	100 mg/kg bw/day	350 mg/kg bw/day	700 mg/kg bw/day	Mean value (range)	Number of control groups
Incidence of foetal external variations	0/353	0/351	1/329	0/350	0/221	-	-
Incidence of foetal visceral malformations (%)	0/185 (0%)	1/181 (0.5%)	1/172 (0.6%)	0/181(0%)	4/118 (3.4%) **	-	-
Litter incidence of foetal visceral malformations (%)	0/25 (0%)	1/25 (4%)	1/24 (4.2%)	0/25 (0%)	3/18 (16.7%) **	-	-
Incidence of foetal visceral variations	7/185 (3.8%)	8/181 (4.4%)	11/172 (6.4%)	2/181 (1.1%)	2/118 (1.7%)	-	-
Incidence of foetal skeletal malformations (%)	9/168 (5.4%)	0/170 (0%) **	5/158 (3.2%)	4/169 (2.4%)	7/110 (6.4%)	-	-
Litter incidence of foetal skeletal malformations (%)	4/25 (16%)	0/25	3/24 (12.5%)	3/25 (12.0%)	4/18 (22.2%)	-	-
Incidence of foetal skeletal variations (%)	122/168 (72.6%)	103/170 (60.6%)	126/158 (79.7%)	150/169 (88.8%) **	106/110 (96.4%) **	-	-
Litter incidence of foetal skeletal variations (%)	24/25 (96.4%)	25/25 (100%)	24/24 (100%)	25/25 (100%)	18/18 (100%)	-	-

[1] Studies performed between 1976-1985.
*($p<0.05$)
** ($p<0.01$)

Pilot reproduction toxicity study in rats (Report No.: S-2758) (see also section 2.6.6.1):

In this pilot study, groups of 8 male and 8 female Sprague-Dawley Crl:CD strain rats were fed diet containing 0, 500, 2000 or 5000 ppm RE-45601 Technical (purity: 83.3%) for 1 week before mating. The doses equal to 0, 20.8, 83.3, 208.3 mg/kg bw/day when corrected for purity of active substance. The vehicle used in study for preparation of diet was Acetone. Females received the diet continuously throughout mating and gestation, and until Day 7 of lactation when they were necropsied. The offspring were exposed to the test material in utero and while nursing until they were sacrificed and necropsied on Day 7 of lactation. Effects on adults and offspring were observed at the maximum dose level of 5000 ppm.

Postnatal growth was affected in this study at doses where no maternal toxicity was observed. Birth weight and pup weight at day 4 did not differ between the groups but pup weight at day 7 (sexes combined) were reduced in all three dose groups (↓9%, ↓9%, and ↓11% in the groups 500, 2000, and 5000 ppm, respectively). Pup weight gain (day 0-7) was also reduced in all dose groups (↓13%, ↓14%, and ↓16% in the groups 500, 2000, and 5000 ppm, respectively). The dams of the high dose group (5000 ppm) had a reduced body weight and bodyweight gain, but no effect on the dams were observed in the lower dose groups (500 and 2000 ppm). The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated). The study was performed in accordance FIFRA Good Laboratory Practice (GLP) Standards. The study is considered as supplementary data (dose range finding study).

Reproduction toxicity study in rats (Report No.: S-2778) (see also section 2.6.6.1):

In the 2-generation study rats (30 males and females/generation (F0 and F1)) were given clethodim at a dietary concentration of 0, 5, 20, 500, and 2500 ppm (equivalent to 0, <0.8, 0.8, 26.7 and 133.7 mg a.s./kg bw/day (F0 generation, sexes combined) and 0, < 0.8, 0.8, 28.3, and 151.2 mg a.s./kg bw/day (F1 generation, sexes combined) (values corrected for purity of test substance). An increase in the number of stillborn F1 pups was observed (14 pups which corresponds to 3.8% of the delivered pups in the highest treatment group compared with 2 pups, i.e. 0.7%, in the control group). The number of F0 females with at least one stillborn pup was 7 (25%) in the 2500 ppm group and 2 (9.1%) in the control group. The number of stillborn F2 pups in the control group was 7 (2.7%), indicating that the control value in the F1 generation may be in the lower range of the spectrum. No increase in stillborn F2 pups was observed. Historical control data was not provided but considering the lack of effect in the F2 pups and that the control value in the F2 generation was higher than the F1 generation, this may be incidental. Postnatal pup weight (day 0, 4, and 21) did not differ between groups in either generation.

Dilation of the renal pelvis was observed in five F1 pups (1.8%) in 4 litters (16.7%) in the high dose group. No incidence of this was observed in the control group. Historical control data was not provided in detail, but it was stated in the report that "In studies conducted in this facility between 1984-1986, the historical control data for this finding ranged between one pup from one litter to seventeen pups in ten litters." According to the applicant, no further details is available with respect to the historical control data. Considering that the kidney has not been identified as target organ in other studies and that there were no indications of renal toxicity in the developmental study performed in the same strain, this finding is not considered to demonstrate teratogenic effect.

Body weights for F1a adult females were significantly reduced during the pre-mating (7-10%) and gestation periods (6-9%) up through day-7 (6%) of lactation. While body weights were reduced for F1a females, body weight gain during gestation was not affected by treatment but corrected maternal body weight during pregnancy calculated as a change in maternal body weight gain compared to controls using data point GD0 and LD1 shows a reduced bodyweight gain of 14% in F1 females at 2500 ppm. Mean food consumption values were significantly reduced on days 0-2, 2-5, and 9-12 of gestation of the F1 females. There was no effect on body weight or food consumption in the F0 females.

The NOAEL for parental toxicity in study is 500 ppm (26.7 mg/kg bw/day) based on reduced body weights noted in both generations at 2500 ppm and reduced absolute prostate and seminal vesicles weights noted in F1 adults at 2500 ppm. NOAEL for reproductive toxicity is 2500 ppm (133.7 mg/kg bw/day, highest dose tested). The NOAEL for offspring toxicity is 500 ppm based on slightly increased number of stillborn noted in F1 pups at 2500 ppm (although unclear relevance). The NOAEL for offspring toxicity is a new value for the renewal procedure. In DAR 2005 the NOAEL for offspring toxicity was set at 2500 ppm. The NOAELs for parental and reproductive toxicity set in previous evaluation (DAR 2005) remains.

The study was performed in general accordance with OECD 416 and with EPA, FIFRA and TSCA Good Laboratory Practice (GLP) Standards. There were some deviations from the current version of the guideline. Endpoints required in OECD 416 (2001) that was not assessed/measured in the study included analysis of sperm parameters, developmental and functional observations of pups, weight of adrenals, brain, liver, pituitary gland, spleen, and thyroid, and histopathology of the vagina. While these limits the scope of the study, they do not affect the reliability. The study is considered acceptable.

Rabbit

Pilot developmental toxicity study in rabbits (Report No.: S-2734):

In this dose range finding study, Chevron RE-45601 (purity: 83.3%) was administered orally by gavage daily on gestational days 7-19 to groups of 8 female rabbits at doses of 0, (control), 50, 150, 300 and 500 mg/kg bw/day (equal to 0, 41.7, 125, 250, and 417 mg/kg bw/day, after correction for purity of technical substance using a correction factor of 1.2). Treatment related effects were associated with mortality (≥300 mg/kg bw/day), clinical signs of dried faeces (≥50 mg/kg bw/day, statistical significant at ≥300 mg/kg bw/day), reduced body weight (≥300 mg/kg bw/day), reduced bodyweight gain (≥150 mg/kg/day), reduced feed consumption during the dosage period (≥50 mg/kg/day, statistically significant at 500 mg/kg bw/day) with a post dosage increase in food consumption compared with the control (≥150 mg/kg/day), increased maternal liver weight and liver/body weight ratio (≥300 mg/kg/day, not statistically significant but ~20% increase), gross pathological findings observed in animals that aborted and/or died (hairball in stomach at ≥300 mg/kg bw/day, gastric ulceration at 500 mg/kg bw/day), abortion (500 mg/kg/day), and premature delivery (one animals at 500 mg/kg/day). There was also a possible increase in resorptions: the number of resorptions was 1.4 in the 300 mg/kg bw/day group compared with the 0.3 in the control. There was none in the highest dose group but only one female was available for assessment in that group. In addition, the foetal body weight was 13% and 32% lower in the 300 and 500 mg/kg bw/day dosage groups, respectively, compared with the control. The study is not suitable for NOAEL setting (low number of animals used and limited parameters investigated). The study was not compared to any guideline since it is a pilot study. It was performed in accordance EPA, FIFRA, and TSCA Good Laboratory Practice (GLP) Standards. The study is considered as supplementary data.

Table 2.6.6.2.1-13: Clinical signs-summary

	Dosage Group (mg/kg/days 7-19 of Presumed Gestation) 0(Vehicle)	50	150	300	500
Rabbits Observed	8	8	8	8	8
Rabbits Pregnant	7	8	8	7	7
Cited Observation:[a]					
Died [87]	0	0	0	2	2[b]
Aborted [85]	0	0	0	0	4[b]**
Naturally Delivered	0	0	0	0	1
Dried Feces [07]	0/0	1/8	3/3	5**/32	7**/71**
Soft or Liquid Feces [30]	1/1	1/2	2/5	2/4	0/0
No Feces Present [07]	0/0	0/0	0/0	1/2	0/0
Alopecia [03]	4/17	4/25	4/33*	3/32*	4/44**
Red Substance in Pan [07]	1/1	0/0	0/0	0/0	1/1

[ ] = Physical Sign Code.
/ = Rabbits/Days.

a. Maximum incidences (rabbits/days) are 8/184, 8/184, 8/184, 8/174 and 8/136, respectively, for the 0(vehicle), 50, 150, 300 and 500 mg/kg/day dosage groups.

b. Rabbit 10516 had a red substance in the cage pan (related to abortion) prior to its death on day 20 of gestation. Rabbit 10519 died following a clonic convulsion on day 8 of gestation; the death was possibly interrelated with an intubation accident, although test substance was not present in the lungs and was present in the stomach at necropsy.

* Significantly different from vehicle control value, at $P<0.05$.
** Significantly different from vehicle control value, at $P<0.01$.

Table 2.6.6.2.1-14: Maternal body weight- summary

DOSAGE GROUP[a]		0 MG/KG/DAY	50 MG/KG/DAY	150 MG/KG/DAY	300 MG/KG/DAY	500 MG/KG/DAY
ANIMALS - TESTED		8	8	8	8	8
PREGNANT	N(%)	7(87.5)	8(100.0)	8(100.0)	7(87.5)	7(87.5)
MATERNAL BODY WEIGHT						
DAY 0	MEAN±S.D.	3.80 ± 0.22	3.76 ± 0.27	3.80 ± 0.23	3.82 ± 0.19	3.86 ± 0.19
DAY 7	MEAN±S.D.	4.04 ± 0.24	3.96 ± 0.26	4.01 ± 0.22	4.07 ± 0.25	4.09 ± 0.22
DAY 10	MEAN±S.D.	4.06 ± 0.22	3.95 ± 0.25	4.01 ± 0.19	3.99 ± 0.18	3.90 ± 0.31 [6]
DAY 13	MEAN±S.D.	4.15 ± 0.23	4.01 ± 0.29	4.04 ± 0.22	3.97 ± 0.25	3.79 ± 0.39 [6]
DAY 16	MEAN±S.D.	4.22 ± 0.22	4.05 ± 0.29	4.01 ± 0.28	3.91 ± 0.39	3.58 ± 0.39 ** [6]
DAY 20	MEAN±S.D.	4.23 ± 0.23	4.06 ± 0.31	4.04 ± 0.24	3.76 ± 0.44 *	3.31 ± 0.40 ** [5]
DAY 24	MEAN±S.D.	4.31 ± 0.23	4.12 ± 0.30	4.14 ± 0.24	3.96 ± 0.56 [6]	3.28 ± 0.80 [3]
DAY 29	MEAN±S.D.	4.34 ± 0.27	4.11 ± 0.36	4.20 ± 0.24	4.22 ± 0.44 [5]	4.41 ± 0.00 [1]
DAY 29C[b]	MEAN±S.D.	3.89 ± 0.31	3.68 ± 0.36	3.82 ± 0.29	3.75 ± 0.33 [5]	3.97 ± 0.00 [1]

Table 2.6.6.2.1-15: Maternal bodyweight changes- summary

DOSAGE GROUP[a]		0 MG/KG/DAY	50 MG/KG/DAY	150 MG/KG/DAY	300 MG/KG/DAY	500 MG/KG/DAY
ANIMALS - TESTED		8	8	8	8	8
PREGNANT	N(%)	7(87.5)	8(100.0)	8(100.0)	7(87.5)	7(87.5)
MATERNAL BODY WEIGHT CHANGE						
DAYS 0- 7	MEAN±S.D.	+0.23 ± 0.13	+0.20 ± 0.14	+0.21 ± 0.04	+0.25 ± 0.11	+0.23 ± 0.08
DAYS 7-10	MEAN±S.D.	+0.03 ± 0.02	-0.01 ± 0.04	+0.00 ± 0.05	-0.07 ± 0.11	-0.16 ± 0.08 * [6]
DAYS 10-13	MEAN±S.D.	+0.09 ± 0.05	+0.06 ± 0.08	+0.02 ± 0.06	-0.02 ± 0.12 *	-0.11 ± 0.10 ** [6]
DAYS 13-16	MEAN±S.D.	+0.07 ± 0.05	+0.04 ± 0.07	-0.03 ± 0.11	-0.06 ± 0.19	-0.20 ± 0.07 * [6]
DAYS 16-20	MEAN±S.D.	+0.01 ± 0.06	+0.01 ± 0.10	+0.03 ± 0.14	-0.15 ± 0.14 *	-0.27 ± 0.08 ** [5]
DAYS 20-24	MEAN±S.D.	+0.08 ± 0.05	+0.06 ± 0.04	+0.10 ± 0.06	+0.20 ± 0.26 [6]	+0.03 ± 0.27 [3]
DAYS 24-29	MEAN±S.D.	+0.04 ± 0.12	-0.01 ± 0.13	+0.06 ± 0.06	+0.07 ± 0.09 [5]	+0.20 ± 0.00 [1]
DAYS 20-29	MEAN±S.D.	+0.11 ± 0.14	+0.05 ± 0.12	+0.16 ± 0.08	+0.35 ± 0.22 [5]	+0.54 ± 0.00 [1]
DAYS 7-20	MEAN±S.D.	+0.20 ± 0.06	+0.09 ± 0.19	+0.02 ± 0.15 *	-0.31 ± 0.47	-0.72 ± 0.18 * [5]
DAYS 7-29	MEAN±S.D.	+0.31 ± 0.18	+0.14 ± 0.17	+0.19 ± 0.14	+0.11 ± 0.42 [5]	+0.09 ± 0.00 [1]
DAYS 0-29[c] [b]	MEAN±S.D.	+0.08 ± 0.21	-0.08 ± 0.24	+0.02 ± 0.21	-0.09 ± 0.40 [5]	-0.07 ± 0.00 [1]

Table 2.6.6.2.1-16: Maternal feed consumption- summary

DOSAGE GROUP[a]		0 MG/KG/DAY	50 MG/KG/DAY	150 MG/KG/DAY	300 MG/KG/DAY	500 MG/KG/DAY
ANIMALS - TESTED		8	8	8	8	8
PREGNANT	N(%)	7(87.5)	8(100.0)	8(100.0)	7(87.5)	7(87.5)
MATERNAL FEED CONSUMPTION (g/day)[b]						
DAYS 0- 7	MEAN±S.D.	173.8 ± 13.5	163.4 ± 33.8 [7]	170.1 ± 12.8	174.1 ± 13.0	178.0 ± 8.6
DAYS 7-10	MEAN±S.D.	177.4 ± 9.8 [6]	166.1 ± 29.1	169.5 ± 11.8	147.7 ± 60.6	82.8 ± 48.8 * [6]
DAYS 10-13	MEAN±S.D.	172.2 ± 16.3	152.1 ± 44.4	153.1 ± 30.6	129.0 ± 67.8	49.1 ± 62.9 * [6]
DAYS 13-16	MEAN±S.D.	168.3 ± 18.9	134.0 ± 54.5	109.8 ± 57.9	85.3 ± 81.8	13.8 ± 21.9 * [6]
DAYS 16-20	MEAN±S.D.	169.2 ± 15.0 [6]	138.5 ± 60.2	114.5 ± 48.2	74.6 ± 89.1	2.0 ± 0.4 * [6]
DAYS 20-24	MEAN±S.D.	160.2 ± 27.4	144.6 ± 36.9 [6]	153.3 ± 31.2 [7]	116.7 ± 70.0 [5]	47.0 ± 74.7 ** [3]
DAYS 24-29	MEAN±S.D.	108.3 ± 56.9	95.0 ± 51.6 [7]	135.6 ± 39.0 [7]	158.4 ± 35.6 [5]	180.8 ± 0.0 [1]
DAYS 20-29	MEAN±S.D.	130.5 ± 41.3	111.0 ± 45.6 [7]	143.5 ± 34.2 [7]	155.7 ± 26.5 [5]	159.7 ± 0.0 [1]

Table 2.6.6.2.1-17: Maternal feed consumption in g/kg of body weight- summary

DOSAGE GROUP [a]		0 MG/KG/DAY	50 MG/KG/DAY	150 MG/KG/DAY	300 MG/KG/DAY	500 MG/KG/DAY
ANIMALS - TESTED		8	8	8	8	8
PREGNANT	N(%)	7(87.5)	8(100.0)	8(100.0)	7(87.5)	7(87.5)
MATERNAL FEED CONSUMPTION [b] (g/Kg/day)						
DAYS 0- 7	MEAN±S.D.	44.5 ± 4.5	42.6 ± 9.6 [7]	43.7 ± 4.5	44.2 ± 4.0	44.9 ± 3.4
DAYS 7-10	MEAN±S.D.	44.2 ± 2.0 [6]	42.0 ± 7.4	42.4 ± 3.7	36.7 ± 14.9	20.2 ± 11.0 A [6]
DAYS 10-13	MEAN±S.D.	42.0 ± 4.1	38.1 ± 10.8	38.0 ± 7.4	32.0 ± 16.5	11.8 ± 14.8 A [6]
DAYS 13-16	MEAN±S.D.	40.2 ± 4.3	32.9 ± 12.8	26.9 ± 13.8	20.7 ± 19.7	3.4 ± 5.2 A [6]
DAYS 16-20	MEAN±S.D.	40.1 ± 2.7 [6]	33.9 ± 14.1	28.2 ± 10.9	18.0 ± 21.3	0.6 ± 0.2 A [5]
DAYS 20-24	MEAN±S.D.	37.4 ± 5.6	34.4 ± 7.3 [6]	37.4 ± 7.2 [7]	29.2 ± 16.7 [5]	11.9 ± 18.3 AA [3]
DAYS 24-29	MEAN±S.D.	24.8 ± 12.3	22.6 ± 11.6 [7]	32.6 ± 9.2 [7]	38.0 ± 8.9 [5]	41.8 ± 0.0 [1]
DAYS 20-29	MEAN±S.D.	30.2 ± 8.7	26.5 ± 9.8 [7]	34.7 ± 8.0 [7]	38.1 ± 6.0 [5]	38.1 ± 0.0 [1]

DAYS refers to the days of gestation.
[ ] = Number of values averaged when fewer than the number of rabbits pregnant per group.
a. Test substance was administered on days 7-19 of presumed gestation.
b. This table is restricted to pregnant rabbits.
A Significantly different from vehicle control value, at P≤0.05.
AA Significantly different from vehicle control value, at P≤0.01.

Table 2.6.6.2.1-18: Selected results in pregnant rabbits exposed to clethodim technical via oral gavage on gestational days 7-19

Parameter	Vehicle control	50 mg/kg bw/day	150 mg/kg bw/day	300 mg/kg bw/day	500 mg/kg bw/day
Number of does	8	8	8	8	8
Number of pregnant does	7	8	8	7	7
Abortions	0	0	0	0	4**
Naturally delivered	0	0	0	0	1 (day 27)
Mortality	0	0	0	2	2
Clinical signs					
Dried faeces23	0/0	1/8	3/3	5**/32	7**/71**
Soft or liquid faeces23	1/1	1/2	2/5	2/4	0/0
Alopecia23	4/17	4/25	4/33*	3/32*	4/44**
Red substance in pan23	1/1	0/0	0/0	0/0	1/1
Necropsy observations					
Paraovarian cyst(s)	5	4	4	6	7
Haemorrhagic lungs	0	0	0	0	1
Ulcerations in cardiac or pyloric regions of the stomach	0	0	0	0	3
Hairball present in stomach	0	0	0	2	2
Liver weight (g)	111	107	116 (↑5%)	132 (↑19%)	133 (↑20%)
Relative liver weight (% of body weight)	2.55	2.61	2.76 (↑8%)	3.13 (↑23%)	3.03 (↑19%)
Reproductive endpoints in animals pregnant and delivered by c-section on day 29					
Number of does	7	8	8	5	1
Maternal body weight, day 29 (kg)	4.34	4.11	4.20	4.22	4.41
Maternal body weight gain, day 7-29 (kg)	+0.31	+0.14	+0.19	+0.11	+0.09
Corrected maternal body weight1 (kg)	3.89	3.68	3.82	3.75	3.97
Corrected maternal body weight gain1, day 0-29 (kg)	+0.08	-0.08	+0.02	-0.09	-0.07

Parameter	Vehicle control	50 mg/kg bw/day	150 mg/kg bw/day	300 mg/kg bw/day	500 mg/kg bw/day
Corpora lutea	10.3	10.4	9.0	10.4	11.0
Implantations	7.0	7.1	5.9	8.0	9.0
Litter size	6.7	7.1	5.9	6.6	9.0
Number of live/dead foetuses	47/0	57/0	47/0	33/0	9/0
Resorptions	0.3	0	0	1.4	0
Does with resorptions	1/7	0/8	0/8	2/5	0/1
Live foetal body weight/litter	49.17	43.15	48.77	42.90 (↓13%)	33.52 (↓32%)

Table 2.6.6.2.1-19: Animals that died during the study:

Exposure (mg/kg bw/day)	Time of death (gestational day)	Signs prior to death	Necropsy results	State of pregnancy
300	21	Weight loss (day 7-20), reduced food consumption, solid or liquid faeces (days 19-20)	Hairball in the stomach and dilated, blood-filled intestinal blood vessels	One early resorption and five foetuses that appeared to have been alive and normal for their developmental ages
300	26	Persistent weight loss (from day 10), reduced food consumption, dried faeces (day 17-23), no faeces (day 24-25).	Small hairball present in stomach and paraovarian cysts.	Two early and two late resorptions and nine foetuses that appeared to have been alive and normal for their developmental ages
500	8	Convulsions occur within approximately seven minutes after intubation: the rabbit died within 15 minutes of intubation. Weight loss and reduced food consumption.	Haemorrhagic lungs. The haemorrhagic lungs may have resulted from convulsive activity: test substance was present in the stomach and not apparent in the lungs. Paraovarian cysts.	Six embryos that appeared alive and normal for their developmental ages at the time of maternal death.
500	20	Weight loss (day 7-19), reduced food consumption, dried faeces (day 13-19),	Paraovarian cysts, numerous ulcerations (>20<40) in the gastric pylorus and a hairball present in the stomach.	Red substance, assumed to be blood and a sign of abortion, was observed in the cage pan on the day death occurred. Five late resorptions and one empty implantation site: the conceptus from this site was presumed to have been aborted and cannibalized by the doe.

Developmental toxicity study in rabbits (Report No.: S-2869):

In the developmental rabbit study, inseminated rabbits were administered 0, 25, 100, or 300 mg/kg bw per day (equal to 0, 20.8, 83.3 and 250 mg/kg bw/day after correction for purity). Maternal toxicity was observed in the top two doses (83.3 and 250 mg/kg bw/day). Reduced food consumption and body weight gain, along with dried faeces were observed in the does; effects similar to those in the rat. Observations of a red substance in the pan was made in the highest dose group (250 mg/kg bw/day; observed in 2 rabbits on three days), something that was also observed in the rat. In the rat developmental study this was interpretated as a sign of abortion; however, since no rabbits in the high group aborted it may be a sign of rectal irritation and bleeding. In addition, uterine weight in the highest exposure group was 10% lower than that of the control.

Table 2.6.6.2.1-20: Selected results in pregnant rabbits exposed to clethodim technical via oral gavage on gestational days 7-19

Parameter	Vehicle control	25 mg/kg bw/day	100 mg/kg bw/day	300 mg/kg bw/day
Number of does inseminated	19	20	20	20
Number of pregnant does	19	18	17	17
Abortions	0	1	0	0
Mortality	0	0	1	0
Naturally delivered	0	0	0	0
Body weight and weight change[1]				
Body Weight, day 0	3.58	3.45	3.64	3.50
Body Weight, day 20	3.93	3.73	3.84	3.60*
Body Weight, day 29	4.02	3.84	3.90	3.84
Corrected body weight[2], day 29	3.58	3.43	3.46	3.45
Days 0-7	0.16±0.06	0.15±0.10	0.14±0.07	0.20±0.10
Days 7-10	0.01±0.06	0.02±0.05	0.01±0.05	0.01±0.04
Days 10-13	0.06±0.08	0.03±0.04	0.04±0.06	0.01±0.09
Days 13-16	0.09±0.06	0.05±0.05	0.02±0.10	0.05±0.10** (↓44%)
Days 16-20	0.02±0.08	0.03±0.08	0.01±0.08[16]	-0.02±0.12
Days 20-24	0.05±0.07	0.08±0.06[17]	0.05±0.08[16]	0.13±0.11
Days 24-29	0.04±0.08	0.04±0.12[17]	0.01±0.10[16]	0.11±0.06
Days 20-29	0.09±0.10	0.11±0.13[17]	0.06±0.13[16]	0.24±0.13** (↑167%)
Days 7-20	0.18±0.1l	0.13±0.10	0.05±0.21[16]	-0.10±0.27** (↓156%)
Days 7-29	0.28±0.14	0.24±0.13[17]	0.11±0.20[16]	0.14±0.19
Days 0-29	0.44±0.18	0.39±0.19[17]	0.24±0.23[16]	0.34±0.23
Days 0-29(corrected)[2]	0.00±0.20	-0.01±0.20[17]	-0.20±0.22[16]	-0.06±0.28
Food consumption[1] (g/day)				
Days 0-7	172.5±11.4	163.4±21.6	165.3±15.2 (↓4%)	167.6±17.7 (↓3%)
Days 7-10	167.9±14.5	162.2±20.0[17]	154.9±24.2 (↓8%)	151.6±24.5(↓10%)
Days 10-13	163.7±17.5	152.9±28.8	141.6±31.6 (↓14%)	127.6±46.6* (↓22%)
Days 13-16	161.1±22.0	147.5±36.2	126.0±58.3 (↓22%)	96.8±66.0** (↓40%)
Days 16-20	156.4±28.8	145.8±41.8	129.3±62.3[16] (↓17%)	98.3±69.2** (↓37%)
Days 20-24	143.4±36.5	147.8±24.1[16]	135.6±43.5[15] (↓5%)	133.4±44.7 (↓7%)
Days 24-29	106.6±36.5[18]	108.2±38.6[17]	90.8±41.0[15] (↓15%)	137.8±31.0 (↑29%)
Days 20-29	122.7±33.4[18]	126.2±25.1[17]	110.3±38.0[15] (↓10%)	135.9±32.9 (↑11%)
Days 7-20	161.8±18.0	151.8±30.5	137.8±44.2[16] (↓15%)	116.9±48.8** (↓28%)
Days 7-29	145.9±19.9[18]	140.6±23.2[17]	125.1±38.5[15] (↓14%)	124.7±39.0 (↓15%)
Days 0-29	152.5±16.2[18]	145.9±21.7[17]	134.4±32.1[15] (↓12%)	135.0±30.8 (↓11%)
Clinical observations[3][4]				
Dried faeces	2/2	1/1	3/18	5/49**
Soft or liquid faeces	2/11	2/3**	6/15	3/13
Alopecia	14/179	11/96**	11/150	10/112**
Red substance in pan	0/0	0/0	0/0	2/3**
Necropsy results				
Paraovarian cyst(s)	12	10	7	10
Ulcerations on fundic region of the stomach	0	0	1	0
External abscess on neck	0	0	1	0
Liver: papillary process appears white in color. Numerous white areas located on each lobe	0	1	0	0
Ulcerated area on external surface of the gall bladder	0	1	0	0

Parameter	Vehicle control	25 mg/kg bw/day	100 mg/kg bw/day	300 mg/kg bw/day
Red fluid-like substance in uterus	0	0	1	0
Agenesis of the left uterine horn	0	0	0	1
Reproductive and fetal endpoints in animals pregnant and delivered by c-section on day 29				
Number of does	19	17	16	17
Uterine weight (g)	440.88	404.34	443.91	395.48 (↓10%)
Corpora lutea	9.6	8.8	10.6	11.4
Implantations	8.3	6.9	7.8	7.1
Litters evaluated	19	17	16	17
Litter size	7.3	6.8	7.3	6.5
Number of foetuses evaluated[5]	140	115	117	111
Number of live/dead foetuses[5]	139/1	114/1	117/0	111/0
Resorptions	0.9	0.2	0.4	0.6
Does with any resorptions	9	3	6	8
Live foetal body weight/litter	44.87	44.21	43.81	44.54
Live foetal body weight/litter – males	45.85[6]	44.96	44.53	43.58[6]
Live foetal body weight/litter – females	44.01	13.54	43.38	44.86
Percent dead or resorbed conceptuses/litter	11.9	4.4	6.1	6.3

This table is restricted to pregnant rabbits. Days refer to the days of gestation.
* p<0.05 different from control; ** p<0.01 different from control
[1][ ]= Number of values averaged when different from number of pregnant does.
[2] Body weight minus gravid uterus weight
[3] / = rabbits/days
[4] Maximum incidences (rabbits/days) are 20/460, 20/453, 20/448, and 20/460 for the 0 (vehicle) ,50, 150, 300, and 500 mg/kg/day dosage groups, respectively.
[5] Observations for dead conceptuses were excluded from statistical analyses
[6] One control and one high dose litter contained no males

Foetal effects in the rabbits were not very pronounced but observed at a maternal dose of 250 mg/kg bw/day. The incidences of misaligned sutures (fontanelle) and nasal irregular ossification were higher compared with the control: the foetal incidence of misaligned sutures was 3.6% in the high dose group and not observed in any control foetus, while the foetal incidence of nasal irregular ossification was 6.3% (statistically significant increase) compared with the 2.2% in the concurrent control and the 0.24% in the historical control. Angulation of the hyoid alae was noted in 6.3% of the high dose foetuses and 1.4% of the control foetuses (mean historical control incidence: 1.29%), an increase that reached statistical significance. While this is a common observation in rabbit foetuses, considering that the incidence was higher than both the concurrent and the historical control, it might have been exacerbated by the treatment. The overall incidence of foetuses with any alterations was 18.7%, 19.3%, 23.9%, and 23.4% in the control, low, mid, and high dose groups, respectively. The same dose in the pilot study, i.e. 250 mg/kg bw, caused a reduction in foetal body weight (↓13%, sexes combined – similar trend in both sexes) and increased resorptions. This was not observed in the full developmental study, in which the foetal body weight was slightly reduced in males (↓5%) and slightly increased in females (↑2%).

In both developmental studies, the developmental pilot (rabbit), and the two-generation studies, foetal effects were observed; however, only in the presence of maternal toxicity, making the distinction between direct and indirect effects on the foetus unclear. Post-natal growth was reduced in the 5-week study in rats (≥500 ppm) at doses where the dams were unaffected, but this was not observed in the full 2-generation study on rats.

The lowest developmental LOAEL was observed in the rabbit and was 250 mg/kg bw/day. At this dose a reduction in foetal body weight and an increased number of resorptions were observed in the pilot, and incidences of misaligned sutures (fontanelle), nasal irregular ossification, and angulation of the hyoid alae were increased in the

main developmental study. The NOAELs of those studies were 83.3 mg/kg bw/day (main) and 125 mg/kg bw/day (pilot). Maternal toxicity was present at the developmental LOAEL (reduced food consumption and body weight gain, along with dried faeces). Deviations from OECD 414 (2001; the 2018 update is not applicable to rabbits): the exposure period ended at day 19 instead of the day prior to termination (shorter exposure period). This does not invalidate the study. The study is considered acceptable.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

Regulation 1272/2008 (CLP) states that "Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency."

Table 2.6.6.2.2-1. Hazard categories for reproductive toxicants (corresponding to table 3.7.1(a) in regulation 1272/2008)

Category	Description
1	Known or presumed human reproductive toxicant. Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily human data (Category 1A) or from animal data (Category 1B).
1A	Known human reproductive toxicant. The classification of a substance in Category 1A is largely based on evidence from humans.
1B	Presumed human reproductive toxicant. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility 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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
2	Suspected human reproductive toxicant. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed 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 the other toxic effects.

In the rat developmental study, foetal effects were observed at the highest dose including reduced litter size, post implantation loss, reduced foetal weight, increase in tail defects and reduced ossification in the presence of marked maternal toxicity including 20% mortality. The foetal effects are considered to be secondary to the maternal toxicity according to the CLP criteria as there is maternal mortality greater than 10% (criteria chapter 3.7.2.4.4). At the dose of 350 mg/kg bw/day, the foetal effects were limited to reduced foetal weight and reduced ossification. The dams at

this dose levels showed maternal toxicity in the form of reduced body weight and bodyweight gain and clinical effects. The limited foetal toxicity is of limited severity and considered to be secondary to the maternal toxicity. The maternal mortality observed in this study was considered relevant for STOT-RE 2 (refer to section 2.6.3).

There were developmental effects in the studies described above; however, the effects were generally observed at doses that caused maternal toxicity. As is stated in under category 2 in the table above, "Such effects shall have been observed 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 the other toxic effects.". The effects observed included reduced foetal weight, increased number of resorptions, certain external and visceral malformations, and retarded/altered ossification processes – these types of effects can be caused by maternal toxicity. In one study, the number of foetuses per litter and the number of litters with viable pups were reduced but these effects occurred at a dose with severe maternal toxicity (including mortality). Pup weight and pup weight gain was reduced in a 5-week study on rats, but this was not evident in the full 2-genaration study despite overlapping doses.

In the main two-generation study (Report S-2758), an increase in the number of stillborn F1 pups was observed (14 pups which corresponds to 3.8% of the delivered pups in the highest treatment group compared with 2 pups, i.e. 0.7%, in the control group). The number of F0 females with at least one stillborn pup was 7 (25%) in the 2500 ppm group and 2 (9.1%) in the control group. The number of stillborn F2 pups in the control group was 7 (2.7%), indicating that the control value in the F1 generation may be in the lower range of the spectrum. No increase in stillborn F2 pups was observed. Historical control data was not provided but the applicant refers to a historical value of 9 stillborn pups from 6 litters cited from one control group in a different 2-generation study performed earlier by the same laboratory. The effect increased with dose however the lack of effect in the F2 pups and the higher control value in the F2 compared to the F1 generation indicate that this may be incidental. The toxicological significance is thus unclear, and data is not considered to provide convincing evidence to fulfil criteria for classification of the substance in category 2.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 63: Summary table of animal studies on effects on or via lactation.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
No specific study available. Refer to assessment above at section 2.6.6.1.			

Table 64: Summary table of human data on effects on or via lactation.

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 65: Summary table of other studies relevant for effects on or via lactation.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

In the reproductive pilot study in rats, combined pup weight at day 7 but not day 0 and combined pup weight gain (day 0-7) were reduced in one all exposure groups (500-5000 ppm; 416.2-4162 ppm when corrected for purity; doses not corrected for dietary intake). This could be attributed to exposure via the breastmilk or a reduction of milk quality but since they were also exposed in utero this cannot be established. In addition, the 2-generation rat study did not indicate any effects on the pups via lactation (the only effect on the pups that was observed was an increased number of stillborn pups at 2500 ppm, a dose level that corresponded to 133.7 mg/kg bw/day in the F0 generation after correction for dietary intake).

There are no epidemiological studies assessing any potential effect of clethodim on lactation/breastmilk or effects when exposure occurs via breastmilk.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

In regulation (EC) No 1272/2008, it is stated that "Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (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 likelihood that the substance is present in potentially toxic levels in breast milk."

It is not possible from the available information (reproductive pilot + 2-generation rat study) to assess effects specifically on the quality of the breast milk or whether the observed effects in the pilot study were induced by exposure via the breast milk.

There is not enough evidence to determine if classification for effects on or via lactation is necessary. No classification is suggested.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity.

Clethodim does not meet the criteria in regulation (EC) No. 1272/2008 (CLP) for classification for reproductive toxicity.

2.6.7 Summary of neurotoxicity

Table 66: Summary table of animal studies on neurotoxicity.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results:** **- NOAEL/LOAEL** **- target tissue/organ** **-critical effect at LOAEL** Bold text=adverse effect	**Reference**
An Oral (Gavage) Acute Neurotoxicity Study of Clethodim in Rats Guidelines followed: OPPTS 870.6200 (1998) OECD 424 (1997) Deviations from current guidelines: None Species: Rat Strain: Charles River CD® (Sprague-Dawley) 3 treatment groups and a control group of 12 rats/sex/group GLP Acceptable	Clethodim TG Purity: 95.4% Oral gavage, single dose 15 days <u>Doses:</u> 0, 10, 100 and 1000 mg/kg bw	NOAEL neurotoxicity: 1000 mg/kg bw NOAEL systemic toxicity: 100 mg/kg bw <u>Effects at 100 mg/kg bw:</u> - reduced foot splay in males (not statistically significant) <u>Effects at 1000 mg/kg bw:</u> - **clinical signs** (↑ soiled fur on day 0 in females, one of these animals also displayed slight salivation) ↓ transient locomotor activity (total and ambulatory counts) in females on day 0 (stat. sign. in first 10 min interval) (considered connected to general toxicity at this group) - reduced foot splay in males (statistically significant at day 7)	[redacted] (2012) Report number: WIL-194041 Vol. 3. B.6.7.1.1 New data for renewal: Yes
A 28-Day Dietary Dose Range-Finding Neurotoxicity Study of Clethodim in Rats Guidelines followed: None (dose range finding study) Deviations from 424 (1997): - fewer animals (5/sex instead of 10) - histopathological examination not performed - FOB performed only during week 3 - haematology and clinical biochemistry parameters were not assessed. Species: Rat Strain: Crl:CD(SD) (Sprague-Dawley) 3 treatment groups and a control group of 5 rats/sex/group GLP Supportive	Clethodim TG Purity: 95.4% Exposure via the diet <u>Doses:</u> 0, 500, 1500 or 5000 ppm (equal to 0, 45, 132, and 441 mg/kg/day for ♂, 0, 51, 155, and 475 mg/kg bw per day for ♀) (it is not clear whether the results from the chemical analysis was used to calculate these values or if only feed consumption was used)	No NOAEL was set in the study* <u>Effects at 5000 ppm:</u> ↓**mean body weights** (Day 28: males:15%, females: 5%) ↓**mean bodyweight gain** (Day 0-28: males: 30%, females: 21% n.s (first week (48%)) ↓brain weight in males, 4%)	[redacted] (2012) Report number: WIL-194039 Vol. 3. B.6.7.1.2 New data for renewal: Yes
A 90-Day Oral Dietary Neurotoxicity Study of Clethodim in Rats Guidelines followed: OPPTS 870.6200 (1998)	Clethodim TG Lot/batch: AS 506r Purity: 95.4%	NOAEL systemic toxicity: 1500 ppm (94 mg/kg bw/d for males and 115 mg/kg bw/d for females) NOAEL neurotoxicity: 5000 ppm (331 mg/kg bw/d for males and 380 mg/kg bw/d for females)	[redacted] (2012) Report number: WIL-194040

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results:** **- NOAEL/LOAEL** **- target tissue/organ** **-critical effect at LOAEL** Bold text=adverse effect	**Reference**
Deviations from OECD 424 (1997): None Species: Rat Strain: Crl:CD(SD) (Sprague-Dawley) 3 treatment groups and a control group of 12 rats/sex/group GLP Acceptable	Exposure via the diet Doses: 0, 500, 1500 and 5000 ppm (equal to 0, 31, 94 and 331 mg/kg bw per day for ♂, 0, 38, 115 and 380 mg/kg bw per day for ♀)	LOAEL systemic toxicity: 5000 ppm (331 mg/kg bw/d for males and 380 mg/kg bw/d for females) LOAEL neurotoxicity: - Effects at 5000 ppm Males: ↓ **mean body weight** (7-11%) ↓ **body weight gain** (day 0-91: 16%) Females: ↓ mean body weight (7-9%) ↓ **body weight gain** (day 0-91: 19%) No neurotoxic effects	Vol. 3. B.6.7.1.3 New data for renewal: Yes

* Study not suitable for NOAEL setting (low number of animals used and limited parameters investigated

Three neurotoxicity studies of varying length were performed on rats: in the acute neurotoxicity study rats (refer to Vol. 3, B.6.7.1.1) were exposed to 10-1000 mg/kg bw via oral gavage, in a 28-day dose range-finding study (refer to Vol. 3, B.6.7.1.2) the animals were administered 45-441 mg test item/kg bw/day via the diet, and one 90-day study (refer to Vol. 3, B.6.7.1.3) in which the animals were administered 31-380 mg test item/kg bw/day via the diet. All studies are new data for the renewal of active substance.

In the acute study (refer to Vol. 3, B.6.7.1.1), no mortality occurred during the study. The incidence of hair loss on forelimbs was increased in animals in the high dose group, and a larger number of females in the highest dose group (1000 mg/kg bw) had soiled fur on study day 0 compared to the control group; one female in the highest dose group with soiled fur also had slight salivation. This was not observed at later time points. There were no significant differences in body weight or body weight gain between the control and test substance-treated groups. Handling, open field, and sensory parameters were not affected. One male in the low dose (10 mg/kg) group displayed a head flick (a shaking head or backward flip of the head) on study day 14 during the open field test. This was not observed in any other animals or groups and is not considered treatment related. Hindlimb foot splay was decreased in males of the high dose (↓8%, ↓12%, ↓30, and ↓24% compared with the control group prior to test initiation, on days 0, 7, and 14, respectively) and middle dose group (↓5%, ↓3%, ↓20, and ↓18% compared with the control group prior to test initiation, on days 0, 7, and 14, respectively. The effect was only statistically significant in high dose males on day 7. The motor activity was highly variable within the 10 minute-time intervals and differed largely between individuals and groups at times; however, the cumulative values did not indicate any clear trends in affected motor activity. There was a tendency towards lower activity in females on day 0 (both total and ambulatory activity in the 0–10-minute interval was statistically significantly decreased; ↓16%) but no clear trend was observed. The RMS agrees with the applicant that this may be connected to general toxicity as this group. Soiled fur + slight salivation was observed in one animal. There was no apparent effect on habituation patterns in the treated animals. Therefore, the effects noted in this study are not considered adverse and effect levels thus represent LOEL and NOELs rather than LOAEL and NOAELs. The NOAEL for neurotoxicity was 1000 mg/kg bw, the highest dose tested. NOAEL for systemic toxicity was considered 100 mg/kg bw/day based on soiled fur in females and salivation in one animal.

Although salivation was observed in one animal only, this effect was also observed in acute oral toxicity study (Report number: S 2498) in the same strain at 800 mg/kg bw/day, and therefore considered reflecting systemic toxicity rather than neurotoxicity. The study was performed in accordance with OECD 424, and with FIFRA and OECD Good Laboratory Practice (GLP) Standards. The study is considered acceptable.

In the 28-day dietary study (refer to Vol. 3, B.6.7.1.2), no deaths occurred, and no clinical signs stood out in the exposed groups. Body weight and/or body weight gain were affected in all dose groups at one interval or more. The final weight at day 28 was 15 % lower in males of the high dose group, and body weight gain was reduced by 16, 14, and 30 % in the low, middle, and high dose males, respectively. A similar trend was observed in females. The absolute weight was not significantly affected in females (a 5% reduction at the top dose) and the overall body weight gain was reduced by 21% in this group, mainly due to a significant decrease of body weight change during the first week (48%). No clear effects on food consumption were observed, but body weight gained as percent of feed consumed was lower in the 5000 ppm males. No treatment related effects on home cage, handling, sensory or neuromuscular observations, or motor activity were observed. Absolute brain weight, but not liver weight in males, was slightly reduced in the top and lowest dose group (↓5% in the low dose and ↓ 4% in the top dose). No effect on brain weight was observed in middle dose group. The study is considered as supportive data (dose range-finder study) (Report number WIL-194039).

In the 90-day study (refer to Vol. 3, B.6.7.1.3), no mortality occurred, and no clear treatment related clinical signs were observed. Body weights of both sexes were lower in the highest dose group than those of the control group throughout the study (7-11% in males and 7-9% in females). Body weights of the low and middle dose groups were comparable to control weights. Body weight gain in the 5000-ppm group (331 mg/kg bw/d ♂ and 380 mg/kg bw/d ♀) was reduced in both sexes (16% in males and 19% in females) over the entire study period, in general due to lower gains during the first month. Food consumption in males was reduced during the first week, potentially indicating palatability issues, but the consumption per kg bw was similar or slightly higher the rest of the study. In females, the food consumption per kg bw was similar to the control group overall. The functional battery revealed no treatment related effect on home cage, handling, open field, sensory, or neuromuscular observations. Physiological observations included lower body weight in both sexes. No clear treatment related trends were observed in the treated animals. Total and ambulatory motor activity counts for the 5000 ppm group females at the study week 7 evaluation was lower than that of the control. The value was also lower than the HCD and the control value was higher than the HCD. No effects on habituation were observed. There were no effects on liver weight, brain weight, or brain length or width but it is noted that relative weights were not reported. No treatment related changes were noted during necropsy. The study follows OECD TG 424 and is considered acceptable (Report number WIL-194040).

In summation, the test item did not induce much toxicity. No mortality occurred in the studies. Some clinical signs were noted, mainly in the acute study. Some cases of soiled fur and one case of salivation in females of the highest dose group (954 mg/kg bw) during the first week of the acute oral gavage study. Increased incidence of hair loss of the forelimbs occurred in the acute oral gavage study, and a similar tendency (higher total incidence but the same number of animals as the control group) was observed in the high dose (380 mg/kg bw/day) females of the high dose group in the 90-day study. Male body weight and body weight gain was reduced in both the 28- and 90-day studies. Females did not appear as affected. The overall body weight gain was reduced in both studies, mainly due

to a reduction in the beginning of the study (first week in the 28-day study and until day 35 in the 90-day study) with values at the end being similar to those of the controls. Food consumption was not clearly affected in either study. The motor activity seen in the acute toxicity study was highly variable within the 10 minute-time intervals and differed largely between individuals and groups at times; however, the cumulative values did not indicate any clear trends in affected motor activity. There was a tendency towards lower activity in females on day 0 (both total and ambulatory activity in the 0–10-minute interval was statistically significantly decreased; ↓16%) but no clear trend was observed. The RMS agrees with the applicant that this may be connected to general toxicity as this group. Hindlimb foots play was reduced in high dose males in acute study, but not in the two longer studies. The significance of this change and in particular, the direction of change, is unclear. A slight but statistically significant reduction (4%) in absolute brain weight was observed in males exposed to 441 mg/kg bw/day for 28 days. In the absence of other indications in all three studies, the effect noted in the FOB assessments and the motor activity is not considered to indicate a neurotoxic potential of the test substance in rats.

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

Please refer to Vol. 4 for information on impurities.

2.6.8.1.1 Clethodim imine sulfone (RE-47719)

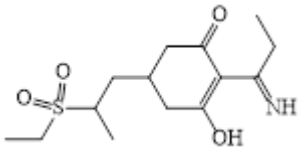


Table 2.6.8.1.1-1. Summary table of studies on clethodim imine sulfone (RE-47719)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Acute oral toxicity study GLP: Yes Guideline: 40 CFR 158.135, Pesticide Assessment Guideline No. 81-1 Deviations from OECD TG 401 (1981): No deviations 5 female Sprague-Dawley rats Acceptable	RE-47719 Purity: 98.6% Lot/batch: SX-1800 Single dose of 1.4 g/kg Vehicle: A solution of 0.7% carboxymethylcellulose (CMC) and 1.0% TWEEN 80 in distilled water	LD_{50} female rats >1400 mg/kg bw	Vol.3, B.6.8.1.1/01 [redacted] (1988a) Report No.: S-3154 New data for the Annex I renewal: No
5-week oral toxicity study GLP: Yes	RE-47719 Purity: 99.3% (no certificate of analysis available)	NOAEL: 1000 ppm (equal to 70.9 mg/kg bw/day)	Vol.3, B.6.8.1.1/02

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Guideline: None Deviations from OECD TG 407: - reactivity to auditory and proprioceptive stimuli was not determined - blood clotting time/potential was not determined - the following organs were not weighed: epididymides, prostate + seminal vesicles with coagulating spleen, and heart - histopathological assessment was not performed for the following organs: cervix, epididymides, prostate + seminal vesicles with coagulating glands), and vagina - highest humidity was slightly above the recommended (78% vs 70%) 10 Sprague-Dawley rats per sex and dose level. Acceptable with limitations	Lot/batch: SX-1800 <u>Target concentrations:</u> 0, 100, 1000 and 8000 ppm Achieved concentrations: 80.8, 871 and 7820 ppm (corresponding to 0, 6.7, 70.9 and 604 mg/kg bw/day for males, and 0, 7.8, 83.7 and 723 mg/kg bw/day for females). <u>Duration of exposure:</u> 5 weeks	<u>Effects observed at 100 ppm (equal to 6.7 (M) and 7.8 (F) mg/kg bw/day):</u> ↓ globulin (M: 17%) ↑ A:G (M: 18%) <u>Effects observed at 1000 ppm (equal to 70.9 (M) and 83.7 (F) mg/kg bw/day):</u> ↓ globulin (M: 17%) ↑ A:G (M: 15%) <u>Effects observed at 8000 ppm (equal to 604 (M) and 723 (F) mg/kg bw/day):</u> ↑ reticulocytes (M: 220%), ↑ liver absolute and relative weights (14-19%) ↑serum cholesterol (M:57%, F:27%) ↓ ALK (F:32%) ↓ ALT (M: 32%) ↑ albumin (M: 9%) ↓ globulin (M: 17%) ↑ A:G (M: 30%) ↑ calcium (M: 5%) ↓ food consumption and bw gain (M: 27%) at day 7, but not at day 14 or 21.	[redacted] (1988a) Report No.: S-3158 New data for the Annex I renewal: No
Reverse mutation assay with and without S9 (Ames test) GLP: Yes Guideline: 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2. Deviations from OECD TG 471 (1997): - no confirmation of bacterial cell density at the time of treatment. Acceptable	RE-44719 Purity: 98.6% Lot/batch: SX-1800 <u>Concentrations:</u> 0, 100, 333, 1000, 3333 and 10000 µg/plate (+/- S9 mix).	RE-44719 does not induce mutations in the *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A) in the presence and absence of a rat liver-derived metabolic activation system (S9-mix). The test material was slightly cytotoxic at 10 mg/plate to TA100 without S-9.	Vol.3, B.6.8.1.1/03 Machado (1988) Report No.: S-3155 New data for the Annex I renewal: No
Chromosomal aberrations assay in Chinese Hamster Ovary (CHO) cells in vitro. GLP: Yes Guideline: 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2. Deviations from OECD TG 473 (2016): - different treatment and fixation times, only 200 instead of 300 metaphases per concentration were analysed for chromosomal aberrations - no short-term exposure in the absence of S9 was performed - no laboratory historical control data was reported. Acceptable with limitations	RE-44719 Purity: 99.3% (stated in the analytical report) or 98.6% (stated in the protocol). (no certificate of analysis is available) Lot/batch: SX-1800 <u>Concentrations:</u> 0, 50, 100, 200 and 400 µg/mL (±S9 mix)	RE-44719 does not induce chromosome aberrations in CHO cells under the conditions tested and was partially insoluble in treatment medium at final concentrations of 380 µg/mL and higher.	Vol.3, B.6.8.1.1/04 Putnam (1988a) Report No.: S-3156 New data for the Annex I renewal: No
Oral teratogenicity and developmental toxicity screen.	RE-47719	No NOAEL was set in study*	Vol.3, B.6.8.1.1/05

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Administration by gavage GLP: Yes Guideline: EPA/FIFRA Pesticide Assessment Guideline 83-3 (Nov 1984). Deviations from OECD TG 414 (2018): - only 10 pregnant females were used in each dose group - no measurement of foetal anogenital distance, thyroid weights and histopathology and assessment of blood thyroid hormone (T4, T3 and TSH) concentrations in the maternal animals. – rats were exposed from days 6-15 (the guideline recommends exposure from days 5-15 but also through the entire period of gestation to the day of caesarean section) 10 pregnant female Sprague-Dawley rats per dose level. Supportive	Purity: 98.6% Lot/batch: SX-1800 <u>Doses:</u> 0, 10, 100 and 700 mg/kg bw/day Vehicle: aqueous 0.7% carboxymethyl cellulose and 1.0% Tween 80 in deionized water <u>Duration of exposure:</u> Day 6 to 15 of gestation (maximum of ten dosages)	<u>Effects observed at 100 mg/kg bw/day:</u> <u>Maternal:</u> ↓ bw gain (Days 6-18: 17%) <u>Effects observed at 700 mg/kg:</u> <u>Maternal:</u> -clinical signs (excess salivation and alopecia) ↓ bw gain (Days 6-9: 77%; Days 6-12: 63%; Days 6-18: 16%) ↓ FC <u>Foetal:</u> ↓ foetal body weight (13%) ↑ incidence of cervical rib (litter incidence: 100% compared to 10% in control; foetal incidence: 38.5% compared to 1.2% in controls) - delayed sternal ossification	[redacted] (1988a) Report No.: S-3157 New data for the Annex I renewal: No

* The study is not suitable for NOAEL setting (low number of animals used, limited parameters investigated)

Results

Clethodim imine sulfone (5-(2-(ethylsulfinyl)propyl)-3-hydroxy-2-(1-iminopropyl) cyclohex-2-en-1- one) is a metabolite found in crops. It was not found in the rat metabolism studies.

All studies with clethodim imine sulfone (RE-47719) were conducted under GLP. The studies were included in the previous EU evaluation (DAR 2005). There are no new data for this metabolite.

The acute oral LD_{50} of clethodim imine sulfone (RE-47719) was estimated to be >1400 mg/kg bw in female rats and thus higher than clethodim which shows an LD_{50} of 1133 mg/kg bw in female rats (Vol. 3, B.6.2.1/01). The study was carried out according to OECD TG 401 and considered acceptable (Vol. 3, B.6.8.1.1/01).

Oral administrations of clethodim imine sulfone (RE-47719) to rats at dietary concentrations of 0, 6.7, 70.9 and 604 mg/kg bw/day for males and 0, 7.8, 83.7 and 723 mg/kg bw/day for females for 35-days resulted in a NOAEL of 70.9 mg/kg bw/day based on increased reticulocytes, liver weights and higher serum cholesterol levels noted at 604 mg/kg bw/day. The study was not conducted in accordance with any guideline and the weight of several organs were not determined, histopathology not performed on prostate and epididymides. Further, no blood clotting test or functional observations were performed. The study is therefore considered acceptable with limitations (B.6.8.1.1/02). The NOAEL (70.9 mg/kg bw/day) following 35-day administration of clethodim imine sulfone (RE-47719) is higher compared to the NOAEL (12.5 mg/kg bw/day) and LOAEL (65.6 mg/kg bw/day) obtained in the 28-day oral toxicity study conducted with clethodim (see Vol. 3, B.6.3.1/01).

The clethodim imine sulfone (RE-47719) gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A) in the presence and absence of a rat liver S9-mix. The

study follows OECD TG 471 with exception for minor deviation (no confirmation of bacterial cell density at the time of treatment). The study was considered acceptable (Vol. 3, B.6.8.1.1/03).

Furthermore, clethodim imine sulfone did not induce structural chromosomal aberrations in Chinese hamster ovary (CHO) cells *in vitro*. The study did not fulfil the requirement of the more recent OECD TG 473 (2016) and the following deviations were noted: different treatment and fixation times, only 200 instead of 300 metaphases per concentration were analysed for chromosomal aberrations, no short-term exposure in the absence of S9 was performed, and no laboratory historical control data was reported. Hence, the RMS considers the chromosomal aberration study to be acceptable with limitations (Vol. 3, B.6.8.1.1/04).

A teratogenicity study with clethodim imine sulfone (RE-47719) was performed at 0, 10, 100 and 700 mg/kg bw/day according to the EPA guideline 40 CFR 158.34, Pesticide Assessment Guideline, No. 83-3 (Nov. 1984). In this study maternal adverse effects were observed at 100 mg/kg bw/day (reduced bodyweight gain (17%)) and 700 mg/kg bw/day (clinical signs of excess salivation and alopecia, and reduced bodyweight gain (77%)). Adverse foetal effects were observed at 700 mg/kg bw/day (reduced foetal body weight (13%), increased incidence of cervical rib and delays in sternal ossification). A number of deviations were observed from the current OECD test guideline, adopted in 2018. Only 10 pregnant female rats were used in each dose group and measurement of foetal anogenital distance, thyroid weights and histopathology as well as assessment of blood thyroid hormone (T4, T3 and TSH) concentrations in the maternal animals were not performed. Furthermore, rats were exposed from days 6-15 (the guideline recommends exposure from days 5-15 but also through the entire period of gestation to the day of caesarean section). The study was considered as supportive data due to major deviations from OECD TG 414. The study was not suitable for NOAEL setting (low number of animals used, limited parameters investigated). However, the result of this study indicates that clethodim imine sulfone (RE-47719) is of lower toxicity compared to the parent compound (LOAEL for developmental toxicity in rats was set at 292 mg/kg bw/day in the developmental toxicity study conducted with clethodim) also taking into account that the effects observed in study were not considered sufficient evidence for a classification of test item for developmental toxicity (Vol. 3, B.6.8.1.1/05).

Overall conclusion:

Clethodim imine sulfone (RE-47719) was less acutely toxic than the parent substance, and did not cause gene mutations in Ames test, nor did it induce structural chromosomal aberrations *in vitro*. A NOAEL of 70.9 mg/kg bw/day was obtained in a 5-week oral toxicity study conducted with clethodim imine sulfone (RE-47719) based on increased reticulocytes, liver weights and higher serum cholesterol levels noted at 604 mg/kg bw/day. Thus, the toxicity of clethodim imine sulfone (RE-47719) following repeated dose administration was considered lower than that of clethodim (LOAEL in the 28-day oral toxicity study conducted with clethodim was set at 54.7 mg/kg bw/day).

In the developmental toxicity screening study in the rat, clethodim imine sulfone induced foetal alterations (increased incidence of cervical rib and delayed sternal ossification) at a dose level (700 mg/kg bw/day, NOAEL 100 mg/kg bw/day) causing maternal toxicity (reduced body weight gain, reduced food consumption and clinical signs such as excess salivation and alopecia). Also, at this dose level foetal weight was reduced (13%). The result of this study indicates that clethodim imine sulfone (RE-47719) is of lower toxicity compared to the parent compound. For clethodim the NOAEL and LOAEL for developmental toxicity in rats were 83.3 and 292 mg/kg bw/day, respectively. At the LOAEL of 292 mg/kg bw/day increased incidence of skeletal variations (incomplete

or unossified vertebrae, unossified 5th or 6th sternebrae) were observed. At the higher dose level of 583 mg/kg bw/day increased incidence of skeletal malformations and visceral malformations were observed in addition, and pup weight was reduced 25%.

The effects on developmental observed in the study conducted with clethodim imine sulfone (RE-47719) were observed in presence of maternal toxicity and considered not sufficient evidence for a classification of test item for developmental toxicity.

Clethodim imine sulfone (RE-47719) did not induce gene mutations or structural chromosome aberrations. However, **a data gap** for genotoxicity was identified since aneuploidy has not been properly assessed, this is accordance to the EFSA document Guidance on aneugenicity assessment (2021) *

*EFSA Scientific Committee (SC), doi: 10.2903/j.efsa.2021.6770, states on page 4 that "The genotoxicity testing strategy indicated in the EFSA Scientific Committee opinion is designed to investigate the genotoxic potential of substances through the detection of three genotoxic endpoints: gene mutations, structural chromosomal aberrations (i.e. clastogenicity) and numerical chromosomal aberrations (i.e. aneuploidy). The testing strategy id developed as a stepwise approach, beginning with a basic battery of *in vitro* tests, comprising:

- A bacterial reverse mutation assay [Organisation for Economic Co-operation and Development (OECD) TG 471, endpoint: gene mutations]; *and*
- an *in vitro* mammalian cell micronucleus (MN) test (OECD TG 487, endpoints: clastogenicity and aneugenicity)."

2.6.8.1.2 Clethodim 5-OH sulfone (RE-51228)

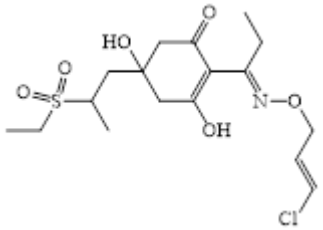


Table 2.6.8.1.2-1: Summary table of studies on clethodim 5-OH sulfone (RE-51228)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Acute oral toxicity study GLP: Yes Guideline: 40 CFR 158.135, Pesticide Assessment Guideline No. 81-1. Deviations from OECD TG 401 (1981): No deviations 5 female Sprague-Dawley rats	RE-51228 Purity: 99.9% Lot/batch: SX-1796 Vehicle: aqueous solution of 0.7% carboxymethylcellulose and 1.0% TWEEN 80 Single doses of 1.4 g/kg	LD_{50} female rats >1400 mg/kg bw	Vol.3, B.6.8.1.2/01 [redacted] (1988b) Report No.: S-3159 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Acceptable			
5-week oral toxicity study GLP: Yes Guideline: None Deviations from OECD TG 407 (2008): - the humidity was slightly out of range (55-74% vs the recommended maximum of 70%) - sensory reactivity to stimuli was not assessed - blood clotting time/potential was not measured - organs not weighed: epididymides, prostate + seminal vesicles with coagulating glands, thymus, spleen, and heart - histopathological analysis of the vagina, epididymides, prostate+seminal vesicles not performed 10 Sprague-Dawley rats per sex and dose level. Acceptable with limitations	RE-51228 Purity: 94.8% Lot/batch: SX-1803 <u>Target concentrations:</u> 0, 100, 1000 and 8000 ppm <u>Achieved concentrations:</u> 0, 73.2, 856 and 7290 ppm (equal to 0, 5.94, 67.7 and 588 mg/kg/day for males, 0, 6.43, 75.5 and 663 mg/kg/day for females)	NOAEL: 73.2 ppm (equal to 5.94 mg/kg/day for males, 6.43 mg/kg/day for females) <u>Target organs and effects:</u> - reductions in haemoglobin in males (7 and 5% in the middle and high dose, respectively) -reductions in haematocrit in males (9 and 6% in the middle and high dose, respectively)	Vol.3, B.6.8.1.2/02 [redacted] (1988b) Report No.: S-3162 New data for the Annex I renewal: No
Reverse mutation assay with and without S9 (Ames test) GLP: Yes <u>Guideline:</u> 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2. <u>Deviations from OECD TG 471 (1997):</u> No information on the purity of the test substance provided and no confirmation of bacterial cell density at the time of treatment. Acceptable with limitations	RE-51228 Purity: not stated Lot/batch: not stated. <u>Concentrations:</u> Exp I: 0.03, 0.10, 0.33, 1.00, 3.33 mg/plate (+/- S9 mix) Exp. II: 0.10, 0.33, 1.00, 3.33, 5.00 mg/plate (+/- S9 mix)	RE-51228 does not induce mutations in the *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A) in the presence and absence of a rat liver-derived metabolic activation system (S9-mix). RE-51228 was cytotoxic to TA98 at ≥3.3 mg/plate and to TA100 at ≥1.0 mg/plate (+S9)	Vol.3, B.6.8.1.2/03 Machado (1987) Report No.: S-3111 New data for the Annex I renewal: No
Chromosomal aberrations assay in Chinese Hamster Ovary (CHO) cells in vitro. GLP: Yes Guideline: 40 CFR 158.135, Pesticide Assessment Guideline No. 84-2. Deviations from current guideline: Does not fulfil the requirement of OECD TG 473 guideline (2016) and limited information is provided on the purity of the test substance. Only 200 instead of 300 metaphases per concentration	RE-51228 Purity: not determined by the testing facility but it is mentioned that the active ingredient in dose=99.9% (no certificate of analysis is available) Lot/batch: SX-1796 <u>Concentrations:</u> 0, 313, 625, 1250 and 2500 µg/mL (-S9 mix)	RE-51228 does not induce chromosome aberrations in CHO cells under the conditions tested. RE-51228 was partially insoluble in test article-treated groups at final concentrations of 625, 1250 and 2500 µg/ml in culture medium.	Vol.3, B.6.8.1.2/04 Putnam (1988b) Report No.: S-3160 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
were analysed for chromosomal aberrations. Different treatment and fixation times compared to OECD TG 473. No short-term exposure in the absence of S9 was performed. No laboratory historical control data reported. Acceptable with limitations			
Oral teratogenicity and developmental toxicity screen. Administration by gavage GLP: Yes Guideline: EPA/FIFRA Pesticide Assessment Guideline 83-3 (Nov 1984). Deviations from OECD TG 414 (2018): Only 10 pregnant females were used in each dose group, no measurement of foetal anogenital distance, thyroid weights and histopathology and assessment of blood thyroid hormone (T4, T3 and TSH) concentrations in the maternal animals. Rats were exposed from days 6-15 (the guideline recommends exposure from days 5-15 but also through the entire period of gestation to the day of caesarean section). 10 pregnant female Sprague-Dawley rats per dose level. Supportive	RE-51228 Purity: 99.9% Lot/batch: SX-1796 Vehicle: aqueous 0.7% carboxymethyl cellulose and 1.0% Tween 80 in deionized water Dose: 0, 10, 100 and 700 mg/kg bw/day Duration of exposure: Day 6 to 15 of gestation. (maximum of ten dosages)	No NOAEL was set in study* <u>Target organ and adverse effects:</u> <u>700 mg/kg bw/day:</u> - clinical signs in dams (rales) -reduced body weight gain (Day 12-16: 19%, n.s.) ↓ gravid uterine weight (Day 28: 4%, n.s.) ↓foetal weight (10%, n.s.)	Vol.3, B.6.8.1.2/05 [redacted] (1988b) Report No.: S-3161 New data for the Annex I renewal: No

* The study is not suitable for NOAEL setting (low number of animals used, limited parameters investigated)

Results

Clethodim 5-OH sulfone, (2-(I-1-(((I-3-chloroallyl)oxy)imino)propyl)-5-(2-(ethylsulfonyl) propyl)-3,5-dihydroxycyclohex-2-en-1-one) is a crop metabolite and also a minor rat metabolite (1% in urine).

All studies with clethodim 5-OH sulfone (RE-51228) were conducted under GLP. The studies were included in the previous EU evaluation (DAR 2005). There are no new data for this metabolite.

The acute oral LD_{50} of clethodim 5-OH sulfone (RE-51228) was estimated to be >1400 mg/kg bw in female rats and thus higher than clethodim which shows an LD_{50} of 1133 mg/kg bw in female rats. The study was carried out according to OECD TG 401 and considered acceptable (Vol. 3, B.6.8.1.2/01)

Oral administrations of clethodim 5-OH sulfone (RE-51228) to rats at dietary target concentrations of 0, 100, 1000 and 8000 ppm (equal to an overall average weekly test material intake of 5.94, 67.7 and 588 mg/kg bw/day in males and to 6.43, 75.5 and 663 mg/kg/day in females) for 35 days resulted in a NOAEL of 5.94 mg/kg bw/day based on changes in haematological parameters noted at ≥67.7 mg/kg bw/day. The haematological changes consisted of

reductions in haemoglobin levels observed in males in the middle-dosage group (7%) and high dosage group (5%), and reductions in haematocrit levels observed in males in the middle-dosage group (9%) and high dosage group (6%). These effects were also observed in repeated dose toxicity studies conducted with the parent substance (effects that often appeared in males at lower doses than females). The NOAEL (5.94 mg/kg bw/day) following 35-day administration of clethodim 5-OH sulfone (RE-51228) is lower compared to the NOAEL (12.5 mg/kg bw/day) obtained in the 28-day oral toxicity study conducted with clethodim; however, the LOAEL (67.7 mg/kg bw/day) following 35-day administration of clethodim 5-OH sulfone (RE-51228) is higher compared to the LOAEL (65.6 mg/kg bw/day) obtained in the 28-day oral toxicity study conducted with clethodim (Vol. 3, B.6.3.1.1/01). Furthermore, it could be noted that other effects in addition were observed at the LOAEL for clethodim (reduced erythrocytes and haemoglobin in both sexes, increased platelets in males, increased liver weight in both sexes and hepatic centrilobular hypertrophy in males) compared with the LOAEL for clethodim 5-OH sulfone. Thus, the general toxicity of clethodim 5-OH sulfone is considered lower or similar to that of clethodim. The study was checked for compliance with OECD TG 407 and following deviations were observed: humidity was slightly out of range (55-74% vs the recommended maximum of 70%), sensory reactivity to stimuli was not assessed, blood clotting time/potential was not measured, organs not weighed (epididymides, prostate + seminal vesicles with coagulating glands, thymus, spleen, and heart), histopathological analysis not performed for the vagina, epididymides, prostate+seminal vesicles, no blood clotting performed. The study is acceptable with limitations (Vol. 3, B.6.8.1.2/02).

The clethodim 5-OH sulfone (RE-51228) gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A) in the presence and absence of a rat liver S9-mix. However, the purity of the test compound was not provided. The study was considered acceptable with limitations (Vol. 3, B.6.8.1.2/03)

Clethodim 5-OH sulfone (RE-51228) did not induce structural chromosomal aberrations in CHO cells in vitro. The chromosomal aberration study does not fulfil the requirement of the more recent OECD TG 473 (2016) and the test item was partially insoluble at the tested concentrations. Hence, the RMS considers the chromosomal aberration study to be acceptable with limitations (Vol. 3, B.6.8.1.2/04).

A teratogenicity study with clethodim 5-OH sulfone (RE-51228) was performed at 0, 10, 100 and 700 mg/kg bw/day according to the EPA guideline 40 CFR 158.34, Pesticide Assessment Guideline, No. 83-3 (Nov. 1984). In this study maternal adverse effects were observed at 700 mg/kg bw/day (clinical signs of rales, reduced bodyweight gain (19%, n.s.) and reduced gravid uterine weight (Day 28: 4%, n.s.)). Adverse foetal effects were observed at 700 mg/kg bw/day (reduced foetal weight (10%, n.s,), however this effect occurred in presence of maternal toxicity. A number of deviations were observed from the current OECD test guideline, adopted in 2018. Only 10 pregnant female rats were used in each dose group and measurement of foetal anogenital distance, thyroid weights and histopathology as well as assessment of blood thyroid hormone (T4, T3 and TSH) concentrations in the maternal animals were not performed. Furthermore, rats were exposed from days 6-15 (the guideline recommends exposure from days 5-15 but also through the entire period of gestation to the day of caesarean section). The study was considered as supportive data due to major deviations from OECD TG 414. The study is not suitable for NOAEL setting (low number of animals used, limited parameters investigated). However, the result of this study indicates that clethodim 5-OH sulfone (RE-51228) is of lower toxicity compared to the parent compound (LOAEL for

developmental toxicity in rats was set at 292 mg/kg bw/day in the developmental toxicity study conducted with clethodim) also taking into account that the observed foetal effect in the presence of maternal toxicity was not considered sufficient evidence for a classification for developmental toxicity (Vol. 3, B.6.8.1.2/05).

Overall conclusion:

Clethodim 5-OH sulfone (RE-51228) was less acutely toxic than the parent substance, and did not cause gene mutations in Ames test, nor did it induce structural chromosomal aberrations in vitro (albeit this study had some limitations, e.g. with solubility). A NOAEL of 5.94 mg/kg bw/day was obtained in a 5-week oral toxicity study conducted with 5-OH sulfone (RE-51228) based on changes in haematological parameters noted at ≥67.7 mg/kg bw/day. Thus, the toxicity of clethodim 5-OH sulfone following repeated dose administration was considered lower or similar to that of clethodim (LOAEL in the 28-day oral toxicity study conducted with clethodim was set at 54.7 mg/kg bw/day). In the developmental toxicity screening study in the rat, clethodim 5-OH sulfone induced reduced foetal weight (10%, n.s) at a dose level of 700 mg/kg bw/day and in the presence of maternal toxicity (reduced bodyweight gain, 19%, n.s.). The result of this study indicates that clethodim 5-OH (RE-51228) is of lower toxicity compared to the parent compound (LOAEL for developmental toxicity in rats was set at 292 mg/kg bw/day in the developmental toxicity study conducted with clethodim). It could also be noted that the effects observed in the study conducted with clethodim 5-OH sulfone (RE-51228) were not sufficient evidence for a classification of test item for developmental toxicity. Thus, the result of the study indicates that clethodim 5-OH sulfone (RE-51228) is of no concern for developmental toxicity, and no further data is needed.

Clethodim 5-OH sulfone (RE-51228) did not induce gene mutations or structural chromosome aberrations. However, **a data gap** for genotoxicity was identified since aneuploidy has not been properly assessed, this is accordance to the EFSA document Guidance on aneugenicity assessment (2021) *.

*EFSA Scientific Committee (SC), doi: 10.2903/j.efsa.2021.6770, states on page 4 that “The genotoxicity testing strategy indicated in the EFSA Scientific Committee opinion is designed to investigate the genotoxic potential of substances through the detection of three genotoxic endpoints: gene mutations, structural chromosomal aberrations (i.e. clastogenicity) and numerical chromosomal aberrations (i.e. aneuploidy). The testing strategy id developed as a stepwise approach, beginning with a basic battery of *in vitro* tests, comprising:

- A bacterial reverse mutation assay [Organisation for Economic Co-operation and Development (OECD) TG 471, endpoint: gene mutations]; *and*
- an *in vitro* mammalian cell micronucleus (MN) test (OECD TG 487, endpoints: clastogenicity and aneugenicity).”

2.6.8.1.3 Clethodim oxazole sulfone (RE-47797)

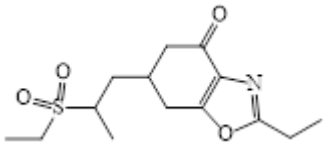


Table 2.6.8.1.3-1: Summary table of studies on clethodim oxazole sulfone (RE-47797)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Reverse mutation assay with and without S9 (Ames test) GLP: Yes Guideline: OECD TG 471 (1997) Deviations from current guideline: None Acceptable	Clethodim oxazole sulfone (RE-47797) Purity: 98.9% Lot/batch: AS582d <u>Concentrations:</u> Exp I: 17, 50, 167, 500, 1667, 5000 µg/L (+/-S9 mix) Exp. II: 17, 50, 167, 500, 1667, 5000 µg/L (+/- S9 mix)	RE-47797 does not induce mutations in the *Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2*uvr*A reverse mutation assay. Toxicity, observed as a reduction in the number of revertant colonies, occurred with TA 1535 in the absence of S9 mix only, at the highest concentration of 5000 µg per plate. No precipitation of the test item was observed in either mutation assay, neither in the presence nor the absence of S9 mix.	Vol.3, B.6.8.1.3/01 Stevenson, (2004) Report No.: S-22789 New data for the Annex I renewal: No
Chromosomal aberrations assay in Chinese Hamster Ovary (CHO) cells in vitro. GLP: Yes Guideline: OECD TG 473 (1997). Deviations from current guideline: Only 100 metaphases per concentration were scored for chromosomal aberrations, OECD 473 (2016) requires 300. Acceptable with limitations	Clethodim oxazole sulfone (RE-47797) Purity: 98.9% Lot/batch: AS582d <u>Concentrations:</u> 1250, 2500 and 5000 µL/mL (±S9 mix)	RE-47797 does not induce chromosome aberrations in CHO cells without metabolic activation. Clastogenic in the presence of S9 mix at 5000 µg/mL	Vol.3, B.6.8.1.3/02 Hart & Stevenson, (2005) Report No.: S-22910 New data for the Annex I renewal: No
Mouse lymphoma cell mutation assay GLP: Yes Guideline: OECD TG 476 (1997) Deviations from OECD TG 490 (2016): - The report does not state whether cell stocks had been cleansed of mutants. - Acceptance and evaluation criteria are inconsistent with OECD 490 (2016) - No precipitation of the test item was seen at the highest concentration.	Clethodim oxazole sulfone (RE-47797) Purity: 98.9% Lot/batch: AS582d <u>Concentrations:</u> <u>Exp. I:</u> 1000, 2000, 3000, 4000, 5000 µg/mL (-S9 mix 4h exposure) 500, 1000, 2000, 3000, 4000, 5000 µg/mL (+S9 mix 4 h exposure)	Clethodim oxazole sulfone (RE-47797) gave statistical significance, when tested for mutagenic activity in mouse lymphoma L5178Y cells, in the presence of S9-mix, at concentrations extending into the toxic range. However, taking the Global Evaluation Factor (GEF) into account for the microwell version of 12x 10^{-6}, shows that the results are not biological relevant since all mutation fraction values were below the GEF. Clethodim oxazole sulfone was not mutagenic in the absence of S9-mix when tested to the predetermined maximum concentration of 5000 µg/mL (4 h exposure) and at concentrations extending into the toxic range (24 h exposure).	Vol.3, B.6.8.1.3/03 Riach, (2009) Report No.: S-22967 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
- No HCD is available. The RTG for the highest dose in experiment one was higher than specified in the TG (27-29% instead of the recommended 10-20%) Acceptable	Exp. II: 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 µg/mL (- S9 mix, 24 h exposure) 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 µg/mL (+S9 mix, 4 h exposure)		
Mouse micronucleus test GLP: Yes Guideline: OECD 474 (1997) Deviations from current guideline (OECD TG 474 (2016): Evidence of that the test article induced toxicity to the bone marrow was not presented. The study is considered supportive unless bone marrow exposure can be demonstrated.	Clethodim oxazole sulfone (RE-47797) Purity: 99.5% Lot/batch: AS582e Doses: Daily doses of 500, 1000 and 2000 mg/kg bw (range finder) and 2000 mg/kg bw (test) Duration of exposure: Two dosages separated by 24 h. Crl:CD-1 (ICR) mice (3 animals of each sex)	Clethodim oxazole sulfone did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice treated up to 2000 mg/kg/day (the maximum dose currently recommended for this study). No evidence of exposure to bone marrow	Vol.3, B.6.8.1.3/04 [redacted] (2007) Report No.: 2749/3-D617 New data for the Annex I renewal: No

Results

Clethodim oxazole sulfone (2-ethyl-6-(2-(ethylsulfonyl)propyl)-6,7-dihydrobenzo[d]oxazol-4(5H)- one) is a soil and rotational crop metabolite (small amounts, not part of the residue definition). It is found in groundwater with a maximum PEC_{gw} of 1.945 µg/L. There are no new data for this metabolite in this report.

The clethodim oxazole sulfone (RE-47797) gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A) in the presence and absence of a rat liver S9-mix. The study follows OECD TG 471 and was considered acceptable (Vol. 3, B.6.8.1.3/01).

Clethodim oxazole sulfone (RE-47797) induced structural chromosomal aberrations in CHO *in vitro* in the presence of a rat liver-derived metabolic activation system (S9 mix). In the absence of S9-mix clethodim oxazole sulfone did not induce structural chromosomal aberrations. It is noted that OECD TG 473 requires 300 metaphases to be scored for chromosomal aberrations but only 100 were scored in the study. The study is considered acceptable with limitations (Vol. 3, B.6.8.1.3/02).

The clethodim oxazole sulfone (RE-47797) gave a negative response, when tested for mutagenic activity, in mouse lymphoma L5178Y cells, in the presence of S9-mix, at concentrations extending into the toxic range. Clethodim

oxazole sulfone was not mutagenic in the absence of S9-mix when tested to the predetermined maximum concentration of 5000 µg/mL (4 h exposure) and at concentrations extending into the toxic range (24 h exposure). No precipitation of the test item was seen at the highest concentrations. The study was conducted in compliance with the outdated OECD TG 476 (1997). The study is considered acceptable (Vol. 3, B.6.8.1.3/03).

The *in vivo* micronucleus test did not indicate clastogenicity/aneuploidy. However, no evidence that the test item induced toxicity to the bone marrow was presented, thus no conclusion could be drawn and follow-up data for this endpoint is needed. The study is only considered supportive unless bone marrow exposure can be demonstrated (Vol. 3, B.6.8.1.3/04).

No studies are available regarding acute and repeated dose toxicity. However, an *in silico* assessment of clethodim oxazole sulfone predicts that it can be considered of no greater toxicological concern than the parent compound. The general toxicity of clethodim and the metabolites was assessed using all endpoints available in Derek Nexus and the profilers relevant to toxicity in the OECD QSAR Toolbox. According to both Derek Nexus (v.6.1.0) and the OECD QSAR Toolbox (v4.4), no unique alerts were identified for clethodim oxazole sulfone when compared to the parent (clethodim) and major rat metabolite (clethodim sulfoxide) (B.6.8.1.10/02).

Overall conclusion:

Clethodim oxazole sulfone was not mutagenic in Ames test. Negative response was observed in the mouse lymphoma assay. Clethodim oxazole sulfone induced structural chromosomal aberrations in CHO *in vitro* in the presence of a rat liver-derived metabolic activation system (S9 mix). Furthermore, the *in vivo* micronucleus test did not indicate clastogenicity/aneuploidy, but no conclusions could be drawn since no evidence for bone marrow exposure was presented. Clethodim oxazole sulfone (RE-47797) was not likely to be of greater toxicological concern than the parent compound based on the QSAR prediction (no unique alerts identified).

A data gap was identified for the endpoint of genotoxicity (i.e. follow-up data for lack of evidence for bone marrow exposure in the mouse micronucleus test)

2.6.8.1.4 Clethodim sulfone (RE-47253)

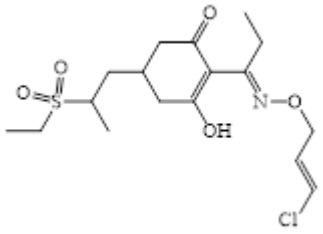


Table 2.6.8.1.4-1: Summary table of studies on clethodim sulfone (RE-47253)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
14-day dose range finding study Oral route	Clethodim sulfone (RE-47253)	No NOAEL was set in study* <u>Target organs and effects:</u>	Vol.3, B.6.8.1.4/01

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results:**	**Reference**
GLP: Yes Guideline: None Species: Rat Strain: Han Wistar (Crl:WI (Han)) 5 animals per sex and dose level Supportive data	Purity: 99.1% Lot/batch: 10975AJT004-2 Doses: 0, 50, 500 and 2500 ppm (equal to 0, 4.1, 40.3 and 183.5 mg/kg/day for males and 0, 4.4, 42.2 and 196.1 mg/kg/day for females) Duration of exposure: 14-days	2500 ppm: - reduced bodyweight gain (M: ↓31%, F: ↓47%)	[redacted] (2020a) Report No.: 510884 New data for the Annex I renewal: Yes
28-day oral toxicity study GLP: Yes Guideline: OECD Guideline 407 (2008). Deviations from OECD TG 407 (2008): No histopathology of the coagulating gland Species: Rat Strain: Han Wistar (Crl:WI (Han)) 5 animals per sex and dose level Acceptable	Clethodim sulfone (RE-47253) Purity: 99.1% Lot/batch: 10975AJT004-2 Dose: 0, 50, 500 and 2500 ppm (equal to 0, 4.1, 39.9 and 211.1 mg/kg/day for males, and 0, 4.2, 42.8 and 207.1 mg/kg/day for females) Duration of exposure: 28-days	NOAEL: 50 ppm (equivalent to 4.1 mg/kg bw/day) Target organs and effects: 500 mg/kg bw/day: ↓ bodyweight gain, males (15%) ↓ blood cell count, females (7%,) ↓ haemoglobin, females (6%) ↓ haematocrit, females (8%,) 2500 ppm: ↓ bodyweight gain, males (16%) ↓ blood cell count, females (8%) ↓ haemoglobin, females (7%) ↓ haematocrit, females (9%) ↑ creatinine, males (39%) ↓ cholesterol, males (35%) ↑ mean liver weights (M: absolute: ↑12%, relative to bw: ↑19.5%, relative to brain weight: ↑14%; F: relative to bw: ↑18%, absolute: ↑15.5%, n.s., relative to brain weight: ↑ 17%, n.s.) -germ cell degeneration in the testis (5/5) -cellular debris and decreased sperm in the epididymis (5/5)	Vol.3, B.6.8.1.4/02 [redacted] (2020b) Report No.: 510900 New data for the Annex I renewal: Yes
Reverse mutation assay with and without S9. GLP Guideline: OECD Guideline 471 (1997). Deviations from current guideline: None Acceptable	Clethodim sulfone (RE-47253) Purity: 99.2% Lot/batch: AS776g Concentrations: 17, 50, 167, 500, 1667 and 5000 µg/plate (with and without S9 mix) Strains: *Salmonella Typhimurium* TA 1536,	Clethodim sulfone gave a mutagenic response in *Salmonella typhimurium (*TA1535 and TA100) in the absence of S9 mix. Clethodim sulfone gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, and TA100) in the presence S9-mix; in *Salmonella typhimurium* (TA1537 and TA98) and *Escherichia coli* (WP2 *uvr*A) in the presence and absence of S9-mix. No toxicity to the bacteria was observed and no precipitation of the test item occurred in either the presence or the absence of S9 mix.	Vol.3, B.6.8.1.4/03 Stevenson (2004) Report No.: 22788 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
	TA 1537, TA 98, TA100 *Escherichia coli* (WP2*uvr*A)		
Reverse mutation in *Salmonella typhimurium* strains TA100 and TA1535 without S9. GLP Guideline: OECD 471 (1997). Deviations from current guideline: Only two strains of *S. thypimurium* was used (according to the guideline at least five strains of bacteria should be used. These should include four strains of *S. thypimurium* (TA1535, TA1537 or TA97a or TA97, TA98 and TA100). In order to detect cross-lining mutagens it may be preferable to include TA102 or to add a DNA repair-proficient strain of *E.Coli*.) Acceptable but limited	Clethodim sulfone (RE-47253) Purity: 99.86% Lot/batch: NC034-Impurity391-005 <u>Concentrations: Exp. 1:</u> 1.6, 8, 40, 200, 1000 and 5000 µg/plate and <u>Exp 2:</u> 156.3, 312.5, 625, 2500 and 5000 µg/plate. Strains: *Salmonella Typhimurium* TA TA 1537, TA100	In TA100 an statistically significant increase in revertant numbers was observed in the absence of metabolic activation. The increase (in experiment 1 at 1.6, 8, 40, 200 but not at 1000 and 5000 µg/plate and only at 625 µg/plate in experiment 2) and thus not concentration dependent or reproducible. No statistically significant increase in revertant numbers was observed at any concentration in neither experiment with TA1535. The test article was completely soluble in the aqueous assay system at all concentrations. No evidence of toxicity was observed.	Vol. 3, B.6.8.1.4/04 Williams (2008) Report No.: 2749/5 New data for the Annex I renewal: No
Chromosomal aberrations assay in CHO cells *in vitro*. GLP Guideline: OECD TG 473 (1997). Deviations from current guidelines: Only 100 metaphases were scored for chromosomal aberrations, OECD 473 (2016) requires 300 Acceptable	Clethodim sulfone (RE-47253) Purity: 99.2% Lot/batch: AS776g <u>Concentrations:</u> <u>Test 1:</u> 156, 313, 625, 1250, 2500 and 5000 µg/mL (+S9 Mix) and 20, 39, 78, 156, 313, 625, 1250, 2500 and 5000 µg/mL (-S9 Mix). <u>Test 2:</u> 1250, 2500, 3000, 4000 and 5000 µg/mL were tested in the presence of S9 mix and 313, 625, 1250, 2500, 4000 and 5000 µg/mL in the absence of S9 mix.	RE-47253 induced structural chromosomal aberrations in CHO cells in vitro in the presence of S9 mix. In the absence of S9 mix clethodim sulfone did not induce structural chromosomal aberrations. Toxicity in the form of reduced cell counts (below 50% of vehicle control), was noted in cultures treated with 5000 µg/mL in the absence of S9 mix and in test 2 in the presence of S9 mix.	Innes (2003) Vol.3, B.6.8.1.4/05 Report No.: 23058 New data for the Annex I renewal: No
Chromosomal aberrations assay in CHO cells *in vitro*. GLP Guideline: OECD TG 473 (1997).	Clethodim sulfone (RE-47253) Purity: 99.86% Lot/batch:	Clethodim sulfone did not induce structural chromosomal aberrations in the presence of S9-mix and in the presence or absence of cofactors in CHO cells in vitro.	Lloyd, (2009) Vol.3, B.6.8.1.4/06

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Deviations from OECD 473 (2016): No long-term exposure. Only 200 metaphases were scored for chromosomal aberrations, OECD 473 (2016) requires 300. Supportive	NC034-Impurity391-005 Concentrations: 392.4, 1654, 2940 and 3920 µg/mL (+S9 mix ± cofactors)		Report No.: 2749/6 New data for the Annex I renewal: No
Mouse lymphoma cell mutation assay GLP Guideline: OECD TG 476 (1997). Deviations from current guideline: OECD 476 has now been superseded by OECD 490 (2016). Acceptance and evaluation criteria are inconsistent with OECD 490 (2016). HCD not presented. Acceptable	Clethodim sulfone (RE-47253) Purity: 99.2% Lot/batch: AS776g Concentrations: Experiment 1, assay 1 (without S9 mix): 62.5, 125, 250, 500, 1000, 2000, 3000 µg/mL and assay 2 (with S9 mix): 250, 500, 1000, 2000, 3000, 4000, 5000 µg/mL. Experiment 2, assay 3 (without S9 mix): 200, 600, 1000, 1400, 1800, 2200, 2600, 3000 µg/mL and assay 4 (with S9 mix): 1400, 2000, 2600, 3200, 3800, 4400, 5000 µg/mL.	Clethodim sulfone gives an equivocal/inconclusive response, when tested for mutagenic activity, in the absence of S9 mix, at concentrations extending into the toxic range. Clethodim sulfone is mutagenic in the presence of S9 mix when tested to the predetermined maximum concentration of 5000 µg/mL (4 h exposure) and at concentrations extending into the toxic range (24 h exposure).	Riach, (2003) Vol.3, B.6.8.1.4/07 Report No.: 22966 New data for the Annex I renewal: No
Mouse lymphoma cell mutation assay GLP Guideline: OECD TG 476 (1997). Deviations from current guideline: OECD 476 has now been superseded by OECD 490 (2016). Acceptance and evaluation criteria are inconsistent with OECD 490 (2016). Supportive	Clethodim sulfone (RE-47253) Purity: 99.9% Batch: NC034-Impurity391-007 and NC034-Impurity391-008 Concentrations: Experiment 1 (3 h treatment with and without S9): 0, 200, 400, 800, 1200, 1600, 2000, 2500, 3000, 3500, 3920 µg/mL. Experiment 2 (3 h treatment with S9, 24 h treatment without S9): -S9 mix: 0, 31.25, 62.5, 125, 250, 375, 500, 625, 750, 1000, 1500 µg/mL and +S9 mix: 0, 250, 500, 1000, 1500,	Upon addition of the test article to the cultures, precipitate was observed at concentrations from 400 µg/mL in the absence and presence of S9. Under the conditions tested clethodim sulfone was negative without rat liver S9 (no conclusion can be drawn with S9 due to precipitation).	Stone, (2009) Vol.3, B.6.8.1.4/08 Report No.: 2749/7 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
	2000, 2500, 2750, 3000 µg/mL. Experiment 3 (24 h treatment without S9): 0, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600, 3920 µg/mL		
In vivo mouse micronucleus assay. GLP Guideline: OECD TG 474 (2016). Deviations from current guideline: None Species: Mouse Strain: CRL:NMRI 5 males (main exp.) 3 males and 3 females (range finder) Acceptable	Clethodim sulfone (RE-47253) Purity: 99.1% Batch: 10975AJT004-2 Dose: 2000 mg/kg bw. Duration of exposure: 2 doses (separated by 24 h)	Clethodim sulfone did not induce micronuclei in the bone marrow micronucleus test of male mice at a dose of 2000 mg/kg body weight (the maximum recommended dose in accordance with current regulatory guidelines). Bone marrow exposure was shown.	[redacted] (2021) Vol.3, B.6.8.1.4/09 Report No.: 2019-32623 New data for the Annex I renewal: Yes
In vivo mouse micronucleus assay. GLP Guideline: OECD TG 474 (1997). Deviations from current guideline: Only 2000 immature erythrocytes per animal were scored for micronuclei (4000 required). No evidence of test article induced toxicity to the bone marrow. Species: Mouse Strain: Crl:CD-1 (ICR) 6 males (main exp.) 3 males and 3 females (range finder) Supportive	Clethodim sulfone (RE-47253) Purity: 99.3% Batch: AS776i Dose: 2000 mg/kg bw. Duration of exposure: 2 doses (separated by 24 h)	Clethodim sulfone induced a small increase in micronuclei in the polychromatic erythrocytes of the bone marrow of mice with 2000 mg/kg/day. The increase (1.5-5 MN PCE/2000 PCE scored) remained within the historical control range. The results are concluded to be equivocal. No evidence of exposure to bone marrow	[redacted] (2007) Vol.3, B.6.8.1.4/10 Report No.: 2749/1-D6172 New data for the Annex I renewal: No
Unscheduled DNA synthesis in mouse liver using an in *vivo/in vitro* procedure. GLP Guideline: OECD 486 (1997)	Clethodim sulfone (RE-47253) Purity: 99.3% Batch: AS776i	Clethodim sulfone was negative in the *in vivo/in vitro* unscheduled DNA synthesis in mouse primary hepatocyte cultures at 2-4 h and 12-14 h after dosing.	[redacted] (2007) Vol.3, B.6.8.1.4/11

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Deviations from current guideline: None Species: Mouse Strain: Crl:CD-1 (ICR) 6 males/group Supportive	Dose: Single doses of 2000 mg/kg bw Sacrifice a nominal 2-4 h and 12-14 h after dosing.		Report No.: 2749/2-D6173 New data for the Annex I renewal: No

* The study is not suitable for NOAEL setting (dose range finding study)

Results

Clethodim sulfone (2-(I-1-(((I-3-chloroallyl)oxy)imino)propyl)-5-(2-(ethylsulfonyl) propyl)-3- hydroxycyclohex-2-en-1-one) is a metabolite in crops and groundwater (max PEC_{gw}: 0.843 µg/L). It is a minor metabolite in rats (≤ 1% in urine). For the renewal of active substance the applicant has submitted three new studies for this metabolite in addition to old data. The following studies are new data for renewal of active substance: 14-d oral dose range finding study in the rat, 28-d oral toxicity study in the rat, and an *in vivo* mouse micronucleus assay. All new and old studies were considered acceptable or supportive.

Oral administration of clethodim sulfone (RE-47253) by diet for 14 days was generally well tolerated in rats at dose levels up to 2500 ppm, equivalent to 183.5 mg/kg/day in males and 196.1 mg/kg/day in females, with no adverse clinical signs but was associated with lower body weight gain in the high dose group when compared with controls (M: ↓31% bw gain, F: ↓47% bw gain). The study was considered as supportive data (dose range finding study) (Vol. 3, B.6.8.1.4/01).

Oral administration of clethodim sulfone (RE-47253) by diet at 0, 50, 500 and 2500 ppm for 28 days (equal to 0, 4.1, 39.9 and 211.1 mg/kg/day for males, and 0, 4.2, 42.8 and 207.1 mg/kg/day for females) was generally well tolerated in rats with no in-life clinical signs at dose levels up to 2500 ppm. Haematology effects in females that received 500 ppm (42.8 mg/kg bw/day) of clethodim sulfone (RE-47253) were limited to lower red blood cell count (7%), haemoglobin (6%) and haematocrit (8%), when compared with controls. Males that received 500 ppm (39.9 mg/kg bw/day) had a lower bodyweight gain (15%) than the control males. Other observed effects were associated with animal's receiving 2500 ppm and included lower bodyweight gain (M: 16%), lower red blood cell count (F: 8%), haemoglobin (F: 7%) and haematocrit (F: 9%), higher creatinine (M: 39%), lower cholesterol (M: 35%), higher liver weights (M: absolute:↑12%, relative to bw: ↑19.5%, relative to brain weight:↑14%; F: relative to bw: ↑18%, absolute: ↑15.5%, n.s., relative to brain weight: ↑ 17%, n.s.), germ cell degeneration in the testis (5/5 individuals), and cellular debris and decreased sperm in the epididymis (5/5 individuals). The NOAEL was 50 ppm (equivalent to 4.1 mg/kg bw/day) based on reduced bodyweight gain noted in males at ≥500 ppm (15-16%), changes in haematological parameters indicating mild anaemia noted in females at ≥ 500 ppm (reduced red blood cell count, haemoglobin and haematocrit levels), changes in biochemical parameters noted in males at 2500 ppm (reduced cholesterol, increased creatinine levels), increased liver weights noted in both sexes at 2500 ppm, and histopathological changes in sperms noted at 2500 ppm. The NOAEL (4.1 mg/kg bw/day) following 28-day

administration of clethodim sulfone (RE-47253) was lower compared to the NOAEL (12.5 mg/kg bw/day) in the 28-day oral toxicity study conducted with clethodim. Furthermore, the LOAEL (39.9 mg/kg bw/day) following 28-day administration of clethodim sulfone (RE-47253) was lower compared to the LOAEL (65.6 mg/kg bw/day) in the 28-day oral toxicity study conducted with clethodim. Thus, it cannot be concluded that the general toxicity of clethodim sulfone (RE-47253) is less toxic than the parent substance based on the available data. The study follows OECD TG 407 except for the fact that no histopathology of the coagulating gland was done. The study was considered acceptable (Vol. 3, B.6.8.1.4/02)

The clethodim sulfone (RE-47253) gave a mutagenic response in *Salmonella typhimurium* TA1535 and TA100 in the absence of S9 mix only, when tested in DMSO up to a predetermined maximum concentration of 5000 µg per plate. The response was detected only with the pre-incubation method. No mutagenic activity was observed with *Salmonella typhimurium* TA 1537 and TA 98 or with *Escherichia coli* WP2*uvr*A. The study follows OECD TG 471. The study was considered acceptable (Vol. 3, B.6.8.1.4/03).

In a repeated experiment in *Salmonella typhimurium* TA1535 and TA100, a statistically significant increase in revertant numbers was observed in TA100 in the absence of metabolic activation. An increase was observed in experiment 1 at 1.6, 8, 40, 200 but not at 1000 and 5000 µg/plate and only at 625 µg/plate in experiment 2 and thus not concentration dependent or reproducible. No statistically significant increase in revertant numbers was observed at any concentration in neither experiment with TA1535. The study follows OECD TG 471 with the exception that only two strains of *S. thypimurium* was used. However, another Ames test is available (Report No.: 22788, presented above) using all recommended test strains. The study was considered acceptable (Vol. 3, B.6.8.1.4/04). Because one of the two Ames test gave a positive response, mutagenicity cannot be ruled out and follow up data is needed for this endpoint.

Clethodim sulfone (RE-47253) gave an equivocal/inconclusive response, when tested for mutagenic activity, in mouse lymphoma L5178Y cells, in the absence of S9 mix, at concentrations extending into the toxic range. Clethodim sulfone was mutagenic in the presence of S9 mix when tested to the predetermined maximum concentration of 5000 µg/mL (4 h exposure) and at concentrations extending into the toxic range (24 h exposure). The study was conducted in accordance with OECD TG 476 (1997) which is superseded by OECD 490 (2016). The study was considered acceptable (Vol. 3, B.6.8.1.4/07).

In a second experiment in mouse lymphoma L5178Y cells clethodim sulfone (RE-47253) was non-mutagenic in the presence and absence of S9 mix when tested at concentrations extending into the toxic range but the RMS notes that upon addition of the test article to the cultures, precipitate was observed at concentrations from 400 µg/mL in the absence and presence of S9. Therefore, the RMS concludes that under the conditions tested clethodim sulfone (RE-47253) was negative without rat liver S9 and that no conclusion can be drawn from this study on mutagenicity in the presence of S9. The study was conducted in accordance with OECD TG 476 (1997). The study was considered as supportive data (Vol. 3, B.6.8.1.4/08). Because of the positive result in the first MLA, follow up data is needed for this endpoint.

Clethodim sulfone (RE-47253) induced structural chromosomal aberrations in CHO cells *in vitro* in the presence of a rat liver-derived metabolic activation system (S9 mix). In the absence of S9 mix clethodim sulfone did not induce structural chromosomal aberrations. The RMS notes that OECD TG 473 requires 300 metaphases to be scored for

chromosomal aberrations but only 100 were scored in the study. The study was considered acceptable (Vol. 3, B.6.8.1.4/05).

In a second experiment, clethodim sulfone (RE-47253) did not induced structural chromosomal aberrations in the presence of S9 mix in CHO cells *in vitro*. The RMS notes that OECD TG 473 requires 300 metaphases to be scored for chromosomal aberrations but only 200 were scored in the study and no long-term exposure was included. The study was considered supportive (Vol. 3, B.6.8.1.4/06). The positive response in the first chromosome aberration test (above) was followed up in vivo (bone marrow micronucleus test).

Clethodim sulfone (RE-47253) induced a small increase in micronuclei in the polychromatic erythrocytes of the bone marrow of mice with 2000 mg/kg/day. The increase (1.5-5 MN PCE/2000 PCE scored) remained within the historical control range (5 MN PCE/2000 PCE scored) and the RMS therefore concludes that the results are equivocal. It should be noted that only 2000 immature erythrocytes per animal were scored for micronuclei (4000 required according to OECD TG 474) and that no evidence of test article-induced toxicity to the bone marrow was presented. The study is considered supportive (CA 5.8.1.4/10).

A second *in vivo* micronucleus study was performed in another strain of mice in which clethodim sulfone was not clastogenic or aneugenic as it did not induce micronuclei in the bone marrow micronucleus test of male mice at a dose of 2000 mg/kg body weight (the maximum recommended dose in accordance with current regulatory guidelines). The study was performed in accordance with OECD TG 474 (2016) without deviations and considered acceptable. Systemic exposure was shown by the presence of the test item in plasma (CA 5.8.1.4/09). Clethodim sulfone (RE-47253) was evaluated as negative in the *in vivo/in vitro* unscheduled DNA synthesis in mouse primary hepatocyte cultures at two time points. The study was conducted in compliance with OECD TG 486 (1997) but considered supportive due to the limitation of this assay.

Overall conclusion

Clethodim sulfone (RE-47253) was not clastogenic or aneugenic but was positive in Ames test and MLA inducing gene mutagenicity. **A data gap** was identified for gene mutations (positive responses in Ames test and MLA need to be followed up).

Regarding general toxicity for the assessment of clethodim sulfone, it cannot be concluded that clethodim sulfone (RE-47253) is less toxic than the parent substance based on the available data. The metabolite is considered qualitatively different from the parent compound since effects on male reproductive organ (cellular debris and decreased sperms in epididymis and germ cell degeneration in testis) were observed in the 28-day oral toxicity study at 2500 ppm (211 mg/kg bw/day). RMS proposes to apply an additional safety factor of 10 in the risk assessment provided that the metabolite clethodim sulfone is not shown to be genotoxic. The NOAEL in the 28-day oral toxicity study conducted with clethodim sulfone (RE-47253) was 4.1 mg/kg bw/day and application of a safety factor for inter- and intraspecies differences of 100, and an additional safety factor of 10 would result in an ADI/AOEL of 0.004 mg/kg bw/day. The magnitude of additional safety factor of 10 is considered sufficient for an extrapolation of study duration (subacute to chronic exposure) and the lack of data for reproductive toxicity.

RMS proposal: the concern for genotoxicity and reproductive toxicity and need for an additional safety factor in the risk assessment to be discussed at expert meeting.

2.6.8.1.5 Clethodim oxazole sulfoxide (RE-47796)

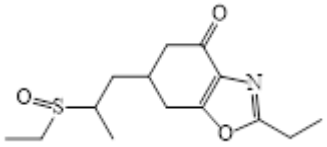


Table 2.6.8.1.5-1: Summary table of studies on clethodim oxazole sulfoxide

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
14-day dose range finding study GLP Guideline: None Species: Rat Strain: Han Wistar Crl:WI (Han) 5 animals per sex and dose level Supportive	Clethodim oxazole sulfoxide Purity: 98.5% Batch: 10976AJT015-1 Doses: 0, 50, 500 and 2500 ppm (equal to 0, 5.5, 56.3 and 270.9 mg/kg/day in males and 0, 5.3, 56.1 and 246.5 mg/kg/day in females) Duration of exposure: 14-days	No NOAEL was set in study* Target organs and effects: No test item related effects observed.	[redacted] (2020c) Vol.3, B.6.8.1.5/01 Report No.: 510533 New data for the Annex I renewal: Yes
28-day oral toxicity study GLP Guideline: OECD TG 407. Deviations from OECD TG 407 (2008): The highest dose level was lower than recommended in the TG (but adequate for comparison with the parent compound) Species: Rat Strain: Han Wistar Crl:WI (Han) 5 animals per sex and dose level Supportive	Clethodim oxazole sulfoxide Doses: 0, 50, 500 and 2500 ppm (equal to 0, 4.3, 41.2 and 211.7 mg/kg/day in males and 0, 4.5, 46.3 and 221.9 mg/kg/day in females) Duration of exposure: 28-days	NOAEL: 2500 ppm (211.7 mg/kg bw/day) Target organs and effects: 500 ppm: ↑ mean uterus weight (absolute weight: ↑ 50%,) 2500 ppm: ↑ mean pituitary gland weight in males (absolute weight: ↑18%) ↑ mean adrenal gland weight in females (absolute weight ↑17%) ↑ mean uterus weight (absolute weight: ↑16%, n.s.).	[redacted] (2021) Vol.3, B.6.8.1.5/02 Report No.: 510549 New data for the Annex I renewal: Yes
Reverse mutation assay with and without S9. GLP Guideline: OECD Guideline 471 (1997). Deviations from current guideline: None Acceptable	Clethodim oxazole sulfoxide Purity: 98.5% Batch: 10976AJT015-1 Doses: 1.7, 5.4, 17, 52, 164, 512, 1600, and 5000 µg/plate	Clethodim oxazole sulfoxide was not mutagenic in the *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) reverse mutation assay and in the *Escherichia coli* (WP2*uvr*A) reverse mutation assay with and without metabolic activation.	Groot (2020) Vol.3, B.6.8.1.5/03 Report No.: 20225638 New data for the Annex I renewal: Yes

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Mouse lymphoma cell mutation assay GLP Guideline: OECD TG 490 (2016). Deviations from current guideline: None Acceptable	Clethodim oxazole sulfoxide Purity: 98.5% Batch: 10976AJT015-1 Doses: 15.6, 31.3, 62.5, 125, 250, 500, 1000, and 2000 µg/mL	Under the conditions tested clethodim oxazole sulfoxide was negative with and without rat liver S9.	Groot (2021) Vol.3, B.6.8.1.5/04 Report No.: 2019-32483 New data for the Annex I renewal: Yes
In vitro micronucleus assay with in peripheral human lymphocytes GLP Guideline: OECD TG 487 (2016). Deviations from current guideline: None Acceptable	Clethodim oxazole sulfoxide Purity: 98.5% Batch: 10976AJT015-1 Doses: 500, 1000, and 2000 µg/mL in the presence and absence of S9 mix	Clethodim oxazole sulfoxide did not induce a relevant increase in the number of mononucleated and binucleated cells with micronuclei in the absence or presence of S9 mix	De Jong (2021) Vol.3, B.6.8.1.5/05 Report No.: 20225640 New data for the Annex I renewal: Yes

* The study is not suitable for NOAEL setting (dose range finding study)

Results

Clethodim oxazole sulfoxide (RE-47796) is a metabolite in crops (small amounts, not part of the residue definition), and ground water (max PEC_{gw}: 0.10 µg/L). It is found in the rat metabolism (≤5% in urine). For the renewal of active substance, the applicant has submitted new studies for this metabolite. The studies were considered acceptable or supportive.

Oral administration of clethodim oxazole sulfoxide (RE-47796) by diet for 14 days was well tolerated in rats at dose levels up to 2500 ppm, equivalent to 270.9 mg/kg/day in males and 246.5 mg/kg/day in females, with no adverse findings. The study is a dose range finding study and considered as supportive data (Vol. 3, B.6.8.1.5/01).

Oral administration of clethodim oxazole sulfoxide (RE-47796) by diet for 28 days was well tolerated in rats with no evidence of toxicity at dose levels up to 2500 ppm (equivalent to 211.7 mg/kg bw/day in males and 221.9 mg/kg bw/day in females). The RMS notes that mean pituitary gland weight was higher in males at 2500 ppm (18% higher for absolute weight), mean adrenal gland weight was higher in females at 2500 ppm (17% higher for absolute weight) and mean uterus weight was higher in females at 500 (50% for absolute weight) and 2500 ppm (16% for absolute weight, n.s.). However, there was no histological correlate to these findings (assessed for high dose level). In addition, there was minimal diffuse follicular cell hypertrophy in the thyroid gland in one animal at 2500 ppm and also a minimal increased haematopoiesis in the spleen at 2500 ppm in one male and one female. The top dose appears

to be too low according to the OECD guideline 407 which includes a limit dose of 1000 mg/kg bw/day and considering that no adverse effects were observed in the dose range finding study (doses up to 270.9 mg/kg/day in males and 246.5 mg/kg/day in females). The NOAEL (211.7 mg/kg bw/day) following 28-day administration of clethodim oxazole sulfoxide was higher compared to the NOAEL (12.5 mg/kg bw/day) obtained in the 28-day oral toxicity study conducted with clethodim. The study was conducted in compliance with OECD TG 407 (2008) with the exception that the highest dose level was too low. Thus, the study was considered as supportive data. However, the toxicity of this metabolite following repeated dose was clearly less than that of the parent compound, thus no further data was needed (Vol. 3, B.6.8.1.5/02).

The clethodim oxazole sulfoxide (RE-47796) gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A) in the presence and absence of a rat liver S9-mix. The study follows OECD TG 471 and was considered acceptable (Vol. 3, B.6.8.1.5/03).

Clethodim oxazole sulfoxide (RE-47796) did not induce an increase in the mutation frequency in in the mouse lymphoma L5178Y test system in the absence and presence of S9-metabolic activation. The study was conducted in accordance with OECD guideline 490 (2016) with no deviations from the test guideline. The study was considered acceptable.

Clethodim oxazole sulfoxide (RE-47796) did not induce an increase in the number of mononucleated and binucleated cells with micronuclei in the absence or presence of S9 mix, in either of the two experiments. The study was conducted in accordance with OECD guideline 487 (2016) with no deviations from the test guideline. The study was considered acceptable.

Overall conclusion:

Clethodim oxazole sulfoxide (RE-47796) was not mutagenic and did not induce an increase in the number of mononucleated and binucleated cells with micronuclei (Ames test, MLA and *in vitro* MN test were all negative). A NOAEL of 211.7 mg/kg bw/day was obtained in a 28- day oral toxicity study in the rat conducted with clethodim oxazole sulfoxide (RE-47796), thus the toxicity of clethodim oxazole sulfoxide (RE-47796) following repeated dose administration was considered lower than that of clethodim.

2.6.8.1.6 DME sulfoxide acid (M17R)

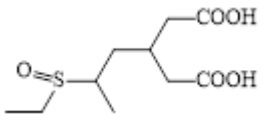


Table 2.6.8.1.6-1: Summary table of studies on DME sulfoxide acid (M17R)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Acute oral toxicity study GLP Guideline: OECD TG 423 (2001)	DME Sulfoxide Acid Purity: 99.51% (HPLC) and 94.09% (minus water content)	The oral LD_{50} value of DME sulfoxide acid was calculated to be >5000 mg/kg bw.	[redacted] (2010a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Deviations from current guideline: Deviations from the minimum level of relative humidity. Species: Rat Strain: Sprague Dawley Crl:CD (SD) Two groups of 3 females Acceptable	Batch: 154-VK-144 Dose: 2000 mg/kg		Vol.3, B.6.8.1.6/01 Report No.:491727 New data for the Annex I renewal: No
28-day oral toxicity study. GLP Guideline: OECD TG 407 (2008). Deviations from current guideline: - 7 animals were necropsied later than after a maximum of 20 h fasting but not longer than 21 h. – A few tissues were not available for histopathology. Reasons for missing a few tissues included that those tissues were not discernible at trimming or were erroneously not collected at necropsy. Missing tissues are listed in raw data and in the pathology report. - The mean analysed concentrations of the pellet diets of Group 2, Group 3 and Group 4 were in the range of 122-127% and higher than the criterion range of 80-120% - a few deviations from the minimum level of relative humidity occurred in the animal room. Species: Rat Strain: Sprague Dawley Crl:CD (SD) 5 animals per sex and dose level Acceptable	DME sulfoxide acid Purity: 99.51% (HPLC) and 94.09% (minus water content) Batch: 154-VK-144 <u>Doses:</u> 0, 200, 1000 and 5000 ppm (equal to 0, 15, 80 and 396 mg/kg bw per day in males, 0, 16, 78, and 407 mg/kg bw per day in females). <u>Duration of exposure:</u> 28-days	NOAEL: 1000 ppm (equal to 80 mg/kg bw per day) <u>Target organs and effects:</u> <u>1000 ppm:</u> - clinical signs (chromodacryorrhoea from day 26 up to 28) <u>5000 ppm:</u> - clinical signs (black staining of the back during the first 3 days) ↑thymus in males (absolute thymus weight: ↑24%, relative thymus weight: ↑25%). ↑adrenal weight in males (relative adrenals weight: ↑20%)	[redacted] (2010b) Vol.3, B.6.8.1.6/02 Report No.: 491728 New data for the Annex I renewal: No
Reverse mutation assay with and without S9. GLP Guideline: OECD Guideline 471 (1997). Deviations from current guideline: None Acceptable	DME sulfoxide acid Purity: 99.51% (HPLC) and 94.09% (minus water content) Batch: 154-VK-144 <u>Doses:</u> 3, 10, 33, 100, 333, 1000, 3330, and 5000 µg/plate	Under the conditions of this assay, DME sulfoxide acid gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2uvrA) in the presence and absence of a rat liver-derived metabolic activation system (S9-mix).	Verspeek-Rip (2009) Vol.3, B.6.8.1.6/03 Report No.: 491725 New data for the Annex I renewal: No
In vitro mammalian chromosome aberration test (human lymphocytes)	DME sulfoxide acid	Both in the absence and presence of S9 mix, DME sulfoxide acid did not induce structural chromosomal	Buskens (2010)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
GLP Guideline: OECD TG 473 (1997). Deviations from current guideline: No deviations are identified from OECD 473 (2016). Acceptable	Purity: 99.51% (HPLC) and 94.09% (minus water content) Batch: 154-VK-144 Doses: 333, 1000 and 2503 µg/mL (± S9 mix, 3 h exposure) and 300, 700 and 1000 µg/mL (± S9 mix, 24 and 48 h exposure)	aberrations and was not clastogenic in human lymphocytes *in vitro.*	Vol.3, B.6.8.1.6/04 Report No.: 491726 New data for the Annex I renewal: No

Results

Metabolite DME sulfoxide acid (3-[(2-ethylsulfinyl) propyl]-pentanedioic acid) is a crop metabolite. The studies were included in the previous EU evaluation (DAR 2005). There are no new data for this metabolite.

The acute oral LD_{50} of DME sulfoxide acid (M17R) was calculated to be >5000 mg/kg bw in female rats. Thus, DME sulfoxide acid (M17R) was of less acutely toxicity than clethodim which shows an LD_{50} of 1133 mg/kg bw in female rats. The study follows OECD TG 423 with minor deviations (occasional deviations of minimum level of relative humidity occurred during the study) and was considered acceptable (Vol.3, B.6.8.1.6/01).

Oral administrations of DME sulfoxide acid (M17R) to rats for 28-days at dietary concentrations of 0, 200, 1000 and 5000 ppm (equal to 0, 15, 80 and 396 mg/kg bw/day for males, 0, 16, 78, and 407 mg/kg bw/day for females) resulted in a NOAEL of 1000 ppm (equal to 80 mg/kg bw/day), based on thymus and adrenal weight changes in males at 5000 ppm (equal to 396 mg/kg bw/day). It is also noted that decreased red blood cell distribution width, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration were observed in males at all doses. However, these changes were small, showed no dose response and were considered to be within normal ranges according to study author (control data given in study report). Thus, of no toxicological significance. Furthermore, statistically significant reduced platelets were noted for females of high dosage level, but without clear dose-response and values within normal control values given in study report. The NOAEL (80 mg/kg bw/day) following 28-day administration of clethodim DME sulfoxide acid (M17R) was higher compared to the NOAEL (12.5 mg/kg bw/day) obtained in the 28-day oral toxicity study conducted with clethodim. Thus, the toxicity of clethodim DME sulfoxide acid (M17R) following repeated dose administration was less than that of the parent compound. The study was conducted in compliance with OECD TG 407 (2008). Deviations identified includes the mean analysed concentrations of the pellet diets of 200, 1000 and 5000 ppm groups were higher than the criterion range of 80-120%. Further, 7 animals were necropsied later than after a maximum of 20 h fasting but not longer than 21 h. A few tissues were not available for histopathology. Reasons for missing a few tissues included that those tissues were not discernible at trimming or were erroneously not collected at necropsy, and a few deviations from the minimum level of relative humidity occurred in the animal room. The RMS concludes that the study integrity was not adversely affected by these deviations. The study was considered acceptable (Vol.3, B.6.8.1.6/02).

The DME sulfoxide acid (M17R) gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2uvrA) in the presence and absence of a rat liver S9-mix. The study follows OECD TG 471 and was considered acceptable (Vol.3, B.6.8.1.6/03).

DME sulfoxide acid (M17R) did not induce structural chromosomal aberrations and was not clastogenic in cultured peripheral human lymphocytes *in vitro*. The study was conducted in compliance with the now outdated OECD TG 473 (1997). However, the results obtained are considered sufficient by the RMS for adequate evaluation and conclusion on clastogenicity. The study was considered acceptable (Vol.3, B.6.8.1.6/04).

Overall conclusion:

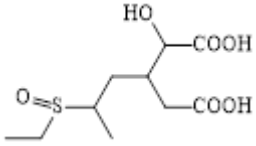
DME sulfoxide acid (M17R) was less acutely toxic than the parent substance and was non-mutagenic *in vitro.* Further, it did not induce structural chromosomal aberrations and was not clastogenic in cultured peripheral human lymphocytes *in vitro*. A NOAEL of 80 mg/kg bw/day was obtained in a 28- day oral toxicity study in the rat conducted with clethodim sulfoxide acid (M17R), thus the toxicity following repeated dose administration was considered lower than that of clethodim.

DME sulfoxide acid (M17R) did not induce gene mutations or structural chromosome aberrations. **A data gap** was identified for genotoxicity since aneuploidy has not been properly assessed, this is accordance to the EFSA document Guidance on aneugenicity assessment (2021) *

*EFSA Scientific Committee (SC), doi: 10.2903/j.efsa.2021.6770, states on page 4 that "The genotoxicity testing strategy indicated in the EFSA Scientific Committee opinion is designed to investigate the genotoxic potential of substances through the detection of three genotoxic endpoints: gene mutations, structural chromosomal aberrations (i.e. clastogenicity) and numerical chromosomal aberrations (i.e. aneuploidy). The testing strategy id developed as a stepwise approach, beginning with a basic battery of *in vitro* tests, comprising:

- A bacterial reverse mutation assay [Organisation for Economic Co-operation and Development (OECD) TG 471, endpoint: gene mutations]; *and*
- an *in vitro* mammalian cell micronucleus (MN) test (OECD TG 487, endpoints: clastogenicity and aneugenicity)."

2.6.8.1.7 Hydroxy 3-[(2-Ethylsulfinyl) propyl]-pentanedioic acid (M14R/M15R)



Metabolite M15R is the dehydro-form of the plant metabolite DME sulfoxide acid and is expected to have similar toxicity to DME sulfoxide acid (M17R). Read across from DME sulfoxide acid (M17R) is proposed for Metabolite M15R.

A data gap was established on aneuploidy for M17R which is also applicable to M15R.

2.6.8.1.8 DME sulfone acid (M18R)

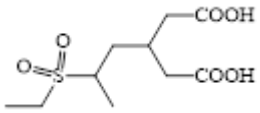


Table 2.6.8.1.8-1: Summary table of studies on DME sulfone acid (M18R)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Acute oral toxicity study GLP Guideline: OECD TG 423 (2001). Deviations from current guideline: None Species: Rat Strain: Sprague Dawley Crl:CD (SD) Two groups of 3 females Acceptable	DME sulfone acid Purity: 99.58% Batch: 151-SRC-178 Dose: 2000 mg/kg	The oral LD_{50} value of DME sulfoxide acid was calculated to be > 5000 mg/kg bw.	[redacted] (2010c) Vol.3, B.6.8.1.7/01 Report No: 491732 New data for the Annex I renewal: No
Reverse mutation assay with and without S9. GLP Guideline: OECD Guideline 471 (1997). Deviations from current guideline: None Acceptable	DME sulfone acid Purity: 99.58% Batch: 151-SRC-178 Doses: 3 to 5000 µg/plate	Under the conditions of this assay, DME sulfone acid gave a non-mutagenic response in *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A) in the presence and absence of a rat liver-derived metabolic activation system (S9-mix).	Verspeek-Rip (2009b) Vol.3. B.6.8.1.7/02 Report No.: 491731 New data for the Annex I renewal: No

Results

DME sulfone acid (3-[(2-ethylsulfonyl) propyl]-pentanedioic acid) is a metabolite found in crops and has a similar structure to the metabolite DME sulfoxide acid (M17R). Given the very close structural similarity it is expected that both metabolites behave similarly. The studies were included in the previous EU evaluation (DAR 2005). There are no new data for this metabolite.

The acute oral LD_{50} of DME sulfone acid (M18R) was calculated to be >5000 mg/kg bw in female rats. Thus, DME sulfone acid (M18R) was less acutely toxic than clethodim which shows an LD_{50} of 1133 mg/kg bw in female rats. The study follows OECD TG 423 and is considered acceptable (Vol.3, B.6.8.1.7/01).

The DME sulfone acid (M18R) gave a non-mutagenic response in Salmonella typhimurium (TA1535, TA1537, TA98 and TA100) and Escherichia coli (WP2uvrA) in the presence and absence of a rat liver S9-mix. The study follows OECD TG 471 and is considered acceptable (Vol.3, B.6.8.1.7/02).

Overall conclusion:

Read across from DME sulfoxide acid (M17R) is proposed for Metabolite M18R. In addition, one Ames test and one acute oral toxicity study conducted with metabolite M18R are available. DME sulfone acid (M18R) was not considered acutely toxic and the Amest test was negative.

However, **a data gap** was established on aneuploidy for M17R which is also applicable to M18R.

2.6.8.1.9 3-chloroallyl alcohol (3-CAA)

Table 2.6.8.1.9-1: Summary table of studies on 3-chloroallyl alcohol (3-CAA)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
In vivo micronucleus/transgenic rodent assay. GLP Guideline: OECD TG 488 (2013) and OECD TG 474 (2016). Deviations from current guideline: None Species: Rat Strain: Fischer 344 homozygous Big Blue® transgenic male 6 males / dose group Not acceptable	3-chloroallyl alcohol (3-CAA) Purity: 96% Batch: DE3-173144-15 Doses: 0, 10, 30 and 100 mg/kg bw/day in drinking water. Duration of exposure: 29-days.	Administration of chloroallyl alcohol in drinking water at doses up to and including a top dose of 100 mg/kg/day was concluded to be negative for the induction of cII mutants in liver and bone marrow and negative for the induction of micronucleated reticulocytes in the peripheral blood of male Fischer 344 Big Blue® rats. However, the decrease in water consumption was considered to be an effect of most likely non-palatable drinking water due to its content of solved 3-CAA which in turn lead to decreased body weight gain. The liver weight increase was approximately 5% and is considered to be insignificant. Hence, MTD was not proven to be achieved in the study.	[redacted] (2020) Vol.3, B.6.8.1.9/01 Report No.: AF97GE.171.BTL New data for the Annex I renewal: Yes
14-day range finding study Non-GLP Guideline: none Deviations from current guideline: Not applicable Species: Rat Strain: Sprague-Dawley 5 males and 5 females/group Acceptable as a dose range finding study	3-chloroallyl alcohol (3-CAA) Purity: 98.1% Batch: 32634-05-23 Doses: 0, 25, 50, 75 and 100 mg/kg bw/day by oral gavage. Duration of exposure: 14-days.	The purpose of this study was to select dose levels for a 28-day repeat-dose study to investigate Pig-a mutations and micronuclei formation. The route of administration was oral gavage, and it was clearly demonstrated that doses of 25, 50, 75, and 100 mg/kg/day for 14 days resulted in mortality in the 75 and 100 mg/kg/day group males and females.	[redacted] (2022) Vol.3, B.6.8.1.9/02 Report No.: Charles River ID 00155013 New data for the Annex I renewal: Yes
28-day repeated dose including toxicokinetics, micronucleus assay and pig-a assay GLP Guidelines: OPPTS 870.5395, OECD TG 407, OECD TG 417, OECD TG 474 and OECD Draft	3-chloroallyl alcohol (3-CAA) Purity: 98.1% Batch: 32634-05-23	A single case of mortality, a male given 50 mg/kg/day found dead on day 3, was observed. Target organs were liver and stomach with a dose-response relationship, increased levels of the liver enzymes AST (38% (M), 62% (F) at 50 mg/kg bw/day) and ALT (264% (M) 148% (F) at 50 mg/kg bw/day) and	[redacted] (2022) Vol.3, B.6.8.1.9/03 Report No.: Charles River ID 00155012

Guideline: Erythrocyte Pig-a Mutation Assay (2021) Deviations from current guidelines: - according to the pig-a assay draft guideline two positive controls are listed but only one (ENU) is found in the pig-a report. - bile acids are not investigated in clinical chemistry <u>Deviations from OECD TG 407:</u> -low number of animals 6/sex/group except for the highest dose group where 8 rats/sex were included (the guidance recommends at least 10 animals (5/sex) should be used at each dose level) -sensory reactivity to stimuli of different types and functional observations were not included in the study -oestrus cycle of females was not determined Species: Rat Strain: Sprague-Dawley 6 males and 6 females/group except in highest dose group where 8 males and 8 females were included and in the toxicokinetic study where 3 males and females were included. Acceptable but limited parameters investigated for toxicology assessment	Doses: 0, 10, 25 and 50 mg/kg bw/day by oral gavage. Duration of exposure: 28-days.	increased liver weights (absolute weight: 12% (M), 43% (F), relative body weight: 25% (M), 47% (F) at 50 mg/kg bw/day). Erosion/ulcer in the non-glandular stomach and hepatocellular degeneration/necrosis were observed at 50 mg/kg bw/day. NOAEL is 25 mg/kg/day. The bioanalysis and subsequent toxicokinetics showed exposure in rat plasma with C_{max} values being similar to or above ten times of the value of the limit of detection for all male and female 3-CAA-treated groups. The micronucleus evaluation showed no evidence of dose groups with a statistically significant increase in micronucleated polychromatic erythrocytes (%MN-PCE) compared to control, nor was there evidence of a dose-response, the results were within the distribution of the historical negative control while the positive control group was statistically significant increased compared to the control group for both males and females. There was no evidence for cytotoxicity of the bone marrow but the exposure of 3-CAA in rat plasma indicates adequate bone marrow exposure. The pig-a study showed no sex difference for either RBCs or RETs. There were no statistically significant differences in mutation frequency between treated groups and the control group and, thus, there was not positive trend. Only one group (group 2) showed elevated RET mutant frequency outside the historical control data 95% quantile. However, this effect seems to be due solely to a single female animal in this treatment group. Thus, this seems to be an outlier value and is not considered to be a relevant effect and can thus be disregarded.	New data for the Annex I renewal: Yes

Results

The metabolite 3-chloroallyl alcohol is the aglycon of the crop metabolite 3-chloroallyl alcohol glucoside. New data are available for this metabolite.

Administration of 3-chloroallyl alcohol (3-CAA) in drinking water at doses up to and including a top dose of 100 mg/kg/day was negative for the induction of cII mutants in liver and bone marrow and negative for the induction of micronucleated reticulocytes in the peripheral blood of male Fischer 344 Big Blue® rats. However, the RMS consider

the decrease in water consumption to most likely be an effect of non-palatable drinking water due to its content of solved 3-CAA which in turn lead to decreased body weight gain. The liver weight increase was approximately 5%, was not statistically significant, and is considered to be insignificant. Hence, MTD was not proven to be achieved in the study and no firm conclusions can be drawn. The study is not acceptable.

Due to the inconclusive results in the transgenic rodent assay, the applicant performed a new combined study. This combination study has several objectives such as to assess the toxicity caused by 3-CAA during 28 days repeated administration, the toxicokinetics of 3-CAA as well as its potential to induce micronuclei in red blood cells and gene mutations in reticulocytes and red blood cells in male and female Sprague-Dawley rats. The top dose was 50 mg/kg/day administered by oral gavage and this dose led to a single case of mortality (one male) demonstrating that maximum tolerated dose was achieved. Target organs were liver and stomach shown by findings of erosion/ulcer in the non-glandular stomach and hepatocellular degeneration/necrosis with a dose-response relationship (and also found in the single dead male), increased levels of the liver enzymes AST and ALT and increased liver weights. Due to the adverse findings in stomach and liver, NOAEL was determined to be 25 mg/kg/day.

The bioanalysis and subsequent toxicokinetics showed evidence of high exposure in rat plasma with C_{max} values being similar to or above ten times of the value of the limit of detection for all male and female 3-CAA-treated groups.

The micronucleus evaluation showed no evidence of dose groups with a statistically significant increase in micronucleated polychromatic erythrocytes (%MN-PCE) compared to control, nor was there evidence of a dose-response, the results were within the distribution of the historical negative control while the positive control group was statistically significant increased compared to the control group for both males and females. There was no evidence for cytotoxicity of the bone marrow but the exposure of 3-CAA in rat plasma indicates adequate bone marrow exposure.

The pig-a study showed no sex difference for either RBCs or RETs. Thus, mutant RBC and mutant RET data were not analysed separately for each sex. There were no statistically significant differences in mutation frequency between treated groups and the control group and, thus, there was no positive trend. Only the lowest dose group (group 2) showed elevated RET mutant frequency outside the historical control data 95% quantile. However, this effect is due solely to a single female animal in this treatment group. Thus, this seems to be an outlier value and is not considered to be a relevant effect and can thus be disregarded.

<u>Further studies conducted with 3-CAA (presented in 1,3-dichloropropene DAR, 2017):</u>

Toxicity studies have been evaluated for the metabolite 3-chloroallyl alcohol (3-CAA) during the active substance approval of 1,3-dichloropropene (Spain, 2017 and EFSA Journal 2018;16(11):5464). Conclusions drawn in DAR (2017) are presented in table below.

In the EFSA conclusion on the peer review of 1,3-dichloropropene (EFSA Journal 2018;16(11):5464) it was concluded that the metabolite 3-CAA with respect to acute toxicity should be classified as Acute Tox 3 H301, and that repeated-dose studies with administration via drinking water resulted in an overall NOAEL of 3 mg/kg bw/day

based on periportal hepatotoxicity and decreased water consumption, while the genotoxic potential could not be concluded in the absence of evidence of bone marrow exposure in the *in vivo* micronucleus assay.

Note: the studies presented in Table below are presented in 1,3-D-DAR (2017) and have not been evaluated by RMS

<table>
<tr><th>Study</th><th>Conclusions (as presented in 1,3-D DAR (2017)</th></tr>
<tr><td>Acute oral toxicity study in Fischer 344 rats (1999)

Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot: 199801576-46

Doses: 100 or 200 mg/kg bw

Acceptable</td><td>The acute oral LD_{50} was approximately 141 mg/kg for male and 91 mg/kg for female rats. According to EC criteria, the metabolite 3-Chloroallyl alcohol should be classified as T, R25 (or Acute tox Cat 3 H301), being more toxic to females than males.

<table>
<tr><th rowspan="2">Dose (mg/Kg)</th><th rowspan="2">Number of animals (per sex/dose)</th><th colspan="2">Number of deaths</th><th colspan="2">Day of death after dosing (number of dead animals)</th></tr>
<tr><th>Male</th><th>Female</th><th>Male</th><th>Female</th></tr>
<tr><td>50</td><td>5</td><td>NA</td><td>0/5</td><td>NA</td><td>0</td></tr>
<tr><td>100</td><td>5</td><td>0/5</td><td>3/5</td><td></td><td>1</td></tr>
<tr><td>200</td><td>5</td><td>5/5</td><td>5/5</td><td>1</td><td>1</td></tr>
</table>
</td></tr>
<tr><td>Acute dermal toxicity study in New Zealand White rabbits (1999)

Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot: 199801576-46

Doses: 200 or 300 mg/kg bw

Acceptable</td><td>The acute dermal LD_{50} was approximately 316 mg/kg for male and 468 mg/kg for female rabbits. According to EC criteria, the metabolite 3-Chloroallyl alcohol should be classified as R24 (or Acute tox 3 H311) “Toxic” being less toxic to females”

<table>
<tr><th rowspan="2">Dose (mg/Kg)</th><th colspan="2">Number of deaths</th></tr>
<tr><th>Male</th><th>Female</th></tr>
<tr><td>200</td><td>0/5</td><td>-</td></tr>
<tr><td>300</td><td>-</td><td>0/5</td></tr>
<tr><td>500</td><td>5/5</td><td>3/5</td></tr>
</table>
</td></tr>
<tr><td>Acute dermal irritation study in New Zealand White rabbits (1999)

Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot: 199801576-46

Two male and one female rabbits were used

Acceptable</td><td>Acute dermal irritation study in New Zealand White rabbits (1999)

The systemic clinical signs consisted of slight erythema and oedema in 2 out of 3 animals at the application site in male and female that was resolved by day 7 (see table 1). In males, eyelids and urogenital area appeared swollen for the study. The application of 3-Chloroallyl alcohol did not have any effect on body weight.

In the light of these observations, the 3-Chloroallyl alcohol seemed no to have a dermal irritation effect.

Individual scores for skin irritation at 24, 48 and 72 h:

<table>
<tr><th rowspan="2">Animal</th><th colspan="3">Erythema</th><th colspan="3">Oedema</th></tr>
<tr><th>24</th><th>48</th><th>72</th><th>24</th><th>48</th><th>72</th></tr>
<tr><td>98A5295</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>98A5296</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>0</td></tr>
<tr><td>98A5297</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>0</td></tr>
</table>
</td></tr>
<tr><td>Dermal sensitization potential study in Hartley albino guineas pigs (1999)

OECD TG 406</td><td>The metabolite 3-Chloroallyl alcohol is considered not to be skin sensitizer.</td></tr>
</table>

Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot.: 199801576-46 Acceptable	<table><tr><td>Group</td><td>No. of animals</td><td colspan="2">Incidence of significant responses (1)</td><td>Ratio* (%)</td></tr><tr><td></td><td></td><td>24 hours</td><td>48 hours</td><td></td></tr><tr><td>Test</td><td>20</td><td>5</td><td>2</td><td>10</td></tr></table>*Ratio= number of animals with positive response/number of animals examined. <table><tr><td>Group</td><td>No. of animals</td><td colspan="2">Incidence of significant responses</td><td>Ratio* (%)</td></tr><tr><td></td><td></td><td>24 hours</td><td>48 hours</td><td></td></tr><tr><td>Test</td><td>20</td><td>0</td><td>0</td><td>0</td></tr><tr><td>Naive Control</td><td>10</td><td>0</td><td>0</td><td>0</td></tr></table>*Ratio= number of animals with positive response/number of animals examined.
4-week repeated dose drinking water toxicity study in Fischer 344 rats (1999) Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot.: 199801576-46, TSN101692 5/sex/group Doses: 0, 10, 30, 100 mg/kg bw/day Acceptable	A decrease in water and feed consumption, decreases in haemoglobin and haematocrit in high-dose male and female rats at the 100 mg/kg/day dose were observed. Small increases in alanine aminotransferase in males and females given 100 mg/kg/day, aspartate aminotransferase in females from the high dose level, and cholesterol in both sexes given ≥30 mg/kg/day were linked to histopathological effects on the liver. Treatment-related histopathologic changes were present in the liver, with minor effects on the kidneys of rats given the mid and high-dose. Based on the results of this study, the NOAEL for 3-chloroallyl alcohol when ingested via the drinking water by rats was 10 mg/kg/day for both sexes.
13-week sub-chronic drinking water toxicity study in Fischer 344 rats (1999) Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot.: 199801576-46, TSN101692 10/sex/group Doses: 0, 3, 10, 30 mg/kg bw/day Acceptable	Based on the results of this study 3-chloroallyl alcohol at doses of 3, 10 or 30 mg/kg/day in drinking water caused decreased water consumption at 3 ppm. Decreases in water consumption was 6.22% and 9.26% in male and female, respectively. At this dose there were no histological findings in target organs (liver and kidney) At 10 mg/kg/day or greater dose levels there were histopathological findings in target organs. Therefore, NOEL was determined to be 0 mg/kg/day and NOAEL was 3 mg/kg/day for both males and females. Histopathological observations (liver, kidney) in the study:

	<table><tr><td></td><td colspan="4">Males</td><td colspan="4">Females</td></tr><tr><td>Dose (mg/kg/day)</td><td>0</td><td>3</td><td>10</td><td>30</td><td>0</td><td>3</td><td>10</td><td>30</td></tr><tr><td>Number of animals examined:</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr><tr><td>Observation</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Liver (No.of tissues examined)</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr><tr><td>Within normal limits</td><td>8</td><td>8</td><td>9</td><td>0</td><td>9</td><td>7</td><td>5</td><td>0</td></tr><tr><td>Architecture altered secondary to diaphragmatic hernia</td><td>0</td><td>1</td><td>1</td><td>2</td><td>1</td><td>2</td><td>4</td><td>0</td></tr><tr><td>Hypertrophy; hepatocyte; periportal</td><td>0</td><td>0</td><td>0</td><td>10</td><td>0</td><td>0</td><td>2</td><td>10</td></tr><tr><td>" " -- very slight</td><td>0</td><td>0</td><td>0</td><td>3</td><td>0</td><td>0</td><td>2</td><td>0</td></tr><tr><td>" " – slight</td><td>0</td><td>0</td><td>0</td><td>7</td><td>0</td><td>0</td><td>0</td><td>10</td></tr><tr><td>Inflammation; chronic; focal</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td><td>0</td></tr><tr><td>" " – sligh</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td><td>0</td></tr><tr><td>Inflammation; chronic; periportal</td><td>0</td><td>0</td><td>0</td><td>10</td><td>0</td><td>0</td><td>0</td><td>8</td></tr><tr><td>" " -- slight</td><td>0</td><td>0</td><td>0</td><td>10</td><td>0</td><td>0</td><td>0</td><td>8</td></tr><tr><td>Inflammation; granulomatous; subserosa; multifocal</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td></tr><tr><td>" " -- slight</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td></tr><tr><td>Necrosis; hepatocyte; multifocal</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr><tr><td>" " -- slight</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr><tr><td>Necrosis; hepatocyte; individual cells; multifocal</td><td>2</td><td>1</td><td>0</td><td>8</td><td>0</td><td>0</td><td>0</td><td>10</td></tr><tr><td>" " -- very slight</td><td>2</td><td>1</td><td>0</td><td>8</td><td>0</td><td>0</td><td>0</td><td>8</td></tr><tr><td>" " -- slight</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td></tr><tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Kidney (No.of tissues examined)</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr><tr><td>Within normal limits</td><td>4</td><td>5</td><td>0</td><td>0</td><td>9</td><td>9</td><td>6</td><td>1</td></tr><tr><td>Cyst; tubule; papilla; focal</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr><tr><td>Degeneration; with regeneration; tubule; cortex</td><td>6</td><td>5</td><td>10</td><td>10</td><td>1</td><td>1</td><td>4</td><td>9</td></tr><tr><td>" " -- very slight</td><td>6</td><td>5</td><td>10</td><td>10</td><td>1</td><td>1</td><td>4</td><td>9</td></tr><tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Nasal tissue (No. of tissues examined)</td><td>10</td><td>0</td><td>0</td><td>10</td><td>10</td><td>0</td><td>0</td><td>10</td></tr><tr><td>Within normal limits</td><td>10</td><td>0</td><td>0</td><td>3</td><td>9</td><td>0</td><td>0</td><td>9</td></tr><tr><td>Slight inflamation;chronic;nasolacrimal duct;unilateral</td><td>0</td><td>0</td><td>0</td><td>2</td><td>1</td><td>0</td><td>0</td><td>0</td></tr><tr><td>Inflamation;chronic;nasolacrimal duct;bilateral</td><td>0</td><td>0</td><td>0</td><td>5</td><td>0</td><td>0</td><td>0</td><td>1</td></tr><tr><td>slight</td><td>0</td><td>0</td><td>0</td><td>4</td><td>0</td><td>0</td><td>0</td><td>1</td></tr><tr><td>moderate</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr></table>
In vitro bacterial reverse mutation assay (1999) Test substance: 3-chloroallyl alcohol, purity: 98%, Lot: 199801576 Acceptable	3-chloroallyl alcohol was non-mutagenic, under the conditions of this study.
In vitro mammalian forward mutation assay (1999) Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot: TSN101692 Acceptable	3-chloroallyl alcohol induced a weak positive response in the mouse lymphoma mutation assay, both in the absence and presence of metabolic activation, under the conditions of this study.
In vivo micronucleus test (1999) Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot: 199801576-46: TSN101692 Study considered as not reliable in the absence of evidence of bone marrow exposure (EFSA Journal 2018;16(11):5464)	3-chloroallyl alcohol was considered negative in the mouse bone marrow micronucleus test, under the conditions of this study.
Oral gavage developmental toxicity probe study in CD rats (1999) Test substance: 3-chloroallyl alcohol, purity: 98%, Lot: TSN101692, ID# 6823-MI, notebook reference # 199801576-46 10 time-mated females/group	Administration of 75 mg/kg bw/day 3-chloroallyl alcohol to pregnant rats resulted in excessive maternal toxicity, manifested as mortality (20%) and clinical signs (decreased activity, cold to touch, rapid or labored respiration, and perioral/perinasal soiling). Subsequently, the surviving rats in this dose group were euthanized on gestation day 7. Administration of 25 mg/kg bw/day induced maternal toxicity, evidenced as decreases in body weight gains and feed consumption on gestation days 6-9 and 9-12,

Doses: 0, 10, 25 or 75 mg/kg bw/day Exposure: days 6 through 20 of gestation Acceptable	as well as significant increases in absolute and relative liver weights (both 19%). No treatment related maternal effects were seen at 10 mg/kg bw/day. No signs of reproductive toxicity (embryonal/foetal effects) were seen at 10 or 25 mg/kg bw/day.
Oral gavage developmental toxicity study in CD rats (1999) Test substance: 3-chloroallyl alcohol, purity: 98.6%, Lot: TSN101692, ID# 6823-MI, notebook reference # 199801576-46 25 time-mated females/group Doses: 0, 3, 10 or 25 mg/kg bw/day Exposure: days 6 through 20 of gestation Acceptable	Materials and methods: "Groups of 25 time-mated female CD rats were administered aqueous solutions of 3-chloroacrylic acid by gavage at targeted doses of 0 (distilled water), 3, 10 or 25 mg/kg/day on days 6 through 20 of gestation. In-life maternal parameters included clinical observations, body weight, body weight gain and feed consumption. On day 21 of gestation, all surviving rats were euthanized and examined for gross pathologic alterations. Liver, kidneys and gravid uterine weights were recorded, along with the number of corpora lutea, implantations, resorptions and live/dead foetuses. All foetuses were weighed, sexed and examined for external alterations. Approximately 1/2 of the foetuses were examined for visceral alterations while skeletal examinations were conducted on the remaining foetuses. Discussion and conclusions: Under the conditions of the present study, gavage administration of 3-chloroallyl alcohol to pregnant rats resulted in maternal toxicity at a dose level of 25 mg/kg bw/day. Mean feed consumption and body weight gain were significantly decreased on gestation days 6-9 at this dose level, with increased absolute and relative liver weights being observed at scheduled necropsy. Reproductive toxicity was observed at the maternal toxic dose of 25 mg/kg bw/day, evidenced as statistically significant decreases in foetal body weights (4% relative to controls) at this dose. No teratogenic effects were observed at any dose level (the low-incidence malformations observed scattered throughout the different groups were within the range of historical controls, and not dose-related, thus being considered incidental). No significant signs of maternal or reproductive toxicity were detected at 3 and 10 mg/kg bw/day. Therefore, the NOAEL for both maternal and developmental toxicity was set at 10 mg/kg bw/day.

Overall conclusion:

Genotoxicity:

3-CAA does not induce gene mutations nor clastogenicity in rats at doses at and below the maximum tolerated dose while adequate exposure of 3-CAA in blood plasma has been achieved (Vol.3, B.6.8.1.9/03).

General toxicity:

3-CAA is more acutely toxic than the parent substance (refer to 1,3-D-DAR, 2017). The acute oral LD_{50} (rat) was 91 mg/kg bw (classification as Acute tox Cat 3, H301) compared to acute oral LD_{50} (mouse) of 1688 mg/kg bw (classification as Acute tox Cat 4, H302) determined for clethodim.

The toxicity profile of 3-CAA shows that the target organs are liver and non-glandular stomach after 28-day repeated dosing. NOAEL was determined to be 25 mg/kg/day based on adverse effects on stomach (erosion/ulcer) and liver (changes in clinical parameters, increased weight, hepatocellular degeneration/necrosis) observed at 50 mg/kg bw/day. The NOAEL (25 mg/kg bw/day) following 28-day administration of 3-CAA was higher compared to the NOAEL (12.5 mg/kg bw/day) obtained in the 28-day oral toxicity study conducted with clethodim based on effects on the liver (increased liver weight, centrilobular hypertrophy) observed at 65.6 mg/kg bw/day. It could however be noted that the findings on liver (necrosis, relevant for STOT-RE 2 classification) noted for 3-CAA are more severe compared to the findings on the liver (hypertrophy) noted for clethodim (Vol.3, B.6.8.1.9/03).

In the 90-day oral toxicity study (refer to 1,3-D-DAR, 2017), where rats were exposed to 3-CAA at dose levels of 0, 3, 10 and 30 mg/kg bw/day, a NOAEL of 3 mg/kg bw/day was obtained based on periportal hepatotoxicity and decreased water consumption observed at ≥10 mg/kg bw/day. This NOAEL (3 mg/kg bw/day) and the LOAEL (10 mg/kg bw/day) are lower compared to the NOAEL (25 mg/kg bw/day) obtained in the 90-day oral toxicity study in the rat conducted with clethodim based on effects on the liver (increased weight and hepatic hypertrophy) observed at 134 mg/kg bw/day. Thus, the toxicity of 3-CAA following repeated dose administration was considered higher than that of clethodim.

Since 3-CAA was not genotoxic in the 28-day repeated dose study (including toxicokinetics, micronucleus assay and pig-a assay), toxicological reference values can be derived for this metabolite (see table below).

The NOAEL of 3 mg/kg bw/day obtained from the 13-week study in the rat (refer to 1,3-D-DAR, 2017), based on periportal hepatotoxicity and decreased water consumption observed at 10 mg/kg bw/day was used for calculation of the ADI after applying a safety factor of 200 (10 for inter-species variability x 10 for intra-species variability x 2 for extrapolation from sub-chronic to chronic study duration).

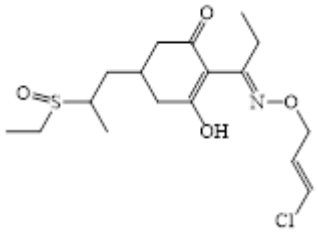
A NOAEL of 10 mg/kg bw/d was obtained from 4-week rat and developmental rat studies (refer to 1,3-D-DAR, 2017), based on effects on liver (periportal hepatotoxicity/increased weight), and used for calculation of the ARfD after applying a safety factor of 100.

Reference value	**Value**	**Study relied upon**	**Safety factor**
ADI	0.015 mg/kg bw/day	13-week rat*	200**
ARfD	0.1 mg/kg bw	4-week rat and developmental rat*	100

*Study presented in 1,3-D-DAR (2017)

**A default conversion factor of 2 was used to extrapolate from sub-chronic to chronic study duration in accordance to the EFSA guidance document (Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data, EFSA Journal 2012;10(3):2579).

2.6.8.1.10 Clethodim sulfoxide (RE-45924)



Clethodim sulfoxide is found in crops, groundwater (max PEC_{gw}: 0.198 µg/L), and in the urine and faeces of rats representing 46-61% and 2-5% of the administered dose in urine and faeces, respectively (see B.6.1.1). Clethodim sulfoxide is considered a major metabolite of clethodim and may therefore be considered to have been assessed by the toxicology studies with the parent compound. Further, in QSAR analyses (Vol. 3, B.6.8.1.10), clethodim sulfoxide was predicted to be inactive for *in vitro* mutagenicity with no misclassified or unclassified features.

2.6.8.1.11 General toxicity and genotoxicity assessment using in silico methods

2.6.8.1.11-01: In silico methods for genotoxicity assessment of groundwater metabolites

Table 2.6.8.1.11-1: Summary table of genotoxicity assessment using *in silico* methods

Method, guideline, deviations if any	Relevant information about the study	Observations /Results	Reference
Genotoxicity assessment of groundwater metabolites of clethodim using *in silico* methods.	The genotoxicity of clethodim and its groundwater metabolites has been assessed. In silico genotoxicity predictions were made using Derek Nexus v.6.0.1 and Leadscope Inc. non-human genetic toxicity model suite v.2.4. Read across was carried out using the OECD QSAR Toolbox v.4.4.	In silico assessment of clethodim groundwater metabolites using Derek Nexus and Leadscope Inc non-human genetic toxicity models predicts that clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide or clethodim oxazole sulfone can be considered to be of no greater genotoxicity concern than the parent.	Pellizzaro and Da Silva-Turner (2020); Vol.3, B.6.8.1.10/01 New data for the Annex I renewal: Yes
General toxicology assessment of groundwater metabolites of clethodim using *in silico* methods.	The toxicity of clethodim and its groundwater metabolites has been assessed. In silico genotoxicity predictions were made using Derek Nexus v.6.0.1. Read across was carried out using the OECD QSAR Toolbox v.4.4.	In silico assessment of clethodim groundwater metabolites predicts that all metabolites can be considered to be of no greater toxicological concern than the parent.	Pellizzaro and Da Silva-Turner (2020); Vol.3, B.6.8.1.10/02 New data for the Annex I renewal: Yes

Results

In silico assessment of clethodim groundwater metabolites predicts that clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide or clethodim oxazole sulfone can be considered to be of no greater genotoxicity and toxicological concern than the parent.

Table 2.6.8.1.11-02: In silico toxicity assessment of metabolite deoxy-M17R

Method, guideline, deviations if any	Relevant information about the study	Observations /Results	Reference
Genotoxicity and general toxicology assessment of deoxy-M17R using *in silico* methods.	The genotoxicity and general toxicity assessment of metabolite deoxy-M17R has been assessed. In silico genotoxicity predictions were made using Derek Nexus v6.2.0 and Leadscope v3.1. OECD QSAR Toolbox (v4.5) was used for read across. The general toxicity of deoxy-M17R has been predicted using all endpoints available in Derek Nexus (v6.2.0), and toxicity profilers available in the OECD QSAR Toolbox (v4.5).	*In silico* assessment of metabolite deoxy-M17R using Derek Nexus and Leadscope predicts no areas of concern. No conclusions could be drawn from the read across used (**data gap**).	Pellizzaro and Hynes (2022); Vol.3, B.6.8.1.10/03 New data for the Annex I renewal: Yes

Read across by the applicant:

*Note text below is text by the applicant***:**

Read-across is the extrapolation of the known toxicological properties of a substance, or a group of substances, to a similar substance which has not been directly tested, or for which the properties are only partially known. If two substances are shown to be structurally similar and/or similar in other ways, the data for the tested substance might be used to estimate unknown properties for the other substance.

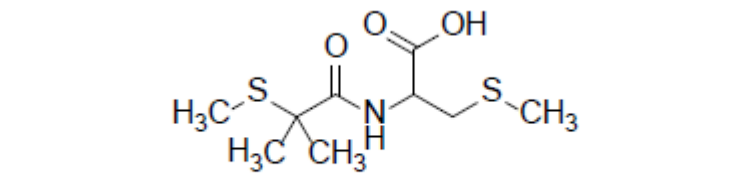
All available databases in the OECD QSAR Toolbox (v4.5) were included in the search for analogy substances that could be used for read across.

Initially, the databases were searched for substances that contained the same organic functional groups as deoxy-M17R (Alkane, branched with tertiary carbon <AND> Carboxylic acid <AND> Sulfide) and no others (i.e. strict). Six substances were found, but these substances were only associated with physical chemical properties data, and no toxicological data was available. Therefore, they were not suitable for read across to deoxy-M17R.

Next the databases were searched for substances that contained the same organic functional groups as deoxy-M17R (Alkane, branched with tertiary carbon <AND> Carboxylic acid <AND> Sulfide), but they could contain other functional groups too. 82 substances were subcategorised to remove substances that have a different protein binding profile to deoxy-M17R. The protein binding profile of deoxy-M17R is:

- Protein binding by OASIS – No alert found
- Protein binding by OECD – No alert found

Only one substance was found to have the same protein binding profile as deoxy-M17R; S-Methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine (Figure 1). S-Methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine was taken forward to check its suitability for read across to deoxy-M17R.



S-Methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine
CAS Number 74407-28-6

Figure 1: Structure of S-Methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine which was identified to be suitable to read across to deoxy-M17R.

S-Methyl-N-(2-methylthio)propionyl)-L-cysteine has been compared to deoxy-M17R using 2D parameters available in the OECD QSAR Toolbox. Similarity (Dice, atom centered fragments, Figure 2), pKa (Acidic pKa OASIS consensus), and logP (logKow) were predicted (Table 3). S-Methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine is 92.3% similar to deoxy-M17R, and has similar pKa and logP values, as well as similar organic functional groups. Therefore, deoxy-M17R and S-methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine are concluded to be similar and the toxicological outcomes of S-methyl-N-(2(methylthio)propionyl)-L-cysteine can be used for deoxy-M17R.

S-Methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine has been tested in the Ames test and gave a negative outcome*

* https://pubchem.ncbi.nlm.nih.gov/bioassay/1259407#sid=363903211

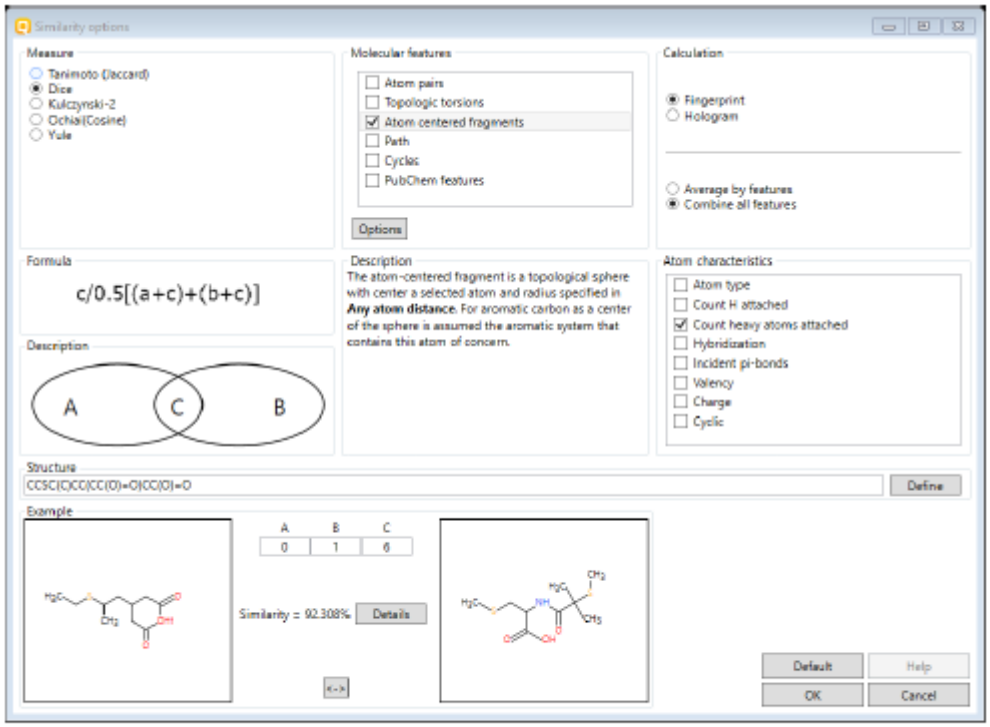


Figure 2: Inputs used to assess the similarity of deoxy-M17R and S-Methyl-N-(2-methyl-2(methylthio)propionyl)-L-cysteine

Table 3: 2D parameters used to compare deoxy-M17R and S-Methyl-N-(2(methylthio)propionyl)-L-cysteine

2D parameter	deoxy-M17R	S-Methyl-N-(2-methyl-2(methylthio)propinoyl)-L-cysteine
Similarity	100%	92.3%
pKa	4.26	3.4
logP	1.64	2.22

Conclusion by applicant:

Deoxy-M17R is a livestock metabolite that has not been tested in any toxicological studies. The toxicity of deoxy-M17R has been investigated using various *in silico* methods.

According to Derek Nexus and Leadscope, deoxy-M17R is non-gneotoxic. In addition, S-methyl-N-(2-methylthio)propionyl)-L-cysteine, which is negative in the Ames test, can be used to read across to deoxy-M17R.

No areas of concern were identified during a general toxicity screening using Derek Nexus and the OECD QSAR Toobox.

In conclusion, deoxy-M17R is of no toxicological concern according to *in silico* methods.

Conclusion and comments (RMS)

The following is stated in EFSA guidance (2016) on the establishment of the residue definition for dietary risk assessment: *"Read across refers to an approach making use of endpoint information, i.e. experimental data on genotoxicity for one or more chemicals (source chemical(s)), to make a prediction for the same endpoint for one or more different chemicals (target chemical(s). The source and target chemical(s) are considered to provoke similar effects related to the assessed endpoints, usually based on structural similarity, and therefore assumed to exhibit similar biological activity"*

Deoxy-M17R has not been tested in any toxicological studies. According to *in silico* methods using Derek Nexus (v6.2.0) and Leadscope (v3.1), deoxy-M17R is non-genotoxic. However, RMS does not agree to the conclusion by the applicant with regards to the read across analysis.

It could be noted that S-methyl-N-(2-methylthio)propionyl)-L-cysteine has not been sufficiently tested for genotoxicity. Ames tests using the strain *S. Typhimurium* are available but these studies are of restricted reliability since they do not include experiments with the presence of S9 mix (experiments without S9 mix only). Furthermore, all relevant genotoxicity endpoints have to be explored (gene mutation, and structural and numerical chromosomal aberrations). This have not been done (Ames test only). Also, it is not relevant to use similarity indices when using arguments for read across.

Thus, a final conclusion on the genotoxic potential could not be drawn. A **data gap** for genotoxicity is identified.

2.6.8.2 Supplementary studies on the active substance

Table 2.6.8.2-1. Summary table of additional studies performed on the active substance

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
A 28-Day Oral (Dietary) Dose Range-Finding Immunotoxicity Study of Clethodim in Female B6C3F1 Mice (GLP) Guidelines followed: OPPTS 870.7800 (1998) Deviations from current guidelines: No positive control Supportive Species: Mice Strain: B6C3F1 Female 8 mice/group GLP Supportive	Clethodim TG, Batch: AS 506r Purity: 95.4% <u>Doses:</u> 400, 2000 and 4000 ppm (equal to 101, 551 and 958 mg/kg bw/day) Clethodim was offered ad libitum in the diet for 28 consecutive days	NOAELsystemic: 400 ppm (101 mg/kg bw/day) LOAELsystemic: 2000 ppm (551 mg/kg bw/day) NOAELimmunotoxicity: 4000 mg/kg bw (958 mg/kg bw/day) LOAELimmunotoxicity: - <u>Effects observed at 2000 ppm (551 mg/kg bw/day):</u> ↑ absolute and relative liver weight (16%) <u>Effects observed at 4000 ppm (958 mg/kg bw/day):</u> ↑ absolute and relative liver weight (41 and 39 %, respectively) ↓ food consumption No evidence of immunotoxicity.	[redacted] (2012a) Report number: WIL-194037 Vol. 3, B.6.8.2/01 New data for the Annex I renewal: Yes
A 28-Day Oral (Dietary) Immunotoxicity Study of Clethodim in Female B6C3F1 Mice Guidelines followed: OPPTS 870.7800 (1998) Deviations from OPPTS 870.7800 (1998): None Mice Strain: B6C3F1	Clethodim TG Purity: 95.4% <u>Doses:</u> 0, 400, 2000 and 4000 ppm (equal to 0, 136, 603 and 1312 mg/kg bw per day) Clethodim was offered ad libitum in the diet for 28 consecutive days	NOAELsystemic: 400 ppm (136 mg/kg bw/day) LOAELsystemic: 2000 ppm (603 mg/kg bw/day) NOAELimmunotoxicity: 4000 ppm (1312 mg/kg bw/day): LOAELimmunotoxicity: - <u>Effects observed at 2000 ppm (603 mg/kg bw/day):</u> ↑ absolute and relative liver weight (17 and 13 %, respectively) ↓ food consumption day 0-7 <u>Effects observed at 4000 ppm (1312 mg/kg bw/day):</u> ↑ absolute and relative liver weight (45 and 42 %, respectively) ↓ food consumption day 0-7 No evidence of immunotoxicity.	[redacted] (2012b) Report number: WIL-194038 Vol. 3, B.6.8.2/02 New data for the Annex I renewal: Yes

Female 10 mice/group GLP Acceptable			
Five-Week Subchronic Feeding Study of High Purity RE-45601 (SX-1718) and RE-45601 Process Neutrals (SX-1717) in Rats No guideline followed. Sprague-Dawley® Crl:CD® (SD) BR 10 rats/sex/group GLP Supportive	High Purity RE-4560, Purity: 96.2% Dose: 6800 ppm (equal 597 mg/kg bw/day for males and 667 mg/kg bw/day for females) Process Neutrals of RE-45601 <u>Dose:</u> 1200 ppm (equal 4.87 mg clethodim/kg bw/day for males and 5.78 mg clethodim/kg bw/day for females) The test items were offered ad libitum in the diet for 5 consecutive weeks	<u>Effects observed rats treated with 6800 ppm clethodim (597 mg/kg bw/day for males and 667 mg/kg bw/day for females):</u> ↓body weight (F: 9-15%) ↓ body weight gain (M: 33%, F: 42%) - mild anaemia (5-7% reductions in erythrocyte, haemoglobin and haematocrit values) ↑ liver weight (M: abs.:12%, rel.: 34%, F: rel. 24%) accompanied by centrilobular hypertrophy. ↓ adrenal weight Males were more severely affected. <u>Effects observed rats treated with 1200 ppm process neutrals (148 and 175 mg Process Neutrals/kg body weight/day containing 4.87 and 5.78 mg clethodim/kg bw/day for males and females, respectively):</u> ↓ body weight (Day 35: M: 6%) ↓ body weight gain (M: 12%) ↑ liver weight (F: abs. and rel.: 10%) -hepatic centrilobular hypertrophy ↓ testis weight (abs. 5% n.s, rel. 6%) In general, animals exposed to clethodim were more severely affected than those exposed to process neutrals. <u>Conclusion:</u> Clethodim alters several health parameters in rats but impurities may contribute partially to some of these results.	[redacted] 1987 Report no. S-2763 Vol. 3. B.6.8.2/03 New data for the Annex I renewal: No
Cytochrome P-450 concentration following 21-day oral administration in male rats. No guideline followed. Rat. Sprague-Dawley Crl:CD® BR 8 males/group GLP Supplementary	RE-45601 Technical (batch SX-1688) Purity: 83.3% 250 mg/kg/day (208 mg/kg/day, corrected for purity)	<u>Effects observed at 208 mg/kg bw/day:</u> ↑ liver weight (M: abs: 21%, rel: 23%) No difference in CYP450 concentration was observed	[redacted] 1989 Report no. S-3055 Vol. 3. B.6.8.2/04 New data for the Annex I renewal: Yes

Immunotoxicity

Two immunotoxicity studies were performed, one dose range finding study and one main study. Both were performed according to OPPTS 870.7800 (1998) with no deviations except that the dose range finding study did not include a positive control. In the studies, female mice were exposed to 0, 400, 2000 and 4000 ppm (corresponding to 101, 551 and 958 mg/kg bw/day in the dose range finding study and 0, 136, 603 and 1312 mg/kg bw per day in the main study). No signs of toxicity except for increased liver weights and lower food consumption were observed. The absolute liver weights in the dose range finding study were 16 and 41% lower than that of the control group in the middle and high dose, respectively. The corresponding relative liver weight values were 16 and 39%. In the main study, the absolute and relative liver weights were 17 and 13 %, respectively, in the middle dose, and 45 and 42 %, respectively, in the high dose.

No immunosuppressant effect was observed in the dose range finding study. There was a statistically significantly higher mean AFC response in the 2000 ppm group (↑54%). There was a similar tendency in the 4000 ppm group, the mean value was 36% higher than that of the control group (not statistically significant) but the value was lower than that of the 2000 ppm group. In the main study, there was a 19-15% reduction in AFC response in the top two doses but there was no dose response, the differences were not statistically significant, and there was an increase in this endpoint in the dose-range finding study (B.6.8.2.1). There was also a statistically significant decreasing trend in relative spleen weight (Jonckheere's Test); however, the differences between the exposed groups and the control were not statistically significant and the mean value of the highest dose group was only 8% lower than that of the control (0.36 vs 0.39). Overall, clethodim does not appear to be immunotoxic at these dose levels.

Process neutrals

A study in which high purity clethodim (RE-45601, 96.2% active ingredient) and RE-45601 Process Neutrals (containing 3.3% RE-45601) were administered ad libitum in the diet to separate groups of 10 rats of each sex for five weeks. The animals were sacrificed on days 36-37. Body weight of males exposed to clethodim was lower than the control from day 7 and throughout the study, resulting in a total weight gain that was 33% lower than that of control males. Males of the process neutrals group was had slightly and not statistically significantly lower body weight from day 7 (3-5%) and a statistically significantly lower body weight at day 35 (↓6%), resulting in a total weight gain that was 12% lower than that of control males. No effect on body weight or body weight gain was observed in females exposed to process neutrals, but both body weight (9-15%) and total body weight gain (42%) was lower in females exposed to clethodim compared with the control group. In males, relative food consumption was unaffected in both except for a 15% reduction in males exposed to clethodim during the first week. Absolute food consumption was lower than the control in both groups (albeit slightly more affected in the clethodim group). Following the same pattern as the body weight, no effects were observed in females exposed to process neutrals, but females exposed to clethodim had a reduced absolute food consumption (g/animal/day) but unaffected relative food consumption. The applicant states that palatability may have been an issue during the study and that this may have affected the results. However, no palatability study is available to confirm this statement.

Mild anaemia was evident in both sexes in the clethodim group (5-7% reduction in erythrocyte, haemoglobin, and haematocrit values). This was not evident in the process neutrals group. Some serum chemistry parameters were slightly affected. Males in the clethodim group had significantly higher total protein (7%) and albumin values (7%), and lower alkaline phosphatase value (16%, not statistically significant). Females exposed to clethodim was

unaffected. Both males and females of the process neutrals group had lower alkaline phosphatase value compared with the control (males 19%, not statistically significant; females 24%). Historical control values were provided for total protein (males), albumin (males) and alkaline phosphatase (females). It is noted that the values in this study for these parameters fall within the historical control range; however, the historical control consists of two studies only and it is not stated when they were performed.

Liver weight was increased in both sexes in the clethodim group (absolute, relative to bw and relative to brain weight). Liver weight was affected in females but not males (except for that relative to bw) exposed to process neutrals. Trace to mild centrilobular hypertrophy was observed in both exposure groups and both sexes, with a higher incidence in males versus females and in animals exposed to clethodim versus process neutrals. The incidence of liver hypertrophy was 10 of 10 males and 8 of 10 females exposed to clethodim, and 6 of 10 males and 3 of 10 females exposed to process neutrals. Adrenal weight (absolute and relative to brain weight) was reduced in both males and females exposed to clethodim. No histopathological lesions were noted. Increased relative (to bw) kidney, testes, and brain weight was observed in the clethodim group, likely due to the decrease in terminal body weight. This is not considered to be of concern. Testis weight, relative to brain weight, was 6% lower in the process neutrals group (absolute weight was 5% lower, not statistically significant). No histopathological lesions were noted. No effect on ovary weights were observed.

The dose of active ingredient given via the process neutrals is low (5-6 mg/kg bw/day) but this level could possibly affect the animals. Treating rats dermally with 8.32 mg clethodim/kg bw for 21 days over a 28-day period caused skin irritation and increased triglyceride levels in females (40%, not statistically significant) (Vol. 3 B.6.3.3). Elevated platelet counts and elevated cholesterol levels (26%, not statistically significant) were observed in male rats exposed orally to 12.5 mg clethodim/kg bw/day for 5 weeks (Vol. 3 B.6.3.1.1). These effects differed from the ones observed in the current study but does indicate that clethodim could affect rats in that dose range.

In conclusion, the active ingredient caused effects on blood, liver, adrenals, and body weight and impurities may contribute partially to these effects. The observed anaemia was only observed in the clethodim group, indicating that this effect was likely not caused in combination with the process neutrals.

CYP450

Male rats were administered 208 mg clethodim/kg bw/day for 21 days via oral gavage. This exposure resulted in increased liver weights but no other signs of overt toxicity. The mean CYP450 concentration, determined in liver samples from the exposed rats, did not statistically differ from that of the control. When the content of cytochrome P-450 is expressed as total nmoles/liver and mg/gram liver, mean values for treated animals are significantly higher those of the control. This is likely a result of increased liver weights in the treated animals.

Table 2.6.8.2-2. Cytochrome P-450 Data (mean±SD)

	Cytochrome P-450			**Protein Concentration**
	nmoles/mg protein	**nmoles/g liver**	**nmoles/liver**	**mg protein/g liver**
Controls	0.99±0.23	29.3±8.0	301±89	29.9±6.0
250 mg/kg	0.92±0.13	33.7±7.6	410±87* (↑36%)	37.1±7.8* (↑24%)

Clethodim did not alter CYP concentrations in male rats at an oral dose of 208 mg/kg bw/day for 21 days. However, the carbon monoxide method does not work well to determine the amount of the CYP3A family and since no specific CYP substrates have been used, CYP3A induction cannot be completely ruled out.

2.6.9 Summary of medical data and information

No medical findings have been reported linked to clethodim in a plant during manufacturing. Plant protection products containing clethodim have been registered in Europe since the beginning of 1990 and are registered in most EU Member States. To the applicant's knowledge, no cases of poisoning incidents among users or the general population have been reported (Rao, 2020).

2.6.10 Toxicological end points for risk assessment (reference values)

Clethodim technical was tested in several repeated dose studies in rats, mice, dogs, and rabbits, including subacute, semi-chronic and chronic studies, reproduction studies, neurotoxicity and immunotoxicity studies.

Route specific AOELs:

The following was stated in DAR 2005: *"As clethodim is extensively metabolised, route-specific toxicity cannot be excluded. Therefore, if available, toxicity studies for the route concerned are considered to calculate route-specific AOELs.*

At the NOAEL (83 mg a.s./kg bw/day) in the dermal toxicity study the estimated area concentration is 0.74 mg/cm2 (assuming a body weight of 0.285 kg and a corresponding 400 cm^2 body surface, and using the reported 8% exposed body surface). No data on dermal absorption at this area concentration is available. Therefore, the dermal NOAEL is not used for the establishment of the AOEL. Local effects were not taken into account.

No repeated dose inhalation toxicity studies were available for the establishment of a route-specific AOEL."

The RMS is of the opinion that this position remains for the renewal of active substance.

Table 67: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Subacute toxicity studies						
Rat (Sprague-Dawley) 10/sex/group	5-week, oral (dietary)	Clethodim technical Lot/batch: SX-1688 Purity: 83.4% Vehicle: Acetone	↑liver weights (M: abs weight 12%, rel to brain weight: 13%; F: rel to brain weight: 14%) - histopathological changes in the liver (centrilobular hypertrophy (M)) ↓haemoglobin (M:4%, F:6%)	12.5	65.6	[redacted] 1986 Report No.: S-2720 Vol. 3, B.6.3.1/01
Mouse (CD-1) 10/sex/group	4-week, oral (dietary)	Clethodim technical Lot/batch: SX-1688 Purity: 84%	↓haemoglobin (M: 4%)	29.7	74.4	[redacted] 1986 Report No.: S-2733 Vol. 3, B.6.3.1/02

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
		Vehicle: Acetone				
Rat (Sprague-Dawley) 6/sex/group	4-week dermal	Clethodim technical Lot/Batch: SX-1688 Purity: 83.2% Vehicle: 0.7% carboxymethyl cellulose (CMC) and 1.0% TWEEN 80 in distilled water	-clinical signs (anogenital discharge in all males (6 animals) and two females) ↑ absolute liver weight (F: 20%) ↑ relative liver weight (F: 22%) ↑ liver weight relative to brain weight (F: 24%) ↑ triglyceride levels (F: 160 %) ↓ BUN (M: 22%, F: 20% n.s.) ↓ BUN/creatinine ratio (M: 32%, F: 21% n.s.)	83.2	832	[redacted] J[redacted] 1987 Report number: S-2848 Vol.3 B.6.3.3/01
Semichronic toxicity studies						
Rat (Sprague-Dawley) 12/sex/group	13-week, oral + 6-week recovery period	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 84% Vehicle: Acetone	↓ bw gain (M: 10%) ↑liver weight (rel) (M and F: 12%) - histopathological changes in the liver (hepatic centrilobular hypertrophy: M: 8/12, F: 2/12)	25	134	[redacted], 1986 Report No.: S-2765 Vol. 3, B.6.3.2/01
Dog (Beagle) 4/sex/group	90-days, oral (gelatine capsules)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 83.3%	-effects on the liver (↑cholesterol (F: 42%), ↑liver weights n.s. (M: abs weight: 16%, rel weight: 12%; F: abs weight: 15%, rel. weight: 6%)	21	62	[redacted]1987 Report No.: S-2759 Vol. 3, B.6.3.2/02
Dog (Beagle) 6/sex/group	1-year, oral (gelatine capsules)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 83.3%	↑liver weight (M: abs. 27%, rel. 16%; F: abs. 34%, rel weight: 25%) - changes in blood chemistry (↑platelet count, M:20% n.s., F: 39%, ↑WBC, F: 27%) -histopathological changes in the sternal bone marrow (hyperplasia: M: 1/6, F: 1/6)	0.83	62	[redacted]1988 Report No.: S-2964 Vol. 3, B.6.3.2/03
Chronic toxicity studies						
Rat (Sprague-Dawley) 65/sex/group 10/sex/group (interim sacrifice, 1 y)	2-year, oral (dietary)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 83% Vehicle: Acetone	- ↑liver weights (F: rel. 18% n.s., (1 y), 12% (2 y), rel to brain weight: F: 24% (1 y))	16	86	[redacted] 1988 Report No.: S-2766 Vol. 3, B.6.5/02
Mouse (CD-1) 60/sex/group	78-weeks, oral (dietary)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 83% Vehicle: Acetone	-effects on the liver (↑liver weights: M: abs. 12% n.s., rel, 17%; histopathological changes (centrilobular hypertrophy (M, F), increased pigment (F)	24	199	[redacted]1988 Report No.: S-2867 Vol. 3, B.6.5/01

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
			and bile duct hyperplasia (M)) -histopathological changes in the lungs (foci of amphophilic alveolar macrophages (M, F)			
Reproduction and teratogenicity studies						
Rat (Albino Crl:COBS/CD 30/sex/group (F0 and F1 generation)	2-generation, oral (dietary)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 83.3% Vehicle: Acetone		Parental: 32.2 Offspring: 32.2 Reproductive: 163	Parental: 163 Offspring: 163 Reproductive: -	[redacted] 1987 Report No: S-2778 Vol. 3, B.6.6.1/02
Rat (Crl:CD 25 animals/group	Teratogenicity, oral (gavage)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 83.3% Vehicle: Carboxymethyl cellulose, Tween 80 aqueous suspension	- clinical signs (excessive salivation, poor condition, red nasal discharge, alopecia, staining ano-genital area) ↓bw gain maternal (GD: 6-15: 15% n.s., GD 15-20: 17%) ↓foetal weight (11%) ↑skeletal variations (incomplete or unossified vertebrae, unossified 5th and/or 6th sternebrae) (foetal: 88.8% compared to 72.6% in control)	Maternal and developmental: 83.3	Maternal and developmental: 292	[redacted] 1987 Report No.: S-2808 Vol. 3, B.6.6.2.2/01
Rabbit (New Zealand White SPF) 19-20 animals/group	Teratogenicity, oral (gavage)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 83.3%	- clinical signs, dams (dried faeces and red substance in pan) ↓bw gain (Day 7-20: +0.05 kg vs +0.18 kg in the control, n.s.)	Maternal: 20.8 Developmental: 83.3	Maternal: 83.3 Developmental: 250	[redacted], 1987 Report No.: S-2869 Vol. 3, B.6.6.2.4/01
Neurotoxicity						
Rat (Crl:CD(SD) 12/sex/group	90-days, neurotoxicity	Clethodim TG (RE-45601) Lot/batch: AS 506r Purity: 95.4% Vehicle: Acetone	↓bw and bw gain Day 0-91: 16%)	94	331	[redacted] 2012 Report No.: WIL-194040 Vol. 3, B.6.7.1.3
Immunotoxicity						
Mouse (B6C3F1) 10 female mice/group	28-days, immunotoxicity (dietary)	Clethodim technical (RE-45601) Lot/batch: SX-1688 Purity: 95.4% Vehicle: Acetone	↑liver weights (abs weight: 17%, rel weight: 13%)	136	603	[redacted] 2012b Report No.: WIL-194038 Vol. 3, B.6.8.2/02

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The calculation of ADI for humans is based on the no-observable effect level (NOAEL) in the most susceptible animal species and with particular respect to the chronic toxicity studies; and an appropriate safety factor which is usually 100.

The lowest NOAEL in the repeated dose-studies was 0.83 mg a.s./kg bw/day that of the 1-year oral study in the dog. However, the LOAEL in the 1-year study is the same as in the 90-day study in the same species, and the NOAEL in that study is 21 mg a.s./kg bw/day. In the DAR (2005), it was argued that *"Since it was concluded that no effect of exposure duration is to be expected after oral exposure to clethodim, the NOAEL of 21 mg a.s./kg bw/day from the 90-day oral toxicity study in dogs might be also be considered for the establishment of the ADI".* However, it is noted that the effect sizes in the one-year study was larger than in the 90-day study and that it cannot be determined if no effect would have been observed with one year exposure to 21 mg/kg bw/day. Because of this, the NOAEL of the 2-year study in rats (16 mg a.s./kg bw/day) should also be considered for the derivation of the ADI. As was determined in the DAR (2005), the ADI is preferably based on a chronic study and the NOAEL of the 2-year rat study is appropriate for calculation of ADI. Application of a safety factor for inter- and intraspecies differences of 100, results in an **ADI of 0.16 mg/kg bw/day** (as in the EFSA conclusion from 2011).

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

Calculation of the ARfD and AAOEL is usually derived from an appropriate acute toxicity study in which a NOAEL for adverse effects can be established. In the absence of a specific study designed to determine this endpoint, it is based on a consideration of the NOAELs for "acute effects" observed in studies ranging from acute to sub-chronic exposure durations. Thus, relevant NOAELs may be derived from studies involving administration of a single dose or from repeat dose studies in which effects are noted during the initial days of dosing.

In the acute oral study in rats (Vol. 3, B.6.2.1/01), salivation, decreased motor activity, unsteady gait, hyperreactivity, lacrimation, clonic convulsions, red nasal discharge, ocular discharge, collapse, reduced food consumption and yellow anogenital stains were observed. Effects occurred at all dose levels (≥666 mg a.s./kg bw) and mortality occurred from 1208 mg a.s./kg bw. Similarly, in the acute oral toxicity study in mice (1250 – 2916 mg a.s./kg bw) (Vol. 3, B.6.2.1/02), effects included hypoactivity, rough coat, hunched appearance, ataxia, tremors, salivation, laboured respiration, and soft faeces and urine stains. Mortality occurred from 1666 mg a.s./kg bw. In the acute neurotoxicity study in the rat (Vol. 3, B.6.7.1.1), the NOAEL systemic was 100 mg/kg bw due to the following effects occurring at 1000 mg/kg bw: transient reduced locomotor activity (total and ambulatory counts) (of unclear toxicological significance) and soiled fur on day 0 (one of these animals also displayed slight salivation) in females and reduced foot splay in males. Since no substance related mortalities were observed in available studies at doses up to 1000 mg/kg bw and no finding indicative of effects elicited by an acute exposure were observed at doses up to 500 mg/kg bw, no ARfD was deemed necessary.

2.6.10.3 Toxicological end point for assessment of occupational, bystander and resident risks – AOEL (acceptable operator exposure level)

The AOEL is based on the most sensitive study of short to medium term toxicity, relevant to a worker exposure season of no more than 3 months.; and an appropriate safety factor which is usually 100.

Examination of the repeat dose toxicity studies shows that the main toxic effects of clethodim comprise changes in haematological parameters, including anaemia, and evidence of liver hypertrophy/toxicity at relatively high dose levels.

An **AOEL of 0.2 mg/kg bw/day** is proposed based on an overall NOAEL of 21 mg/kg bw/day from the 90-day dog study and an assessment factor of 100. No correction for oral absorption is required. In this study effects on the liver (increased liver weights and increased cholesterol) were observed at a dose level of 62 mg/kg bw/day.

In the EFSA conclusion from 2011, an AOEL of 0.2 mg/kg bw/day was set based on the 90-day dog study as well as on the 1-yr dog study, and an assessment factor of 100.

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

No AAOEL was deemed necessary (refer to section 2.6.10.2)

2.6.10.5 Drinking water limit

The maximum admissible concentration of an active substance is **0.1 µg/L** (according to Directive 89/778/EEC).

A health-based limit (adult) of 0.96 mg/L (960 µg/L) can be derived assuming 20% of the ADI, water consumption of 2 L/day and bodyweight of 60 kg. The calculation of this value is:

C_{max} water= (ADI x 20% x Bodyweight)/2L = (0.16 x 0.2x 60 kg)/2L = 0.96 mg/L. Since this value is higher than the maximum permissible groundwater concentration of 0.1 µg/L, the C_{max} water calculated should not be used.

A health-based limit (infant) of 0.213 mg/L (213 µg/L) can be derived assuming 20% of the ADI, water consumption of 0.75 L/day and bodyweight of 5 kg. The calculation of this value is:

C_{max} water= (ADI x 20% x Bodyweight)/0.75L = (0.16 x 0.2x 5 kg)/0.75L = 0.213 mg/L (213 µg/L). Since this value is higher than the maximum permissible groundwater concentration of 0.1 µg/L, the C_{max} water calculated should not be used.

2.6.11 Summary of product exposure and risk assessment

No acute AOEL has been set or is proposed for clethodim (refer to section 2.6.10). Therefore, an acute exposure assessment was not performed. The acceptable operator exposure level (AOEL) for clethodim (0.2 mg/kg bw/day) will not be exceeded under practical conditions of use without the use of personal protective equipment. The systemic exposure to workers, bystanders and residents will be within acceptable levels of the proposed systemic

AOEL of clethodim. Therefore, the exposure of operators, workers, residents and bystanders for clethodim is acceptable (see Vol.3, B.6.5 PPP).

Table 2.6.11-1: Summary of estimated operator exposure to clethodim (longer term)

Model data	Level of PPE	Total absorbed dose (mg/kg/day)	% of systemic AOEL
Tractor mounted boom spray application outdoors to low crops-sugar beet			
Application rate		0.3 kg a.s./ha	
Spray application (AOEM; 75th percentile) Body weight: 60 kg	Work wear (arms, body and legs covered) Mixing/Loading and Application.	0.0221	11.06

Table 2.6.11-2: Summary of estimated resident exposure to clethodim (longer term)

Model data		Total absorbed dose (mg/kg bw/day)	% of systemic AOEL
Field crop (boom) sprayer application outdoors to low crops-sugar beet Buffer zone: 2-3 m Drift reduction technology: no DFR: 3 µg/cm^2/kg a.s./ha DT_{50}: 30 days			
Number of applications and application rate		1 × 0.3 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0041	2.03
	Vapour (75th perc.)	0.0011	0.54
	Deposits (75th perc.)	0.0007	0.34
	Re-entry (75th perc.)	0.0051	2.53
	Sum (mean)	0.0078	3.92
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0010	0.48
	Vapour (75th perc.)	0.0002	0.12
	Deposits (75th perc.)	0.0002	0.10
	Re-entry (75th perc.)	0.0028	1.41
	Sum (mean)	0.0031	1.54

Table 7.2.3-1: Exposure model for intended uses

Critical uses	Sugar beet (max. 1 × 2.5 L product/ha)
Model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 calculator version: 30/03/2015

Table 7.2.3-2: Estimated worker exposure to clethodim

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)	% of systemic AOEL
Crop inspection Outdoor Work rate: 2 h/day[1], DT_{50}: 30 days[2] DFR: 3 µg/cm^2/kg a.s./ha[2] Interval between treatments: Not applicable			
Number of applications and application rate		1 × 0.3 kg a.s./ha	

Body weight: 60 kg	Potential exposure TC: 12500 cm^2/person/h	0.0696	18.8
	Work wear (arms, body and legs covered) TC: 1400 cm^2/person/h	0.0300	2.1
Hand-harvesting sugar beet Outdoor Work rate: 8 h/day[1], DT_{50}: 30 days[2] DFR: 3 µg/cm^2/kg a.s./ha[2] Interval between treatments: Not applicable			
Number of applications and application rate		1 × 0.3 kg a.s./ha	
Body weight: 60 kg	Potential exposure TC: 5800 cm^2/person/h	0.0696	34.8
	Work wear (arms, body and legs covered) TC: 2500 cm^2/person/h	0.0300	15.0

[1] 2 h/day for crop inspection tasks and 8 h/day for hand harvesting tasks
[2] EFSA Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products [EFSA Journal 2014;12(10):3874 [55 pp.]

2.7 RESIDUES

2.7.1 Summary of storage stability of residues

There was one old study (CA 6.1/01, TSR5068SGBT) investigating the stability of clethodim and 5-Hydroxy clethodim sulfone in sugar beets, which was previously evaluated in the DAR. The RMS did not find this acceptable, as the study was not performed according to current OECD guideline, and a common moiety method was used for analyses, without any justification. Additionally, this analytical method was not acceptably validated.

New studies were submitted to address the storage stability of clethodim, clethodim sulfoxide, clethodim sulfone, M14R, M17R, M18R and 3-chloroallyl alcohol glucoside in plant commodities, covering all the required categories.

Storage stability was investigated in homogenised plant matrices unless stated otherwise in Table 2.7.1-1. In all supervised residue trials (see Vol. 1, 2.7.4 and Vol. 3, B.7.3), field samples were first stored deep-frozen as whole commodity and were then homogenised at the analytical laboratory before extraction. In accordance with OECD guideline 506 (paragraph 15), a homogenate is likely to represent a worst case versus the use of a whole commodity, i.e. storage stability data conducted in homogenised samples are considered acceptable. The storage stability of residues in the available trials was sufficiently demonstrated, unless otherwise stated in Vol. 1, 2.7.4 and Vol. 3, B.7.3.

In animal commodities, there were only two older studies (CA 6.1/12, Weissenburger, 1989, ADC 1124 and CA 6.1/13, Lear, 1989, 129-003) submitted. According to the RMS, these studies had several deviations and deficiencies compared to current guidelines. In the study with cow (CA 6.1/12), the spiking level in milk was too low (4X LOQ), there was no information about extraction procedures and the validation of the analytical method was not acceptable. Likewise, in the study with poultry a common moiety method, which was not acceptably validated, was used for analyses. In the common moiety method, clethodim-like residues were determined as DME, 5-hydroxy clethodim sulfone-like residues determined as DME-OH and S-methyl clethodim like residues determined as S-methyl DME, which according to the RMS does not address the stability of individual components. These studies were therefore considered as supportive only.

Similarly to plants, sample work-up procedures of animal matrices included homogenisation prior to fortification and freezing in the storage stability studies. In the livestock feeding studies, samples obtained during the study were either stored frozen as whole commodity, first as whole commodity followed by storage as homogenised sample, or as homogenised sample (see study summaries in Vol. 3, B.7.4 for details). A homogenate is likely to represent a worst case versus the use of a whole commodity.

The overview of the available data is presented in Table 2.7.1-1 below. Analytical methods used in the storage stability studies were considered acceptable and fit for purpose to address storage stability, except 6.1/01 and the studies in animal matrices.

Table 2.7.1-1. Overview of storage stability of clethodim and its metabolites in different plant matrices

Storage stability commodity category	Matrix/commodity	Demonstrated storage duration (months)	Reference/comment
Clethodim			
High water content	Sugar beet leaves	6 (-20 °C)	CA 6.1/01 (determined as equivalents)

Storage stability commodity category	Matrix/commodity	Demonstrated storage duration (months)	Reference/comment
	Alfalfa	<1 (-18 ˚C)	CA 6.1/02
	Sugar beet leaves	<1 (-18 ˚C)	CA 6.1/09
	Onion bulbs	<1 (-18 ˚C)	CA 6.1/11
High oil content	Rape seeds	6.5 (-18 ˚C)	CA 6.1/05 (6.5 months was the longest storage period)
	Rape seeds	n.d. (-18 ˚C)	CA 6.1/06 (storage interval 0 and 9 months, residues declined to 62%)
	Rape seeds	6 (-18 ˚C)	CA 6.1/11
High protein content/dry	Peas (dry seeds)	9 (-18 ˚C)	CA 6.1/06
	Peas (dry seeds)	9 (-18 ˚C)	CA 6.1/11
High starch content	Sugar beet roots	11 (-20 ˚C)	CA 6.1/01 (determined as equivalents)
	Potato tubers	<1 (-18 ˚C)	CA 6.1/03
	Sugar beet roots	<1 (-18 ˚C)	CA 6.1/11
High acid content	Grapes	<1 (-18 ˚C)	CA 6.1/07
	Grapes	<1 (-18 ˚C)	CA 6.1/11
Clethodim sulfoxide			
High water content	Alfalfa	6 (-18 ˚C)	CA 6.1/02
High oil content	Rape seeds	6.5 (-18 ˚C)	CA 6.1/05 (6.5 months was the longest storage period)
	Rape seeds	9 (-18 ˚C)	CA 6.1/06
High protein content/dry	Peas (dry seeds)	9 (-18 ˚C)	CA 6.1/06
High starch content	Potato tubers	6 (-18 ˚C)	CA 6.1/03 (6 months was the longest storage period)
	Potato tubers	9 (-18 ˚C)	CA 6.1/06
High acid content	Grapes	9 (-18 ˚C)	CA 6.1/06
Clethodim sulfone			
High water content	Sugar beet leaves	9 (-20 ˚C)	CA 6.1/01 (determined as equivalents)
	Alfalfa	3 (-18 ˚C)	CA 6.1/02
High oil content	Rape seeds	6.5 (-18 ˚C)	CA 6.1/05 (6.5 months was the longest storage period)
	Rape seeds	n.d. (-18 ˚C)	CA 6.1/06 (storage interval 0 and 9 months, residues declined to 65%)
High protein content/dry	Peas (dry seeds)	9 (-18 ˚C)	CA 6.1/06
High starch content	Sugar beet roots	11 (-20 ˚C)	CA 6.1/01 (determined as equivalents)
	Potato tubers	6 (-18 ˚C)	CA 6.1/03 (6 months was the longest storage period)
	Potato tubers	9 (-18 ˚C)	CA 6.1/06
High acid content	Grapes	9 (-18 ˚C)	CA 6.1/06
Clethodim equivalents (sum of clethodim, clethodim sulfoxide and clethodim sulfone expressed as clethodim)			
High water content	Alfalfa	2 (-18 ˚C)	CA 6.1/04
	Sugar beet leaves	6 (-18 ˚C)	CA 6.1/09
	Onion bulbs	9 (-18 ˚C)	CA 6.1/11
High oil content	Rape seeds	9 (-18 ˚C)	CA 6.1/11
High protein content/dry	Peas (dry seeds)	9 (-18 ˚C)	CA 6.1/11
High starch content	Potato tubers	3.4 (-18 ˚C)	CA 6.1/04 (3.4 months was the longest storage period)
	Sugar beet roots	9 (-18 ˚C)	CA 6.1/11
High acid content	Grapes	9 (-18 ˚C)	CA 6.1/11
M14R, M15R			
High water content	Onion bulbs	12 (-18 ˚C)	CA 6.1/10
High oil content	Rape seeds	9 (-18 ˚C)	CA 6.1/10
High starch content	Sugar beet root	12 (-18 ˚C)	CA 6.1/10
High protein content/dry	Pea seeds (dry)	12 (-18 ˚C)	CA 6.1/10
High acid content	Grapes	9 (-18 ˚C)	CA 6.1/10
M16R, M17R			
High water content	Sugar beet leaves	9 (-18 ˚C)	CA 6.1/09
High oil content	Rape seeds	9 (-18 ˚C)	CA 6.1/06
High starch content	Potato tubers	9 (-18 ˚C)	CA 6.1/06
High protein content/dry	Peas (dry seeds)	9 (-18 ˚C)	CA 6.1/06
High acid content	Grapes	9 (-18 ˚C)	CA 6.1/06
M18R, M19R			
High water content	Sugar beet leaves	9 (-18 ˚C)	CA 6.1/09

Storage stability commodity category	Matrix/commodity	Demonstrated storage duration (months)	Reference/comment
High oil content	Rape seeds	9 (-18 ˚C)	CA 6.1/06
High starch content	Potato tubers	9 (-18 ˚C)	CA 6.1/06
High protein content/dry	Peas (dry seeds)	9 (-18 ˚C)	CA 6.1/06
High acid content	Grapes	9 (-18 ˚C)	CA 6.1/06
M14A, M15A (3-CA glucoside)			
High water content	Alfalfa	9 (-18 ˚C)	CA 6.1/08
High oil content	Rape seeds	6 (-18 ˚C)	CA 6.1/08
High starch content	Sugar beet root	3 (-18 ˚C)	CA 6.1/08
High protein content/dry	Peas dry seeds	3 (-18 ˚C)	CA 6.1/08
High acid content	Grapes	15 (-18 ˚C)	CA 6.1/08
No defined group	Pea straw	9 (-18 ˚C)	CA 6.1/08

Studies in grey were considered not acceptable
n.d. = not determined

In plant commodities, parent clethodim was demonstrated to be stable in high-oil content commodities for up to 6 months and in high-protein content/dry commodities for at least 9 months. Clethodim was shown to be unstable in high-water, high-starch and high-acid commodities and was quickly (in less than one month) partially or completely degraded into clethodim sulfoxide. Therefore, it is reasonable to demonstrate the stability of clethodim as sum of clethodim and clethodim sulfoxide expressed as clethodim which is covered by the proposed residue definitions for monitoring and risk assessment.

Clethodim (determined as sum of clethodim, clethodim sulfoxide and clethodim sulfone expressed as clethodim) was demonstrated to be stable in high-water content commodities from 2 up to 9 months (2 months in alfalfa, 6 months in sugar beet leaves, and 9 months in onion bulbs) and for up to 9 months in the other commodity groups (high oil-, high starch-, high protein and high acid content).

Clethodim sulfoxide is stable in high-starch content, high-acid content, high-protein content (dry) and high-oil content commodities for at least 9 months, and in high-water content commodities for at least 6 months when stored frozen at -18°C or below.

Clethodim sulfone is stable in high-starch content, high-acid content, and high-protein content (dry) for at least 9 months, in high-oil content for at least 6.5 months, and in high-water content commodities for at least 3 months when stored frozen at -18°C or below.

M14R is demonstrated to be stable in commodities of high-oil content and high acid content for at least 9 months and in high-water content, high-protein content and high-starch content commodities for at least 12 months, when stored frozen at -18°C or below.

The metabolites M17R and M18R are stable in high-water content, high-starch content, high-acid content, high-protein content (dry) and high-oil content commodities for at least 9 months when stored frozen at -18°C or below.

The metabolite M14A/M15A (3-Chloroallyl alcohol glucoside) was shown to be stable in high-acid content commodities for at least 15 months, in high-water content commodities for up to 9 months, in high-oil content commodities for up to 6 months and in high-protein and high starch content commodities for up to 3 months,. In pea straw, 3-chloroallyl alcohol glucoside was found to be stable for at least 9 months when stored frozen at -18°C or below.

Table 2.7.1-2. Overview of storage stability of clethodim and its metabolites in different animal commodities

Storage stability commodity category	Matrix/commodity	Demonstrated storage duration (months)	Reference/comment
Clethodim			
Ruminant	Milk	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Fat	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Kidney	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Liver	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Muscle	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
Poultry	Egg	2 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Fat	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Gizzard	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Liver	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Muscle	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
S-Methyl clethodim sulfoxide			
Ruminant	Milk	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Fat	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Kidney	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Liver	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Muscle	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
Poultry	Egg	2 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Fat	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Gizzard	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Liver	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Muscle	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
5-hydroxy clethodim sulfone			
Ruminant	Milk	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Fat	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Kidney	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Liver	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
	Muscle	5 (-20 ˚C)	CA 6.1/12 (Weissenburger, 1989)
Poultry	Egg	2 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Fat	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Gizzard	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Liver	1.4 (-18 ˚C)	CA 6.1/13 (Lear, 1989)
	Muscle	0.7 (-18 ˚C)	CA 6.1/13 (Lear, 1989)

Studies in grey were considered not acceptable

Neither of the two storage stability studies of residues in animal commodities were considered acceptable by the RMS, due to deviations from the guideline and that the analytical methods used were not acceptably validated. Thus, it is the opinion of the RMS that the storage stability of the residues could not be demonstrated. Nevertheless, the results indicate that residues were shown to be stable under frozen conditions (<-18°C) for at least 1.4 months in poultry tissue (except 5-hydroxy clethodim sulfone in muscle where a decline below 70% was observed, and therefore it was stable only for 22 days), 2 months in egg and 5 months in ruminant commodities and milk. The applicant stated that no new study is submitted or required to address the storage stability of clethodim and its metabolites in animal commodities. The RMS agrees with regards to the representative uses, since no residues above the LOQ are expected in animal commodities. However, when considering other uses leading to quantifiable residues in animal commodities, the storage stability would need be demonstrated.

There was no information regarding storage stability of the residues in honey, and this is not considered necessary for the representative crops.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

2.7.2.1 Plants

Metabolism of clethodim was investigated in four crop groups: root and tuber vegetables (carrot), oilseed/pulses (cotton and soybean), leafy vegetables (spinach) and fruit crops (tomato). It was observed that no single pathway is expected to be exclusive for a crop group. However, it is the RMS opinion that the two old studies (from 1988) with carrots, cotton and soybean have several deficiencies and can only be considered supportive. There are other studies with root and tuber vegetables (carrots), but there are no acceptable studies in pulses and oilseeds. Thus these results are regarded as indicative only, but still the representative uses on sugar beet and onion are sufficiently covered by the available metabolism studies.

Table 2.7.2.1-1. Overview of metabolism studies of clethodim in plants

Plant category	Crop	Application	Application rate	Reference/comment
Fruit	Tomato	Spraying/Outdoor	375 g as/ha	6.2.1/08 (Osterman, Kandala, 2022)
Root crops	Carrot	Foliar spraying/Indoor	2x280 g as/ha (560 g in total)	6.2.1/01 (Chen, 1988a)
	Carrot	Spraying/Indoor	2x280 g as/ha (560 g in total)	6.2.1/02 (Chen, 1988b)
	Carrot	Spraying/Outdoor	624-638 g as/ha	6.2.1/03-05 (Dohn et al, 2009)
Leafy crops	Spinach	Spraying/Outdoor	539-569 g as/ha	6.2.1/06 (Dohn et al, 2012)
Pulses and oilseeds	Soybean	Foliar spraying/Indoor	2x280 g as/ha (560 g in total)	6.2.1/01 (Chen, 1988a)
	Soybean	Spraying/Indoor	2x280 g as/ha (560 g in total)	6.2.1/02 (Chen, 1988b)
	Cotton seeds	Foliar spraying/Indoor	2x280 g as/ha (560 g in total)	6.2.1/01 (Chen, 1988a)
	Cotton seeds	Spraying/Indoor	2x280 g as/ha (560 g in total)	6.2.1/02 (Chen, 1988b)

Grey text indicate that the study is considered supportive only

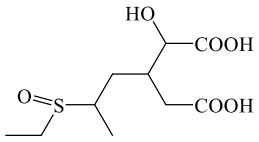
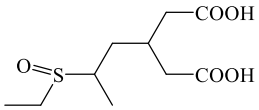
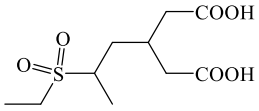
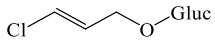
In all four groups clethodim is extensively metabolised and not detected or present at low amounts in mature crops. The one major metabolic pathway, observed in all groups, is sulfoxidation to clethodim sulfoxide followed by further oxidation to clethodim sulfone. Clethodim sulfoxide and clethodim sulfone conjugates were also identified as major or minor metabolites in all crops except in tomato, where these conjugates were not observed. Another pathway is elimination of chloroallyl moiety, leading to the formation of clethodim imine and 3-chloroallyl metabolites, including 3-chloroallyl alcohol glucoside (M14A/M15A).

In the metabolism studies in carrot and cotton and soybean, which were performed under indoor conditions (CA 6.2.1/01-02), no clethodim was detected in any of the plant parts except in carrot roots (0.8-1.1% of TRR; 0.003-0.007 mg/kg). Major metabolites (>10% of TRR) were clethodim sulfoxide (in carrot leaves, carrot roots and soybeans), imine sulfoxide (in soybean leaves, in carrot leaves and in cotton leaves), 5-hydroxy sulfone (in soybeans and carrot roots) and conjugates of clethodim sulfoxide (in soybean beans and leaves). Other identified metabolites are clethodim sulfone, imine sulfone, 5-hydroxy sulfoxide and aromatic sulfone. No ring-opened metabolites M14R/M15R, M16R/M17R and M18R/M19R have been identified. It is suggested that these metabolites are formed as a result of photolytic reaction, while the studies were performed indoor, where access to light can be a limitation. However, since clethodim imine metabolites were detected in these studies, cleavage of the chloroallyl group must have occurred and potentially, metabolites M14R/M15R, M16R/M17R and M18R/M19R could be formed assuming

photolysis under natural daylight conditions. M17R is formed from clethodim by cleaving the hydroxycyclohexenone ring, and further degraded to M14R by hydroxylation and M18R by sulfoxidation.

In carrot roots grown outdoors (CA 6.2.1/03), clethodim was detected at very small amounts. Clethodim sulfoxide and clethodim sulfone were present at significant levels (0.029-0.032 mg/kg, 18-24% TRR and 0.011-0.013 mg/kg, 7.0-9.9% TRR) in mature roots. The most abundant other components observed were M17R (13% TRR), M3A ((11% TRR) and M18R 8.8% TRR). The absolute concentration of M3A, M17R and M18R had decreased to 0.02 mg/kg in mature carrot.

Spinach plants were grown under outdoor conditions and a similar metabolic profile as the outdoor study on carrot was found. Metabolites occurring at significant levels were M14R (0.476 mg/kg, 14.2% TRR), M16R (1.16 mg/kg, 34.6% TRR), M19R (0.418 mg/kg, 12.5% TRR) (equivalent to M15R, M17R and M18R in carrot) and M14A (3-chloroallyl alcohol glucoside, 0.785 mg/kg, 22.7% TRR) which was the minor metabolite M15A in carrot. However, clethodim sulfoxide was only minor with levels up to 6.8% TRR. Notably, the metabolite codes are different in the outdoor studies on carrot and spinach, codes representing the same structures are displayed by EFSA (EFSA Journal 2019;17(5):5706) as M14R/M15R, M16R/M17R, M18R/M19R and M14A/M15A. For clarity these compounds are presented in the following table:

Compound identifier	Name in study/assessment report and SMILES	Structure	Comment
M14R/M15R	Hydroxy 3-[(2-Ethylsulfinyl) propyl]-pentanedioic acid CC(S(CC)=O)CC(C(O)C(O)=O)CC(O)=O		M15R in carrot M14R in spinach
M16R/M17R	3-[(2-Ethylsulfinyl) propyl]-pentanedioic acid CC(S(CC)=O)CC(CC(O)=O)CC(O)=O		M17R in carrot M16R/M17R in spinach
M18R/M19R	3-[(2-Ethylsulfonyl) propyl]-pentanedioic acid CC(S(CC)(=O)=O)CC(CC(O)=O)CC(O)=O		M18R in carrot M19R in spinach
M14A/M15A	3-Chloroallyl alcohol glucoside Cl/C=C/CO[C@H](O[C@@H]1CO)[C@H](O)[C@H]([C@@H]1O)O		M15A in carrot M14A/M15A in spinach

At this point it is important to note that metabolite M19R (spinach) was wrongly attributed to a metabolite where "the phenyl-ring was intact" in the framework of the EFSA review of all existing MRLs for clethodim (EFSA Journal 2019;17(5):5706), and is comparable, but not identical to the metabolite M19R significant only in carrot foliage.

In the new metabolism study, conducted on tomatoes grown under outdoor conditions (CA 6.2.1/08), the major metabolic pathways of clethodim are oxidation at the ethyl-thio-group, elimination of the chloroallyl side chain and oxidative cleavage of the cyclohexanedione ring. Clethodim sulfone and clethodim sulfoxide lose the chloroallyl portion of the molecule and form corresponding oxazoles and imines. Major metabolites were clethodim sulfone, clethodim sulfoxide, clethodim oxazole sulfoxide, M14R/M15R (hydroxy pentanedioic acid) and its glucoside. A highly polar fraction RT3/RT4 was characterised by TLC and found to contain significant portions of malic acid and citric acid. Although, a direct comparison with the also polar fraction M3/4A found in the carrot and spinach

metabolism studies could not be drawn, it is highly likely that these fractions are identical and are composed of compounds formed in the Krebs cycle, which includes small organic acids such as malic acid and citric acid, and both polar fractions are therefore attributable to complete breakdown of the allyl moiety of clethodim and natural incorporation. This fraction is therefore considered not relevant for risk assessment.

The results from the outdoor studies in carrot and spinach and the new tomato study indicated that the clethodim ring can be opened by a photolysis reaction seen in the outdoor trials (based on formed imine metabolites) to form the pentanedioic acids M14R/M15R, M16R/M17R and M18R/M19R. These metabolites were not identified in the older metabolism studies on carrot, cotton and soybean which were performed indoor. On the other hand, the presence of clethodim imine metabolites were reported in the older studies and therefore cleavage of the chloroallyl group seems to have occurred and potentially allyl-metabolites such as M3/4A and M14A/M15A could also have been formed in addition to metabolites M14R/M15R, M16R/M17R and M18R/M19R.

Although the clethodim oxazole and clethodim imine moieties were also formed in tomato grown outdoors at low levels, 3-chloroallyl alcohol (free or conjugated) was not detected, neither in the fruit nor in the leaves, which is consistent with the findings in carrots, cotton and soybean grown indoors. However, 3-chloroallyl alcohol glucoside (M14A/M15A) was found at low levels in carrot roots (0.004 mg/kg; 3.1% TRR) and carrot foliage (0.027 mg/kg; 3.6% TRR) grown outdoors and at significant levels in spinach grown outdoors (0.785-1.089 mg/kg; 21.1-22.7% TRR).

Although the same metabolites were potentially identified in metabolism studies in carrot, spinach, and tomato, there were clear quantitative differences, especially with regards to the amount of M14A/M15A (3-chloroallyl alcohol glucoside) in spinach (leafy crops) as mentioned above. The proposed metabolic pathway in carrots grown outdoors (CA 6.2.1/03) is presented in figure 2.7.2.1-1 as an example of the more complex metabolism, including photolytic degradation.

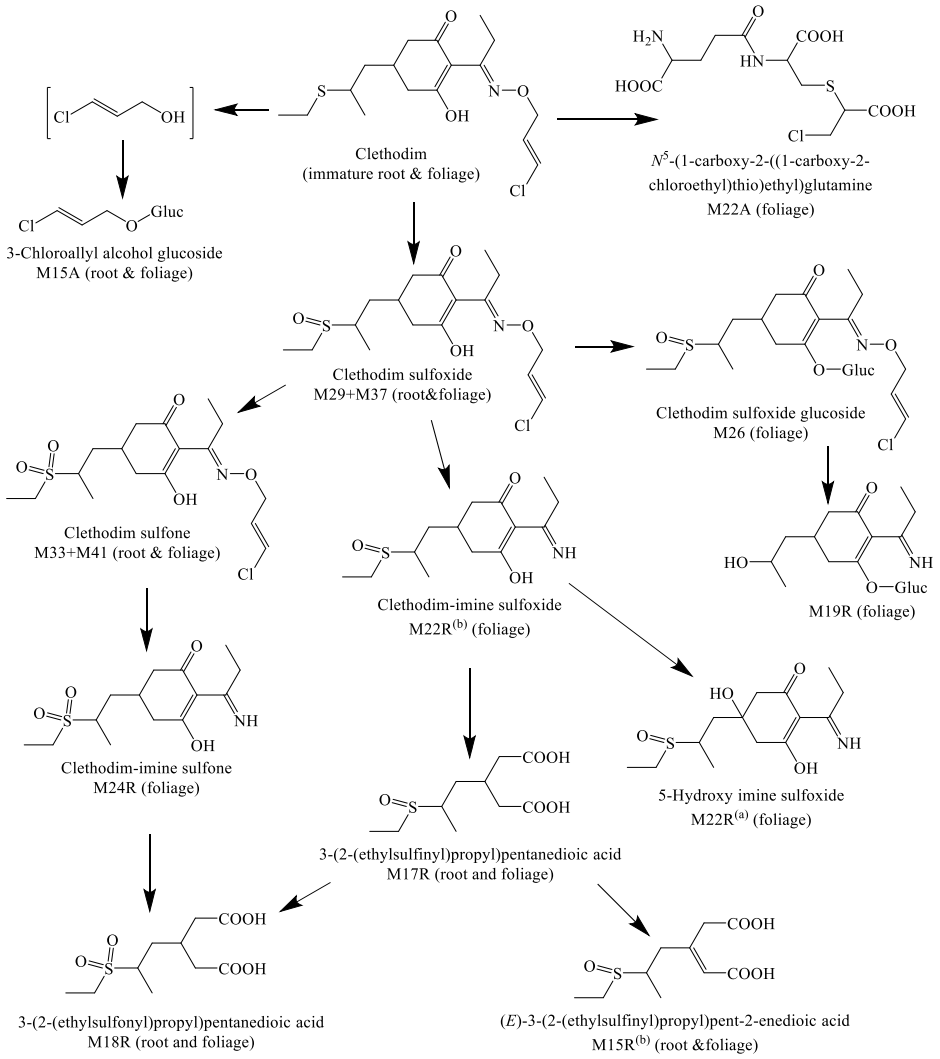


Figure 2.7.2.1-1: Proposed metabolic pathway for clethodim in carrot grown outdoors

2.7.2.2 Animals

The livestock metabolism of clethodim was investigated in poultry (laying hen) and in lactating ruminants (goat) with both clethodim and with M17R as a representative of pentanedioic acid like metabolites.

The RMS considered that the hen study (Lee, 1988, 6.2.2/01) had too many deviations from the current guideline, and the results can therefore only be regarded as supportive.

After five daily doses of [ring-4,6-^{14}C]-clethodim at 3.5 mg/day (27 mg/kg diet, 2.1 mg/kg bw/day), 78% of the total dose was recovered in excreta, 1.9% in tissues and 0.1% in eggs.

Radioactive residues in tissues were highest in kidney (1.2 mg/kg) and liver (0.7 mg/kg) and in the GI tract (2.8 mg/kg). Residue levels in skin, heart, fat, reproductive organs, gizzard, thigh muscle and breast muscle were all within the range of 0.1-0.3 mg/kg. Residue levels in eggs were less than 0.22 mg/kg (maximum at day 4 in egg white). Radioactivity levels in egg yolk and egg white did not reach a plateau within the 4-day study period. Clethodim was detected in all tissues and egg white/yolk at levels ≤0.03 mg/kg and at 0.20 mg/kg in fat. Major metabolites were identified as clethodim sulfoxide (15 - 82% of TRR; 0.01-0.51 mg/kg) and clethodim sulfone (10

- 38% of TRR; <0.01-0.33 mg/kg). No other metabolites were identified. Results for a higher dose indicated an increase in residue levels in tissues and eggs roughly proportional to the dose.

The first goat study ([redacted], 1988, 6.2.3/01) was considered supportive, and the results are therefore regarded as indicative. Following three daily administrations of [propyl-1-^{14}C]-clethodim at 42.6 mg/day (three capsules/day containing 14.2 mg, one capsule on the fourth day), equivalent to 24 mg/kg diet as received and 1.2 mg/kg bw/day (55N for cattle), 56% of the total dose was excreted in urine, 34% in the faeces, 0.14% in milk and 0.6% in tissues.

Radioactive residues in tissues were highest in liver (0.41 mg/kg) and kidney (0.38 mg/kg). Blood contained 0.17 mg/kg. Residue levels in heart, muscle and fat were all within the range of 0.033-0.079 mg/kg. Residue levels in milk did not exceed 0.049 mg/kg, but the RMS considers that it cannot be concluded that a plateau was reached due to the short duration of the study. Clethodim was detected at high levels in blood, liver and day 2 urine and traces were detected in kidney, subcutaneous fat, day 1, 3 and 4 urine, and milk sample at sacrifice. The major metabolites were clethodim sulfoxide, S-methyl sulfoxide and S-methyl (urine only). Other observed minor metabolites (<5% TRR) were clethodim sulfone (blood, liver and urine), imine sulfoxide (blood, liver, kidney, subcutaneous fart, urine and faeces), 5-hydroxy sulfone (blood and faeces) and 5-hydroxy sulfoxide (urine and faeces). Radioactivity in the unextractable milk fraction was shown to be incorporated into lactose.

The metabolites S-methyl sulfoxide and S-methyl are directly formed from clethodim. Since no clethodim is expected in animal feed, these metabolites are also not expected in edible animal products.

In the Article 12 MRL review, EFSA (2019) highlighted that: “During the peer review, no residue definition for animal commodities was proposed because the animal dietary burden was below the trigger value. In the present review, based on the two metabolism studies [Rose, Suzuki, 1988 and Lee, 1988] the following residue definition for monitoring and risk assessment is tentatively proposed: sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim. Since additional metabolites in plant commodities were considered relevant additional studies are needed […]. It is noted that in the available metabolism study livestock was fed with the parent compound only, which according to the residue trials, is not expected to be present in crops fed to livestock. Furthermore, no studies investigating the livestock metabolism of metabolites proposed for inclusion in the residue definitions for risk assessment are available. Therefore, the proposed residue definition for livestock should be considered tentative only, and additional livestock metabolism studies investigating the relevant metabolites found in plant, are still required.” The residue definition for risk assessment RA1 contains two major groups of metabolites:

1) Clethodim like metabolites comprising clethodim, clethodim sulfoxide and clethodim sulfone. These metabolites are covered by the existing livestock metabolism studies since clethodim is easily converted into clethodim sulfoxide and clethodim sulfone as demonstrated in the available animal metabolism studies.

2) Pentanedioic acid like metabolites comprising M14R/M15R, M16R/M17R and M18R/M19R. These metabolites are plant unique metabolites and a new animal metabolism study on lactating goats was provided using [methyl-^{14}C]-M17R as a representative compound of this group of metabolites.

In the new study ([redacted] 2022, R00239, 6.2.3/02), a single goat was orally dosed with [methyl-^{14}C]-M17R (0.415 mg/kg bw/day, 32N for cattle) once daily for five consecutive days.

M17R and its metabolites were rapidly excreted by lactating goats. The majority of the administered dose (AD) (69.9%) was recovered in the urine, with an additional 6.7% of the AD in the faeces. Milk contained a total of 0.012% of the AD reaching a maximal level (0.0017 mg/kg M17R equivalents) after 3 days of dosing, and plateaued at about 0.0012 mg/kg for the remainder of the dosing period. The distribution of radioactive residues in the skim milk and cream fractions was approximately equal with 44% and 56% distribution in the skim milk and cream, respectively. Only 0.057% of the AD was recovered in the edible tissues. The overall recovery of the administered dose was 87.7%.

The highest residue levels in tissues were found in kidney (0.207 mg/kg) and liver (0.016 mg/kg). The residues in all types of muscle and fat were <0.01 mg/kg M17R equivalents and therefore not further analysed.

Residues in liver and kidney were identified as the major compounds parent M17R and deoxy-M17R. Only kidney contained an unknown metabolite at a low level (<10% TRR). Parent M17R was found at 71% TRR (0.013 mg/kg) in liver and 26% TRR (0.055 mg/kg) in kidney, deoxy-M17R accounted for 19% TRR (0.003 mg/kg) in liver and 68% TRR (0.143 mg/kg) in kidney. The residue level in the PES was 9.9% TRR (0.0017 mg/kg) and 2.9% TRR (0.006 mg/kg) for liver and kidney, respectively.

It was concluded that M17R primarily metabolises through the reduction of the sulfoxide to form the sulfide metabolite (deoxy-M17R). The study is considered sufficient to establish the metabolic pathway and the fate of residues after uptake of M17R by ruminants, and could be extrapolated to the other pentanedioic acid metabolites.

2.7.3 Definition of the residue

The current residue definition for monitoring (Regulation (EU) No 839/2008) is “Clethodim (sum of Sethoxydim and Clethodim including degradation products calculated as Sethoxydim)”. Since sethoxydim is a standalone active substance that is no longer approved in Europe, the residue definition for clethodim is proposed to be changed.

The available crop metabolism studies indicate that clethodim is extensively metabolised by sulfoxidation into clethodim sulfoxide followed by further oxidation to clethodim sulfone. Metabolism studies in plants conducted under outdoor conditions indicate a photolytic opening of the clethodim ring to form the pentanedioic acid metabolites M14R (M15R in carrot), M16R/M17R and M19R (M18R in carrot) as major metabolites.

Another pathway is elimination of chloroallyl moiety, leading to the formation of clethodim imine and 3-chloroallyl metabolites, including 3-chloroallyl alcohol glucoside (M14A/M15A), which is mainly formed in leafy crops (spinach). This metabolite was not detected in the GAP compliant residue trials with the representative crops. 3-chloroallyl alcohol was also shown to be formed by complete degradation of clethodim sulfone in high temperature hydrolysis (CA 6.5.1/03).

It has previously been concluded that clethodim sulfoxide and clethodim sulfone are sufficient marker components for monitoring residues of clethodim.

Based on the current assessment, the general toxicity of the metabolites clethodim sulfoxide, M14R/M15R, M16R/M17R and M18R/M19R is covered by the parent clethodim. Regarding general toxicity for the assessment of clethodime sulfone, the RMS considers that it cannot be concluded that this metabolite is less toxic than the parent substance based on the available data (see 2.6.8.1.4). The current assessment of the metabolite 3-Chloroallyl alcohol

(the aglycon of M14A/M15A) found that it is not genotoxic. However, based on toxicity data from the DAR of 1,3-dichloropropene the systemic toxicity of M14A/M15A is not the same as for the parent clethodim and specific toxicological reference values are proposed (see 2.6.8.1.9). Thus, it is considered that it is necessary to have an individual residue definition for risk assessment for the metabolite M14A/M15A (3-chloroallyl alcohol glucoside). Therefore, the following residue definitions for monitoring and risk assessment are provisionally proposed for plant commodities:

- Monitoring residue definition (plants): Sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim;
- Risk assessment residue definition RA1 (plants): Sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R, and M18R/M19R, expressed as clethodim;
- Risk assessment residue definition RA2 (plants): M14A/M15A

However, these proposed residue definitions are pending the conclusion of the toxicological assessment of clethodim sulfone. If it is concluded that its toxicity is not covered by clethodim, a separate residue definition for risk assessment may be needed. The RMS also identified a data gap for genotoxicity for clethodim sulfone (positive responses in Ames and MLA need to be followed up) and for M17R, since aneuploidy has not been properly assessed. The later data gap is also applicable for M14R/15R and M18R/M19R, since read across from M17R was proposed. The final residue definitions are therefore pending the outcome of the toxicological assessment.

For processed commodities, it could not be concluded based on available data and the representative uses if a separate residue definition would be necessary.

The metabolism in rotational crops was similar to the one in primary crops but would need to be more properly investigated in a new study. It is tentatively concluded that a separate residue definition is not necessary.

Animal commodities

In previous EU evaluations based on the two metabolism studies in poultry and goat (CA 6.2.2/01 and CA 6.2.3/01) the following residue definition for monitoring and risk assessment was tentatively proposed: sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim". It was also concluded that an evaluation of the livestock metabolism of the pentanedioic acid metabolites M14R/M15R, M16R/M17R and M18R/M19R was pending.

A new ruminant metabolism study on the plant unique metabolite M17R (CA 6.2.3/02) was submitted and evaluated. This study demonstrated that based on the calculated dietary burden for the pentanedioic acid metabolites at the representative uses, the residues of M14R/M15R, M16R/M17R and M18R/M19R and the proposed deoxy-M17R metabolite are predicted to be well below 0.01 mg/kg (*i.e.*, non-detectable or < LOQ) in any livestock commodity. Therefore, these metabolites are considered to be not relevant for inclusion in the residue definitions for monitoring or risk assessment purposes in animal commodities. However, if livestock is exposed to feed items with higher residue levels of pentanedioic acid metabolites, resulting in a significant dietary burden, it may be considered to include the deoxy metabolites in the residue definition. An *in silico* toxicity assessment of deoxy-M17R was submitted by the applicant at a later stage, and was evaluated. The RMS does not agree with the applicant that this

assessment indicates that this metabolite is of no toxicological concern, and a data gap for genotoxicity was identified.

The following residue definition for monitoring and risk assessment is provisionally proposed for animal commodities:

- Monitoring and risk assessment residue definition (animals): Sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim

Similarly to the situation for plants, these proposed residue definitions are pending the conclusion of the toxicological assessment of clethodim sulfone and a final conclusion on the genotoxic potential of deoxy-M17R.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Clethodim is a herbicide. The field of use is proposed on sugar beet, bulb onion and garlic in the EU. The representative formulated product is an emulsifiable concentrate (EC) formulation containing 120 g as/L used for the control of annual and perennial grasses. Detailed study summaries are available in Volume 3, B.7.3.1 and in Appendix G. The extraction efficiency of the analytical methods used was investigated in a separate study (6.10.2/10, Wiesner, 2020, report no S19-0144), which is evaluated and presented in B.5.2.1, and was considered sufficiently demonstrated.

Sugar beet

cGAP NEU and SEU: 300 g as/ha, BBCH 12-33, PHI not applicable (spraying overall)

A total of 39 residue trials were conducted on sugar beet during 2005-2019, 21 trials in Northern Europe and 18 trials in Southern Europe. All trials conducted before 2018 were performed according to a GAP that was used for the previous active substance approval which was focused on a PHI of 56 days after application. For renewal the GAP is now focused on an application timing of BBCH 12-33. Since the previous GAP with a PHI of 56 days resulted in application to a significantly later crop growth stage, these trials are generally more critical compared to the representative uses in this submission. Additionally, not all metabolites included in the residue definition for risk assessment were analysed in these earlier studies. Therefore, studies performed before 2018 were considered supportive (except two trials in 2015, 6.3.1/09), and were not used for MRL calculation and the consumer risk assessment. In total 8 trials (harvest) performed in NEU and 9 trials (harvest) in SEU during 2018 and 2019 were available and acceptable.

Clethodim is stable for less than 1 month in samples of sugar beet tops and roots. In all the acceptable residue trials, samples were stored deep-frozen for less than 1 month (9-15 days), but due to the low stability, it could not be excluded that clethodim was degraded during this time. However, as residues were determined as total clethodim equivalents (sum of clethodim, clethodim sulfoxide, clethodim sulfone, expressed as clethodim according to the residue definition for enforcement, and including also M14R, M17R and M18R, according to the residue definition for risk assessment) and storage stability for these were at least 6 months in sugar beet tops (high water content commodities) and 9 months in sugar beet roots. Also 3-chloroallyl alcohol glucoside was shown to be stable for up to 9 months in sugar beet tops and root. Therefore, the samples were considered stored in line with the demonstrated

periods of storage stability. Extraction efficiency was also sufficiently demonstrated in a cross-validation study evaluated in B.5 (CA 6.10.2-01, Wiesner 2020).

In the trials performed during 2005-2015, with an application at a later BBCH (up to 49) and PHI of 56 days, residues of clethodim determined as total equivalents were below LOQ in the majority of samples, but were quantified in some samples of sugar beet root (up to 0.044 mg/kg) and at levels up to 0.22 mg/kg in tops with leaves. However, these data were considered supportive only, except two trials with an application at BBCH 33-35, which were acceptable.

In the trials supporting the current critical GAP, all residues in sugar beet roots according to the residue definitions for monitoring and risk assessment were below the respective LOQs of 0.005 mg/kg (clethodim, clethodim sulfoxide, clethodim sulfone), 0.01 mg/kg (M14R, M17R, M18R) and 0.05 mg/kg (3-chloroallyl alcohol glucoside). Residues of clethodim and 3-chloroallyl alcohol glucoside were also below the respective LODs (0.0015 and 0.015 mg/kg). M14R was analysed in four trials per region, which were conducted in 2019, and all residues were below the LOQ of 0.01 mg/kg, and also below the LOD (0.003 mg/kg). The results are summarised in Table 2.7.4-1.

In sugar beet leaves, total clethodim equivalents according to the proposed definition for enforcement (sum of clethodim, clethodim sulfoxide, and clethodim sulfone) was <0.014-0.036 mg/kg in NEU and <0.014-0.018 mg/kg in SEU. The residues according to the residue definition for risk assessment (sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R and M18R/M19R, expressed as clethodim) was <0.06-0.31 mg/kg in NEU and <0.06-0.21 mg/kg in SEU. The residues of M14A/M15A (3-chloroallyl alcohol glucoside) were analysed in 8 trials from NEU and 7 acceptable trials from SEU and were below the LOQ (0.05 mg/kg) and also the LOD (0.015 mg/kg).

In Northern and Southern Europe, eight and seven field trials on sugar beet, respectively, were performed in the growing seasons 2018/19 and were considered acceptable for MRL purposes and for risk assessment. All residues in sugar beet roots according to the residue definitions for monitoring and risk assessment are below the respective LOQs of 0.005 mg/kg (clethodim, clethodim sulfoxide, clethodim sulfone), 0.01 mg/kg (M14R, M17R, M18R) and 0.05 mg/kg (3-chloroallyl alcohol glucoside). Thus, since this is a <LOQ situation, the number of trials in SEU is considered sufficient, even if there is not eight acceptable trials for this major crop. M14R was analysed in four trials per region, which were conducted in 2019, and all residues were below the LOD of 0.003 mg/kg. The zero residue situation demonstrated for M14R has therefore been extended to the trials conducted during 2018 (S18-00165-01 to -08) to give eight trials per region compliant with the full residue definition for risk assessment.

Bulb onions and garlic

cGAP NEU and SEU: 240 g as/ha, BBCH 12-19, PHI not applicable (spraying overall)

In total, twenty residue trials on onion have been conducted throughout Europe using applications of clethodim at rates generally within ±25% of the appropriate critical GAP of 240 g as/ha.

Ten field trials in Northern and Central Europe and ten in Southern Europe on onion were performed in the growing seasons 2018 - 2020 and were considered acceptable for MRL purposes and for risk assessment. Although six trials (4x N-EU and 2x S-EU) were performed at a more critical growth stage compared to GAP, these trials are considered

acceptable since in five trials residues at harvest were all below the LOQ and the residue level of the sixth trial is well within the range of residues obtained from GAP-compliant trials on bulb onion.

Total residues in onion bulbs according to the residue definition for monitoring were within the range of <0.014 - 0.023 mg/kg and according to the residue definition for risk assessment are within the range of <0.06 - 0.21 mg/kg, respectively. Residues of clethodim, M18R and 3-chloroallyl alcohol glucoside (M14A/M15A) were always below the respective LOD (0.0015, 0.003, and 0.015 mg/kg). M14R was analysed in six trials each in Northern Europe and in Southern Europe, which were conducted in 2019/20, and all residues were below the LOD of 0.003 mg/kg. The zero residue situation demonstrated for M14R has therefore been extended to the trials conducted during 2018 and 2020 (S18-01121-01 to -08) to give ten trials in the northern zone and ten trials in the southern zone compliant with the full residue definition for risk assessment. The results are summarised in Table 2.7.4-1.

The results from bulb onion can also be extrapolated to garlic, according to guideline SANTE/2019/12752.

Table 2.7.4-1: Available data for residues of clethodim in sugar beet and onion according to the residue definition for risk assessment (RA1)

Trial	Clethodim (mg/kg)	Clethodim sulfoxide (mg/kg)	Clethodim sulfone (mg/kg)	M14R/ M15R (mg/kg)	M16R/ M17R (mg/kg)	M18R/ M19R (mg/kg)	Total residue according to:	
							MO	RA1
Sugar Beet Roots (N-EU)								
S18-08165-01	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-02	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-03	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-04	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08161-01	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08161-02	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08161-03	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08161-04	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
						STMR:	0.01	0.06
						HR:	0.01	0.06
Sugar Beet Tops with Leaves (N-EU)								
S18-08165-01	<0.005	<0.005	<0.005	(<0.01)	0.06	0.02	<0.014	0.14
S18-08165-02	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-03	<0.005	0.005	0.018	(<0.01)	0.16	0.03	0.026	0.31
S18-08165-04	<0.005	<0.005	<0.005	(<0.01)	0.10	0.03	<0.014	0.21
S18-08161-01	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08161-02	<0.005	<0.005	<0.005	<0.01	0.02	<0.01	<0.014	0.07
S18-08161-03	<0.005	<0.005	<0.005	<0.01	0.02	<0.01	<0.014	0.07
S18-08161-04	<0.005	0.012	0.019	<0.01	0.13	0.02	0.033	0.26
						STMR:	0.01	0.11
						HR:	0.033	0.31
Sugar Beet Roots (S-EU)								
S15-03505-06	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S15-03505-08	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-05	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-06	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-07	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-08	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08161-05	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08161-06	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08161-07	*<0.005*	*<0.005*	*<0.005*	*<0.01*	*<0.01*	*<0.01*	*<0.014*	*<0.06*
S18-08161-08	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
						STMR:	0.01	0.06
						HR:	0.01	0.06

Table 2.7.4-1: Available data for residues of clethodim in sugar beet and onion according to the residue definition for risk assessment (RA1)

Trial	Clethodim (mg/kg)	Clethodim sulfoxide (mg/kg)	Clethodim sulfone (mg/kg)	M14R/ M15R (mg/kg)	M16R/ M17R (mg/kg)	M18R/ M19R (mg/kg)	Total residue according to:	
							MO	RA1
Sugar Beet Tops with Leaves (S-EU)								
S15-03505-06	<0.005	<0.005	0.007	(<0.01)	0.04	0.01	0.016	0.10
S15-03505-08	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-05	<0.005	<0.005	<0.005	(<0.01)	0.02	<0.01	<0.014	0.07
S18-08165-06	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-07	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08165-08	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08161-05	<0.005	<0.005	0.008	<0.01	0.10	0.03	0.017	0.21
S18-08161-06	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08161-07	*<0.005*	*<0.005*	*<0.005*	*<0.01*	*<0.01*	*<0.01*	*<0.014*	*<0.06*
S18-08161-08	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
						STMR:	0.01	0.06
						HR:	0.017	0.21
Bulb Onion (N-EU)								
S18-01121-01	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-01121-02	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-01121-03	<0.005	0.007	0.007	(<0.01)	<0.01	<0.01	0.018	0.06
S18-01121-04	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-08160-01	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-02	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-04	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-05	<0.005	0.005	<0.005	<0.01	<0.01	<0.01	0.014	0.06
S18-08160-06	<0.005	0.009	0.010	<0.01	0.02	<0.01	0.023	0.07
S20-00082-01	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
						STMR:	0.01	0.06
						HR:	0.023	0.07
Bulb Onion (S-EU)								
S18-01121-05	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-01121-06	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-01121-07	<0.005	<0.005	<0.005	(<0.01)	<0.01	<0.01	<0.014	<0.06
S18-01121-08	<0.005	<0.005	0.007	(<0.01)	<0.01	<0.01	0.016	0.06
S18-08160-07	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-08	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-09	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-11	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-12	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
S18-08160-13	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.014	<0.06
						STMR:	0.01	0.06
						HR:	0.016	0.06

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

For clethodim the feeding of sugar beet tops with leaves and processed wastes including sugar beet dried pulp, ensiled pulp and molasses are relevant for livestock.

The exposure of livestock to residues of clethodim was estimated using the Animal Model 2017, and both according to the proposed residue definition for monitoring and for risk assessment, since only tentative conversion factors could be determined. Input values for the model are summarised in Table 2.7.5-1 and Table 2.7.5-2.

Based on the available metabolism study in rotational crops, no residues above the LOQ are anticipated in root/tuber crops due to crop rotation and therefore only data from the primary crop residue trials are considered in the dietary burden. In rotational wheat immature plant, straw, and hulls, TRR levels of 0.57-0.93 mg/kg indicate that residues may be expected to be present in feed items. As no individual metabolite was identified and expected at residue levels above the LOQ, residues in rotational cereal forage were not considered in the dietary burden calculations.

The Northern EU data set is considered to represent the worst-case situation with all total residues according to the residue definitions at the LOQ in sugar beet roots and an STMR of 0.01 and 0.11 mg/kg and an HR of 0.033 and 0.31 mg/kg (RD-MO and RD-RA1 respectively) in sugar beet tops with leaves. Since residues of M14A/M15A, which is proposed to have a separate residue definition for risk assessment, was not detected (<LOD), no calculation was performed. The input data for the dietary burden calculation is shown in Table 2.7.5-1 and 2.7.5-2.

Table 2.7.5-1: Input values (RD-MO) for the dietary burden calculation

Feed Commodity	Median dietary burden		Maximum dietary burden	
	Input Value (mg/kg)	Comment	Input Value (mg/kg)	Comment
Enforcement residue definition: Sum of clethodim, clethodim sulfoxide, and clethodim sulfone, expressed as clethodim				
Sugar beet (tops)	0.04	STMR x CF[1)]	0.12	HR x CF[1)]
Sugar beet (dried pulp)	0.03	LOQ (root) x CF[1)] x PF[2)]	0.03	LOQ (root) x CF[1)] x PF[2)]
Sugar beet (ensiled pulp)	0.03	LOQ (root) x CF[1)] x PF[2)]	0.03	LOQ (root) x CF[1)] x PF[2))]
Sugar beet (molasses)	0.03	LOQ (root) x CF[1)] x PF[2)]	0.03	LOQ (root) x CF[1)] x PF[2))]

STMR: Supervised trials median residue (according to the residue definition for monitoring)
HR: Highest residue (according to the residue definition for monitoring)

1) A tentative conversion factor of 3.5 was used for sugar beet tops, derived from metabolism study in carrot leaves, and a tentative CF of 2.5 was used for sugar beet roots
2) PF: Processing factor (raw commodity to processed commodity). A <LOQ residue situation is assumed for all metabolites of the residue definition for risk assessment in sugar beet roots. Therefore, a processing factor for sugar beet dried pulp, ensiled pulp and molasses is not appropriate and therefore set to 1.

Table 2.7.5-2: Input values (RD-RA1) for the dietary burden calculation

Feed Commodity	Median dietary burden		Maximum dietary burden	
	Input Value (mg/kg)	Comment	Input Value (mg/kg)	Comment
Risk assessment residue definition (RA1): Sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R and M18R/M19R, expressed as clethodim				
Sugar beet (tops)	0.11	STMR	0.31	HR
Sugar beet (dried pulp)	0.06	LOQ (root) x PF[1)]	0.06	LOQ (root) x PF[1)]
Sugar beet (ensiled pulp)	0.06	LOQ (root) x PF[1)]	0.06	LOQ (root) x PF[1)]
Sugar beet (molasses)	0.06	LOQ (root) x PF[1)]	0.06	LOQ (root) x PF[1)]

STMR: Supervised trials median residue (according to the risk assessment residue definition)
HR: Highest residue (according to the risk assessment residue definition)
1) PF: Processing factor (raw commodity to processed commodity). A <LOQ residue situation is assumed for all metabolites of the residue definition for risk assessment in sugar beet roots. Therefore, a processing factor for sugar beet dried pulp, ensiled pulp and molasses is not appropriate and therefore set to 1.

The results of the dietary burden calculations are shown in Table 2.7.5-3 and Table 2.7.5-4. The calculated dietary burdens based on the residue definition for monitoring were found to exceed the trigger value of 0.004 mg/kg bw for cattle and sheep (all diets). The calculated dietary burdens based on the residue definition for risk assessment (RA1) were found to exceed the trigger value of 0.004 mg/kg bw for all groups of livestock, except swine. The highest dietary burden was calculated for ruminants (0.022 mg/kg bw/d), followed by sheep (0.013 mg/kg bw/d) and poultry (0.005 mg/kg bw/d). Based on the available information, rotational crops have no effect on the dietary burden, since residues are expected to be below the LOQ.

Table 2.7.5-3: Results of the dietary burden calculation with residue levels expressed according to the residue definition for monitoring

Relevant groups	Dietary burden expressed in				Most critical diet[1]	Most critical commodity[2]		Trigger (0.004 mg/kg bw/day) exceeded
	mg/kg bw/day		mg/kg DM					
	Median	Max.	Median	Max.				
Cattle (all diets)	0.004	0.008	0.11	0.22	Dairy cattle	Sugar beet	tops	Yes
Cattle (dairy only)	0.004	0.008	0.11	0.22	Dairy cattle	Sugar beet	tops	Yes
Sheep (all diets)	0.002	0.005	0.04	0.11	Lamb	Sugar beet	tops	Yes
Sheep (ewe only)	0.001	0.004	0.04	0.11	Ram/Ewe	Sugar beet	tops	No
Swine (all diets)	0.000	0.001	0.02	0.06	Swine (breeding)	Sugar beet	tops	No
Poultry (all diets)	0.001	0.002	0.01	0.03	Poultry layer	Sugar beet	tops	No
Poultry (layer only)	0.001	0.002	0.01	0.03	Poultry layer	Sugar beet	tops	No

[1] When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"

[2] The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

Table 2.7.5-4: Results of the dietary burden calculation with residue levels expressed according to the residue definition for risk assessment (RA1)

Relevant groups	Dietary burden expressed in				Most critical diet[1]	Most critical commodity[2]		Trigger (0.004 mg/kg bw/day) exceeded
	mg/kg bw/day		mg/kg DM					
	Median	Max.	Median	Max.				
Cattle (all diets)	0.012	0.022	0.30	0.56	Dairy cattle	Sugar beet	tops	Yes
Cattle (dairy only)	0.012	0.022	0.30	0.56	Dairy cattle	Sugar beet	tops	Yes
Sheep (all diets)	0.005	0.013	0.12	0.30	Lamb	Sugar beet	tops	Yes
Sheep (ewe only)	0.004	0.010	0.12	0.30	Ram/Ewe	Sugar beet	tops	Yes
Swine (all diets)	0.001	0.003	0.06	0.15	Swine (breeding)	Sugar beet	tops	No
Poultry (all diets)	0.002	0.005	0.02	0.07	Poultry layer	Sugar beet	tops	Yes
Poultry (layer only)	0.002	0.005	0.02	0.07	Poultry layer	Sugar beet	tops	Yes

[1] When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"

[2] The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

Two livestock feeding studies for clethodim were submitted, one in cattle (CA 6.4.2/01, [redacted], 1989) and one in hen (CA 6.4.1/01, [redacted] 1988). The RMS considered these studies to be supportive due to deviations regarding analytical methods and that the storage stability of residues was not demonstrated. Residues were determined as clethodim equivalents using a common moiety method which is not compatible with the proposed residue definition. Nevertheless, the studies are presented and the results can be used as indicative.

Table 2.7.5-3 Overview of available livestock feeding studies

Study	Compound administrated	Compounds analysed	Feeding level (expressed as total clethodim equivalents) [mg/kg bw/d]	N-level compared to estimated dietary burden representative uses
Poultry				
CA 6.4.1/01, 1998 Supportive	Clethodim (5%) & clethodim sulfoxide (95%)	Clethodim, S-methyl clethodim sulfoxide and 5-hydroxy clethodim sulfone	1.04	208 N
			3.05	610 N
			9.58	1916 N
Ruminants				
CA 6.4.2/01, 1989 Supportive	Clethodim (5%) & clethodim sulfoxide (95%)	Clethodim, S-methyl clethodim sulfoxide and 5-hydroxy clethodim sulfone	0.65	30 N
			1.89	85 N
			5.73	260 N

In both studies, animals were dosed for 28 consecutive days with a diet fortified with a mixture of 5% clethodim and 95% clethodim sulfoxide. According to the applicant, this forms the major component of the residue in root crops and foliage, but according to the RMS the metabolite M17R in sugar beet tops is the major component.

The lowest dose used in the poultry study was 1.04 mg/kg bw/day (clethodim equivalents), which is a factor of 208 higher than the maximum estimated intake by poultry of 0.005 mg/kg bw/day. With a dietary burden calculated according the lower GAP rate (1x 120 g as/ha) the lowest dose rate is a factor of 520 higher than the maximum estimated intake by poultry of approximately 0.002 mg/kg bw/day. The results of the hen feeding study at the lowest dose, indicated that no residues above the LOQ in eggs and tissues were observed. At the estimated intake dose level, independent of the GAP considered, residues in all poultry tissues and egg are expected to be <LOQ.

The lowest dose used in the ruminant study was 0.65 mg/kg bw/day (clethodim equivalents), which is a factor of 30 higher than the maximum estimated intake by cattle of approximately 0.022 mg/kg bw/day. With a dietary burden calculated according the lower GAP rate (1x 120 g as/ha) the lowest dose rate is a factor of 81 higher than the maximum estimated intake by cattle of approximately 0.008 mg/kg bw/day. The results of the livestock feeding study at the lowest dose, indicated that no residues above the LOQ in milk and tissues were observed, with the exception of the total DME residues in liver (HR= 0.059 mg/kg) and kidney (HR= 0.051 mg/kg). At the estimated intake dose level, independent of the GAP considered, residues in these organs are expected to be <LOQ.

2.7.6 Summary of effects of processing

2.7.6.1 Nature of residues

The nature of residues has been investigated with ^{14}C-clethodim, ^{14}C-clethodim sulfoxide (with cyclohexyl-labels) and ^{14}C-clethodim sulfone (with allyl-label) in accordance with OECD guideline No. 507 (CA 6.5.1/01-03). In these hydrolysis studies it was shown that the substances are extensively degraded to clethodim oxazole, clethodim oxazole sulfoxide and 3-chloroallyl alcohol, respectively, under processing conditions simulating pasteurisation, baking/brewing/boiling and sterilisation.

In Persch, 2013 (CA 6.5.1/01, S12-00895) after processing simulating pasteurisation, clethodim was the main residue, but also clethodim oxazole was a major component with 14% formed. With processing conditions representing baking, boiling and brewing and sterilisation, clethodim was extensively degraded to clethodim

oxazole, which was formed with amounts of 80% and 96%, respectively. An additional degradation product, clethodim trione was also formed with amounts of 5.4% and 3.8%, respectively.

In Bloβ, 2018 (CA 6.5.1/02, S18-02073) with clethodim sulfoxide under conditions representative of pasteurisation, the degradation product clethodim oxazole sulfoxide was formed with an amount of 89%. Under conditions representative of baking, brewing, boiling and sterilisation, clethodim oxazole sulfoxide was formed with amounts of 94% and 98%, respectively, and an additional degradation product, clethodim trione sulfoxide with amounts of 6.9%, 5.5% and 2.7%, respectively.

In Bloβ, 2018 (CA 6.5.1/03, S18-02074) with clethodim sulfone, under conditions representative of pasteurisation, baking, brewing, boiling and sterilisation, trans-3-chloroallyl alcohol was formed at an amount of 99-102%. Under conditions representative of sterilisation also a second product M3 was formed at a very low amount of 1.7-1.9%.

These results indicate that clethodim oxazole sulfoxide, clethodim oxazole sulfone and 3-chloroallyl alcohol could be present in processed commodities, if the raw agricultural commodity contains residues at significant levels. Clethodim oxazole is unlikely to be present since the parent compound is never seen to be present at detectable levels in the raw commodity.

2.7.6.2 Distribution of residues in peel and pulp

Not required. The representative crops are not separated into peel and pulp.

2.7.6.3 Magnitude of residues in processed commodities

As residues in sugar beet root, onion and garlic raw agricultural commodities (RAC) are below 0.1 mg/kg (based on the total residue definition for risk assessment) and the chronic exposure does not exceed 10% of the ADI, no further considerations about the effect of processing on the magnitude of residues is required. However, one processing study on sugar beet previously submitted for active substance approval was reassessed (CA 6.5.3/01, Lai, 1992, TSR5068SGBT), and was considered supportive. Residues determined as the common moieties DME and DME-OH in sugar beet roots and sliced roots, dehydrated pulp and refined sugar were all below the LOQ and processing factors were therefore not estimated. The mean residue in molasses determined as DME was 0.28 mg/kg and an indicative processing factor of 2.8 was estimated.

2.7.7 Summary of residues in rotational crops

Since several soil metabolites were found to be persistent in soil, metabolism studies in rotational crops are needed to be able to determine the nature and extent of potential residue accumulation in rotational crops.

A study was submitted, in which the metabolism of clethodim related residues was studied in carrot, lettuce and wheat grown in rotation after application of [ring-4,6-^{14}C]-clethodim to bare soil at an exaggerated rate of 1.1 kg as/ha (3.7-4.6N) (CA 6.6.1/01, Gaddamidi, 1988, MEF-0036).

The total radioactive residue was below 0.05 mg/kg in carrot roots and leaves, wheat grain and in mature lettuce leaf (except for the 30 days PBI). In carrot leaf, lettuce leaf (30 days PBI) and wheat straw and hull, the TRR ranged from 0.053-0.65 mg/kg.

Clethodim imine sulfoxide was significant in lettuce leaf (30 days PBI) and in carrot leaves (366 days PBI). The significant soil metabolites clethodim oxazole sulfoxide and clethodim oxazole sulfone were minor and found only at low levels in all crops grown in rotation. Other metabolites were all below 10% TRR and 0.01 mg/kg, clethodim was not detected in any crop.

The metabolites imine sulfoxide, clethodim oxazole sulfoxide and clethodim oxazole sulfone are soil metabolites of clethodim. Their occurrence in rotational crops is considered to be due to the uptake by plant roots.

Taking into account the exaggerated application rate any identified metabolite is not expected to exceed the trigger value of 0.01 mg/kg in food items, when compared to the cGAPs under consideration. However, since residues in rotational wheat immature plant, straw, and hulls were found at TRR levels of 0.57-0.93 mg/kg this indicate that residues may be present in feed items (considering a fallow period of 120 days).

There were deviations from OECD 502 in the study. The application rate was higher than in the intended cGAP (3.7-4.6N) and the recoveries (33-106% TRR) indicated that the extraction was incomplete and the residue levels were likely underestimated. It is also noted that the %TRR that was identified was low. Additionally, in this study, the active substance was only labelled in one position; [Ring-4,6-^{14}C]-clethodim was investigated, and it could be questioned if an allyl-label would also be needed to be able to track all significant moieties or degradation products. Finally, the study was performed in a greenhouse, and since clethodime is photolytically degraded, it may not be representative of the residues that are formed and taken up by plants in field conditions. For example, the metabolites CBA and CAA were found at significant levels in a photolysis study with allyl-labelled clethodim on soil surface,

The applicant stated that based on the findings of the confined rotational crop metabolism study a field rotational crop study is not considered necessary. The RMS is of the opinion that a new rotational crop metabolism study should be requested, to investigate the nature of residues in rotational crops. Based on these results, it could be decided if a field rotational crop study is considered necessary.

2.7.8 Summary of other studies

2.7.8.1 Effects on the residue level in pollen and bee products

The evaluation of residues in pollen and bee products is not necessary since according to EU technical guidelines (SANTE/11956/2016 rev. 9) the representative crops under consideration (sugar beet, onion and garlic) are considered as not melliferous.

2.7.8.2 Extraction efficiency

An extraction efficiency study (S19-00144, Wiesner and Xu, 2020) was submitted as other study in section 6 of the dossier. The RMS evaluated this study, but it is presented in Vol.3, B.5.2.1 and also in 2.5 Methods of analysis of Vol. 1.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

The dietary exposure of clethodim and estimated risk for consumers has been calculated using the toxicological endpoints presented in 2.6.10.1 and 2.6.10.2. The ADI 0.16 mg/kg bw/day has been used. An acute reference dose (ARfD) is not deemed necessary. Furthermore, the STMRs and HRs (residue definition for monitoring) for the representative uses, and EFSA PRIMo rev 3.1 have been used to calculate the consumer exposure. Since the residue definitions are pending further assessment of the toxicological profile of metabolites, it is not possible to determine conversions factors. A tentative conversion factor of 2.5 according to residue definition for risk assessment 1 was applied, as derived from metabolism studies and also proposed by EFSA in the review of MRLs (EFSA, 2019). The input values for the consumer risk assessment can be found in table 2.7.9-1.

Table 2.7.9-1 Clethodim - Residue input values for the consumer risk assessment

Commodity	Chronic exposure		Acute exposure	
	STMR value (mg/kg)	Comment	HR value (mg/kg)	Comment
Risk assessment residue definition (RA1): Sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R and M18R/M19R, expressed as clethodim				
Onion (bulb)	0.025	$STMR_{Mo}$ x CF (2.5)	Not applicable, no ARfD allocated for clethodim	
Garlic	0.025	$STMR_{Mo}$ x CF (2.5)		
Sugar beet (root)	0.025	$STMR_{Mo}$ x CF (2.5)		

For clethodim, the TMDI is maximally 0.1% of the ADI (NL child), with sugar beet roots as the highest contributor, as presented in Annex 1 (Livestock dietary burden). This indicates that no chronic risk is expected for any of the European consumer groups. A calculation of the IESTI is not required, since no ARfD was considered necessary. However, it has to be emphasised that this consumer risk assessment is only provisional, pending the conclusion of the toxicological assessment of clethodim sulfone and a conclusion on the genotoxic potential for clethodim sulfone and the metabolites proposed to be included in the residue definition for risk assessment, M17R, M14R/15R and M18R/M19R.

The metabolite 3-chloroallyl alcohol (the aglycon of M14A/15A) is a common metabolite with the active substance 1,3-dichloropropene. Toxicity studies were evaluated for the metabolite 3-chloroallyl alcohol during the active substance approval of 1,3-dichloropropene (Spain, 2017 and EFSA Journal 2018;16(11):5464) but the evaluation was not finalised because the genotoxic potential of 3-chlorolallyl alcohol could not be concluded. New studies were performed and submitted and the current assessment found that it is not genotoxic. Therefore the following toxicological reference values could be proposed for 3-chloroallyl alcohol; ADI 0.015 mg/kg bw/day and ARfD 0.1 mg/kg bw. The input values for the consumer risk assessment can be found in table 2.7.9-2.

Table 2.7.9-2. M14A/M15A - Residue input values for the consumer risk assessment

Commodity	Chronic exposure		Acute exposure	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Risk assessment residue definition (RA2): M14A/M15A (3-chloroallyl alcohol glucoside)				
Onion (bulb)	0.02	LOQ x CF (0.05 x 0.36)	0.02	LOQ x CF (0.05 x 0.36)
Garlic	0.02	LOQ x CF (0.05 x 0.36)	0.02	LOQ x CF (0.05 x 0.36)
Sugar beet (root)	0.02	LOQ x CF (0.05 x 0.36)	0.02	LOQ x CF (0.05 x 0.36)

CF: Conversion factor: M14A/M15A (3-chloroallyl alcohol glucoside) to 3-chloroallyl alcohol (aglycone): MW (aglycon)/MW (glucoside) = 92.52 / 254.66 = 0.36

For M14A/M15A (3-chloroallyl alcohol glucoside), the TMDI is maximally 1% of the ADI (NL child), with sugar beet roots as the highest contributor, as presented in Annex 2 (PRIMo). For the acute exposure, the highest IESTI was 0.4% of the ARfD (onions, BE toddlers).

These results indicate that there is no unacceptable chronic or acute risk to human health from the consumption of commodities treated with clethodim according to the representative uses.

2.7.10 Proposed MRLs and compliance with existing MRLs

The current residue definition for monitoring is "Clethodim (sum of Sethoxydim and Clethodim including degradation products calculated as Sethoxydim)", but it is no longer considered relevant. Moreover, this definition differs from the proposed residue definition for monitoring, which is "Sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim" for both plant and animal commodities. These proposed residue definitions are pending the conclusion of the toxicological assessment of clethodim sulfone.

The EU MRLs for clethodim in sugar beet roots, onion bulbs and garlic were set at 0.5 mg/kg in Annex II of Commission Regulation (EC) No 839/2008 of 31 July 2008 amending Regulation (EC) 396/2005.

Sugar beet and bulb onions are both major crops in both northern and southern Europe, requiring eight residue trials in each zone to set an MRL. An overview of the results from available trials are presented in Table 2.7.10-1.

Table 2.7.10-1 Overview of the residue trials data relevant for MRL setting based on the critical GAP

Crop	Region/ Indoor[1)]	Results from supervised residue trials (mg/kg)	Calculated MRL (mg/kg)	HR (mg/kg)[2)]	STMR (mg/kg)[3)]	CF[4)]
Sugar beet roots	NEU	Mo.: 8x < 0.014	0.015*	0.01	0.01	n/a
		RA1.: 8x <0.06	-	0.06	0.06	2.5
		RA2.: 8x <0.05	-	0.05	0.05	-
	SEU	Mo.: 9x < 0.014	0.015*	0.01	0.01	n/a
		RA1.: 9x <0.06 #		0.06	0.06	2.5
		RA2.: 7x <0.05		0.05	0.05	-
Sugar beet tops	NEU	Mo.: 6x <0.014, 0.026, 0.033	(0.05)	0.033	0.01	n/a
		RA1.: 2x <0.06; 2x 0.07, 0.14, 0.21, 0.26, 0.31		0.31	0.11	3.5
		RA2.: 8x <0.05		0.05	0.05	-
	SEU	Mo.: 6x <0.014, 0.014, 0.016, 0.017	(0.03)	0.017	0.01	n/a
		RA1.: 6x <0.06; 0.07, 0.10, 0.21		0.21	0.06	3.5
		RA2.: 7x <0.05		0.05	0.05	-
Onion (bulb) Garlic (extrapolated from onion)	NEU	Mo.: 7x < 0.014; 0.014, 0.018, 0.023	0.03	0.023	0.01	n/a
		RA1.: 7x 0.06, 2x 0.06, 0.07		0.07	0.06	2.5
		RA2.: 10x <0.05		0.05	0.05	-
	SEU	Mo.: 9x < 0.014; 0.016	0.02	0.016	0.01	n/a
		RA1.: 9x <0.06, 0.06		0.06	0.06	2.5
		RA2.: 10x <0.05		0.05	0.05	-

1) NEU: Outdoor trials conducted in northern Europe, SEU: Outdoor trials conducted in southern Europe, Indoor: indoor EU trials or Country code: if non-EU trials

2) Highest residue (HR) according to the residue definition for risk assessment based on residue definition for risk assessment 1 (sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R and M18R/M19R, expressed as clethodim) and according to the residue definition for risk assessment based on residue definition for risk assessment 2 (M14A/M15A)

3) Supervised trials median residue (STMR) according to the residue definition for risk assessment based on residue definition for risk assessment 1 (sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R and M18R/M19R, expressed as clethodim) and according to the residue definition for risk assessment based on residue definition for risk assessment 2 (M14A/M15A)

4) Conversion factor (CF) for risk assessment according to EFSA (EFSA Journal 2019;17(5):5706) and results from metabolism studies. Tentative, since the residue definition is pending further toxicological assesment.
*: Indicates that the MRL is set at the limit of analytical quantification (LOQ)

EU MRLs for clethodim in animal commodities were set at 0.05* - 0.2 mg/kg in Annex II of Commission Regulation (EC) No 839/2008 of 31 July 2008 amending Regulation (EC) 396/2005. Based on the representative uses, no residues above the LOQ are expected in animal commodities.

2.7.11 Proposed import tolerances and compliance with existing import tolerances

Not applicable.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

2.8.1.1 Route of degradation in soil

For the renewal, a total of three studies are available investigating the degradation of radiolabelled clethodim in soil under aerobic conditions. In Mamouni, 2006a (A00426) and Pack, 1990 (MEF-0015/0016/8914823) both [ring-4,6-^{14}C] and [allyl-2-^{14}C]clethodim was studied in a total of four soils. In addition, in Pack, 1988a (MEF-0014/8721028), [Propyl-1-^{14}C]clethodim was studied in one soil. With regards to route of degradation, for the first approval there was a confirmatory data requirement to further assess the formation of the minor metabolite clethodim oxazole sulfoxide. This was addressed by Persch, 2012 (S12-00097) in which non-radio labelled clethodim was studied in three additional soils. All studies are considered acceptable for the route of degradation of clethodim under aerobic conditions. In Mamouni, 2006a (A00426), clethodim and its metabolites were extracted with acetonitrile/water (4:1; v/v) for 30 minutes by shaking at about 250 rpm, up to three times. An additional Soxhlet extraction using the same solvents under reflux for four hours released an additional 4.6-8.2%AR. In the other studies, the soils were extracted with methanol (4x) and two times with an aqueous $CaSO_4$ solution or with methanol/water (4:1, v/v) by shaking for 1 hour.

The proposed degradation pathway is shown in Figure 2.8.1.1-1. Under aerobic, non-sterile conditions the major pathway of transformation is the oxidation of sulphur in two steps through clethodim sulfoxide (max 73.4%AR; mean of replicates) and clethodim sulfone (max 42.2%; mean of replicates from non-radiolabelled; 33.3%AR; mean of replicates from radio-labelled) respectively and further to CO_2 (max 34-69% AR) and bound residues (max 20-55%AR). As a parallel minor pathway clethodim sulfone and clethodim sulfoxide also degrades via cyclisation and elimination of the allyl-group into clethodim oxazole sulfoxide (max 6.0%AR; mean of replicates) and clethodim oxazole sulfone (max 10.0%AR; mean of replicates). Found traces of other metabolites also shows that the clethodim sulfoxide and clethodim sulfone can degrade through clethodim sulfoxide imine, chloroallyl alcohol and chloroacrylic acid (CAA).

Clethodim oxazole sulfoxide was detected at 5%AR or above in Pack, 1988 (max 6.1% single replicate, 5% x 2), Pack 1990 (max 5.3%) and Mamouni, 2006a (A00426) (max 5.1%). In the non-radiolabelled study Persch, 2012, clethodim oxazole sulfoxide was not found above LOD in any of the three soils used. Nevertheless, for precautionary reasons this metabolite is considered further in the risk assessment.

In Pack, 1990, an unknown metabolite (peak 18), was observed with both labels, at individual max of 6.0% (day 60) and 6.2% (day 14) for the ring and allyl-label respectively. For the allyl-label this unknown metabolite was present at ≥5% at two consecutive time points (day 14 and day 30). This unknown metabolite was discussed already at the peer-review of the DAR, with reference to Report PRAPeR 32_02 clethodim_Fate where it was concluded that "[..]because newer studies are available that cover a wide enough range of soil conditions, in this case further requests on the unknown compound are not necessary. Also keeping in mind that the metabolite only occurred in one soil." The RMS considers this as a valid conclusion also for the current renewal.

In addition to the aerobic degradation in soil, one old study on photolysis on soil surface is available (Mamouni, 2006b; RCC Study number A00437). It showed an extensive and rapid degradation of radiolabelled clethodim. The ring label gave a low level of mineralisation (max 2.7%AR) and a high level of bound residues (max 73.5% AR). The allyl-label on the other hand showed higher level of mineralisation (max 40%AR) and a lower level of bound residues (max 31.1%AR). The ring-label showed one major metabolite, clethodim sulfoxide (max 53.7%AR). For, the allyl-label, the major metabolite formed was also clethodim sulfoxide (max 60.4%AR). In addition, two other metabolites were found >10%AR, being CAA (max 18.1%) and 2-[3-Chloroallyloxyimino] butanoic acid (CBA) (max 18.7%). These two metabolites where not detected in the studies on aerobic degradation in soil at levels requiring further assessment, but they need to be added to the residue definition for soil based on the findings in the photolysis on soil.

Furthermore, one old study on anaerobic degradation of ring-labelled clethodim in soil is available (Pack, 1998; MEF-0063 / 8819578). It included an aerobic phase for one day followed by an anaerobic phase for 62 days. Due to the rapid degradation of the parent during aerobic conditions, clethodim sulfoxide was the major metabolite formed at day 1 (max 81.8%). The degradation during the anaerobic phase was only assessed after 30 days and 62 days of incubation. After 30 days of anaerobic incubation two major metabolites >10%AR were detected, being clethodim imine (max 44.2%) and clethodim imine sulfoxide (max 15.2%). Given that clethodim imine was the major metabolite formed and as clethodim was already transformed into clethodim sulfoxide to a major extent during the aerobic phase it appears as if clethodim sulfoxide was reduced to clethodim during anoxic conditions which then was degraded through the imine pathway. Since, the anaerobic phase was only sampled after 30 days of anaerobic conditions, the relevance of these findings cannot be fully judged. Nevertheless, since the current renewal do not include autumn/winter applications as representative uses, these two major anaerobic metabolites has not been further considered.

In conclusion, the following soil metabolites need to be further considered:

- clethodim sulfoxide (max 73.4%)
- clethodim sulfone (max 42.2% from non-radiolabelled lab study)
- CBA (max 18.7% from photolysis on soil)
- CAA (max 18.1% from photolysis on soil)
- clethodim oxazole sulfone (max 10.0%)
- clethodim oxazole sulfoxide (max 6.0%)

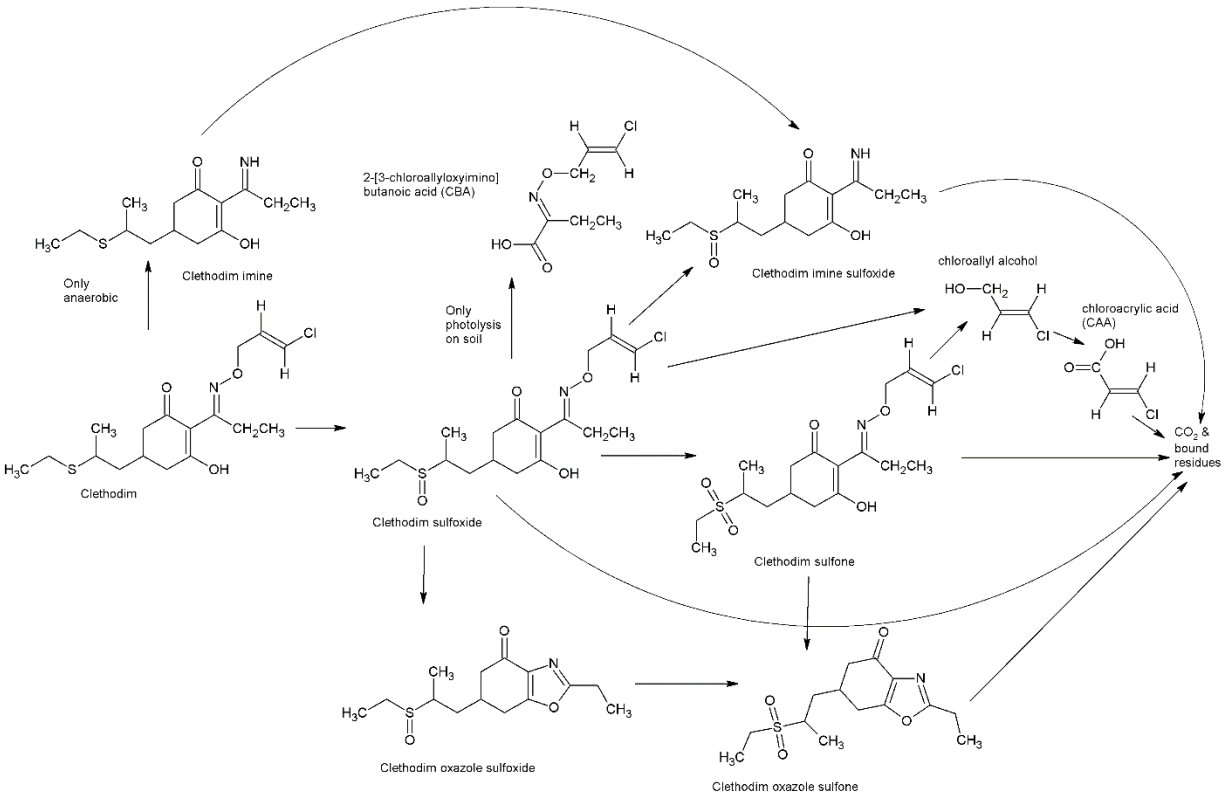


Figure 2.8.1.1-1. Proposed degradation pathway of clethodim in soil.

2.8.1.2 Rate of degradation in soil

For the rate of degradation of the parent compound in aerobic soil a total of four old studies are available and these were already discussed for the route of degradation. For the purpose of renewal, a kinetic re-evaluation performed in accordance with FOCUS Degradation kinetics report (2006, 2014) was provided (Jarvis & Jones, 2021; 1602214.UK0-4483). The model used for the kinetic evaluation was CAKE version 3.3. (IRLS). This new report supersedes the kinetic report provided for the first approval (Darriet *et al*, 2007). Acceptable trigger and modelling endpoints could be derived for the parent in all the studies except for Persch, 2012 (S12-00097) in which the data points were too few especially in the early phase of the degradation. From the remaining three studies, acceptable trigger and modelling endpoints could also be derived for the major metabolites clethodim sulfoxide and clethodim sulfone. Additionally, acceptable trigger and modelling endpoints were derived for the metabolite clethodim oxazole sulfoxide from Mamouni, 2006a (A00426) and Pack, 1988a (MEF-0014/8721028)

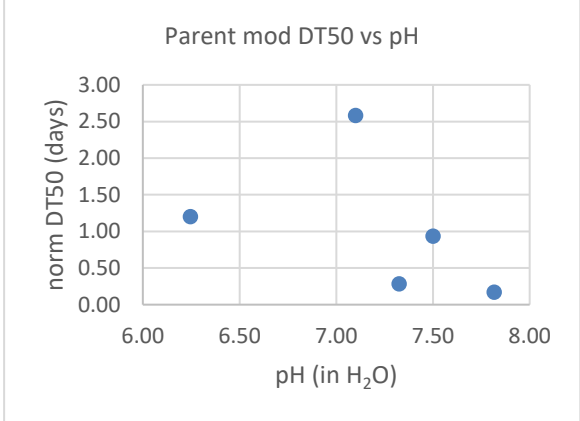
A total of four metabolite dosed studies are available. In Class, 2009 (B 1460 G), the degradation of non-radiolabelled clethodim oxazole sulfone was studied in three soils. The study was evaluated and accepted in the previous DAR, and it is deemed acceptable also for the renewal. The concentration of the metabolite was quantified using a validated LC-MS/MS method. The kinetic evaluation was performed within the study report using only SFO, but this was complemented by biphasic model runs done by RMS. In Turk, 2012 (13917.6136), the degradation of radiolabelled CBA was studied in three soils. This study was evaluated and deemed acceptable as confirmatory data for the previous approval, and it is considered acceptable also for the renewal. The remaining two studies are considered new for the purpose of renewal. In Schubert, 2016 (103391173), the degradation of radiolabelled CAA

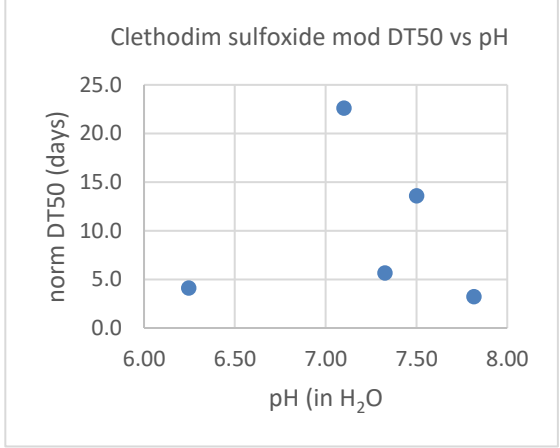
was studied in three soils. The kinetic evaluation was done within the study report, but it was re-evaluated by RMS to fully comply with the Focus Guidance (2006, 2011). The RMS notes that there are some indications of problems with the performance of the study which resulted in the total recovery being outside the recommended range (90-110%) on several occasions, probably due to loss of CO_2 and/or inhomogeneous distribution of non-extractable residues, and rather large scatter of the replicate data. Nevertheless, due to the very rapid degradation (mod DT50 0.1-0.3 days) the results are still considered acceptable. Finally, in Piskorsky, 2019 (20180095), the degradation of radiolabelled clethodim oxazole sulfoxide was studied in three soils. The kinetic evaluation was done within the study report in accordance with Focus guidance (2006, 2011). Biphasic models were selected as best fit model for trigger endpoints as well as for modelling endpoints, which was agreed by the RMS. In addition to the accepted endpoints for clethodim oxazole sulfoxide, which was derived from the parent only fitting, pathway fits were also performed with the best fit models for the downstream metabolite clethodim oxazole sulfone. This resulted in acceptable trigger and modelling endpoints for clethodim oxazole sulfone in two out of the three soils. In addition to the endpoints derived for CAA from the study submitted within this application, already peer-reviewed and agreed endpoints for this substance are available from EFSA conclusion/LoEP for (EZ)-1,3-dichloropropene (2018). All these endpoints are pooled together and a new geomean is calculated in agreement with the procedure previously used for common metabolites from several active substance (e.g. the sulfonyl ureas).

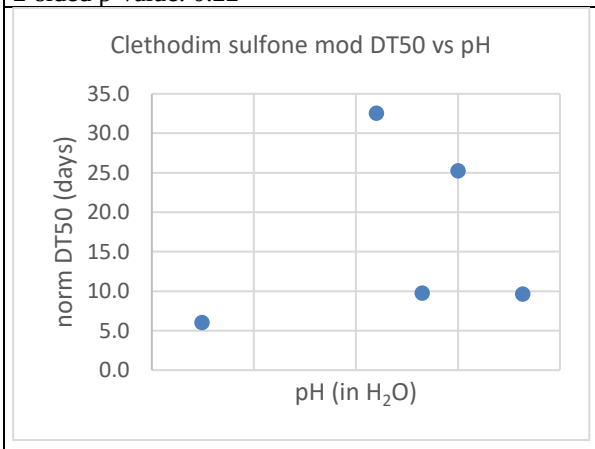
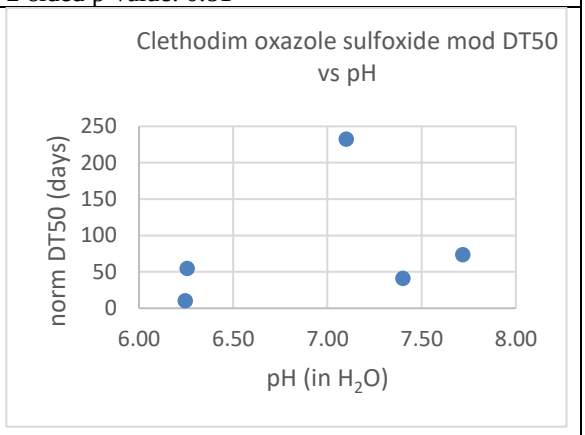
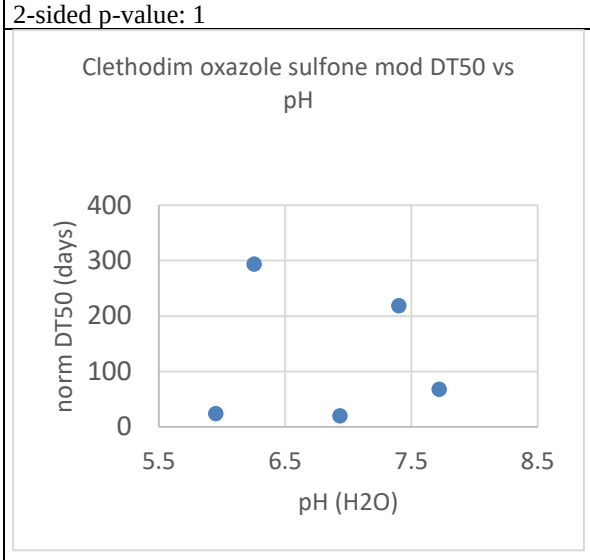
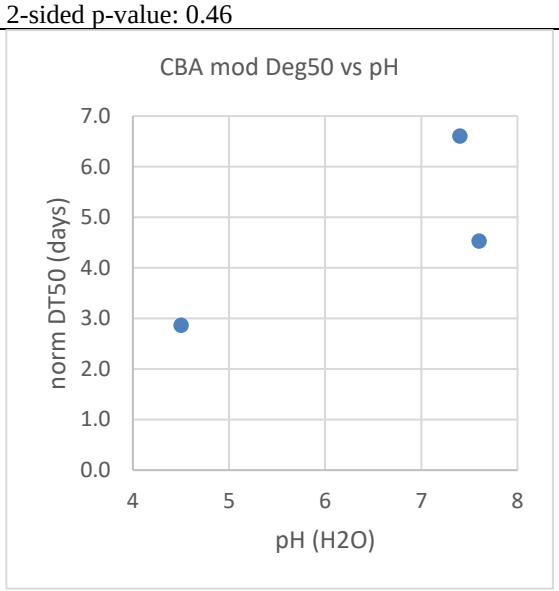
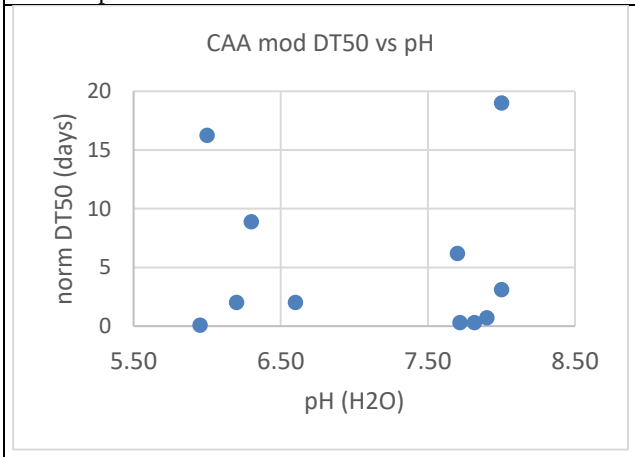
The derived persistence and modelling endpoints accepted by the RMS are summarised in Tables 2.8.1-1 through 2.8.1-7 below and the acceptable formation fractions are shown in Table 2.8.1-8. For reasons explained in Volume 3, not all the conclusions drawn by the author of the kinetic evaluation were agreed upon by the RMS.

pH dependency of degradation

Clethodim and its major metabolites, clethodim sulfoxide and clethodim sulfone, all contains the enol-group which could be deprotonated. The pKa for clethodim (see section 2.2) is 4.47 and it is likely that it is similar for the major metabolites. This pKa is at a relevant pH for acidic soils. Moreover, clethodim is shown to be prone to aqueous hydrolysis at pH 5 whereas it is stable at pH 7 and pH 9. Altogether, these factors could potentially lead to a pH-dependency of the degradation. In figure 2.8.1.2-1, the normalised DT50 are plotted against pH and the corresponding linear correlation coefficients and Kendall rank correlation coefficients are reported for each plot. As shown, no real conclusions can be made on the pH-dependency mainly because there is almost no data for acidic soils.





Correlation coefficient (r): -0.42 Kendall tau: -0.60 2-sided p-value: 0.22	Correlation coefficient (r): 0.04 Kendall tau: -0.20 2-sided p-value: 0.81
	
Correlation coefficient (r): 0.22 Kendall tau: 0 2-sided p-value: 1	Correlation coefficient (r): 0.29 Kendall tau: 0.40 2-sided p-value: 0.46
	
Correlation coefficient (r): -0.04 Kendall tau: 0 2-sided p-value: 1	Correlation coefficient (r): 0.80 Kendall tau: -* 2-sided p-value: -*
	
Correlation coefficient (r): -0.06	

Kendall tau: 0.09 2-sided p-value: 0.75	

Figure 2.8.1.2-1. Plots of norm DT50 vs pH for clethodim and its metabolites. * Not meaningful to run Kendall rank correlation since the number of data point was only 3.

Table 2.8.1.2-1. Summary of kinetic evaluation of laboratory data on aerobic degradation of clethodim in soil.

Study	Soil	pH (H_2O)**	best fit model	DT_{50}, days	DT_{90}, days	$\chi 2$, error-%	Modelling endpoint			
							model	factor to normalise moisture	factor to normalise temp	DT_{50}, days
Mamouni, 2006a (A00426)	Montesquieu	7.8	FOMC	0.24	2.4	22.3 (SFO) 17.2 (FOMC)	SFO (0.21 days)	0.80	1.0	0.17
	Mechtildhausen	7.3	SFO	0.36	1.2	12.1	SFO	0.79	1.0	0.28
	Speyer 2.2	6.3	FOMC	0.19	3.9	19.4	FOMC (DT90/ 3.32)	1.0	1.0	1.2
Pack, 1988a (MEF-0014/8721028)	Greenville*	7.1	SFO	2.5	8.4	5.9	SFO	0.64	1.61	2.6
Pack, 1990 (MEF-0015/0016/8914823)	Greenville*	7.5	SFO	1.1	3.6	4.8	SFO	0.53	1.61	0.9
Persch, 2012 (S12-00097)	Speyer 2.2	6.0	-***	-***	-***	-***	-***	-***	-***	-***
	Speyer 2.4	7.9	-***	-***	-***	-***	-***	-***	-***	-***
	Speyer 5M	7.9	-***	-***	-***	-***	-***	-***	-***	-***
Normalised DT_{50} correlation with pH (r):†		**-0.42**	**Not pH dependant**				**Geometric mean (n=5): (days)**			**0.54**
Worst case DT_{50} (persistence trigger): (days)			**2.5**							

* The characteristics of the Greenville soil in the two studies are significantly different and they are regarded as two different soils (i.e. they are directly included in the overall mean).
** pH values have been used as reported in case no media is defined or recalculated when reported as values in CaCl2 (i.e. according to eq. 3-9 in draft guidance on pH dependent degradation and adsorption in soil for groundwater leaching assessment, April 2021)
***Due to rapid degradation and few sampling points the RMS considers the data set too small to derive degradation endpoints from this study.

Table 2.8.1.2-2. Summary of kinetic evaluation of laboratory data on aerobic degradation of the metabolite clethodim sulfoxide in soil.

Study	Soil	pH (H_2O)**	best fit model	DT_{50}, days	DT_{90}, days	$\chi 2$, error-%	Modelling endpoint			
							model	factor to normalize moisture	factor to normalise temp	DT_{50}, days
Mamouni, 2006a (A00426)	Montesquieu	7.8	FOMC-SFO	3.4	11.3	20.6 (SFO-SFO) 17.2 (FOMC-SFO)	SFO-SFO (4.0 days)	0.80	1.0	3.2
	Mechtildhausen	7.3	SFO-SFO	7.2	24	10.5	SFO-SFO	0.79	1.0	5.7
	Speyer 2.2	6.3	DFOP-decline fit from max†	4.1	51.4	14.2	DFOP-decline fit from max†	1.0	1.0	4.1

Study	Soil	pH (H_2O)**	best fit model	DT_{50}, days	DT_{90}, days	χ2, error-%	Modelling endpoint			
							model	factor to normalize moisture	factor to normalise temp	DT_{50}, days
Pack, 1988a (MEF-0014/8721028)	Greenville*	7.1	SFO-SFO	21.9	72.7	7.8	SFO-SFO	0.64	1.61	22.6
Pack, 1990 (MEF-0015/0016/8914823)	Greenville*	7.5	SFO-SFO	16	53	4.0	SFO-SFO	0.53	1.61	13.6
Persch, 2012 (S12-00097)	Speyer 2.2	6.0	-***	-***	-***	-***	-***	-***	-***	-***
	Speyer 2.4	7.9	-***	-***	-***	-***	-***	-***	-***	-***
	Speyer 5M	7.9	-***	-***	-***	-***	-***	-***	-***	-***
Normalised DT_{50} correlation with pH (r):		**0.04**	**Not pH dependant**				**Geometric mean (n=5): (days)**			**7.4**
Worst case DT_{50} (persistence trigger): (days)			**21.9**							

*: The characteristics of the Greenville soil in the two studies are significantly different and they are regarded as two different soils (i.e. they are directly included in the overall mean).
** pH values have been used as reported in case no media is defined or recalculated when reported as values in $CaCl_2$ (i.e. according to eq. 3-9 in draft guidance on pH dependent degradation and adsorption in soil for groundwater leaching assessment, April 2021).
*** Due to rapid degradation and few sampling points the RMS considers the data set too small to derive degradation endpoints from this study.
† Decline fit performed by RMS since pathway fit resulted in poor visual fit and a significant overestimation of the degradation

Table 2.8.1.2-3. Summary of kinetic evaluation of laboratory data on aerobic degradation of the metabolite clethodim sulfone in soil.

Study	Soil	pH (H_2O)**	best fit model	DT_{50}, days	DT_{90}, days	χ2, error-%	Modelling endpoint			
							model	factor to normalise moisture	factor to normalise temp	DT_{50}, days
Mamouni, 2006a (A00426)	Montesquieu	7.8	FOMC-SFO	12.1	40.1	31.0 (SFO-SFO) 30.2 (FOMC-SFO)	SFO-SFO (12 days)	0.80	1.0	9.6
	Mechtildhausen	7.3	SFO-SFO	12.4	41.1	7.4	SFO-SFO	0.79	1.0	9.7
	Speyer 2.2	6.3	SFO-decline fit from max†	6.0	19.9	25.5	SFO-decline fit from max†	1.0	1.0	6.0
Pack, 1988a (MEF-0014/8721028)	Greenville*	7.1**	SFO-SFO	31.5	105	29.5	SFO-SFO	0.64	1.61	32.5
Pack, 1990 (MEF-0015/0016/8914823)	Greenville*	7.5**	SFO-SFO	29.7	98.7	19.2	SFO-SFO	0.53	1.61	25.2
Persch, 2012 (S12-00097)	Speyer 2.2	6.0	-***	-***	-***	-***	-***	-***	-***	-***
	Speyer 2.4	7.9	-***	-***	-***	-***	-***	-***	-***	-***
	Speyer 5M	7.9	-***	-***	-***	-***	-***	-***	-***	-***
Normalised DT_{50} correlation with pH (r):		**0.22**	**Not pH dependant**				**Geometric mean (n=5): (days)**			**13.6**
Worst case DT_{50} (persistence trigger): (days)			**31.5**							

*: The characteristics of the Greenville soil in the two studies are significantly different and they are regarded as two different soils (i.e. they are directly included in the overall mean).

** pH values have been used as reported in case no media is defined or recalculated when reported as values in $CaCl_2$ (i.e. according to eq. 3-9 in draft guidance on pH dependent degradation and adsorption in soil for groundwater leaching assessment, April 2021).
*** Due to rapid degradation and few sampling points the RMS considers the data set too small to derive degradation endpoints from this study.
† Decline fit performed by RMS since pathway fit resulted in poor visual fit and a significant overestimation of the degradation

Table 2.8.1.2-4. Summary of kinetic evaluation of laboratory data on aerobic degradation of the metabolite clethodim oxazole sulfoxide in soil.

Study	Soil	pH (H_2O) *	best fit model	DT_{50}, days	DT_{90}, days	$\chi 2$, error -%	Modelling endpoint			
							model	factor to normalise moisture	factor to normalise temp	DT_{50}, days
Mamouni, 2006a (A00426)	Montesquieu	7.8	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d.
	Mechtildhausen	7.3	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d.
	Speyer 2.2	6.3	FOMC - decline fit from max†	10.2	126	6.2	FOMC- decline fit from max†	1.0	1.0	10.2
Pack, 1988a (MEF-0014/8721028)	Greenville	7.1	SFO-SFO	225	747	16.4	SFO-SFO	0.64	1.61	232
Pack, 1990 (MEF-0015/0016/8914823)	Greenville	7.5	-**	-**	-**	-**	-**	-**	-**	-**
Persch, 2012 (S12-00097)	Speyer 2.2	6.0	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d
	Speyer 2.4	7.9	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d
	Speyer 5M	7.9	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d
Piskorski, 2019 (20180095) Applied as parent	South Witham	7.4	DFOP	3.1	71	1.7	DFOP (k=0.017)	1.0	1.0	40.8
	RefSol-01-A-05	6.3	DFOP	5.6	158	4.7	DFOP (k=0.008)	0.63	1.0	54.5
	Speyer 6S	7.7	DFOP	27.2	272	3.1	DFOP (k=0.006)	0.64	1.0	73.4
Normalised DT_{50} correlation with pH (r):		**0.29**	**Not pH dependant**				**Geometric mean (n=5): (days)**			**52.2**
Worst case DT_{50} (persistence trigger): (days)			**225**							

* pH values have been used as reported in case no media is defined or recalculated when reported as values in $CaCl_2$ (i.e. according to eq. 3-9 in draft guidance on pH dependent degradation and adsorption in soil for groundwater leaching assessment, April 2021).
**: The RMS did not accept the fit as it significantly overestimates the degradation.
† Decline fit performed by RMS since pathway fit resulted in poor visual fit and a significant overestimation of the degradation
†† Since only decline fit was accepted, no modelling endpoint can be determined.
n.d.: metabolite not detected in the data set

Table 2.8.1.2-5. Summary of kinetic evaluation of laboratory data on aerobic degradation of the metabolite clethodim oxazole sulfone in soil.

Study	Soil	pH (H_2O)*	best fit model	DT_{50}, days	DT_{90}, days	$\chi 2$, error-%	Modelling endpoint			
							model	factor to normalise moisture	factor to normalise temp	DT_{50}, days
Mamouni, 2006a (A00426)	Montesquieu	7.8	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d.
	Mechtildhausen	7.3	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d.
	Speyer 2.2	6.3	-**	-**	-**	-**	-**	-**	-**	-**
Pack, 1988a (MEF-0014/8721028)	Greenville	7.1*	-***	-***	-***	-***	-***	-***	-***	-***

Study	Soil	pH (H_2O)*	best fit model	DT_{50}, days	DT_{90}, days	χ^2, error-%	Modelling endpoint			
							model	factor to normalise moisture	factor to normalise temp	DT_{50}, days
Pack, 1990 (MEF-0015/0016/8914823)	Greenville	7.5*	-***	-***	-***	-***	-***	-***	-***	-***
Persch, 2012 (S12-00097)	Speyer 2.2	6.0	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d
	Speyer 2.4	7.9	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d
	Speyer 5M	7.9	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d	n.d
Piskorski, 2019 (20180095) Applied as clethodim oxazole sulfoxide	South Witham	7.4	DFOP-SFO	219	729	6.2	DFOP-SFO	1.0	1.0	219
	RefSol-01-A-05	6.3	DFOP-SFO	468	1550	5.4	DFOP-SFO	0.63	1.0	294
	Speyer 6S	7.7	-***	-***	-***	-***	-***	-***	-***	-***
Class, 2009 (B 1460 G) Applied as parent	LUFA 2.3	6.9	SFO	20	66	8.5	SFO	0.85	1.0	17
	LUFA 2.2	6.0	SFO	24	79	6.4	SFO	1.0	1.0	24
	LUFA 6S	7.7	SFO	68	227	7.3	SFO	0.61****	1.0	41 ****
Normalised DT_{50} correlation with pH (r):		**-0.04**	**Not pH dependant**				**Geometric mean (n=4): (days)**			**64.1**
Worst case DT_{50} (persistence trigger): (days)			**468**							

* pH values have been used as reported in case no media is defined or recalculated when reported as values in $CaCl_2$ (i.e. according to eq. 3-9 in draft guidance on pH dependent degradation and adsorption in soil for groundwater leaching assessment, April 2021).
**: Neither trigger nor modelling endpoints could be determined since the decline phase was not reached and as the pathway fit for the precursors were not accepted.
*** The fit was not statistically acceptable
**** Please see RMS comments to the normalised DT50 41 d in Vol 3 CA, B.8.1.1.4.
n.d.: metabolite either not detected or at too low levels in the data set

Table 2.8.1.2-6. Summary of kinetic evaluation of laboratory data on aerobic degradation of the metabolite 2-[3-chloroallyloxyimino] butanoic acid (CBA) in soil.

Study	Soil	pH (H_2O)	best fit model	DT_{50}, days	DT_{90}, days	χ^2, error-%	Modelling endpoint			
							model	factor to normalize moisture	factor to normalise temp	DT_{50}, days
Turk, 2012 (13917.6136) Applied as parent	A1 UK soil	4.5	SFO	4.4	14.5	8.2	SFO	0.65	1.0	2.9
	Horn soil	7.4	SFO	6.7	22.4	6.5	SFO	0.99	1.0	6.6
	Sevelen soil	7.6	SFO	4.8	15.8	10	SFO	0.94	1.0	4.5
Normalised DT_{50} correlation with pH (r):		**0.80***	**Potentially pH dependant***				**Geometric mean (n=3): (days)**			**4.4***
Worst case DT_{50} (persistence trigger): (days)			**6.7**							

* Only three data points are available so the pH -dependency cannot be fully evaluated. Since the metabolite is an acid a pH-dependency can possibly be expected. Nevertheless, since the difference in the longest norm DT50 and the geomean is rather small, the choice of input for modelling is not anticipated to have a significant impact on the modelling outcome.

Table 2.8.1.2-7. Summary of kinetic evaluation of laboratory data on aerobic degradation of the metabolite trans-3-chloroacrylic acid (CAA) in soil.

Study	Soil	pH (H_2O)*	best fit model	DT_{50}, days	DT_{90}, days	χ^2, error-%	Modelling endpoint			
							model	factor to normalize moisture	factor to normalise temp	DT_{50}, days
Schubert, 2016 (103391173) Applied as parent	LUFA 2.2	6.0	HS	0.1	1.4	16.2 (SFO) 10.4 (HS)	SFO (0.14)	1.0	1.0	0.1
	LUFA 2.4	7.8	SFO	0.3	0.8	18.2	SFO	1.0	1.0	0.3
	LUFA 6S	7.7	SFO	0.3	1.1	13.1	SFO	1.0	1.0	0.3
Already peer-reviewed values taken from the EFSA conclusion on active substance (EZ)-1,3-dichloropropene										
Applied as (EZ)-1,3-dichloropropene	Marcham soil (M585, UK)	7.7	SFO-SFO	6.2	20.6	28.2	SFO-SFO	-	-	6.2

Study	Soil	pH (H_2O)*	best fit model	DT_{50}, days	DT_{90}, days	$\chi 2$, error-%	Modelling endpoint			
							model	factor to normalize moisture	factor to normalise temp	DT_{50}, days
	Charently soil (M584, France)	6.3	SFO-SFO	10.1	33.61	20.9	SFO-SFO	-	-	8.9
	Thessaloniki soil (M583, Greece)	8.0	SFO-SFO	19	63.1	24.7	SFO-SFO	-	-	19
	Cuckney soil (M579, UK)	6.0	SFO-SFO	18.6	61.7	23.4	SFO-SFO	-	-	16.3
Applied as (EZ)-3-chloroallyl alcohol.	Marcham soil (M622, UK)	7.9	SFO-SFO	0.7	2.4	13	SFO-SFO	-	-	0.7
	Charently soil (M608, France)	6.2	SFO-SFO	2.0	6.7	22.2	SFO-SFO	-	-	2.0
	Thessaloniki soil (M607, Greece)	8.0	SFO-SFO	3.1	10.3	17	SFO-SFO	-	-	3.1
	Cuckney soil (M609, UK)	6.6	SFO-SFO	2.0	6.8	21	SFO-SFO	-	-	2.0
Normalised DT_{50} correlation with pH (r):		**-0.06**	**Not pH dependant**				**Geometric mean (n=11): (days)**			**1.9**
Worst case DT_{50} (persistence trigger): (days)			**19**							

* pH values have been used as reported in case no media is defined or recalculated when reported as values in $CaCl_2$ (i.e. according to eq. 3-9 in draft guidance on pH dependent degradation and adsorption in soil for groundwater leaching assessment, April 2021).

Table 2.8.1.2-8. Summary of formation fractions from kinetic modelling of clethodim and its metabolites.

Soil	Formation fraction estimates in each soil				
	clethodim→ clethodim sulfoxide	Clethodim sulfoxide → clethodim sulfone	Clethodim sulfoxide → Clethodim oxazole sulfoxide	Clethodim sulfone→Clethodim oxazole sulfone	Clethodim oxazole sulfoxide→clethodim oxazole sulfone
Montesquieu	0.83	0.64	-	-	-
Mechtildhausen	0.89	0.63	-	-	-
Speyer 2.2	-	-	-	-	-
Greenville, Pack, 1988a	0.80	0.27	0.09	0.34	-
Greenville, Pack, 1990	0.83	0.39	0.12	-	-
Speyer 2.2	-	-	-	-	-
Speyer 2.4	-	-	-	-	-
Speyer 5M	-	-	-	-	-
South Witham	-	-	-	-	0.68
RefSol-01-A-05	-	-	-	-	0.83
Speyer 6S	-	-	-	-	0.92
Formation fraction for PEC modelling Arithmetic mean	**0.84 (n=4)**	**0.48 (n=4)**	**0.11 (n=2)**	**0.34 (n=1)**	**0.81 (n=3)**

-: The formation fraction is not acceptable, or the metabolite was not formed at sufficient levels

The study on the degradation of radiolabelled clethodim under anaerobic conditions (Pack, 1988b; MEF-0063 / 8819578) could not be used to calculate rates due to the rapid degradation during the aerobic phase (first day).

The soil photolysis study (Mamouni, 2006b; RCC Study number A00437), gave a net irradiation degradation half-live of 0.15 days (corresponding to 0.7 days of natural sunlight at latitudes 30-40°N) for clethodim which is not significantly different to the half-lives determined for aerobic degradation in soil. However, in this specific study a half-life of 2.7 days was determined for the dark control, which indicates that soil photolysis may have an influence on the degradation in soil.

No acceptable field dissipation studies are available (for further details on previous submitted non-acceptable studies; see Volume 3 Annex B.8 (AS)) and the applicant did not consider this requirement to be triggered (i.e. they state that all laboratory trigger DT_{50} values are <60 days for clethodim and metabolites). However, since the normalised lab DT50, at 20 C and pF 2.0 is >60 days for the metabolites clethodim oxazole sulfoxide and clethodim oxazole sulfone in one or more soils, field dissipation studies are set as a data gap.

2.8.1.3 Assessment in relation to the P-criteria for soil

The criteria for persistence in soil, as stated in Annex II to Reg (EC) 1107/2009, are DT_{50} 120 days (PBT) and 180 days (POP and vPvB). It is assumed that these criteria represent a constant rate of degradation over the decline curve, i.e. that single first order (SFO) kinetics has been assumed implicitly when the criteria were defined. Therefore, to allow a comparison against the criteria also for results derived by other kinetic models than SFO it is considered appropriate to divide FOMC/DFOP DT_{90}s by 3.32 in agreement with the DG SANCO Working Document (2012)[1]. The resulting trigger values used in the comparison are shown in the table below:

Table 2.8.1.3-1. Summary of trigger DT_{50} for clethodim used in the comparison with the P-criteria

Soil	Model	DT_{90}/3.32	DT_{50}
Montesquieu	FOMC	0.7	-
Mechtildhausen	SFO	-	0.4
Speyer 2.2	FOMC	1.2	-
Greenville Pack, 1988a	SFO	-	2.6
Greenville Pack, 1990	SFO	-	1.1

The aerobic degradation of clethodim in soil is very rapid and it is concluded that it does not fulfil any of the criteria for persistence (PBT/ vPvB /POP).

With regards to the main metabolites, it should be stressed that the criteria only apply to the parent substance. However, the comparison itself give useful information on the persistence of metabolites and it is thus presented below. The major metabolites clethodim sulfoxide and clethodim sulfone had acceptable DT50's derived from laboratory data in five soils, of which none exceeds the criterion for PBT/ vPvB /POP. For the minor metabolite clethodim oxazole sulfoxide trigger values were available from two soils from parent dosed studies, where the DT50's where 38 (DT90/3.32) and 225 days respectively. In addition, DT50's for three soils from metabolites dosed studies were available where criteria for PBT/ vPvB /POP were not met for any of the soils. In conclusion, the persistence of this metabolite can be seen as low to moderate. For the minor metabolite clethodim oxazole sulfone, DT50's from, two soils from parent dosed studies and an additional three soils from metabolite dosed studies are available. The data from the parent dosed studies gave DT50's in the range of 219-468 days which clearly exceeded the criterion for PBT and vPvB/POP. On the other hand, the data from the metabolite dosed studies gave DT50's in the range of 20-68 days, which indicate that the endpoints derived from the parent dosed studies may be over conservative. The metabolite CBA, only found in significant amounts in the soil photolysis studies, had DT50's from three soils from a metabolite dosed study. It showed a rapid degradation (DT50's in the range of 4.4-6.7 days) and the metabolite does not fulfil the criteria for PBT/ vPvB /POP. Finally, for CAA (also an exclusive soil

[1] DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides", Brussels, 25.09.2012 – rev. 3

photolysis metabolite), data from a total of 11 soils are available, which gave DT50's in the range of 0.3-19 days. Consequently, the criteria for PBT/ vPvB /POP are not met for this metabolite either.

2.8.1.4 Adsorption to soil

Batch experiments to determine the potential for adsorption to soils were available for clethodim, and the major metabolites clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone. For metabolite CAA (3-chloroacrylic acid), which is a common metabolite with (EZ)-1,3-dichloropropene, the already peer-reviewed data as presented in EFSA Conclusion (2018) are reported here. They were not further discussed.

Some of the studies were submitted to support the first approval of the parent compound (Völkel 2006 a; A00448), and it was previously evaluated in the AR (2007), or in the DAR 2005, or in Addendum to the DAR 2006. Some of the studies on single metabolites were also evaluated before (Völkel 2006 b–g, report numbers A58667, A32815, A58680, A32826, A58691, A34391; Kang 2012, report number 13917.6137). Some studies on metabolites were never evaluated before (Lee, 2021 a, b, report numbers AU-2019-28, AU-2019-29A; Völkel 2022_amendment of Beyer, 2018, report number 20180079; Völkel, 2022 a, b, report numbers 20210247, 20200587).

The results from the adsorption experiments are summarized in the following tables. All data are for room temperature. All are isotherm K_F and $K_{F,OC}$ and are derived for the reference concentration 1 mg/L and in the units liter water per kg dry weight (L_w/kg_{dw}) or liter water per kg organic carbon (L_w/kg_{OC}). Our results may differ marginally from those presented by the Applicant, mainly due to rounding off and number of significant digits used for the calculations. For some studies however, the result from individual soils were considered as unreliable, and the RMS has not included these in Volume 1 and LoEP. Main reasons for exclusion were too low mass balance, and/or too low stability. The RMS quantified these factors using the criteria set in EFSA (2017)[2], for instance the RMS investigated if the K_F × soil:solution ratio was higher than 0.3 and the "Boesten ratio" $K_{F,E}$ / K_F was lower than 1.2. If these criteria were not met, and the indirect method was used, the RMS generally excluded the soil from the data set.

All studies used the OECD 106 batch experiments and covered a sufficient number of soils with a range of properties (texture). All studies also covered a sufficiently large range in concentrations (typically a factor 100), although the absolute concentration in some studies were high (up to 10–12 mg/L), which possibly challenges the scope of the guideline (OECD 106 §5) to investigate environmental conditions, since for toxic pesticides such high concentrations are never expected under the uses applied for (GAP). The RMS notes that 10–12 mg/L is high enough to increase dissolved organic carbon (DOC)[3] in the test system, and in some cases also the pH. The RMS also questions if a sufficient range for pH soil is included in the studies, but we also note that this is a generic issue for all PPP-dossiers, and not a specific concern for the clethodim dossier.

[2] EFSA (European Food Safety Authority), 2017. Technical report on the outcome of the pesticides peer review meeting on the OECD 106 evaluators checklist. EFSA supporting publication 2017:EN-1326. 18 pp. doi:10.2903/sp.efsa.2017.EN-1326

[3] The RMS also note that the particle separation methods are different in the studies, some uses ultracentrifugation. This creates different supernatants, with lower amount of colloids after ultracentrifugation (OECD 106 §6, and Annex 5). Modern research methods for studying sorption makes use of passive samplers for determining the dissolved phase in a more consistent manner (e.g. Jahnke et al., Environ. Sci . Technol. 46, 2012).

An analysis of the potential pH dependency of sorption was not available in any of the study reports, and consistently, pKa values were never reported in spite of the recommendation in OECD 106, §13f. Where possible, RMS tried to investigate the influence of soil pH. We looked for correlations of $^{ads}K_{F,OC}$ versus pH (but not log $^{ads}K_{F,OC}$ versus pH), we plotted the data and looked for any type of relationship (linear, sigmoidal, U-shaped or inverted U). For the acid CBA the RMS also speculated in what pKa the test items may have, based on comparison to known values for analogue compounds.

For the active substance, clethodim, there is one single study (Völkel 2006 a; A00448). The RMS found that a linear negative relationship of $K_{F,OC}$ versus pH was significant (p = 0.004, R^2 = 0.99, Nsoils = 4, Excel AddIn Analysis ToolPak, regression). The $K_{F,OC}$ increased from 7–17 L_w/kg_{OC} at pH 7 up to 161–203 L_w/kg_{OC} at pH 5, in essence at least by a factor 9. The RMS therefore propose to divide the sorption data into two groups soils (N = 2 at low pH, and N = 2 at high).

For adsorption at normal pH the K_F at 1 mg/L was 0.222 and 0.288, with a geomean of 0.253 (0.194–0.329) $L_w/kg_{dw,}$ (confidence interval of geomean). The PPP-procedure (from e.g. prosulfocarb) is to use worst case (lowest) instead of average when only two points are available, hence value to use is 0.222 L_w/kg_{dw}. The $K_{F,OC}$ was 6.98 and 17.3 with a geomean of 11.0 (8.45–14.3) L_w/kg_{OC} (confidence interval of geomean), and its lowest of the two points to use is thereby 6.98 L_w/kg_{OC}. Adsorption was non-linear so the arithmetic mean "1/n" of 0.95 should be used for the Freundlich non-linearity constant at normal pH.

For adsorption at low pH the K_F at 1 mg/L was 4.07 and 3.70, with a geomean of 3.88 (2.67–5.63) $L_w/kg_{dw,}$ (confidence interval of geomean). PPP-procedure (from e.g. prosulfocarb) is to use worst case (lowest) instead of average when only two points are available, hence value to use is 3.70 L_w/kg_{dw}. The $K_{F,OC}$ was 203 and 161 with a geomean of 181 (125–262) L_w/kg_{OC} (confidence interval of geomean), and its lowest of the two points to use is thereby 161 L_w/kg_{OC}. Adsorption was linear (confidence interval of the slope overlapped 1) so the arithmetic mean "1/n" of 1.00 should be used for the Freundlich non-linearity constant at low pH.

As a result, the $K_{F,OC}$ for the group normal soil pH is a factor 23 higher than for the low soil pH.

Table 2.8.1.4-1. Clethodim: Adsorption Freundlich isotherm, Freundlich exponent ("1/n"), Kd and organic carbon normalised Kd.

Clethodim							
Soil Type (b)	**OC %**	**Soil pH (a)**	**K_d (mL/g)**	**K_{doc} (mL/g)**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**
sandy loam	2.30	5.60			3.70	161	0.999
Loam	1.28	7.37			0.222	17.3	0.931
clay loam	4.13	7.55			0.288	6.98	0.964
silt loam	2.00	5.36			4.07	203	1.034
Geometric mean (if not pH dependent)					(0.721)	(44.6)	
Arithmetic mean (if not pH dependent)							(0.982)
pH dependence, Yes			Low pH K_{Foc} = 161, 1/n = 1.00, N = 2 Normal pH K_{Foc} = 6.98, 1/n = 0.95, N = 2				

(a) Measured in 0.01 M calcium chloride solution
(b) All soils from (Völkel 2006 a; A00448)

As indicated above, data from the EFSA conclusion of 1,3-dichloropropene (2018) are presented in Table 2.8.1.4-2.

Table 2.8.1.4-2: Kfoc values for 3-chloroacrylic acid, already peer-reviewed and agreed, taken from the EFSA conclusion of the active substance (EZ)-1,3-dichloropropene (2018).

3-CAA							
Soil Type	**OC %**	**Soil pH[a)]**	**K_d (mL/g)**	**K_{doc} (mL/g)**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**
Sandy Loam (Bertie County/COBB)	0.66	5.9	0.115	17.5	0.106	16.1	0.883
Clay loam (Grand Forks/M536)	4.76	6	<0.01	<0.01	NC	NC	NC
Loamy sand Wake County/M537)	0.41	6	0.0518	12.6	0.0409	9.97	0.872
Silty clay loam (Charentilly/M547)	1.07	6.3	<0.01	<0.01	<0.00278	0.259	0.426
Loam (Fresno, M528)	0.81	7	0.00887	1.10	<0.0129	0.16	0.961
Silt Loam (Thessaloniki/M546)	1	7.9	0.0200	1.99	<0.0241	2.41	1.18
Clay (Faringdon/M549)	3.22	7.9	<0.01	<0.01	NC	NC	NC
Sandy clay loam (Marcham/M548)	1.25	8	<0.01	<0.01	NC	NC	NC
Silt loam, M504	0.9	8.2	0.00691	0.767	<0.0143	1.6	0.907
Geometric mean (n=6)					-	**1.72**	
Arithmetic mean (n=6)							**0.872**
pH dependence			slight pH dependence *[slightly higher at higher pH]*				

[a] medium not stated

Regarding the metabolite clethodim sulfoxide, there are three studies (Völkel 2006 b,c, reports number A58667, A32815, and Völkel 2022 b, report number 20200587), of which two studies presents the isotherm for $K_{F,oc}$ (the third A32815 is just pre-studies). The RMS decided to exclude Völkel 2006 b,c (A58667, A32815), because the quality criteria indicate very uncertain data ($K_{F,E}/K_F$ ratios are 2.3–4.7, and the Efsa Checklist recommend caution for values above 1.2. Plotting data for the two isotherm studies together indicates a systematic difference, with significantly higher Koc values in Völkel 2006 b (A58667), since the confidence interval for the points never overlap (Figure 2.8.1.4-1). The RMS interpret this as if degraded substance is erroneously counted as sorbed. Therefore, the RMS propose (Table 2.8.1.4-3) that sorption data for clethodim sulfoxide should be based exclusively on Völkel 2022 b (20200587).

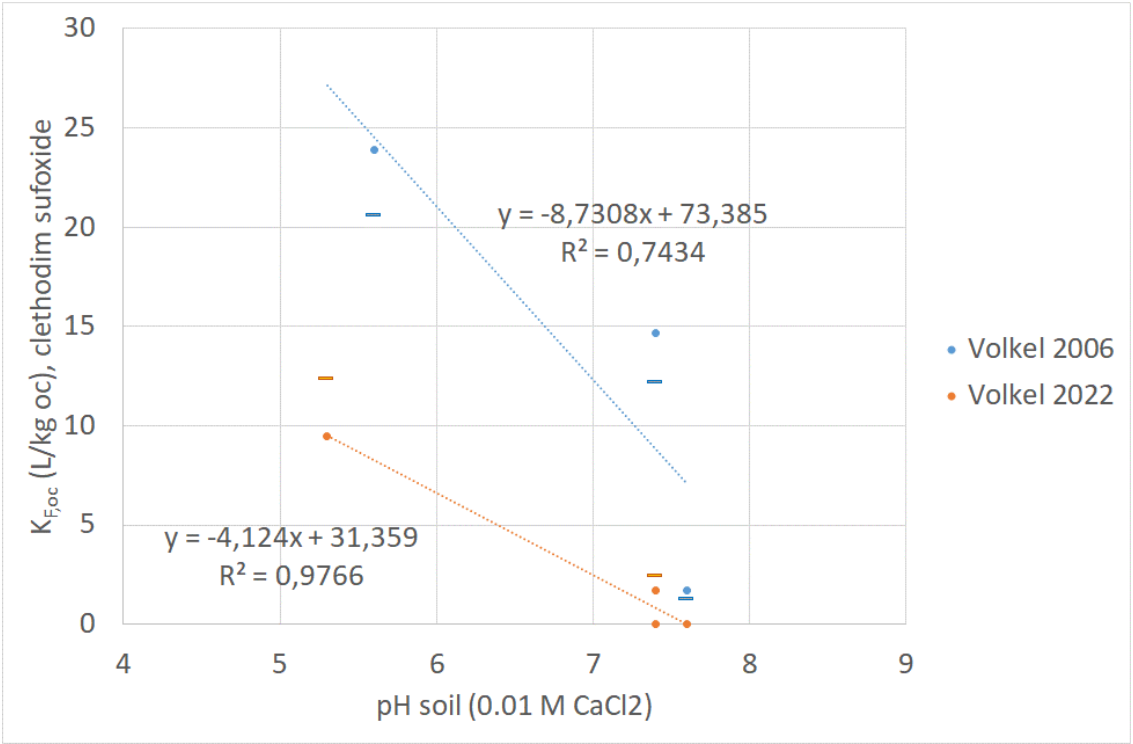


Figure 2.8.1.4-1. $K_{F,oc}$ for clethodim sulfoxide from two isotherm studies. Lower 95% confidence limit for Völkel 2006 (A58667) blue dash, upper confidence limit for Völkel 2022 (UPL/2021/0339) red dash.

Table 2.8.1.4-3: Clethodim sulfoxide: Adsorption Freundlich isotherm, Freundlich exponent ("1/n"), Kd and organic carbon normalised Kd.

Clethodim sulfoxide							
Soil Type	**OC %**	**Soil pH (a)**	**K_d (mL/g)**	**K_{doc} (mL/g)**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**
Clay (St Bauzille) d	1.4	7.4			0	0	-
Sandy loam (Longwood) d	1.2	7.6			0	0	-
Clay (Sout Witham) d	2.8	7.4			0.049	1.7	1.292
Loam (Kenslow) d	3.6	5.3			0.342	9.5	0.956
Geometric mean (if not pH dependent)					(0.129)b	(4.1)b	
Arithmetic mean (if not pH dependent)							1.00 c
pH dependence, Yes			Low pH K_{Foc} = 9.5, 1/n = 1.00, N = 1 Normal pH K_{Foc} = 1.2, 1/n = 1.00, N = 3				

(a) Measured in 0.01 M calcium chloride solution
(b) Geomean for the two sorption isotherms which were above zero
(c) RMS recommend setting 1/n equal to one, due to lack of robust evidence of concentration dependency
(d) Völkel 2022 (UPL/2021/0339)

Regarding the metabolite clethodim sulfone, there are three studies (Völkel 2006 d,e, report numbers A58680, A32826, and Lee 2021 a, report number AU-2019-28), of which two studies presents the isotherm for $K_{F,oc}$ (A32826 is only pre-studies). The RMS propose to use all four soils from Lee 2021 a (AU-2019-28), and one soil from Völkel 2006d (A58680). For these five soils, $K_{F,OC}$ did not have statistically significant (p = 0.056, R^2 = 0.75, N= 5, Excel AddIn Analysis ToolPak, regression) linear correlation on soil pH. However, the RMS still propose to assume $K_{F,OC}$ was pH-dependent. Primarily because the regression should better have been made in relation to the expected sigmoidal relation, which is not how we did it (we do not know pKa). pKa/b values should be possible to derive using QSAR-tools. Therefore the RMS presents $K_{F,OC}$ grouped into low pH and normal pH. For the low pH group, there are only two values, and the PPP-agreement (from e.g. prosulfocarb) is to use worst case (lowest) instead of average when only two points are available, hence $K_{F,OC}$ value to use is 15.9 L_w/kg oc.

Table 2.8.1.4-4: Clethodim sulfone: Adsorption Freundlich isotherm, Freundlich exponent ("1/n"), Kd and organic carbon normalised Kd.

Clethodim sulfone							
Soil Type	**OC %**	**Soil pH (a)**	**K_d (mL/g)**	**K_{doc} (mL/g)**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**
Sandy loam (Longwoods) (b)	1.6	7.5			0.132	8.3	1.16
Clay (St Bauzille) (b)	1.4	7.4			0.159	11	1.04
Loam (Kenslow) (b)	3.6	5.3			0.704	24	0.828
Clay (South Witham) (b)	2.8	7.4			0.0376	1.3	0.785
Sandy loam (Speyer 2.2) (c)	2.3	5.6			0.366	15.9	0.774
Geometric mean (if not pH dependent)					(0.183)	(8.5)	
Arithmetic mean (if not pH dependent)							0.92
pH dependence, Yes			Low pH K_{Foc} = 15.9, 1/n = 1.00, N = 2 Normal pH K_{Foc} = 4.9 1/n = 1.00, N = 3				

(a) Measured in 0.01 M calcium chloride solution
(b) Lee 2021 a (AU-2019-28)
(c) Völkel 2006d (A58680)

Regarding the metabolite clethodim oxazole sulfone, there are three studies (Völkel 2006 f, g, study numbers A58691, A34391, and Lee 2021 b, study number AU-2019-29A), of which two studies presents the isotherm for $K_{F,OC}$ (A34391is only pre-studies). The RMS propose to use all four soils from Lee 2021 b (AU-2019-29A), but none from Völkel 2006 f, g (A58691, A34391) due to low mass-balances and non-convincing data on stability (low stability and sparse chromatographic information). For the four Lee soils (AU-2019-29A), $K_{F,OC}$ did not have statistically significant linear correlation on soil pH (p = 0.31, R^2 = 0.48, N= 4, Excel AddIn Analysis ToolPak,

regression), but considering the many functional groups, many of which has potential to change the molecules charge and hydrophobicity with pH (e.g. the oxazole group may be basic). The RMS proposes to consider pH-dependency as being established, and therefore to group sorption data into the two groups low pH and normal pH. This gives $K_{F,OC}$ groups which differs by a factor 2.3 (230% higher at normal pH). For the normal pH the slope of the isotherm should be set to one, since the confidence interval for the arithmetic mean of the three slopes did overlap with 1. For the acidic pH, there is only one isotherm, and its slope was significantly lower than 1, so we propose to use its arithmetic mean (0.918). For the normal pH, we used the geometric mean for $K_{F,OC}$ (96.3 L_w/kg_{OC}).

Table 2.8.1.4-5: Clethodim oxazole sulfone: Adsorption Freundlich isotherm, Freundlich exponent ("1/n"), Kd and organic carbon normalised Kd.

Clethodim oxazole sulfone							
Soil Type	**OC %**	**Soil pH (a)**	**K_d (mL/g)**	**K_{doc} (mL/g)**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**
Sandy loam (Longwoods) (b)	1.6	7.5			0.9878	61.6	0.964
Clay (St Bauzille) (b)	1.4	7.4			1.736	124	0.996
Loam (Kenslow) (b)	3.6	5.3			1.518	42.2	0.918
Clay (South Witham) (b)	2.8	7.4			2.283	117	0.958
Geometric mean (if not pH dependent)					(1.630)	(86.2)	
Arithmetic mean (if not pH dependent)							(0.989)
pH dependence, Yes			Low pH K_{Foc} = 42.2, 1/n = 0.918, N = 1 Normal pH K_{Foc} = 96.3, 1/n = 1.00, N = 3				

(a) Measured in 0.01 M calcium chloride solution
(b) Lee 2021 b, AU-2019-29A

Regarding the metabolite clethodim oxazole sulfoxide, there is one study on soil sorption isotherm (Beyer 2018, study number 20180079). The RMS propose to use all three soils in that study. For the three Beyer soils, $K_{F,OC}$ did not have statistically significant linear correlation on soil pH (p = 0.14, R^2 = 0.95, N= 3, Excel AddIn Analysis ToolPak, regression), but the RMS do see a weak pH dependency (higher $K_{F,OC}$ at lower pH), but in the studied pH range, the $K_{F,OC}$ only differs by 20%. In the absence of more datapoints and a pKa value, the RMS therefore propose to use averaged sorption isotherm values for the three soils.

Table 2.8.1.4-6: Clethodim oxazole sulfoxide: Adsorption Freundlich isotherm, Freundlich exponent ("1/n"), Kd and organic carbon normalised Kd.

Clethodim oxazole sulfoxide							
Soil Type	**OC %**	**Soil pH (a)**	**K_d (mL/g)**	**K_{doc} (mL/g)**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**
Sandy clay loam (Sout Witham) (b)	2.8	7.4			2.956	105.6	0.934
Clay (Stenson) (b)	3.8	5.8			4.761	125.3	0.947
Silt loam (Am Fischteish) (b)	1.6	5.2			2.020	126.3	0.920
Geometric mean (if not pH dependent)					3.05	118.6	
Arithmetic mean (if not pH dependent)							0.934
pH dependence, No			-				

(a) Measured in 0.01 M calcium chloride solution
(b) Beyer 2018, 20180079

Regarding the metabolite CBA, there are two studies (Kang, 2012 study number 13917.6137 and Völkel, 2022a study number 20210247). Both uses 14-labelled test item, but Kang does not fulfil many critical quality criteria (stability is not convincingly demonstrated, detection limits are not presented, new appearing chromatographic peaks are not discussed, R2 for isotherm regression are too low, slope of isotherm is extraordinary low, and in two of three soils the check "Kd × soil:solution ratio" is too low for indirect method to be acceptable). The RMS therefore solely uses the data from Völkel 2022a (20210247). For the four soils, $K_{F,OC}$ did have statistically significant linear correlation with soil pH (p = 0.002, R2 = 0.995, N= 4, Excel AddIn Analysis ToolPak, regression), and RMS sees a strong pH dependency (higher $K_{F,OC}$ at lower pH), in the studied pH range. A literature pKa value for a similar

compound, the unsubstituted butanoic acid is around 4.6, so the RMS therefore propose that the negative linear relation in the range reflects a change in test item composition, with a higher share of hydrophobic neutral CBA-species at lower pH, giving stronger hydrophobic interaction with the organic matter, and more anionic species at higher pH, which are less hydrophobic, and also repels from the soils natural organic matter which may be slightly negatively charged in this pH range. The RMS therefore proposes to consider pH-dependency as fully established, and to group sorption data into the two groups low pH and normal pH. This gives $K_{F,OC}$ groups which differs by a factor 5.3 (530% higher at low pH).

Table 2.8.1.4-7: CBA: Adsorption Freundlich isotherm, Freundlich exponent ("1/n"), Kd and organic carbon normalised Kd.

CBA							
Soil Type (b)	**OC %**	**Soil pH (a)**	**K_d (mL/g)**	**K_{doc} (mL/g)**	**K_F (mL/g)**	**K_{Foc} (mL/g)**	**1/n**
Clay (St Bauzille)	1.4	7.4			0.049	3.5	1.14
Sandy loam (Longwoods)	1.2	7.6			0.019	1.6	0.89
Clay (South Witham)	2.8	7.4			0.074	2.7	1.03
Loam (Kenslow)	3.6	5.3			0.493	14	0.96
Geometric mean (if not pH dependent)					(0.076)	(3.8)	
Arithmetic mean (if not pH dependent)							(1.01)
pH dependence, Yes			Low pH K_{Foc} = 14 1/n = 1.00, N = 1 Normal pH K_{Foc} = 2.62, 1/n = 1.00, N = 3				

(a) Measured in 0.01 M calcium chloride solution
(b) All from Völkel, 2022 study number 20210247

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

This section has been written both to present degradation data necessary for comparison with the CLP criteria and to fulfil the requirements under Regulation (EC) No 1107/2009. The comparison with the CLP criteria is presented in section 2.9.2.4.2 (Long-term aquatic hazard (including bioaccumulation potential and degradation)).

One new study was provided on aerobic mineralisation of clethodim in surface water to comply with new data requirements. Additionally, one aqueous photolysis study from the open literature was provided as supportive information. All other studies and data points covering the degradation of clethodim in the aquatic environment were covered with studies submitted and accepted during the Annex I inclusion. These studies are still considered valid by the RMS SE. Degradation kinetics for clethodim and its metabolites in the water/sediment system were evaluated according to FOCUS DegKinetics Report (2006, 2014).

The proposed route of degradation in water is shown in the figure below. The RMS has modified the applicant's proposal slightly to include all available information (see Vol. 3, CA, B.8.2): additional metabolites found in some studies are added with a blue rectangular frame, blue arrows are added for additional pathways and the metabolites that are clearly only formed as photolytic degradation products or from biodegradation processes are marked with yellow or green dashed frames, respectively.

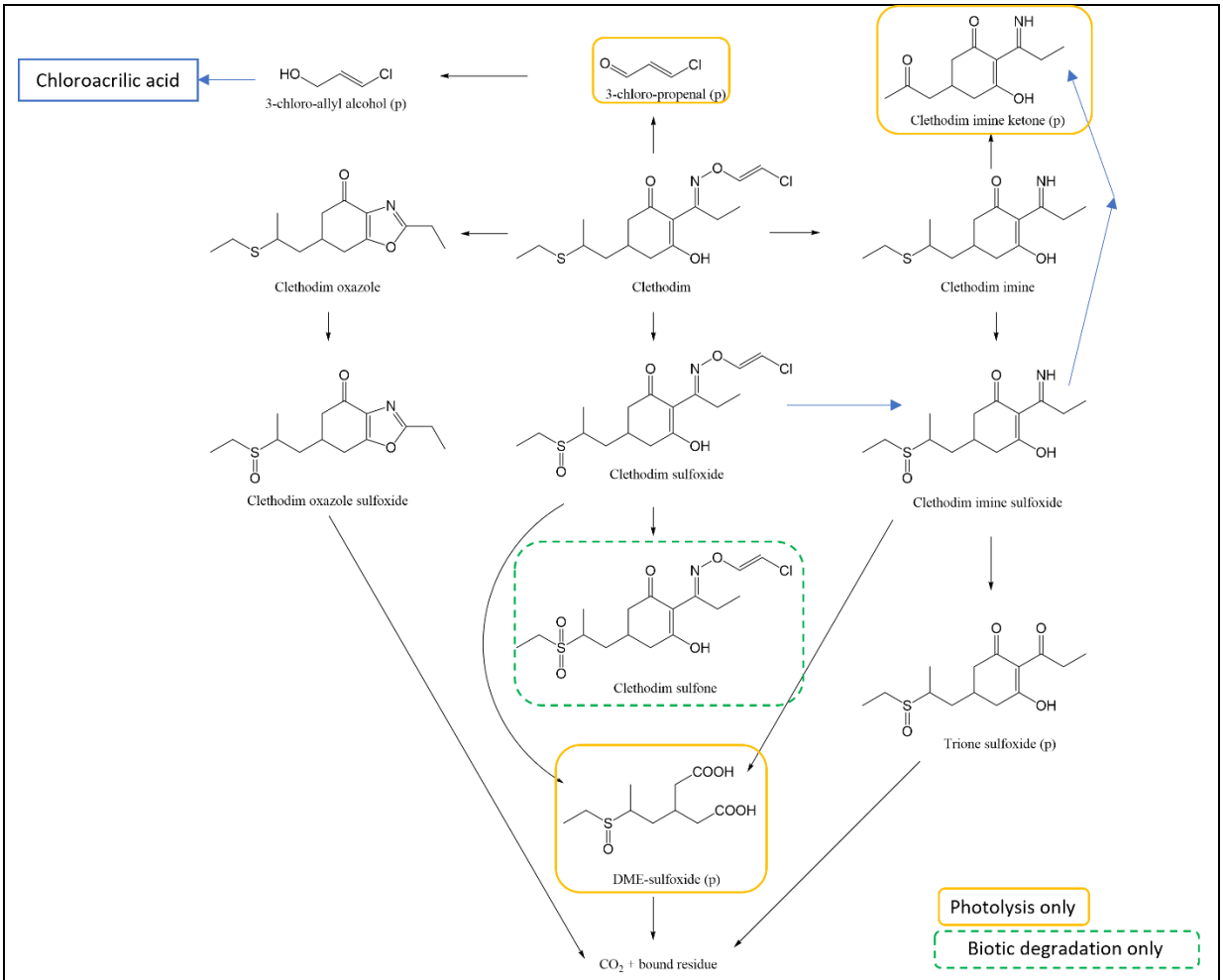


Figure 2.8.2-1. Proposed degradation pathway of clethodim in water. The scheme was taken from the notifier but modified by the RMS to include all available information on the route of degradation in water.

2.8.2.1 Rapid degradability of organic substances

An overview of all studies that are considered relevant for the aquatic compartment are summarised in the table below. The studies are further presented in the sections that follow. See Vol. 3, B.8.2 (CA) for additional information.

Table 68. Summary of relevant information on rapid degradability.

Method	Results	Key or Supportive study	Remarks	Reference
Hydrolysis Guideline: US EPA N:161-1, OECD TG No. 111 Deviations: None that would invalidate the study: sterility not confirmed at the end of the study, no tests at	Clethodim hydrolysed under acidic conditions (pH 5) at environmentally relevant temperatures (25 °C), with estimated half-lives of 28-54 days. Clethodim corresponded to 43.1% AR (propyl-labelled) and 64.9% AR (allyl labelled) at the end of the study at 25 ± 0.1 °C. Two major metabolites were formed, oxazole (max. 50.5% AR after 32 days, propyl-labelled) and chloroallyl-alcohol (max. 30.7% AR after 30 days, allyl-labelled).	Key study Study considered acceptable for evaluation of hydrolytic degradation	Radiolabelled Clethodim (propyl & allyl label). Test concentration: 5 mg/L (propyl-^{14}C-clethodim) and 10 mg/L (allyl-^{14}C-clethodim). Endpoints: route of degradation and major metabolites (>10% AR). Validity criteria met?: yes, apart from that	Pack, 1988c Report no. MEF-0013 / 8703899 Vol. 3 CA B.8.2.1.1

Method	Results	Key or Supportive study	Remarks	Reference
pH 4, only at pH 5, nor at higher temperatures, only at 25 °C, were performed.	At neutral or higher pH at 25 °C, clethodim was stable to hydrolysis. At pH 7 and 9, at the end of the study at 25 ± 0.1 °C, clethodim corresponded to 91% AR (propyl-labelled, both pH) and 93.4% AR (allyl-labelled, pH 7). Mineralization was not measured. Studies at higher temperatures were not performed.		sterility was not confirmed at the end of the study. However, comparison with studies under aerobic suggests that other metabolites would have been formed if biodegradation would have occurred (mainly clethodim sulfoxide and clethodim sulfone).	
Aqueous photolysis Guideline: U.S. EPA N:161-2 OECD TG No. 316 Deviations: Shorter test duration for most studies, but an irradiated 30-day study at pH 7 was performed fulfilling the recommendation in OECD TG No 316. Daily sunlight exposure was 10 hours instead of 12 hours as recommended in OECD TG No. 316.	Clethodim was photochemically degraded under natural sunlight (10-hour daily exposure with average intensity of 20.3 kW/cm^2) at all tested pH-levels (pH 5, 7, 9) with estimated effective photolysis-DT50s of 1.7, 6.8 and 9.6 days at pH 5, 7, and 9 at 25 ± 1 °C. Sensitization enhanced the degradation. Several metabolites were formed at > 10% AR: DME sulfoxide (RE-52453), imine sulfoxide (RE-47718), clethodim sulfoxide (RE-45924), imine (RE-47686), and imine ketone. Mineralization was very low (CO_2 was <1 % AR in all samples). Quantum yield was not determined.	Key study Study considered acceptable	Radiolabelled Clethodim [ring-4,6-^{14}C] Test concentration: 10 mg/L Endpoints: route of degradation and major metabolites (>10% AR). Validity criteria met?: yes.	Chen, 1988a Report no. MEF-0024 Vol 3CA B.8.2.1.2
Aqueous photolysis Guideline: U.S. EPA N:161-2 OECD TG No. 316 Deviations: Shorter test duration for most studies, but an irradiated 30-day study at pH 7 was performed fulfilling the recommendation in OECD TG No 316. Daily	Clethodim was photochemically degraded under natural sunlight (10-hour daily exposure with average intensity of 20.5 kW/cm^2) at all tested pH-levels (pH 5, 7, 9) with estimated effective photolysis-DT50s of 1.5, 4.1 and 6.0 days at pH 5, 7, and 9, and at 25 ± 1 °C. Sensitization enhanced the degradation. Several metabolites were formed at > 10% AR: clethodim sulfoxide (RE-45924), chloroallyl alcohol and 3-chloropropenal. Mineralization reached 24.8% AR (sum of CO_2 trapped and in solution) after 30 days under natural sunlight at pH 7. Quantum yield was not determined.	Key study Study considered acceptable.	Radiolabelled Clethodim [allyl-2-^{14}C] Test concentration: 10 mg/L Endpoints: route of degradation and major metabolites (>10% AR). Validity criteria met?: yes.	Chen, 1988b Report no. MEF-0025 Vol 3CA B.8.2.1.2

Method	Results	Key or Supportive study	Remarks	Reference
sunlight exposure was 10 hours instead of 12 hours as recommended in OECD TG No. 316				
Aqueous photolysis published literature	Clethodim was rapidly degraded under simulated sunlight (250-750 Wm^{-2}) and natural sunlight with half-lives below 5 hours. Clethodim in a formulation degraded even quicker with DT50s <1.5h. Some mineralization occurred (not quantified in the study, authors only state that clethodim was not completely mineralized). Four metabolites were identified: clethodim imine, clethodim sulfoxides, clethodim imine sulfoxide and clethodim imine ketone	Supportive	Non-radiolabelled clethodim analytical standard and clethodim in formulation (Centurion Plus, 120 g/L) Test concentration 5 mg/L	Villaverde (2018), published literature Vol 3CA B.8.2.1.2
Ready biodegradability <u>Guideline:</u> OECD 301D EEC guidance C.4-E (closed bottle test) <u>Deviations:</u> None.	The biodegradation of technical clethodim was high with 55.9% of ThOD after 7 days and 133-138% ThOD measured after 14-28 days. The reference substance (sodium benzoate) was degraded by 72.5% ThOD after 7 days and 104-131% thereafter (DAT 14-28). No inhibitory effect in the mixture of reference substance and clethodim was observed. It was concluded that clethodim is readily biodegradable.	Key study The study was considered acceptable.	The high degradation rates (>100 %) for both clethodim and the reference substance were explained by a high bacterial density in the inoculum that caused a high oxygen consumption and self-digestion of the inoculum. This was also used to explain the oxygen depletion in the blank control, which exceeded the maximum values given in OECD 301D. All other validity criteria of the tests were fulfilled.	Dengler (2002), Report no. 20011424/01-AACB Vol 3CA B.8.2.2.1
Aerobic Mineralisation in surface water <u>Guideline:</u> OECD 309 <u>Deviations:</u> Uncertainty in the mass balance of individual samples.	Mineralization in the pelagic test was low with 6% AR at low test concentration (9.1 µg/L) and 1.4% AR at high test concentration (90.6 µg/L). Half-lives of 14.9 and 23.5 days were calculated for low and high test concentration. 87.3% AR of the reference substance (benzoic acid) was mineralized after 11 days, indicating a valid test. Clethodim sulfoxide was formed reaching average maximum of 86.5% at low concentration and 77.3 at high concentration. No other individual metabolite was detected >10% AR.	Key study Study considered acceptable.	Radiolabelled (ring labelled) Uncertainty in the mass balance of individual samples in the test with low test concentration at day 21 and 28 are not considered to invalidate the study. No other deviations were noted.	Irmer (2020), Report no. S17-08723 Vol 3CA B.8.2.2.2
Aerobic water/sediment <u>Guideline:</u>	Clethodim dissipated from the water phase from ca 92% AR at day 0 to < 2% after 42 days (allyl) or 56 days (ring), mainly due to degradation. The	Key study	Radiolabelled (ring- and allyl) Test concentration:	Mamouni (2006c), Report no. A00450

Method	Results	Key or Supportive study	Remarks	Reference
OECD 308 EPA N, 162-4 <u>Deviations:</u> None that would invalidate the study. Study duration was longer than 100 days in the ring test, and only single samples were taken in the allyl-test.	maximum observed in sediment was 12% AR after 7 days, which also decreased in all test systems to < 2 % AR after 90 days. SFO DegT50 of clethodim in total water/sediment-system was 9.2 days (river) and 13.1 days (pond), resulting in a geomean of 11.0 days. Several metabolites were detected that classify as 'major metabolites' in the total system, i.e. >10% AR or >5% at two consecutive sampling dates: Clethodim sulfoxide, clethodim sulfone, clethodim imine, clethodim imine sulfoxide as well as an unknown, non-characterised metabolite M20. Major metabolites in the water phase: clethodim sulfoxide (>10% AR), clethodim sulfone (>10% AR) and clethodim imine sulfoxide. Major metabolites in the sediment phase: Clethodim sulfoxide, clethodim imine (>10% AR), clethodim imine sulfoxide, M20 (if characterised as one substance). DT50s were estimated with pathway-fits for all metabolites in agreement with relevant guidance on kinetic assessments, resulting in half-lives of 19-52 days and, thus, more persistent in aquatic environments than the parent. DT50s in the total system > 40 days were obtained for clethodim imine (51 days), and clethodim sulfone (52 days). No degradation rates were estimated for the unknown metabolite M20. Significant mineralization occurred in both pond and river system, with a maximum of 43.7 % AR formed in the pond test system after 90 days (allyl-labelled experiment).	Study considered acceptable.	0.06 mg/L River and pond water test systems were used with aerobic conditions in water and anaerobic conditions in sediment. Temperature: 20 ± 2 °C. No duplicated samples were taken in the experiments with the allyl-labelled clethodim. The results were pooled with the results from the ring-labelled study for estimating half-lives. Thus, for that purpose, sufficient data points were available.	Vol 3CA, B.8.2.2.3 Lee & Jarvis (2020), Report no. 1602214.UK0-5305 B.8.2.2.3
Aerobic water/sediment <u>Guideline:</u> BBA, part IV, 5-1 (1990) <u>Deviations from OECD 308:</u> Acclimation phase of 60 days, exceeding the guideline stipulated 4 weeks. LOD/LOQ not reported.	Clethodim dissipated from the water phase from 70.5% AR at day 0 to < 1% after 121 days, mainly due to degradation. Hardly any clethodim was detected in sediment (max. 2.4% after 103 days, not-detected after 121 days). SFO DegT50 of clethodim in total water/sediment-system was 22.1 days. Three metabolites were identified as major in total system, i.e. occurring at >10% AR or >5% at two consecutive sampling dates: clethodim sulfoxide, clethodim imine sulfoxide and clethodim imine. The two sulfoxide compounds were also classified as major in water, while the two imine compounds were classified as major in sediment.	Key study Study considered acceptable for classification purpose and for exposure assessments with the parent only. Study also considered acceptable for exposure assessment of the major metabolites based on	Radiolabelled (ring) Test concentration: 0.077 mg/L Pond water test systems were used with aerobic conditions in water and anaerobic conditions in sediment. Temperature: 20 ± 2 °C. Concentration of clethodim at day 0 is only 70%, suggesting uncertainty in the assessment and handling of the samples. However, degradation	Heintze (1998, amended 2005). Report no. 97245/01-CUWS Vol 3CA, B.8.2.2.3

Method	Results	Key or Supportive study	Remarks	Reference
	Mineralization reached a maximum of 15.4 % AR after 121 days (and 18.3% AR after 196 d).	maximum occurrences, but not acceptable for exposure assessments with parent and metabolite together.	rate estimates may still be acceptable for classification purposes considering that the estimates will be rather conservative. The too long acclimation phase does not seem to have invalidated the results, as the sediment showed good viability after 120 days and the physico-chemical parameters measured before and after the study were unchanged.	

2.8.2.1.1 Ready biodegradability

One study on ready biodegradability was available (Dengler, 2002, Report no. 20011424/01-AACB). The study was conducted in accordance with OECD 301D (closed bottle test). The reference substance (sodium benzoate) was significantly degraded within 7 days (72.5% ThOD). Also, high degradation of technical clethodim was observed during the first 7 days, with 55.9% ThOD. For both substances, clethodim and sodium benzoate, degradation increased to >100% ThOD after 14-28 days. This was correlated to a high cell number and high bacterial density used in the inoculum, resulting in a high oxygen consumption and self-digestion of the inoculum causing the exceedance of the theoretical oxygen demand. This may indicate that the amount of inoculum (0.1 mL/L) was rather high for the batch of effluent used, despite being within the limits given in the guideline (0.05-5 mL/L). The oxygen depletion in the blank control exceeded 1.5 mg dissolved oxygen/L after 28 days. This was explained by the above discussed high bacterial density, which is seen as a further investigation of the experimental techniques. All other validity criteria were fulfilled, i.e. no inhibitory effect was seen in the tests with a mixture of reference substance and clethodim, and the residual concentrations of oxygen in the test bottles were above the minimum value indicated in the test guideline.

The study was already available for the first approval. It is still deemed to be acceptable, despite the high oxygen depletion as the overall test results were consistent and all other validity criteria fulfilled. The conclusion of the study is that clethodim is readily biodegradable.

2.8.2.1.2 BOD_5/COD

No BOD_5/COD test was available.

2.8.2.2 Other convincing scientific evidence

Relevant data on abiotic degradation were available (hydrolysis, see 2.8.2.2.5, and aquatic photolysis, see 2.8.2.2.6).

Other data of relevance for classification and labelling were one study on biodegradation in surface water (see 2.8.2.2.1) and two studies on biodegradation in water/sediment systems (see 2.8.2.2.4). Additionally, studies on biodegradation in soil were available (see 2.8.1.1 and 2.8.1.2).

2.8.2.2.1 Aquatic simulation tests

Irmer (2020), Report no. S17-08723, investigated the rate of mineralization of [ring-4,6, 14C]-clethodim in surface water with a pelagic test according to OECD TG no. 309. The two concentrations (9.1 µg/L and 90.6 µg/L) were in line with recommendations in the guideline. Mineralization was low with a maximum of 6.0 % AR at low test concentration and 1.4% at the high test concentrations after 68 and 60 days, respectively. The overall mass balances were acceptable, even if individual samples at the low test concentration were outside the range of 90-110 % AR. Clethodim degraded during the course of the study with no detection at the end of the study at low test concentration and 10% AR in the high test concentration. Half-lives (SFO) were estimated to be 14.9 days at low test concentration and 23.9 days at high test concentration, in accordance with FOCUS DegKinetics (2006, 2014).

Clethodim sulfoxide was the main metabolite, reaching average maximum values of 86.5 % AR (low concentration, 14 days) and 77% AR (high concentration, 60 days). One unknown metabolite (M5) was detected >10 % AR in both low and high test concentration, but further characterised as consisting of 17 components, none exceeding 5% AR. After 11 days, 87.3% AR of the reference substance, benzoic acid, was mineralized, indicating that the test was valid.

A chiral analysis of clethodim was performed for all samples were clethodim was detected. It was concluded that E-clethodim is dominating and that the R/S-ratio for E-clethodim was constant and 1:1 throughout the study duration. For the Z-isomer of clethodim, the R:S ratio was less constant, but as the overall concentrations were low (max. 5.6% at low test concentration in 2 samples, otherwise <2%), the analysis of the R:S ratio must be considered uncertain. Please refer to section 2.13, and in particular section 2.13.6 for a further discussion on the isomeric composition of clethodim and this study.

The study was provided for the purpose of renewal and deemed acceptable.

Two studies were available that investigated the route and rate of clethodim in aerobic water/sediment systems; Mamouni (2006c), Report no. A00450, and Heintze (1998, amended 2005, Report no. 97245/01-CUWS). Both studies were available already for the previous approval. For the purpose of renewal, a kinetic re-assessment was performed following FOCUS DegKinetics (2006, 2014) by Lee & Jarvis (2020), Report no. 1602214.UK0-5305. Mamouni (2006c), Report no. A00450, included ring- and allyl-labelled clethodim in its study and used river (River Rhine) and pond (Pond Möhlin) test systems at 20 °C. In Heintze (1998, 2005, Report no. 97245/01-CUWS), ring-labelled clethodim was investigated in a pond system at 20 °C (denoted as 'Pond II' in the summary table for clethodim below). Mineralization was significant in all experiments, with a maximum of 32.3 % AR reached after 174 days in the river test system (Mamouni, 2006c), Report no. A00450. Radioactivity in the water phase decreased continuously throughout the study (from 99% AR to 15%-30% AR after 90-120 days, Mamouni, 2006c, Report no. A00450), while radioactivity increased in the sediment phase reaching around 36-54 % AR in sediment after 120 days. In Heintze (1998, 2005, Report no. 97245/01-CUWS), clethodim was only present at roughly 70% in the sample at day 0. The remaining radioactivity was identified as its metabolite clethodim sulfoxide. In both studies,

clethodim dissipated, mainly via degradation, from the water phase, while only small quantities of clethodim were detected in the sediment (maximum of 12 % AR after 7 days in Mamouni (2006c), Report no. A00450, max. 2.4% in Heintze, 1998, 2005, Report no. 97245/01-CUWS). DT50s were estimated to 9-13 days in Mamouni (2006c), Report no. A00450. In Heintze (1998, 2005, Report no. 97245/01-CUWS), a half-live of 22 days was estimated, which is probably relatively slower due to the uncertainty of the measurements at day 0.

Several major metabolites were identified: Clethodim sulfoxide and Clethodim sulfone (both mainly in water phase), Clethodim imine (mainly in sediment), Clethodim imine sulfoxide (both in water and sediment) and unknown, non-characterised, metabolite M20 (detections of 2 x ≥5% AR only in sediment phase).

Summary of the degradation rates estimated for parent and metabolites in the water/sediment studies as well as maximum occurrences are presented in the following tables.

Table 2.8.2.2.1-1: Degradation rates estimated for clethodim

Parent	Distribution river system: Max in water 96.1 % at day 0. Max. sed 11.1 % after 7 d. Distribution pond system: Max in water 96.5 % at day 0. Max. sed 12.0 % after 7 d									
Water / sediment system	pH water phase	pH sed (a)	t. °C	DT_{50} /DT_{90} (days) whole sys. (c)	St. (χ2)	DT_{50} /DT_{90} (days) water	St. (χ2)	DT_{50} /DT_{90} (days) sed	St. (χ2)	Method of calculation
River Rhine	8.18	7.18	20 ± 2	9.2 / 30.6	18.8	n.d.	-	n.d.	-	SFO
Pond Möhlin	8.24	7.15	20 ± 2	13.2 / 43.8	15.6	n.d.	-	n.d.	-	SFO
Pond II [(d)]	7.8	7.84[(b)]	20 ± 2	22.1 / 73.4	5.96	n.d.	-	n.d.	-	SFO
Geometric mean at 20°C[b)]				13.9						

n.d. not determined
(a) Measured in calcium chloride solution
(b) Medium for pH determination not reported.
(c) Normalised using a Q10 of 2.58
(d) Results from this study can be used for classification purposes and risk assessment of the parent only.

Table 2.8.2.2.1-2: Degradation rates estimated for clethodim sulfoxide

Clethodim sulfoxide	Distribution river system: Max in water 57.8 % after 14d. Max. sed 5.2 % after 21 d. Max in total system 61.5 % after 14 days. Distribution pond system: Max in water 37.2 % after 21 d. Max. sed 5.3 % after 21 d. Max in total system 42.5 % after 21 days. kinetic formation fraction (k_f/k_{dp}): 0.747 river system, 0.487 pond system. Arithmetic mean ffM: 0.617 (from parent)									
Water / sediment system	pH water phase	pH sed (a)	t. °C	DT_{50} /DT_{90} (days) whole sys. (b)	St. (χ2)	DT_{50} /DT_{90} (days) water	St. (χ2)	DT_{50} /DT_{90} (days) sed	St. (χ2)	Method of calculation
River Rhine	8.18	7.18	20 ± 2	30.6 / 102	21.9	n.d.	-	n.d.	-	SFO-SFO
Pond Möhlin	8.24	7.15	20 ± 2	26.1 / 86.7	34.1	n.d.	-	n.d.	-	SFO-SFO
Geometric mean at 20°C[b)]				28.3						

n.d. not determined
(a) Measured in calcium chloride solution
(b) Normalised using a Q10 of 2.58

Table 2.8.2.2.1-3: Degradation rates estimated for clethodim imine

Clethodim imine	Distribution river system: Max in water 1.4 % after 7d. Max. sed 18.4 % after 33 d. Max in total system 19.2 % after 33 days. Distribution pond system: Max in water 2.1 % after 7 d. Max. sed 35.8 % after 33 d. Max in total system 36.4 % after 33 days. Kinetic formation fraction (k_f/k_{dp}): 0.253 river system, 0.512 pond system. Arithmetic mean ffM: 0.383 (from parent)									
Water / sediment system	pH water phase	pH sed (a)	t. °C	DT_{50} /DT_{90} (days) whole sys. (b)	St. (χ2)	DT_{50} /DT_{90} (days) water	St. (χ2)	DT_{50} /DT_{90} (days) sed	St. (χ2)	Method of calculation
River Rhine	8.18	7.18	20 ± 2	51.0 / 169	13.7	n.d.	-	n.d.	-	SFO-SFO

Pond Möhlin	8.24	7.15	20 ± 2	46.9 / 156	15.2	n.d.	-	n.d.	-	SFO-SFO
Geometric mean at 20°C[b)]				48.9						

n.d. not determined
(a) Measured in calcium chloride solution
(b) Normalised using a Q10 of 2.58

Table 2.8.2.2.1-4: Degradation rates estimated for clethodim imine sulfoxide

Clethodim imine sulfoxide	Distribution river system: Max in water 7.1 % after 33 d. Max. sed 3.3 % after 90 d. Max in total system 8.3% after 56 days. Distribution pond system: Max in water 4.3 % after 33 d. Max. sed 8.3 % after 90 d. Max in total system 10.6 % after 90 days. kinetic formation fraction (k_f/k_{dp}): 0.307 river system (from clethodim sulfoxide); 1 river system (from clethodim imine). Arithmetic mean ffM: 0.307 (from clethodim sulfoxide), 1 (from clethodim imine)									
Water / sediment system	pH water phase	pH sed (a)	t. °C	DT_{50} /DT_{90} (days) whole sys. (b)	St. ($\chi 2$)	DT_{50} /DT_{90} (days) water	St. ($\chi 2$)	DT_{50} /DT_{90} (days) sed	St. ($\chi 2$)	Method of calculation
River Rhine	8.18	7.18	20 ± 2	14.8 / 49.1	15.5	n.d.	-	n.d.	-	SFO-SFO
Pond Möhlin	8.24	7.15	20 ± 2	-(c)		n.d.	-	n.d.	-	-
Geometric mean at 20°C[b)]				14.8						

n.d. not determined
(a) Measured in calcium chloride solution
(b) Normalised using a Q10 of 2.58
(c) kinetic fit was statistically not acceptable, and the peak not covered in the visual fit.

Table 2.8.2.2.1-5: Degradation rates estimated for clethodim sulfone

Clethodim sulfone	Distribution river system: Max in water 9.5 % after 68 d. Max. sed 2.9 % after 68 d. Max in total system 12.4 % after 68 days. Distribution pond system: Max in water 10.4 % after 68 d. Max. sed 3.1 % after 68 d. Max in total system 13.5 % after 68 days. kinetic formation fraction (k_f/k_{dp}):0.277 river system. Arithmetic mean ffM: 0.277 (from clethodim sulfoxide)									
Water / sediment system	pH water phase	pH sed (a)	t. °C	DT_{50} /DT_{90} (days) whole sys. (b)	St. ($\chi 2$)	DT_{50} /DT_{90} (days) water	St. ($\chi 2$)	DT_{50} /DT_{90} (days) sed	St. ($\chi 2$)	Method of calculation
River Rhine	8.18	7.18	20 ± 2	52.5 / 174	28.6	n.d.	-	n.d.	-	SFO-SFO
Pond Möhlin	8.24	7.15	20 ± 2	-(c)	46.9	n.d.	-	n.d.	-	SFO-SFO
Geometric mean at 20°C[b)]				52.5						

n.d. not determined
(a) Measured in calcium chloride solution
(b) Normalised using a Q10 of 2.58
(c) kinetic fit was statistically and visually too poor and, therefore, not considered acceptable.

Table 2.8.2.2.1-6: Degradation rates estimated for unknown metabolite M20

Unknown metabolite M20	Distribution river system: Max in water 2.7 % after 56 d. Max. sed 3.0 % after 120 d. Max in total system 5.7 % after 90 days. Distribution pond system: Max in water 2.8 % after 56 d. Max. sed 6.0 % after 90 d. Max in total system 8.8 % after 90 days. kinetic formation fraction (k_f/k_{dp}): n.d.									
Water / sediment system	pH water phase	pH sed (a)	t. °C	DT_{50} /DT_{90} (days) whole sys. (b)	St. ($\chi 2$)	DT_{50} /DT_{90} (days) water	St. ($\chi 2$)	DT_{50} /DT_{90} (days) sed	St. ($\chi 2$)	Method of calculation
River Rhine	8.18	7.18	20 ± 2	n.d.	-	n.d.	-	n.d.	-	SFO-SFO
Pond Möhlin	8.24	7.15	20 ± 2	n.d.	-	n.d.	-	n.d.	-	SFO-SFO
Geometric mean at 20°C[b)]										

n.d. not determined
(a) Measured in calcium chloride solution
(b) Normalised using a Q10 of 2.58

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

No field dissipation studies were available (see section 2.8.1.1). No monitoring data was available either (see section 2.8.4).

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Inherent or enhanced biodegradability tests were not provided.

2.8.2.2.4 Soil and sediment degradation data

Soil degradation data are presented in sections 2.8.1.1 (route of degradation) and 2.8.1.2 (rate of degradation). Sediment degradation data are presented in section 2.8.2.2.1.

2.8.2.2.5 Hydrolysis

Hydrolysis of clethodim was investigated in one study, Pack (1988c), Report no. MEF-0013 / 8703899, in sterilized aqueous buffer solutions at pH 5, 7 and 9 (propyl-labelled), and pH 5 and 7 (allyl-labelled) at 25 ± 0.1 °C. Under acidic conditions, at pH 5, hydrolysis was observed and the two major clethodim oxazole and chloroallyl-alcohol were formed with maximum occurrences of 50.5% AR and 30.7 %AR, respectively. At pH 7 and 9, clethodim was deemed stable to hydrolysis with 91-93.4% AR corresponding to the parent substance at the end of the study (30-32 days). Clethodim sulfoxide, present as an impurity in the starting material, was found at low levels in nearly all samples (maximum levels per test ranged between 1.2 and 4.7% AR). Only low levels (≤4.7% AR) of unidentified compounds were detected during the tests.

The estimated DT50s were 28 d (propyl-labelled) and 54 d (allyl-labelled) at pH 5, and 297-499 d at pH 7 and 9.

The study was available also for the first approval and the RMS deems it to be acceptable also for the renewal. This is despite that the tests were not performed at pH 4 as it is unlikely that other metabolites would have been formed compared to the ones formed at pH 5 and despite that tier-1-testing at 50 °C was not performed, as hydrolysis was observed at 25 °C under acidic conditions. The full assessment is provided in Vol. 3 (CA), B.8.2.1.1.

2.8.2.2.6 Photochemical degradation

In total, three studies that investigated the direct aquatic photolysis of clethodim were available. Two studies had already been available for the first approval. These studies investigated the photolysis of [ring-4,6-^{14}C]-clethodim (Chen, 1988a), Report no. MEF-0024, and [allyl-2-^{14}C]-clethodim (Chen, 1988b), Report no. MEF-0025, respectively. The new study, Villaverde (2018), was available from the open literature and investigated the photodegradation potential of clethodim and a formulation containing clethodim in aquatic environments.

The two old studies (Chen, 1988a, Report no. MEF-0024, and Chen, 1988b, Report no. MEF-0025) were performed outdoors at 25 ℃ for up to 2.5 days (pH 5), 30 days (pH 7) and 10-14 days (pH 9), and the test substance exposed to natural sunlight at Richmond, California, USA (37.6°N, 122.2°W) during year 1988. The average duration of exposure to sunlight was 10 hours at an average intensity of ca. 20.3-20.5 kW/cm^2. Incident light intensity was

measured continuously using a photometer, showing a mean daily exposure of 4-31 kW/cm^2. Clethodim was extensively photochemically degraded during the course of the study at all 3 pH-levels. In the irradiated 30-day-studies at pH 7, clethodim decreased from 94.9-99.0% AR at day 0 to 1.2-3.5% AR after 15 days and was not detected thereafter. Several degradation products were identified at >10% AR: clethodim sulfoxide (RE-45924), cholorallyl alcohol, chloropropenal, DME sulfoxide (RE-52453), clethodim imine sulfoxide (RE-47718), clethodim imine (RE-47686), and clethodim imine ketone. For most of them, formation peaked at 3-21 days after treatment (DAT) and was reduced after. Only the formation of metabolite DME sulfoxide increased throughout the studies at pH 7, with a maximum of 48.9% AR reached by the end of the study.

Table 2.8.2.2.6-1: Summary of the photochemical degradation

Metabolite	Max. occurrence [%AR] [a]	DAT [days]	Occurrence after 30 days [%AR]
Clethodim sulfoxide	14.2	3	0
Clethodim imine	12.4 [b]	7	0
Clethodim imine sulfoxide	23.0	21	19.5
DME sulfoxide	48.9	30	48.9
Clethodim imine ketone	11.8	15	9.5
Chloroallyl alcohol	31.3	15	29.2
3-chloro-propenal	21.8	10	19.4

a The maximum occurrences were all obtained from the 30-day study at pH 7.
b Detected at a maximum of 18.2% AR at pH 5. As hydrolysis is not minimized at that pH, the value was disregarded.

The effective photolysis rates of clethodim were estimated to be < 10 days (1.7-9.6 days depending on pH for ring-labelled clethodim and 1.5-6 days for allyl-labelled clethodim, with higher DT50s under neutral and alkaline conditions, where hydrolysis is minimized). Sensitization enhanced the degradation at all pH-levels with effective photolysis rates below 1 day (max. 0.61 d). Significant (and measurable) mineralization occurred only in the study with allyl-labelled clethodim, with a maximum of 24.8 % AR present as CO_2 (in NaOH trap and in solution). Quantum yield was not calculated.

For both studies by Chen (1988a), Report no. MEF-0024, and Chen (1988b), Report no. MEF-0025, the degradation of the metabolites was rather briefly discussed, no degradation rates were calculated for any of the metabolites. For the two metabolites clethodim sulfoxide and clethodim imine, degradation is observed during the studies. However, for all other metabolites (clethodim imine sulfoxide, clethodim imine ketone, DME sulfoxide, chloroallyl alcohol and 3-chloropropenal), no or very limited degradation is observed.

Aqueous photolysis was investigated in the study by Villaverde (2018) both with simulated sunlight (xenon arc lamp with radiation intensity of 250, 500 and 750 W/m^2, internal temperature was 25 ± 1 °C) and with natural sunlight exposure. The outdoor experiments under natural sunlight were performed in the Madrid area during the summer period with mean solar radiation intensities of 70 W/m^2 - 412 W/m^2 during the day, and temperatures ranging from 20 to 46 °C. The tests were performed with different types of water, such as ultrapure, mineral, ground and surface water. It was not explicitly stated, but the figures suggested the study duration was 12 hours. Dark control experiments were performed in parallel confirming that the test conditions were chosen in a way that hydrolysis was minimized. Clethodim showed high photodegradation in aqueous solutions both under natural sunlight and simulated sunlight but was not completely mineralized. Four different degradation products were identified (if isomers are not listed separately): clethodim sulfoxide, clethodim imine, clethodim imine ketone and clethodim imine sulfoxide. The estimated photodegradation rate of (technical) clethodim was below 5 hours for all investigated conditions, and below 1.5 h for clethodim in the formulation.

The two studies by Chen (1988a), Report no. MEF-0024, and Chen (1988b), Report no. MEF-0025, were available also for the first approval and the RMS deems them to be acceptable also for the renewal. This is despite that the daily sunlight exposure was less than the recommendations in OECD TG no. 316, as clear photodegradation was observed and major degradation products were identified. It should also be mentioned that the kinetic assessment was not performed according to FOCUS DegKinetics Guidance (2006, 2014). This is not considered an issue as the estimated degradation rates are not used for any exposure assessment. The study by Villaverde (2018) is new. It is considered supportive information, as it provides valuable information, but did not follow strict guidelines, nor is a method validation available for this study with non-radiolabelled test substance. The study was not performed under GLP.

The full assessment of all three studies is provided in Vol. 3 (CA), B.8.2.1.2.

The applicant suggested that the two metabolites formed in the photolysis study by Chen (1988b), Report no. MEF-0025, namely chloroallyl alcohol and 3-chloropropenal, are not included in the definition of residues. The RMS questioned this, and the applicant provided the following arguments: “The presence of significant levels of imine metabolites in the biotic (water sediment) studies indicates that cleavage of N-O bond occurred and hence this would have resulted in the formation of chloroallyl alcohol and/or 3-chloropropenal. The fact that these metabolites were not observed in the water sediment studies where clethodim was labeled in the allyl ring confirms that chloroallyl alcohol and 3-chloropropenal degraded rapidly and hence were not requiring risk assessment (i.e. the results from the abiotic studies were shown to be superseded by the results in the biotic studies). Some initial methodology work also showed very rapid degradation of chloroallyl alcohol in aquatic systems and hence confirmed our view.”

The RMS agrees that the findings of imine metabolites in the water/sediment study suggests that it is likely that chloroallyl alcohol and/or 3-chloropropenal were formed at the same time, too. However, it must be noted that none of these two metabolites were included as reference items in the water/sediment study by Mamouni (2006c), Report no. A00450, and, thus, could not be identified in the study. Only a degradation product of them, trans-3-chloroacrylic acid, was included as a reference item, and not detected in the study. Several minor metabolites (individually not exceeding 1.6% in total river system, or 3.6% in total pond system) were in fact detected in the study but those did not need to be further characterised. From the EFSA conclusion on 1,3-dichloropropene, an active substance that also transforms into 3-chloroallyl alcohol and 3-chloroacrylic acid, additional information can be obtained. Both substances are found to be stable to hydrolysis but were degraded in water/sediment systems (metabolite dosed studies) with DT50 in total systems of 1.2 days (chloroallyl alcohol) and 5.63 days (chloroacrylic acid), respectively. Thus, for chloroallyl alcohol, the overall conclusion of the applicant can be accepted. In principle, aldehyde-groups are rather reactive. This may suggest that 3-chloropropenal may be a transient metabolite in biotic aquatic systems. However, in order to be precautionary and in lack of further specific evidence, the RMS proposes that 3-chloropropenal is included in the ‘Definition of residues requiring further assessment’. We welcome comments from EFSA and other MS on this issue and our proposal.

2.8.2.2.7 Other / Weight of evidence

No other data that could be of relevance for the classification and labelling were available.

2.8.2.3 Assessment in relation to the P-criteria for water and sediment

The criteria for persistence in water and sediment, as stated in Annex II to Regulation (EC) 1107/2009, are: Water: DT_{50} 40 days (fresh water in PBT), 60 days (POP, marine water in PBT, and all water in vPvB), Sediment: DT_{50} 120 days (fresh water sediment in PBT), 180 days (POP, marine sediment in PBT, and all sediments in vPvB).

Clethodim is slowly hydrolysed at pH 5 (DT50 of 32-54d) or not at all hydrolysed at pH 7 or 9. Clethodim is subject to photolytic degradation with estimated half-lives of 6 days or less at environmentally relevant pH (5-9). Clethodim undergoes biotic degradation, with estimated half-lives of 14.9-23.9 d as obtained from the aerobic mineralization study in a fresh water system at two different application rates (low and high, respectively). DegT50s estimated for the total water-sediment system were similar, with 9.2-22.1 days and a geomean of 13.8 days at reference temperature of 20 °C. The DegT50 for clethodim in the water-sediment system should be compared to the criteria for water, as clethodim is considered mobile to moderately mobile and a maximum of 11% was found in sediment. Thus, clethodim is not considered to fulfil the criteria for persistence with respect to water and sediment. Additionally, the results from the ready biodegradability test suggesting that clethodim is readily biodegradable.

The RMS acknowledges that the criteria only applies to the parent in the frame of EU Reg. no. 1107/2009, however, it can be noted that all major water/sediment metabolites have longer total system-DegT50s and are more persistent in the aquatic water-sediment-system than the parent. One metabolite, clethodim sulfone, would exceed the trigger persistence in the relevant compartment; it has a total system DegT50 of 52 days and mainly occurs in the water phase suggesting that the trigger for water of 40 days should be used. The other metabolite with DegT50 above 40 days, namely clethodim imine, with DegT50 of 51 d, mainly occurs in the sediment phase and should, thus, be compared to the trigger values for sediment.

2.8.3 Summary of fate and behaviour in air

2.8.3.1 Hazardous to the ozone layer

Table 69: Summary table of studies on hazards to the ozone layer.

Method	Results	Remarks	Reference
No studies available.			

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

There were no specific data available on the potential hazard to the ozone layer. However, clethodim is based on its properties (low volatility, fast degradation in air) not expected to undergo long-range transport. In addition, it is not included in Annex I or Annex II to Regulation (EC) 1005/2009.

2.8.3.1.2 Comparison with the CLP criteria

Since clethodim is not expected to undergo long-range transport and is not included in Annex I or Annex II to Regulation (EC) 1005/2009 it is concluded that it does not fulfil the classification criteria for ‘hazardous to the ozone layer’.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Data conclusive but not sufficient for classification.

2.8.3.1.4 Conclusion regarding potential for short- and long-range transport

The vapour pressure of clethodim is 2.7 x 10^{-5} Pa (20 °C) and the Henry's law constant 1.8 x 10^{-6} Pa/m^3/mol. Considering these properties, clethodim has the potential to reach the air if foliar applied since the trigger of 10^{-5} Pa at 20ºC set by the FOCUS guidance Air (2008) is exceeded. Given the proposed foliar use the potential short-range transport of the active substance might have to be considered in the risk assessment.

The atmospheric half-life for reaction with hydroxyl radicals was estimated to <1 hour (Lee & Jarvis, 2020b, 1602214.UK0-6964) assuming an average daily air concentrations of hydroxyl radicals of 1.5 x 10^6/cm^3 (12-hr day). Thus, long-range transport is not expected.

The RMS concludes that no further data on fate of clethodim in air is required.

The RMS considers that clethodim does not fulfil the POP criteria for long-range transport since DT_{50} air is < 2 days.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

Monitoring data for clethodim was not presented by the applicant and claimed not to be available.

2.8.5 Definition of the residues in the environment requiring further assessment

Substances for which further exposure/risk assessment is considered necessary are listed in the below table.

Table 2.8.5-1. RMS proposal for Definition of the residue for exposure and risk assessment.

Compartment	Residue	Justification
Soil	Clethodim	by default
	Clethodim sulfoxide	Up to 73.4% of applied radioactivity in aerobic soil (lab)
	Clethodim sulfone	Up to 42.2% of applied (non-labelled) in aerobic soil (lab)
	CBA	Up to 18.7% of applied radioactivity in soil photolysis
	CAA	Up to 18.1% of applied radioactivity in soil photolysis
	Clethodim oxazole sulfone	Up to 10.0% of applied radioactivity in aerobic soil (lab)
	Clethodim oxazole sulfoxide	Up to 6.0% of applied radioactivity in aerobic soil (lab)
Groundwater	Clethodim	by default
	Clethodim sulfoxide	From soil
	Clethodim sulfone	From soil
	CBA	From soil
	CAA	From soil
	Clethodim oxazole sulfone	From soil
	Clethodim oxazole sulfoxide	From soil
Surface water	Clethodim	by default
	Clethodim sulfoxide	From soil, up to 61.5% of applied radioactivity in total water/sediment system (up to 57.8% of applied radioactivity in water phase and 5.3% of applied radioactivity in sediment phase)
	Clethodim sulfone	From soil, up to 13.5% of applied radioactivity in total water/sediment system (up to 10.4% of applied radioactivity in water phase and 3.1% of applied radioactivity in sediment phase)

Compartment	Residue	Justification
	Clethodim imine	Up to 36.4% of applied radioactivity in total water/sediment system (up to 2.1% of applied radioactivity in water phase and 35.8% of applied radioactivity in sediment phase), up to 12.4% of applied radioactivity in photolysis study
	Clethodim imine sulfoxide	Up to 21.7% of applied radioactivity in total water/sediment system (2x ≥5% and up to 7.1% of applied radioactivity in water phase and up to 15.5% of applied radioactivity in sediment phase), up to 23.0% of applied radioactivity in photolysis study
	Unknown metabolite M20	2x ≥5% and up to 8.8% of applied radioactivity in total water/sediment system (up to 2.8% of applied radioactivity in water phase and 2x ≥5% and up to 6.0% of applied radioactivity in sediment phase)
	DME sulfoxide	Up to 48.9% of applied radioactivity in photolysis study
	Clethodim imine ketone	Up to 11.8% of applied radioactivity in photolysis study
	3-chloro-propenal	Up to 21.8% of applied radioactivity in photolysis study
	CBA	From soil
	CAA	From soil
	Clethodim oxazole sulfone	From soil
	Clethodim oxazole sulfoxide	From soil
Sediment	Clethodim	by default
	Clethodim sulfoxide	Up to 61.5% of applied radioactivity in total water/sediment system (up to 57.8% of applied radioactivity in water phase and 5.3% of applied radioactivity in sediment phase)
	Clethodim sulfone	up to 13.5% of applied radioactivity in total water/sediment system (up to 10.4% of applied radioactivity in water phase and 3.1% of applied radioactivity in sediment phase)
	Clethodim imine	Up to 36.4% of applied radioactivity in total water/sediment system (up to 2.1% of applied radioactivity in water phase and 35.8% of applied radioactivity in sediment phase, respectively),
	Clethodim imine sulfoxide	Up to 21.7% of applied radioactivity in total water/sediment system (2x ≥5% and up to 7.1% of applied radioactivity in water phase and up to 15.5% of applied radioactivity in sediment phase),
	Unknown metabolite M20	2x ≥5% and up to 8.8% of applied radioactivity in total water/sediment system (up to 2.8% of applied radioactivity in water phase and 2x ≥5% and up to 6.0% of applied radioactivity in sediment phase)
Air	Clethodim	by default

2.8.6 Summary of exposure calculations and product assessment

The PEC-calculations were made for all relevant compartments (soil, groundwater, and surface water/sediments) considering the following representative uses:

- Sugar beet, 1 x 120 g a.s/ha, BBCH 12-33
- Sugar beet, 1 x 300 g a.s/ha, BBCH 12-33
- Onions (vegetables, bulb), 1 x 120 g a.s./ha, BBCH 12-19
- Onions (vegetables, bulb), 1 x 240 g a.s./ha, BBCH 12-19

2.8.6.1 PEC soil

PECsoil (Initial PECsoil after application within one season, and actual and TWA PECsoil after 1, 2, 4, 7, 14, 21, 28, 50, and 100 days) were calculated for clethodim and its metabolites clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, 2-[3-chloroallyloxyimino]butanoic acid (CBA) and trans-

3-chloroacrylic acid (CAA). The calculations were made assuming earliest possible application according to the GAP using the lowest interception (i.e., sugar beet 20%, onion 10%). However, the RMS does not agree with the input parameters used in the calculations except for metabolites CAA and CBA. For the other substances, several triggering endpoints proposed by the RMS are more worst-case compared to the input values used in the calculations. Moreover, the lab DT50 values for metabolites clethodim oxazole sulfoxide and clethodim oxazole sulfone indicate that the risk for accumulation in soil (PECacc) needs to be investigated. The RMS sets field dissipation studies as a data gap for these metabolites, since the normalised lab DT50, at 20 °C and pF 2.0 are >60 days in one or more soils (see 2.8.1.2).

The RMS concludes that updated PECsoil calculations are required that consider the endpoints agreed on during the peer review. New PECsoil calculations, including PECsoil initial, PECsoil TWA and, where triggered PECacc, are required for all intended uses (data gap).

2.8.6.2 PEC groundwater

The 80th percentile PECgw of the active substance clethodim and its metabolites clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, 2-[3-chloroallyloxyimino]butanoic acid (CBA) and trans-3-chloroacrylic acid (CAA) were calculated in FOCUS PEARL (v 4.4.4) and FOCUS PELMO (v 5.5.3). Calculations with FOCUS MACRO (Châteaudun) are missing. The application dates were chosen using AppDate tool version 3.06 for an early application at BBCH stage 12, in accordance with the representative GAP, and the crop interception values chosen correspondingly in agreement with the relevant guidance (EFSA GD on DegT50, 2014).

Based on the provided results, PECgw for the parent clethodim and the photolysis metabolites CAA and CBA were <0.001 µg/L for all scenarios and uses. Metabolites clethodim sulfoxide (max. 0.198 µg/L), clethodim sulfone (max. 1.778 µg/L) and clethodim oxazole sulfone (max. 0.684 µg/L) were predicted to exceed the parametric drinking water limit of 0.1 µg/L. Further, clethodim oxazole sulfoxide was predicted to be present at max. 0.1 µg/L. These four metabolites were, thus, further considered in the assessment of relevance in groundwater (see 2.12).

However, the PECgw calculations are not considered acceptable for several reasons, and a data gap is proposed for updated modelling (see below).

Input parameters: The input parameters used are not in line with the endpoints determined by the RMS or not in agreement with relevant guidance. According to the FOCUS Generic GD on GW (2014), a PUF/TSCF of 0 should be used for all compounds unless a TSCF calculated with the Briggs equation is applicable. The argument provided by the study author "Clethodim is a systemic herbicide. Value published in previous EFSA conclusion (EFSA, 2011)." is, thus, not considered sufficient to justify a PUF of 0.5. The vapour pressure for clethodim used in the provided PECgw-calculations does not agree with the value of 2.68×10^{-5} Pa determined in the latest study (see Volume 3, B2 (CA)). Further, the RMS does not agree with the choice of several degradation and adsorption endpoints in the calculations. Most DT_{50} and K_{Foc} proposed by the RMS are more conservative than the once considered in the calculations. The RMS concludes that adsorption is pH-depended for clethodim and suggests that the pH-dependence should be considered, i.e., by calculating PECgw for two contrasting pH (5.1 and 8.0) and reporting results from both runs. Also, adsorption has shown to be pH-dependent for most of the metabolites,

however, the adsorption is generally low for these metabolites. The RMS, therefore, suggests that the pH dependence of the metabolites does not need to be further considered and the geometric mean K_{Foc} and arithmetic mean 1/n from all acceptable soils is used instead.

Metabolic pathway: In the applicant's simulations, metabolite clethodim oxazole sulfone is formed directly from clethodim sulfoxide, which differs from the proposed degradation pathway (see Vol 1, 2.8.1.1) were clethodim oxazole is formed via clethodim sulfone and via clethodim oxazole sulfoxide. Further, for simulation in FOCUS PELMO, the simulations were split into two pathways (A and B). The RMS does not agree with this modelling set-up and concludes that the simulations should follow the pathway as outlined in Volume 1, section 2.8.1.1, i.e., that clethodim oxazole sulfone is formed from clethodim sulfone and clethodim oxazole sulfoxide. Considering the formation fractions proposed by the RMS it is then not necessary to split the pathway in FOCUS PELMO into two simulations.

Both photolysis metabolites CAA and CBA were simulated as the parent compound with the application rate being adjusted for molecular weight and the maximum occurrence in the photolysis study. This is considered acceptable by the RMS.

It is difficult to estimate which impact these deviations from the RMS proposal have on the resulting PECs. Thus, the RMS proposes a data gap for the applicant to provide updated PECgw calculations considering the endpoints and pathway agreed on during the peer review. New calculations for all intended uses are required using all relevant models (FOCUS PEARL, PELMO and MACRO).

The calculations were presented in Jones, B. & Jarvis, T. (2021, report no. 1602214.UK0-2996), see summary in Volume 3 CP B.8 under section B.8.3.

2.8.6.3 PEC surface water and sediment

PECsw and PECsed at FOCUS Steps 1-2 were calculated for clethodim and its metabolites clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfone, clethodim oxazole sulfoxide, clethodim imine, clethodim imine sulfoxide, DME sulfoxide and imine ketone, 2-[3-chloroallyloxyimino]butanoic acid (CBA) and trans-3-chloroacrylic acid (CAA) with the FOCUS Steps 1-2 calculator, version 3.2. The calculations were performed for all intended uses considering a reasonable worst-case (minimal crop cover, early application) in accordance with the GAP. The RMS does, however, not agree with the choice of several endpoints in the calculations. For several compounds DT_{50}, Koc or max. occurrence proposed by the RMS are different to the once considered in the calculations and are often more conservative. Moreover, the RMS concluded to add metabolite 3-chloro-propenal to the list of metabolites requiring a surface water assessment and the unknown metabolite M20 requiring an assessment for surface water and sediment. The FOCUS SW calculations at Steps 1 and 2 were therefore rerun by the RMS (see Volume 3 CP B.8.5.1).

Step 3 modelling was performed for the parent clethodim only. PECsw and PECsed were calculated for all intended uses, using FOCUS SWASH 5.3 with SPIN 2.2 and the operational models FOCUS MACRO 5.5.4, FOCUS PRZM 4.3.1 and FOCUS TOXSWA 5.5.3. All scenarios where the intended crops are implemented were presented. For each use, the application window was set to start at BBCH 12 based on the software AppDate (v. 3.06). The application dates were subsequently selected by the Pesticide Application Timer (PAT) internally by the model.

Step 4 modelling was performed for the parent clethodim considering different mitigation options. The results are presented in the modelling study but were not considered necessary to demonstrate acceptable use and are, thus, not summarised in Volume 3 CP B.8.

However, some input parameters used are not in line with the endpoints determined by the RMS or in agreement with relevant guidance. Like for the PECgw calculations, a PUF of 0.5 was used in the calculations which is not considered acceptable by the RMS. Instead, the RMS proposes to use a default of 0. Further, the DT_{50} and Koc used in the calculations are different to the RMS proposal. The difference in this input values is not expected to change the outcome of the risk assessment. However, for completion the RMS proposes a data gap for the applicant to provide updated PECsw calculations for clethodim at Step 3 considering the endpoints agreed on during the peer review.

Note that, in accordance with FOCUS guidance Air (2008) the contribution of clethodim from deposition after volatilisation needs to be quantified and added to the deposition from spray drift if risk mitigation measures (Step 4 calculations) are required to pass the aquatic risk assessment.

Modelling at FOCUS STEP 1-2 is presented in study Lee, R. & Jarvis, T. (2020b, report no.: 1602214.UK0-2662) and at STEP 3-4 in study Lee, R. & Jarvis, T. (2020c, report no.: 1602214.UK0-3557). See Volume 3 CP B.8, section 8.5 for details.

2.8.6.4 PEC air

Based on its vapour pressure of 2.68 x 10^{-5} Pa (20 °C) and the Henry's law constant of 1.8 x 10^{-6} Pa/m^3/mol at 20ºC and pH 7, clethodim has the potential for short-rang transport after volatilisation from plant surfaces. The RMS concludes that deposition following volatilisation needs to be considered in the terrestrial and aquatic risk assessment if drift mitigations are required to pass the respective risk assessment. Given that the atmospheric half-life for reaction with hydroxyl radicals was estimated to < 1 hour (Lee & Jarvis, 2020b, 1602214.UK0-6964) and, thus, below the trigger of 2 days, no long-range transport is expected.

2.8.6.5 Other routes of exposure

There are no other routes of exposure to be considered if the product is used according to good agricultural practice.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

The available data on avian and mammal toxicity of technical clethodim are summarised in the table below. Data used for the risk assessment are marked in bold.

Table 2.9.1-1. Summary of all terrestrial vertebrates data for clethodim (a.s.)

Species	Method[d]	Results	Remarks	Reference
Birds				
Colinus virginianus (northern bobwhite)	OECD 223	**LD_{50} > 1660 mg a.s./kg bw/d [mm]**	Acute, Oral (1-day)	[redacted] (1986a) Report no.: 162-165 Vol 3 CA B.9.1.1.1
Colinus virginianus (northern bobwhite)	OECD 205	LC_{50} >1244 mg a.s./kg bw/d **[mm]**	Dietary, Short-term (5-day)	[redacted] J. (1986b) Report no.: 162-166 Vol 3 CA B.9.1.1.2
Anas platyrhynchos (mallard duck)	OECD 205	LC_{50} >851 mg a.s./kg bw/d [TWA]	Dietary, Short-term (5-day)	[redacted] *et al.* (1986) Report no.: 162-167 Vol 3 CA B.9.1.1.2
Colinus virginianus (northern bobwhite)	OECD 206	NOEL = 326 mg a.s./kg bw/d (males) NOEL = 258 mg a.s./kg bw/d (females) [TWA]	Dietary, Reproductive (pilot) (6 weeks)[b]	[redacted] *et al.* (1987a) Report no.: 162-176 Vol 3 CA B.9.1.1.3
Colinus virginianus (northern bobwhite)	OECD 206	NOEL = 18 mg a.s./kg bw/d (males) **NOEL = 17 mg a.s./kg bw/d (females)**[a] **[mm]**	Dietary, Reproductive (22 weeks)	[redacted] *et al.* (1988a) Report no.: 162-183 Vol 3 CA B.9.1.1.3
Anas platyrhynchos (mallard duck)	OECD 206	NOEL = 133 mg a.s./kg bw/d (males) NOEL = 122 mg a.s./kg bw/d (females) **[mm]**	Dietary, Reproductive (pilot) (6 weeks)[b]	[redacted] *et al.* (1987b) Report no.: 162-177 Vol 3 CA B.9.1.1.3
Anas platyrhynchos (mallard duck)	OECD 206	NOEL = 82 mg a.s./kg bw/d (males) NOEL = 89 mg a.s./kg bw/d (females) **[mm]**	Dietary, Reproductive (19 weeks)	[redacted] *et al.* (1988b) Report No.: 162-184 Vol 3 CA B.9.1.1.3
Mammals				
Rat (Sprague-Dawley)	OECD 401	**LD_{50} = 1133 g a.s./kg bw (females) [nom]** LD_{50} = 1358 mg/kg bw (males)	Acute, Oral (14 days)	[redacted] (1986) Report No.: S 2498 Vol 3 CA B.6.2.1
Mouse (CD-1)	OECD 401	LD_{50} = 1688 mg/kg bw (females) LD_{50} = 1787 mg/kg bw (males)	Acute, Oral (14 days)	[redacted] 1986 Report number: 2107-143 Vol.3. B.6.2.1/02
Rat (Han Wistar)	OECD 423	LD_{50} > 2000 mg product/kg bw (females)	Acute, Oral (14 days)	[redacted] (2008a) Report number: 082148 Vol 3 CP 7.1.1/01
Rat (Sprague-Dawley)	OECD 453	NOAEL = 16 mg a.s./kg bw/d (males) [c] [nom]	Chronic, Oral (2 years)	[redacted] (1988) Report No.: S-2766 Vol 3 CA B.6.5/02
Rat (Sprague-Dawley)	OECD 416	LOAEL = 250 mg/kg bw/d NOAEL n.d.[e]	Oral, chronic (1 week before mating until day 7 of lactation) [b]	[redacted] (1986) Report no. S-2758 Vol 3 CA 6.6.1./01
Rat (Sprague-Dawley)	OECD 416	NOAEL = 32.2 mg/kg bw/day (parental toxicity) NOAEL = 163 mg/kg bw/day (reproductive toxicity)	Oral, chronic (two-generation)	[redacted] (1987) Report no. S-2778 Vol 3 CA 6.6.1/02

Species	Method[d]	Results	Remarks	Reference
Rat (Sprague-Dawley)	OECD 414	LOAEL = 250 mg/kg bw/d (decreased pup weight) NOAEL n.d. [e]	Oral gavage, single daily dose on gestational days 6-15 [b]	[redacted] (1986) Report number: S-2807 Vol 3 CA 6.6.2.1/01
Rat (Sprague-Dawley)	OECD 414	NOAEL = 83.3 mg/kg bw/day (maternal and developmental toxicity)	Oral gavage, single daily dose on gestational days 6-15	[redacted] (1987) Report number: S-2808 Vol 3 CA 6.6.2.2/01
Rabbit (New Zealand White)	OECD 414	LOAEL = 125 mg/kg bw/day (reduced body weight gain) NOAEL n.d. [e]	Oral gavage, single daily dose on gestational day 7-19 [b]	[redacted] (1986) Report number: S-2734 Vol 3 CA 6.6.2.3/01
Rabbit (New Zealand White)	OECD 414	**NOAEL maternal = 20.8 mg/kg bw/day** NOAEL developmental = 83.3 mg/kg bw/day	Oral gavage, single daily dose on gestational day 7-19	[redacted] (1987) Report number: S-2869 Vol 3 CA 6.6.2.4/01

[a] NOEL used for risk assessment as lower than the LD_{50}/10 in line with EFSA's Bird and Mammal Guidance Document (EFSA/2009/1438)
[b] Pilot study, supportive information only
[c] this endpoint was used in the Applicant's risk assessment. The RMS has used the value of 20.8 mg/kg bw/day instead, as it was generated from a study that followed one of the test guidelines recommended in EFSA 2009.
[d] the method listed here refers to the most relevant test guideline used for determining the validity of the study
[e] study not suitable for NOAEL setting (low number of animals used and limited parameters investigated)
[mm] mean measured concentration; [nom] nominal concentration; [TWA] time-weighted average

Details on the effects on mammals are given in sections 2.6.2. and 2.6.6. of this volume. The respective studies are summarised in Vol 3 CA B6.

2.9.2 Summary of effects on aquatic organisms

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 70. Summary of relevant information on bioaccumulation.

Method	Species	Results	Key or Supportive study	Remarks	Reference
US EPA 165-4	*Lepomis macrochirus* (Bluegill)	Max BCF[a] = 3.5 L/kg (whole fish) and 2.0 L/kg (edible tissue)	key	28-day, bioaccumulation	[redacted] (1987) Report No.: 35636 Vol 3 CA B.9.2.2.3
40 CFR158.130 Pesticide Assessment Guideline Subdivision N. 165-7	*Lepomis macrochirus* (Bluegill)	BCF <0.96 [b] (whole fish)	supportive	28-day, bioaccumulation	[redacted] & [redacted] (1988) Report No.: MEF-0020 Vol 3 CA B.9.2.2.3

[a] not normalised for lipids (no lipid data)
[b] concentrations in fish were <LOQ, so BCF was estimated by the RMS by considering the concentration in fish to be equal to LOQ (0.0364 mg/kg)

2.9.2.1.1 Estimated bioaccumulation

Table 2.9.2.1-1. Log Pow values for clethodim based on pH

pH range	Log Pow
4	3.4
7	2.3 – 2.8
9	1.7 – 1.9

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

Given the available data on log Pow (max 3.4 at acidic pH 4) and experimental BCF in fish 3.5, clethodim is not expected to bioaccumulate.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

Table 71. Summary of relevant information on acute aquatic toxicity.

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
Fish						
US EPA 72-1 (1982)	*Oncorhynchus mykiss* (rainbow trout)	Clethodim	**LC_{50} = 25 mg a.s./L (mm)**	key	96 hours, static	[redacted] (1986a) Report No.: 34968 Vol 3 CA B.9.2.1
OECD 203 (1992), Directive 92/69/EEC, Annex Part C1 (1992)	*Oncorhynchus mykiss* (rainbow trout)	Clethodim sulfoxide	**LC_{50} > 100 mg/L (nom)**	key	96 hours, static	[redacted] (2005) Report No.: 25012230 Vol 3 CA B.9.2.1
OECD 203 (1992), Commission Directive 92/69/EEC, Annex Part C, C.l: "Acute Toxicity for Fish" 1992	*Oncorhynchus mykiss* (rainbow trout)	Clethodim 120	**LC_{50} = 8.98 mg product/L (nom)** (1.21 mg a.s./L(nom))	key	96 hours, static	[redacted] (2006) Report No.: 30704230 Vol 3 CP B.9.3.1
US EPA 72-1 (1982)	*Lepomis macrochirus* (bluegill)	Clethodim	LC_{50} > 33 mg a.s./L (mm)	key	96 hours, static	Swigert, J.P [redacted]) Report No.: S-2839 Vol 3 CA B.9.2.1
Invertebrates						
U.S. EPA 72-2 (1982)	*Daphnia magna* (waterflea)	Clethodim	**EC_{50} > 100 mg a.s./L (nom)**	key	48 hours, static	Forbis, A.D. (1986) Report No.: 34969 Vol 3 CA B.9.2.4
OECD 202 (2004) Commission Directive 92/69/EEC, Annex Part C, C.2: "Acute Toxicity for Daphnia", 1992	*Daphnia magna* (waterflea)	Clethodim 120	**EC_{50} = 29.4 mg product/L (nom)** (3.9 mg a.s./L (nom))	key	48 hours, static	Vinken R. & Wydra V. (2006a) Report No.: 30703220 Vol 3 CP B.9.3.1

2.9.2.2.1 Acute (short-term) toxicity to fish

Relevant endpoints are available from acute (96 h) studies on rainbow trout and bluegill. The sensitivity of the two species to clethodim a.s. was similar. The data on rainbow trout show that the metabolite clethodim sulfoxide was less toxic than the parent compound clethodim (by a factor of 4), whereas the formulated product Clethodim 120 was more toxic than the active substance (by a factor of 20).

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

Relevant endpoints for clethodim a.s. and formulated product are available from acute (48 h) studies on *Daphnia magna*. The data show that the product Clethodim 120 was more than 25 times more toxic than the active substance.

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

Please refer to Section 2.9.2.3.3 'Chronic toxicity to algae or aquatic plants' where both acute (short-term) and chronic toxicity to algae and aquatic plants are discussed.

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

No further acute data on other aquatic organisms are available, nor required for this evaluation.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

Table 72. Summary of relevant information on chronic aquatic toxicity.

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
Fish						
US EPA OPPTS 850.1000 (1996) US EPA OPPTS 850.1400 (1996) ASTM Standard E1241-05	*Cyprinodon variegatus* (sheepshead minnow)	Clethodim	**NOEC = 4.2 mg a.s./L (mm)** EC_{10} n.d.	key	34-day ELS flow through	[redacted] et al. (2011) Report No.: 263A-127 Vol 3 CA B.9.2.2.1
OECD 229 (2012) US EPA OPPTS No. 890.1350 (2009)	*Pimephales promelas* (fathead minnow)	Clethodim	No effects (test concentrations: 0.1-10 mg/L, nom)	key	21-day FSTRA flow through	[redacted] et al. (2020) Report No.: 443A-166A Vol 3 CA B.9.2.3
Amphibians						
OECD 231 (2009) US EPA OPPTS No. 890.1100 (2009)	*Xenopus laevis* (African clawed frog)	Clethodim	No effects (test concentrations: 0.23-23 mg/L, nom)	key	21-day AMA, flow through	[redacted] (2021) Report No.: 443A-165 Vol 3 CA B.9.2.3
Invertebrates						
OECD 211 (2008) Commission Regulation (EC) No 440/2008, Annex, Part C, C.20.: "Daphnia magna Reproduction Test" 2008	*Daphnia magna* (waterflea)	Clethodim 120	Reproduction **NOEC = 1.0 mg product/L** (0.14 mg a.s./L) EC_{10} = 1.69 mg product/L (0.23 mg a.s./L) Survival NOEC = 3.2 mg product/L (0.44 mg a.s./L) EC_{10} = 1.38 mg product/L (0.19 mg a.s./L)	key	21 d, semi static	Kuhl, R. & Wydra, V. (2011) Report No.: 62161221 Vol 3 CP B.9.3.2

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
			(nom)			
Draft OECD 219 (Feb 2000) Streloke, M. and Köpp, H. (1995): Proposal for a BBA-Guideline	*Chironomus riparius* (common non-biting midge)	Clethodim imine	NOEC = 10 mg/L (nom) EC_{10} n.d.	key	28-day, semi-static (spiked water)	Stäbler, D. (2003) Report No.: 20031106/01-ASCr Vol 3 CA B.9.2.5
Algae						
OECD 201 (2006:2011)	*Pseudokirchneriella subcapitata* (green algae)	Clethodim	**E_rC_{50} = 35.1 mg a.s./L** E_rC_{10} = 17.9 mg a.s./L E_yC_{50} = 20.0 mg a.s./L E_yC_{10} = 11.8 mg a.s./L NOEC = 4.98 mg a.s./L (mm)	key	72 hours	Siche, O. & Mollandin, G. (2020a) Report No.: 140061210 Vol 3 CA B.9.2.6.1
OECD 201 (1984) OECD 201 (revised 2004) Commission Directive 92/69/EEC, Annex Part C, C3: "Algal Inhibition Test" 1992	*Pseudokirchneriella subcapitata* (green algae)	Clethodim 120	**E_rC_{50} > 10.0 mg product/L** (>1.35 mg a.s/L) E_rC_{10} = 4.879 mg product/L (0.66 mg a.s/L) E_yC_{50} = 5.97 mg product/L (0.80 mg a.s/L) E_yC_{10} = 2.428 mg product/L (0.33 mg a.s./L) NOEC = 0.98 mg product/L (0.13 mg a.s./L) (nom)	key	72 hours	Vinken R. & Wydra V. (2006b) Report No.: 30701210 Vol 3 CP B.9.3.1
OECD 201 (2006:2011)	*Navicula pelliculosa* (diatom)	Clethodim	E_rC_{50} > 61.3 mg a.s./L E_rC_{10} = 21.2 mg a.s./L E_yC_{50} = 49.3 mg a.s./L E_yC_{10} = 3.12 mg a.s./L NOEC = 0.651 mg a.s./L (mm)	key	72 hours	Siche, O. & Mollandin, G. (2020b) Report No.: 140061218 Vol 3 CA B.9.2.6.2
OECD 201 (1984) OECD 201 (revised 2004) Commission Directive 92/69/EEC, Annex Part C, C3: "Algal Inhibition Test" 1992	*Anabaena flos-aquae* (cyanobacteria)	Clethodim 120	E_rC_{50} = 15.86 mg product/L (2.14 mg a.s/L) E_rC_{10} = 9.004 mg product/L (1.22 mg a.s/L) NOEC (growth rate) = 8.8 mg product/L (1.19 mg a.s/L) E_yC_{50} = 7.43 mg product/L (1.00 mg a.s/L) E_yC_{10} = 3.277 mg product/L (0.44 mg a.s/L) NOEC (yield) = 0.95 mg product/L (0.13 mg a.s/L) (nom)	key	72 hours	Vinken R. & Wydra V. (2006c) Report No.: 30702210 Vol 3 CP B.9.3.1
Aquatic plants						
US EPA 123-2 (1989)	*Lemna gibba* G3 (duckweed)	Clethodim	Fronds E_rC_{50} = 3.78 mg a.s./L E_rC_{10} = 0.323 mg a.s./L NOEC (growth rate) = 0.670 mg a.s./L E_yC_{50} = 1.09 mg a.s./L E_yC_{10} = 0.174 mg a.s./L NOEC (yield) = 0.286 mg a.s./L	key	7-day, static	Rhodes, J.E. & Hughes, J.S. (1991) Report No.: B765-01-1 Vol 3 CA B.9.2.7

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
			(gmm)			
Pesticide Assessment Guidelines, Subdivision J Hazard Evaluation: Wildlife and Aquatic Organisms.	*Lemna gibba* G3 (duckweed)	Clethodim	Fronds E_rC_{50} > 4.83 mg a.s./L E_rC_{10} n.d. NOEC (growth rate) = 4.83 mg a.s./L NOEC (phytotoxicity) =1.77 mg a.s./L (gmm)	key	14-day, static	Grimstead, S.R. et al., (1991) Report No.: 162A-115A Vol 3 CA B.9.2.7
OECD 239 (2014), Ring test protocol for *Glyceria maxima*, July 17, 2018	*Glyceria maxima*	Clethodim	Fresh weight **E_rC_{50} = 0.0886 mg a.s./L** E_rC_{10} = 0.00066 mg a.s./L E_yC_{50} = 0.0342 mg a.s./L E_yC_{10} = 0.00007 mg a.s./L NOEC = 0.027 mg a.s./L (total leaf length and fresh weight) (twa)	key	14-day, semi static	Armbruster, H. (2020) Report No.: 136151245 Vol 3 CA B.9.2.7
OECD 221 (2004) US EPA 712-C-96-156: OPPTS 850.4400 (1996)	*Lemna gibba* (duckweed)	Clethodim sulfoxide	Fronds **E_rC_{50} > 100 mg/L** E_rC_{10} = 30.57 mg/L E_yC_{50} = 75.6 mg/L E_yC_{10} = 14.56 mg/L NOEC = 9.77 mg/L Biomass E_rC_{50} > 100 mg/L E_yC_{50} = 88.4 mg/L E_yC_{10} = 24.13 mg/L NOEC = 31.25 mg/L (nom)	key	7-day, static	Pawlowski, S. (2006) Report No.: 25011240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	Clethodim sulfone	Fronds **E_rC_{50} = 76.7 mg/L** E_rC_{10} = 8.46 mg/L E_yC_{50} = 21.6 mg/L E_yC_{10} = 5.70 mg/L NOEC (growth rate) = 3.2 mg/L Biomass E_rC_{50} > 100 mg/L E_rC_{10} = 5.49 mg/L E_yC_{50} = 37.8 mg/L E_yC_{10} = 5.74 mg/L NOEC = 10 mg/L (nom)	key	7-day, static	Seeland-Fremer, A. & Wydra, V. (2019a) Report No.: 136041240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	Clethodim oxazole sulfoxide	Fronds and Biomass **E_rC_{50} > 100 mg/L** E_yC_{50} > 100 mg/L E_rC_{10} and E_yC_{10} > 100 mg/L NOEC = 100 mg/L (nom)	key	7-day, static	Siche & Mollandin (2020c) Report No.: 136141240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	Clethodim oxazole sulfone	Fronds and Biomass **E_rC_{50} > 100 mg/L** E_yC_{50} > 100 mg/L	key	7-day, static	Seeland-Fremer, A. & Wydra, V.

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
			E_rC_{10} and E_yC_{10} > 100 mg/L NOEC = 100 mg/L (nom)			(2019b) Report No.: 136051240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	Clethodim imine	Fronds **E_rC_{50} = 45.4 mg/L** E_rC_{10} = 14.4 mg/L E_yC_{50} = 19.2 mg/L E_yC_{10} = 4.23 mg/L Biomass E_rC_{50} = 50.9 mg/L E_rC_{10} = 19.5 mg/L E_yC_{50} = 24.8 mg/L E_yC_{10} = 7.93 mg/L NOEC = 3.24 mg/L (mm)	key	7-day, semi static	Wieth, F. & Wydra, V. (2019a) Report No.: 136061240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	Clethodim imine sulfoxide	Fronds **E_rC_{50} > 100 mg/L** E_rC_{10} = 16.0 mg/L E_yC_{50} = 42.5 mg/L E_yC_{10} = 7.34 mg/L NOEC = 10 mg/L Biomass E_rC_{50} > 100 mg/L E_rC_{10} = 13.6 mg/L E_yC_{50} = 32.1 mg/L E_yC_{10} = 8.46 mg/L NOEC = 32 mg/L (nom)	key	7-day, static	Wieth, F. & Wydra, V. (2019b) Report No.: 136071240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	Clethodim imine ketone	Fronds **E_rC_{50} > 100 mg/L** E_yC_{50} > 100 mg/L NOEC = 32 mg/L Biomass E_rC_{50} dry weight > 100 mg/L (nom) E_yC_{50} dry weight > 100 mg/L NOEC = 100 mg/L (nom)	key	7-day, semi static	Wieth, F. & Emnet, P. (2019a) Report No.: 136131240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	Clethodim M17	Fronds and Biomass **E_rC_{50} > 100 mg/L** E_yC_{50} > 100 mg/L E_rC_{10} and E_yC_{10} > 100 mg/L NOEC = 100 mg/L (nom)	key	7-day, static	Wieth, F. & Emnet, P. (2019b) Report No.: 136121240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* (duckweed)	CBA	Fronds **E_rC_{50} > 100 mg/L** E_rC_{10} = 38.0 mg/L E_yC_{50} > 100 mg/L E_yC_{10} = 9.58 mg/L NOEC = 10 mg/L Biomass	key	7-day, static	Seeland-Fremer, A. & Wydra, V. (2019c) Report No.: 136081240 Vol 3 CA B.9.2.7

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
			E_rC_{50} > 100 mg/L E_rC_{10} > 100 mg/L E_yC_{50} > 100 mg/L E_yC_{10} = 13.8 mg/L NOEC = 32 mg/L (nom)			
OECD 221 (2006)	*Lemna gibba* (duckweed)	CAA	Fronds **E_rC_{50} = 10.1 mg/L** E_rC_{10} = 2.23 mg/L E_yC_{50} = 5.09 mg/L E_yC_{10} = 1.91 mg/L Biomass E_rC_{50} >100 mg/L E_rC_{10} = 1.88 mg/L E_yC_{50} = 9.02 mg/L E_yC_{10} n.d. NOEC n.d. (nom)	key	7-day, static	Seeland-Fremer, A. & Wydra, V (2019d) Report No.: 136091240 Vol 3 CA B.9.2.7
OECD 221 (2006)	*Lemna gibba* G3 (duckweed)	Clethodim 120	Fronds E_rC_{50} = 104.17 mg product/L (14.06 mg a.s/L) E_rC_{10} = 2.49 mg product/L (0.34 mg a.s/L) E_yC_{50} = 12.92 mg product/L (1.74 mg a.s/L) E_yC_{10} = 0.91 mg product/L (0.12 mg a.s/L) Biomass **E_rC_{50} = 90.98 mg product/L** (12.28 mg a.s./L) E_rC_{10} = 3.34 mg product/L (0.45 mg a.s/L) E_yC_{50} =11.82 mg product/L (1.6 mg a.s/L) E_yC_{10} = 1.36 mg product/L (0.18 mg a.s/L) NOEC = 1 mg product/L (0.14 mg a.s./L) (nom)	key	7-day, static	Vinken, R. & Wydra, V. (2007) Report No.: 35071240 Vol 3 CP B.9.3.1

2.9.2.3.1 Chronic toxicity to fish

Relevant data for the active substance clethodim are available for sheepshead minnow (34-day Early Life Stage test) and for fathead minnow (21-day Fish Short Term Reproduction Assay). The most sensitive endpoint was the growth of sheepshead minnow, with a NOEC of 4.2 mg a.s./L.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

Relevant data are available from chronic tests with the active substance and formulated product on *Daphnia magna* (21d). Data on the metabolite clethodim imine are available for *Chironomus riparius* (28d). The data on *D. magna*

show 350-fold higher toxicity of the formulated product Clethodim 120 than of the active substance clethodim (but note that the latter endpoint is not considered acceptable by the RMS).

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Relevant data are available from 72 h tests with green algae, diatoms and blue-green algae, and from 7 or 14-day tests with two species of aquatic plants (*Lemna gibba* and *Glyceria maxima*). *Glyceria maxima* was the most sensitive of the tested aquatic species, by up to two orders of magnitude. The study on *G. maxima* had a semi-static design with renewal of test media every 2-3 days. As this is a rooted macrophyte, the test was performed in the presence of sediment, which could potentially lead to an under-estimation of the exposure during the test due to dissipation and subsequent accumulation of the substance in the sediment. Clethodim was measured in the sediment from the highest test concentration (1000 µg a.s./L) only at the end of the 14-day exposure period and was found in small concentrations, representing only 4% of the nominal concentration. The recovery of clethodim in water was 67 – 98 % in fresh media and 56 – 81 % in aged media. Thus it appears that exposure occurred mainly through the water phase and the study can therefore be considered relevant also for classification purposes. The study fulfils the validity criteria of OECD 239, except for the mean CV for yield, where the acceptable limit is slightly exceeded (*i.e.*, 35.8 % instead of max 35%). Since this deviation is only minor and variability in shoot weight is a common problem in this type of test, the RMS considers that overall, the study is valid.

The data on the green algae *Pseudokirchneriella subcapitata* show that the formulated product Clethodim 120 is more toxic than the active substance clethodim by a factor of at least 25. The sensitivity of the cyanobacteria *Anabaena flos-aquae* to the formulated product is comparable to that of the green algae. The sensitivity of the diatom *Navicula pelliculosa* to clethodim technical is slightly lower than that of the green algae (i.e., by a factor of 1.7).

Growth stimulation of toxin-producing cyanobacteria (*Raphidiopsis raciborskii* and *Microcystis aeruginosa*) has also been shown at 1 mg/L for the clethodim product Poquer in the open literature study by Breda-Alves et al 2020. It is unclear how this product compares to the representative one.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

No further acute data on other aquatic organisms are available, nor required for this evaluation.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 73. Summary of information on acute aquatic toxicity relevant for classification.

Method	Species	Test material	Results	Remarks	Reference
US EPA 72-1 (1982)	*Oncorhynchus mykiss* (rainbow trout)	Clethodim	**LC_{50} = 25 mg a.s./L (mm)**	96 hours, static	[redacted] (1986a) Report No.: 34968 Vol 3 CA B.9.2.1
U.S. EPA 72-2 (1982)	*Daphnia magna* (waterflea)	Clethodim	**EC_{50} > 100 mg a.s./L (nom)**	48 hours, static	Forbis, A.D. (1986) Report No.: 34969 Vol 3 CA B.9.2.4

Method	Species	Test material	Results	Remarks	Reference
OECD 239 (2014), Ring test protocol for *Glyceria maxima*, July 17, 2018	*Glyceria maxima*	Clethodim	**ErC_{50} fresh weight= 0.0886 mg a.s./L (twa)** EyC_{50} shoot height = 0.0146 mg a.s./L (twa)	14-day, semi static	Armbruster, H. (2020) Report No.: 136151245 Vol 3 CA B.9.2.7

From the available acute data on aquatic organisms, the most sensitive species tested is *Glyceria maxima*, with an E_rC_{50} of 0.0886 mg a.s./L. Based on these results ($E_rC_{50} \leq 1$ mg/L), clethodim fulfils the criteria for Aquatic Acute Category 1, H400 and the M-factor is 10 as 0.01 mg/L < $E_rC_{50} \leq 0.1$ mg/L (CLP Table 4.1.3.).

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

Table 74. Summary of information on long-term aquatic toxicity relevant for classification.

Method	Species	Test material	Results	Remarks	Reference
US EPA OPPTS 850.1000 (1996) US EPA OPPTS 850.1400 (1996) ASTM Standard E1241-05	*Cyprinodon variegatus* (sheepshead minnow)	Clethodim	**NOEC = 4.2 mg a.s./L (mm)**	34-day ELS flow through	[redacted] et al. (2011) Report No.: 263A-127 Vol 3 CA B.9.2.2.1
OECD 239 (2014), Ring test protocol for *Glyceria maxima*, July 17, 2018	*Glyceria maxima*	Clethodim	**E_rC_{10} = 0.00066 mg a.s./L (twa)** [growth rate based on fresh weight] NOEC = 0.0267 mg a.s./L (twa) [yield and growth rate based on both total leaf length and fresh weight]	14-day, semi static	Armbruster, H. (2020) Report No.: 136151245 Vol 3 CA B.9.2.7
US EPA 165-4	*Lepomis macrochirus* (Bluegill)	Clethodim	Max BCF* = 3.5 L/kg (whole fish) and 2.0 L/kg (edible tissue)	28-day, bioaccumulation	[redacted] (1987) Report No.: 35636 Vol 3 CA B.9.2.2.3
OECD TG no. 301D (Closed Bottle Test)	-	Clethodim	Clethodim: 55.9% of ThOD after 7d, 133-138% of ThOD thereafter (14d-28d) Reference substance: 72.5% ThOD after 7d and 104-131% ThOD thereafter (14d-28d). Clethodim is readily biodegradable.	Test valid, confirmed by high degradation of sodium benzoate. No inhibitory effects. Please also refer to Table 68.	Dengler (2002), Report No.: 20011424/01-AACB Vol 3 CA B.8.2.2.1

Clethodim is not expected to bioaccumulate, since an experimental BCF in fish of only 3.5 was reported.

Long term aquatic data are available for two trophic levels: fish and primary producers (algae and macrophytes). Clethodim is evaluated according to the flow-chart in CLP regulation (EG) 1272/2008, Annex I: Figure 4.1.1. For the present situation where adequate chronic toxicity data are available for two trophic levels, it is required to assess both:

(a) according to the criteria given in Table 4.1.0(b)(ii) (rapidly degraded), and

(b) according to the criteria given in Table 4.1.0(b)(iii),

and the substance is to be classified according to the most stringent outcome.

Thus:

a) According to Table 4.1.0(b)(ii) clethodim is classified based on the most sensitive species *Glyceria maxima* (E_rC_{10} of 0.00066 mg/L), which results in a classification as Chronic 1 (endpoint is ≤ 1 mg/L). Although *Glyceria* is not a pelagic species, it is more sensitive than *Lemna* by a factor 55 and thus leads to a more stringent classification.

b) According to Table 4.1.0(b)(iii) clethodim does not fulfil the criteria for chronic aquatic hazard since the substance is rapidly biodegradable (see results from OECD TG 301D test in the table above) and the BCF is below 500. The surrogate approach due to the lack of valid chronic data on invertebrates is thus not applicable in this case.

Since the outcome from assessment a) is more stringent, clethodim is classified as Chronic 1, H410 and an M factor of 10 applies, as the E_rC_{10} of 0.00066 mg/L for *Glyceria maxima* is in the range 0.0001 mg/L < E_rC_{10} ≤ 0.001 mg/L (CLP Table 4.1.3.)

2.9.2.5 Conclusion on classification and labelling for environmental hazards

In conclusion, Clethodim fulfils the criteria for classification as Aquatic Acute 1 (Very toxic to aquatic life) and Aquatic Chronic 1 (Very toxic to aquatic life with long lasting effects). The applicable M-factors are 10 for both acute and chronic classifications.

2.9.3 Summary of effects on arthropods

Data are available for 5 arthropod species, summarised in the table below. More details on the studies, as well as the RMS's evaluation are presented in the summaries in Volume 3CA Section B.9.3 for the active substance and Vol. 3CP, Section B.9.5 for the formulated product.

Table 2.9.3-1. Summary of endpoints for arthropods; all values refer to nominal concentrations

Species	Test material	Results	Remarks*	Reference
Bees				
	Clethodim	**LD_{50} > 215.1 µg a.s./bee** (oral)	Acute (48 hours), oral and contact	Berg, C. (2020) Report No.: 140061035

Species	Test material	Results	Remarks*	Reference
Apis mellifera (honeybees, adults)		**LD_{50} > 199.2 µg a.s./bee** (contact)		Vol 3 CA B.9.3.1.1
Apis mellifera (honeybees, adults)	Clethodim	LDD_{50} = 10.64 µg a.s./bee/day	Chronic (10 days), oral	Kimmel, S. (2016a) Report No.: 20160123 Vol 3 CA B.9.3.1.2
Apis mellifera (honeybees, larvae)	Clethodim	LD_{10} = 1.1 µg a.s./larva NOED = 0.8 µg a.s./larva	Acute (8 days), oral	Kimmel, S. (2016b) Report No.: 2016030 Vol 3 CA B.9.3.1.3
Apis mellifera (honeybees, adults)	Clethodim 120	**LD_{50} > 100 µg product/bee (> 14 µg a.s./bee)** (oral)	Acute (48 hours), oral and contact	Szentes, C. (2003) Report No.: 2912/03 Vol 3 CP B.9.5.1
		LD_{50} > 100 µg product/bee (> 14 µg a.s./bee) (contact)		
Non-target arthropods other than bees				
Aphidius rhopalosiphi	Clethodim 120 EC[a]	**LR_{50} = 27.6 g a.s./ha** ER_{50}= 11.5 g a.s./ha	Glass plate (2D exposure regime, 14 days)	Bützler, R. (2020a) Report No.: 141411001 Vol 3 CA B.9.3.2
Aphidius rhopalosiphi	Select 120[b]	LR_{50} > 325.5 g a.s./ha **ER_{50} > 325.5 g a.s./ha**	Extended laboratory test (3D exposure regime, 14 days)	Hirth, N. (2003) Report No.: 20021309/01-NEAp Vol 3 CP B.9.5.2
Typhlodromus pyri	Clethodim 120 EC[a]	**LR_{50} = 7.8 g a.s./ha** ER_{50} > 7.8 g a.s./ha	Glass plate (2D exposure regime, 14 days)	Bützler, R. (2020b) Report No.: 141411063 Vol 3 CA B.9.3.2
Typhlodromus pyri	Select 120[b]	LR_{50} = 3.7 g a.s./ha **ER_{50} > 3.5 g a.s./ha**	Extended laboratory test (2D exposure regime, 2 weeks)	Adelberger, I. (2003) Report No.: 20021309/01-NETp Vol 3 CP B.9.5.2
Typhlodromus pyri	Select 240 + adjuvant[c]	LR_{50} = 3.6 g a.s./ha ER_{50} > 4.8 g a.s./ha	Extended laboratory test (2D exposure regime, 14 days)	Röhlig, U. (2001) Report No.: 00 10 48 063 Vol 3 CP B.9.5.2
Typhlodromus pyri	Select 240 EC[d]	LR_{50} < 384 g a.s./ha (fresh residues); **> 384 g a.s./ha** (4,7 & 14 d aged residues) ER_{50} > 11 g a.s./ha (fresh residues); > 384 g a.s./ha (4,7 & 14 d aged residues)	Aged residue laboratory test (3D exposure regime, 14 days)	Warmers C. (2005) Report No.: 20051242/01-NETp Vol 3 CP B.9.5.2
Chrysoperla carnea	Select 1 EC[e]	LR_{50} > 325.5 g a.s./ha **ER_{50} > 325.5 g a.s./ha**	Extended laboratory test (2D exposure regime, 9 weeks)	Hirth, N. (2004) Report No.: 20021309/01-NECc Vol 3 CP B.9.5.2
Aleochara bilineata	Clethodim 120 EC	**ER_{50} > 976 g a.s./ha**	Extended laboratory test (2D exposure regime, 77 days)	Berg, C. (2020) Report No.: 141411071 Vol 3 CP B.9.5.2

[a] Representative formulation H1231bc; Clethodim 120 EC (Clethodim 120 g/L)
[b] Select 120: 120 g/L, 14% clethodim
[c] Select + adjuvant is a 1:2 v/v mixture with Select 240 (25% clethodim) and Para Sommer (75% paraffin oil) and was considered as a representative formulation for clethodim (EFSA Conclusion 2011)
[d] Select 240 EC (nominally 240 g/L clethodim) was considered as a representative formulation for clethodim (EFSA Conclusion 2011)
[e] Select 1 EC: 14% clethodim
* note that the information provided in this column refers to the duration of the test, which for some studies is not the same as the length of exposure. See study summaries in Vol 3CA/CP for details
Endpoints in **bold** have been used in the risk assessment

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Data are available for 4 species of soil meso- and macrofauna, summarised in the table below. More details on the studies, as well as the RMS's evaluation are presented in the summaries in Volume 3CA Section B.9.4 for the active

substance and Vol. 3CP, Section B.9.7 for the formulated product. The most sensitive species to the active substance clethodim was the earthworm *Eisenia andrei*, with a NOEC for reproduction of 27 mg a.s./kg soil dw. The metabolite CAA was 8-fold more toxic than the parent compound, with a NOEC of 3.2 mg CAA/kg soil dw. The formulated product Clethodim 120 EC was 2, 7 and 42 times more toxic than clethodim technical to earthworms, mites and collembolans, respectively.

Table 2.9.4-1. Summary of endpoints for soil meso- and macrofauna

Species	Test material	Results	Remarks	Reference
Earthworms				
Eisenia andrei	Clethodim	NOEC = 54 mg a.s./kg soil dw[b] **NOEC$_{corr}$ = 27 mg a.s./kg soil dw**[a]	56 days (chronic) (10% peat)	Straube, D. (2020a) Report No.: 140061022 Vol 3 CA B.9.4.1
Eisenia fetida	Clethodim sulfoxide	LC_{50} > 1000 mg/kg soil dw **LC_{50} corr > 500 mg kg soil dw**[a]	14 days (acute) (10% peat)	Stäbler, D. (2003) Report No.: 20031112/01-NLEf Vol 3 CA B.9.4.1
Eisenia andrei	Clethodim oxazole sulfoxide	**NOEC = 25 mg/kg soil dw**[b]	56 days (chronic) (10% peat)	Straube, D. (2019) Report No.: 136141022 Vol 3 CA B.9.4.1
Eisenia fetida	Clethodim oxazole sulfone	**NOEC = 10 mg/kg soil dw**[b]	56 days (chronic) (10% peat)	Lührs, U. (2006) Report No.: 31592022 Vol 3 CA B.9.4.1
Eisenia andrei	CBA	NOEC = 6.4 mg/kg soil dw[b] **NOEC$_{corr}$ = 3.2 mg/kg soil dw**[a]	56 days (chronic) (10% peat)	Pavić, B. (2019a) Report No.: 136081022 Vol 3 CA B.9.4.1
Eisenia andrei	CAA	**NOEC = 3.2 mg/kg soil dw**	56 days (chronic) (10% peat)	Pavić, B. (2019b) Report No.: 136091022 Vol 3 CA B.9.4.1
Eisenia fetida	Clethodim 120	NOEC = 196.9 mg product/kg dw (25.6 mg a.s/kg soil dw[b]) **NOEC$_{corr}$=12.8 mg a.s/kg soil dw**[a]	56 days (chronic) 5 % peat content	Witte, B (2011) Report No.: 62162022 Vol 3 CP B.9.7.1
Eisenia andrei and *Eisenia fetida*, sub-species *E. fetida andrei*	Clethodim	Mean BAFk: 1.20	21 days exposure + 21 days elimination	Schöbinger, U. (2012) Report No.: S11-03866 Vol 3 CA B.9.1.3
*Aporrectodea caliginosa, A. rosea, Lumbricus terrestris, L. rubellus, Tanylobous sp. and Epilobous sp.**	Clethodim 120 g/L EC (TM-20015)	DT_{50} for residues in pooled earthworms: Clethodim: 0.5 days Clethodim sulfoxide: 2.33 days Clethodim sulfone: 5.91 days	28 days	Hamberger, A (2012) Report No.: S11-03863 Vol 3 CA B.9.1.3
Other non-target soil meso- and macrofauna				
Folsomia candida	Clethodim	NOEC = 171 mg a.s./kg soil dw (mortality and reproduction) EC_{10} = 169.9 mg a.s./kg soil dw [reproduction] **(EC_{10} corr = 84.95 mg a.s./kg soil dw)**[a]	28 days (5% peat)	Straube, D. (2020b) Report No.: 140061016 Vol 3 CA B.9.4.2
Hypoaspis aculeifer	Clethodim	NOEC = 47.6 mg a.s./kg soil dw[b] [reproduction] EC_{10} = 61.7 mg a.s./kg soil dw	14 days (5% peat)	Straube, D. (2020c) Report No.: 140061089 Vol 3 CA B.9.4.2

Species	Test material	Results	Remarks	Reference
		(NOECcorr = 23.8 mg a.s./kg soil dw)[a]		
Folsomia candida	Clethodim oxazole sulfoxide	**NOEC = 100 mg/kg soil dw**[a]	28 days (10% peat)	Lührs, U. (2005) Report No.: 25001016 Vol 3 CA B.9.4.2
Folsomia candida	Clethodim sulfone	**NOEC = 85.7 mg/kg soil dw**	28 days (5% peat)	Pavić, B. (2020) Report No.: 153041016 Vol 3 CA B.9.4.2
Folsomia candida	Clethodim 120 EC	NOEC = 29.4 mg product/kg soil dw (3.88 mg a.s./kg soil dw) [reproduction] **NOECcorr = 1.94 mg a.s./kg soil dw**[a]	28 days (5% peat)	Straube, D. (2020c) Report No.: 141411016 Vol 3 CP B.9.7.2
Hypoaspis aculeifer	Clethodim 120 EC	NOEC = 52.9 mg product/kg soil dw (6.98 mg a.s./kg soil dw) [reproduction] **(NOECcorr = 3.49 mg a.s./kg soil dw)**[a]	14 days (5% peat)	Straube, D. (2020d) Report No.: 141411089 Vol 3 CP B.9.7.2

[a] Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002. Clethodim sulfoxide and CBA have a log Pow > 2
[b] Highest concentration tested
*taxa identified in samples of natural populations of earthworms in the field
dw = dry weight
Endpoints in **bold** have been used for the risk assessment

2.9.5 Summary of effects on soil nitrogen transformation

Data on soil nitrogen transformation are available for clethodim, 4 of its metabolites and the formulated product. Less than 25% inhibition of N transformation was observed in all tests. More details on the studies, as well as the RMS's evaluation are presented in the summaries in Volume 3CA Section B.9.5 for the active substance and Vol. 3CP, Section B.9.9 for the formulated product.

Table 2.9.5-1. Summary of endpoints for soil nitrogen transformation

Species	Test material	Results	Remarks	Reference
Soil microorganisms	Clethodim	<25% effects at 2.741 mg/kg soil dw	28-day	Reis, K-H. (2005) Report No.: 24991080 Vol 3 CA B.9.5
Soil microorganisms	Clethodim 120	<25% effect at 2.0 mg a.s./kg soil dw	28-day	Feil, N. (2009) Report No.: 50271080 Vol 3 CP B.9.9
Soil microorganisms	Clethodim oxazole sulfone	<25% effect at 0.1 mg/kg soil dw	70-day	Reis, K-H. (2007) Report No.: 31591080 Vol 3 CA B.9.5
Soil microorganisms	Clethodim oxazole sulfoxide	<25% effect at 0.14 mg/kg soil dw	28-day	Hammesfahr, U. (2019a) Report No.: 136141080 Vol 3 CA B.9.5
Soil microorganisms	CBA	<25% effect at 0.35 mg/kg soil dw	57-day	Hammesfahr, U. (2019b) Report No.: 136081080 Vol 3 CA B.9.5
Soil microorganisms	CAA	<25% effect at 0.175 mg/kg soil dw	28-day	Hammesfahr, U. (2019c) Report No.: 136091080 Vol 3 CA B.9.5

2.9.6 Summary of effects on terrestrial non-target higher plants

Data on the formulated product Centurion Pro are available for 6 species of terrestrial higher plants. More details on the studies, as well as the RMS's evaluation are presented in the summaries in Volume 3CA Section B.9.6. The most sensitive endpoint was vegetative vigour in corn.

Table 2.9.6-1. Summary of endpoints on terrestrial plants

Species	Test material	Results	Remarks	Reference
Corn, oat, onion, radish, carrot, soybean	Centurion Pro[a]	**ER_{50} > 270.4 g a.s./ha** (all species tested)	Seedling emergence[b] 21 days	Fiebig, S. (2003a) Report No.: TNK86941 Vol 3 CA B.9.6.2
Corn, oat, onion, radish, carrot, soybean	Centurion Pro[a]	ER_{50} = 4.7 g a.s./ha (corn)[d] ER_{50} = 10.8 g a.s./ha (oats)[d] ER_{50} > 270.4 g a.s./ha (all other species tested)	Vegetative vigour[c] 21 days	Fiebig, S. (2003b) Report No.: TNW86942 Vol 3 CA B.9.6.2
Lolium perenne and *Echinochloa crus-galli*	clethodim	**ER_{50} = 3.37 g/ha** *(E. crus-galli)* ER_{50} = 6.67 g/ha (*L. perenne*)	Vegetative vigour[c], Biomass 21 days	Balluff, M. (2003a)[e] Report No.: 20033008/S1-FGVV Vol 3 CA B.9.6.2
	clethodim sulfoxide	ER_{50} = 16.49 g/ha *(E. crus-galli)* ER_{50} = 24.86 g/ha (*L. perenne*)		
Lolium perenne and *Echinochloa crus-galli*	clethodim sulfone	ER_{50} = 12.47 g/ha *(E. crus-galli)* ER_{50} = 23.2 g/ha (*L. perenne*)	Vegetative vigour[c], Biomass 21 days	Balluff, M. (2003b)[e] Report No.: 20033009/S1-FGVV Vol 3 CA B.9.6.2
Lolium perenne and *Echinochloa crus-galli*	clethodim oxazole sulfone	ER_{50}: >320 g/ha (both species)	Vegetative vigour[c], Biomass 21 days	Balluff, M. (2003c)[e] Report No.: 20033010/S1-FGVV Vol 3 CA B.9.6.2
Lolium perenne and *Echinochloa crus-galli*	clethodim	ER_{50} = 9.57 g/ha *(E. crus-galli)* ER_{50} = 21.0 g/ha (*L. perenne*)	Vegetative vigour[c], Biomass 21 days	Bützler, R. and Kowalczyk, F. (2020)[e] Report No.: 153191087 Vol 3 CA B.9.6.2
	clethodim oxazole sulfoxide	ER_{50} >320 g a.s./ha (both species)		

[a] Formulation details: The tested formulation is Centurion Pro: clethodim 135.2 g/L
[b] Seedling emergence = pre-emergence exposure
[c] Vegetative vigour = post-emergence exposure
[d] Based on fresh weight (most sensitive parameter)
[e] Studies submitted for assessment of metabolite relevance for groundwater.
Endpoints in **bold** have been used for the risk assessment

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No further studies required.

2.9.8 Summary of effects on biological methods for sewage treatment

The available data show no inhibition of the biological activity of activated sludge at a test concentration of 100 mg clethodim/L. More details on the study, as well as the RMS's evaluation are presented in the summary in Volume 3CA Section B.9.8.

Test system	Test material	Results	Key or Supportive study	Remarks	Reference
Activated sludge	Clethodim	ER_{50} > 100 mg/L NOEC = 100 mg/L	key	3 hours	Dengler, D (2002) Report No.: 20011424/01-AAHT Vol 3 CA B.9.8

2.9.9 Summary of product exposure and risk assessment

Overall, an acceptable risk to the environment is demonstrated for Clethodim 120 EC for all the proposed uses. Details of the risk assessments for clethodim and Clethodim 120 EC are summarised for all wildlife groups in the relevant sections below. The formulation used for aquatic testing, Clethodim 120 (TM-20015) is considered comparable to the representative formulation Clethodim 120 EC (H1231bc). Clethodim 120 appears to be more toxic than clethodim to fish, *Daphnia* and algae but not to aquatic plants. Since these effects are formulation-specific, it is considered appropriate for renewal of approval of the active substance to use endpoints for the active substance, rather than the formulation, for aquatic risk assessments.

2.9.9.1 Risk assessment for birds and other terrestrial vertebrates

Risk assessments for terrestrial vertebrates are conducted in accordance with EFSA guidance (2009) for the intended uses of clethodim detailed within the GAP and presented in Vol 3CP, B.9.2.

A comprehensive data set of acute oral, dietary and reproduction studies with the active substance is available. In addition, an acute oral study with Clethodim 120 (TM-20015 considered comparable to the representative formulated product Clethodim 120 EC (H1231bc)) is available in mammals. An acceptable acute risk is demonstrated in the screening assessment for both birds and mammals for all the proposed uses. TER_{lt} are above the trigger value of 5 for applications of 120 g a.s./L in sugar beet, onions and garlic in the screening assessment for mammals only. For all other scenarios, an acceptable long-term risk is demonstrated at the first tier for both birds and mammals. An acceptable acute and chronic risk to mammals and birds through exposure via drinking water (puddles) is demonstrated based on the worst-case screening assessment.

The risk assessment of secondary poisoning through earthworm-eating and fish-eating birds and mammals is necessary for clethodim for acidic conditions only (log P_{ow} of 3.4). In all cases, an acceptable risk is demonstrated at the first tier and therefore the risk of bioaccumulation from the proposed uses of clethodim is considered low in birds and mammals. All the potentially relevant metabolites are not considered a secondary poisoning risk as their log P_{ow} values are all below three.

2.9.9.2 Risk assessment for aquatic organisms

Aquatic risk assessments as presented (refer to Vol 3 CP, B.9.4) are conducted in accordance with EFSA guidance (2013). Laboratory data are available for fish, aquatic invertebrates, algae and macrophytes with the active substance and the formulated product Clethodim 120 (TM-20015, considered comparable to the representative formulated product Clethodim 120 EC (H1231bc)). Metabolites identified as requiring risk assessment in the aquatic compartment are: clethodim sulfoxide, clethodim sulfone, clethodim imine sulfoxide, clethodim imine, DME

sulfoxide (M17), imine ketone (clethodim imine ketone), 3-chloropropenal, CBA, CAA, clethodim oxazole sulfone, clethodim oxazole sulfoxide and the unknown metabolite M20.

New metabolite studies are available for macrophytes only (*Lemna gibba*), as they represent one of the most sensitive taxonomic groups. However, a fish acute and a *Chironomus riparius* spiked water study are available with clethodim sulfoxide and clethodim imine respectively. For fish the metabolite was less toxic than the parent compound, but for invertebrates no direct comparison can be made since different species were tested for the metabolite and parent compound.

An acceptable risk for all representative uses was demonstrated for all aquatic groups based on the laboratory derived acceptable concentrations (RAC) and Step 2 PEC_{sw} values modelled for relevant exposure scenarios. An acceptable risk from exposure to potentially relevant metabolites was confirmed based on FOCUS modelling Step 2 (clethodim sulfone, M17, unknown M20 and 3-chloropropenal) and Step 1 (all other metabolites) and for the worst-case application of 300 g a.s./ha in sugar beet. An acceptable risk from the formulated product for exposure via spray drift was also confirmed using FOCUS Step 1 drift values (also based on worst-case application).

2.9.9.3 Risk assessment for bees and other non-target arthropods

Bee risk assessments as presented (refer to Vol 3 CP, B.9.6.) are performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev. 2 (final), October 17, 2002).

The acute and contact toxicity studies demonstrate clethodim and Clethodim 120 (considered comparable to the representative formulated product Clethodim 120 ED (H1231bc)) to be of low toxicity to bees. An acceptable risk to honeybees is demonstrated at the first tier from all the proposed uses of clethodim. Chronic adult and larval honeybee studies are submitted in line with data requirements, however risk assessment schemes for such studies are not provided under the current guidance. Based on EFSA 2013, a guidance that has not yet been noted by the Standing Committee on Plants, Animals, Food and Feed, the risk assessment for honeybees requires further refinement. Moreover, bumblebees and solitary bees should also be addressed.

Risk assessments for other non-target arthropods than bees are also presented in Vol 3 CP, B.9.6, and performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

Glass plate studies are available for both *Typhlodromus pyri* and *Aphidius rhopalosiphi* and the representative formulated product Clethodim 120 EC. Higher tier extended laboratory studies with *A. rhopalosiphi* (Select 120, 14 % clethodim) and *Chrysoperla carnea* (Select 1 EC, clethodim 14%) are available alongside higher tier extended laboratory studies including an aged residue study with *T. pyri* (Select 120, 14% clethodim; Select, 25% clethodim; Select 240, 240 g/L clethodim). Formulations used for the extended studies are considered sufficiently similar to the representative formulation to enable read across.

An acceptable off-field risk is demonstrated at the first tier for all proposed uses. An acceptable in-field risk is demonstrated at the higher tier for all proposed uses. However, short-term adverse effects may occur in-field with data demonstrating the potential for recovery/recolonization within an acceptable time-period.

2.9.9.4 Risk assessment for earthworms and other non-target soil meso- and macrofauna

Non-target soil meso and macro-fauna risk assessments as presented (refer to CP 10.4.) are performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev. 2 (final), October 17, 2002).

Chronic data on earthworms, collembola and soil mites are available for the active substance and on collembola and soil mites for the representative formulated product Clethodim 120 EC. Chronic earthworm data are available with Clethodim 120 (TM-20025 considered comparable to Clethodim 120 EC (H1231bc)). Six potentially relevant soil metabolites are identified as needing risk assessment: clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, CBA (trans-3-chloroacrylic acid) and CAA (2-[3-chloroallyloxyimino]butanoic acid).

Chronic earthworm studies are available for all metabolites except for clethodim sulfoxide and clethodim sulfone. As clethodim degrades rapidly to clethodim sulfoxide (DT_{50}: 0.3 day) and then to clethodim sulfone (DT_{50}: 3.7 days), these metabolites are present during chronic studies with clethodim. Nonetheless, as a precaution, the RMS considers that both clethodim sulfoxide and clethodim sulfone are more toxic than the parent compound by a factor of 10.

Data on two metabolites are available for *Folsomia candida*, namely clethodim oxazole sulfoxide and clethodim sulfone. For the remaining metabolites, the toxicity is assumed to be 10 times higher than for the parent compound. No metabolite data are available for *Hypoaspis aculeifer*, so the toxicity of metabolites is also assumed to be 10 times larger than that of the parent compound at this screening step.

The chronic risk to non-target soil meso- and macrofauna is considered low from the proposed uses of clethodim; this is demonstrated at the first tier.

2.9.9.5 Risk assessment for terrestrial non-target higher plants

Terrestrial non-target higher plant risk assessments as presented (refer to Vol 3CP B.9.11) are performed in accordance with the recommendation of the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev. 2 final, 2002). The risk assessment is performed based on endpoints from a vegetative vigour and seedling emergence study on *Echinochloa crus-galli* and the active substance clethodim. An acceptable risk is demonstrated at the first-tier for seedling emergence at the highest application rate of 300 g a.s./ha in sugar beet. However, for all proposed uses, a higher tier assessment is required for vegetative vigour as the TERs are < 5. Deposition of clethodim after volatilisation was also considered in the calculation of $PER_{off\text{-}field}$ by the RMS, in accordance with the FOCUS Air guidance (2008).

The following alternative risk mitigation measures are required:

Crop scenario	Mitigation measures
300 g a.s./ha sugar beet	50% drift reduction technology and a 10 m buffer strip 75% drift reduction technology and a 5 m buffer strip 90% drift reduction technology and a 5 m buffer strip
240 g a.s./ha onion and garlic	50% drift reduction technology and a 10 m buffer strip 75% drift reduction technology and a 5 m buffer strip 90% drift reduction technology
120 g a.s./ha sugar beet, onion and garlic	10 m buffer strip 50% drift reduction technology and a 5 m buffer strip 75% drift reduction technology and a 5 m buffer strip 90% drift reduction technology

2.9.9.6 Risk assessment for soil microorganisms

Soil nitrogen transformation risk assessment as presented (refer to Vol 3CP B.9.10) is performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev. 2 (final), October 17, 2002).

Data are available for the active substance and the formulated product Clethodim 120 (TM-20025 considered comparable to Clethodim 120 EC (H1231bc)). The risk to soil nitrogen transformation is considered low from the worst-case use of clethodim (300 g a.s./ha in sugar beet); this is demonstrated at the first tier. Six potentially relevant soil metabolites are identified as needing risk assessment: clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, CBA and CAA. Nitrogen transformation studies with metabolites are available except for clethodim sulfoxide and clethodim sulfone. Given that there were no effects in the study with the active ingredient at concentrations 8.5 times higher than the representative PEC, low risk from the two metabolites tested, and that the formation rates of clethodim sulfoxide and clethodim sulfone are 74.6% and 43.9%, respectively, it is considered that there is a sufficient margin to assume a low risk also for these metabolites. Extrapolating the data from the active substance using a factor 10 also shows acceptable risk for these two metabolites.

2.9.9.7 Risk assessment for biological methods for sewage treatment

The results of a study on the effects of clethodim on the respiration of aerobic wastewater bacteria showed the 3-hour EC_{50} to be greater than 100 mg/L, the highest concentration tested, thus, indicating no concern over effects on biological methods for sewage treatment.

2.10 ENDOCRINE DISRUPTING PROPERTIES

2.10.1 Gather all relevant information.

The mammalian toxicology studies for clethodim cover a range of study types including subacute, subchronic, chronic, developmental, and reproductive toxicity studies in mammalian species including rat, mouse, dog, and rabbit. The relevant regulatory non-mammalian toxicology studies for clethodim include studies in amphibians as well as studies in birds and fish. Furthermore, *in vivo* mechanistic studies (Uterotrophic assay and Hershberger assay) and *in vitro* mechanistic studies (*in vitro* aromatase and steroidogenesis assays) are available as well as *in silico* mechanistic data (ER, AR, TR).

No studies within the scope of the Guidance and relevant to identification of ED properties were deemed to be relevant following evaluation of the hits from search of the open literature.

The following studies and assays were considered in the ED assessment of Clethodim:

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
Mammalian Repeat Dose Toxicity Studies				
1	5-weeks oral (dietary) Rat (Sprague Dawley) 10/sex/group Acceptable	GLP OECD 407 (1995) <u>Deviations from OECD 407 (2008):</u> - exposure for 5 weeks, not 4 - weight of epididymis, thymus, spleen and heart was not determined - blood clotting potential was not measured - functional observations were not performed - histopathology on bone marrow was not performed - humidity (72%) slightly above recommended acceptable value of 70% in the guideline	Yes - weight of epididymis	[redacted] (1986) Report No: S-2720 Vol. 3, B.6.3.1/01
2	4-week oral dietary Mouse (CD-1 ICR-derived) 10/sex/group	GLP OECD 407 (1995) <u>Deviations from OECD 407 (2008):</u> - clinical and functional observations were not performed	No	[redacted] (1986) Report No.: S-2733 Vol. 3, B.6.3.1/02

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
	Acceptable	- blood clotting potential was not determined - thymus, spleen and heart were not weighed - histopathology on bone marrow was not performed.		
3	90 days oral (dietary) Rat (Wistar) Acceptable	GLP OECD 408 (1998) <u>Deviations from OECD 408 (2018):</u> Parameters/endpoints not examined in this study include: - blood measurements of thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH) - plasma/serum measurements of low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and other hormones (on a case-by-case basis) - weights of prostate and seminal vesicles with coagulating glands as a whole, pituitary and thyroid gland - determination of oestrus cycle stage of all females at necropsy - enumeration of cauda epididymis sperm reserves, sperm morphology or sperm motility (optional) - histopathology of coagulation glands and male mammary glands - sensory reactivity and functional observations were not performed. The weights of the epididymides, thymus, spleen, heart and uterus - blood clotting potential - histopathology on bone marrow - humidity (78%) above recommended acceptable value of 70%	Yes - Parameters/endpoints not examined in this study include: - blood measurements of thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH) - plasma/serum measurements of low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and other hormones (on a case-by-case basis) - weights of prostate and seminal vesicles with coagulating glands as a whole, pituitary and thyroid gland - determination of oestrus cycle of all females at necropsy - enumeration of cauda epididymis sperm reserves, sperm morphology or sperm motility (optional) - histopathology of coagulation glands and male mammary glands - sensory reactivity and functional observations - weights of epididymides, thymus, spleen, and uterus	[redacted] (1986) Report No.: S-2765 Vol. 3, B.6.3.2/01
4	90 days oral (gelatine capsules) Dog (Beagle) 4/sex/group Acceptable	GLP statement (but no certificate) In general accordance with OECD 409 (1998) <u>Deviations from OECD 409 (1998):</u> - the weights of the epididymides, thymus, spleen and uterus were not determined. - histopathology on the bone marrow was not performed.	Yes - weights of epididymides, thymus, spleen and uterus were not determined.	[redacted] (1987) Report No: S-2759 Vol. 3, B.6.3.2./02
5	1 year oral (gelatine capsules) Dog (Beagle) 6/sex/group	GLP OECD 452 (1998) <u>Deviations from OECD TG 452 (2018):</u>	No	[redacted] (1988) Report No.: S-2964 Vol. 3, B.6.3.2/03

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
	Acceptable	- no histopathologic evaluation of the harderian gland and lacrimal gland - ornithine decarboxylase was not determined. - the temperature and humidity varied greatly and were outside of the recommended range		
6	2 years oral (dietary) Rat (Sprague-Dawley) 65/sex/group 10/sex/group (sacrificed at interim sacrifice, 1 y) Acceptable	GLP OECD 453 (1981) <u>Deviations from current OECD 453 (2018):</u> - prothrombin time and activated partial thromboplastin time were not measured - weight of thyroid, epididymis, heart, spleen, and uterus were not measured - coagulating gland, vagina, and lacrimal gland were not fixed and/or examined - the humidity varied a lot and was outside of the recommended range	Yes - weight of thyroid, epididymis, spleen, and uterus were not measured - coagulating gland and vagina were not fixed and/or examined	[redacted] (1988a) Report No.: S-2766 Vol. 3, B.6.5./02
7	18 months oral (feeding) Mouse (Crl:CD-1(ICR)BR) 60/sex/group Acceptable	GLP OECD 451 (1981) <u>Deviations from current OECD 451 (2018):</u> Organs not harvested/assessed: coagulating gland, lacrimal gland, mammary glands from males (note that it is only required if visibly dissectible, no information on this)	No	[redacted] (1988) Report No.: S-2867 Vol. 3, B.6.5/01
8	Dose range-finding developmental toxicity, gavage Rat (Sprague-Dawley) 10 mated females/group	GLP EPA 83-3 <u>Major deviations from a full OECD TG 414 (2018):</u> - ten dams/group, TG recommends 20 to achieve at least 16 animals with implantation sites - the exposure period ended at day 15 instead of the day prior to termination (day 19) - anogenital distance in foetuses, thyroid weight, thyroid histopathology, and blood thyroid hormone levels (T4, T3 and TSH) in the maternal animals - it is noted that there were indications of SDA viral infections in some dams at gestation day 20. This was noted in 1, 2, 2, 3, and 2 females in the 0, 50, 150, 300, and 500 mg/kg bw/day group, respectively	Not applicable. Pilot study	[redacted] (1987a) Report No.: S-2807 Vol. 3, B.6.6.2.1/01

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
9	Developmental toxicity rat, gavage Rat (Sprague-Dawley) 25 mated/group	GLP EPA 83-3 Deviations from current OECD 414 (2018): The following endpoints were not assessed: - anogenital distance in foetuses - thyroid weight, thyroid histopathology, and blood thyroid hormone levels (T4, T3 and TSH) in the maternal animals. The exposure period ended at day 15 instead of the day prior to termination (shorter exposure period).	Yes: - anogenital distance in foetuses was not measured - thyroid weight, thyroid histopathology, and blood thyroid hormone (T4, T3 and TSH) concentrations were not measured in the maternal animals.	[redacted] (1987) Report No.: S-2808 Vol. 3, B.6.6.2.2/01
10	Dose range-finding, gavage Rabbit (New Zealand White) 8/group	GLP EPA 83-3 Major deviations from a full OECD 414 (2018): - eight dams/group, TG recommends 20 to achieve at least 16 animals with implantation sites - the exposure period ended at day 19 instead of the day prior to termination (day 28)	Not applicable. Pilot study	[redacted](1987a) Report No.: S-2734 Vol. 3, B.6.6.2.3/01
11	Developmental toxicity, gavage Rabbit (New Zealand White) 19-20/group	GLP EPA 83-3 Deviations from OECD 414 (2001; the 2018 update is not applicable to rabbits): the exposure period ended at day 19 instead of the day prior to termination (shorter exposure period).	No	[redacted] (1987) Report No.: S- 2869 Vol. 3, B.6.6.2.4/01
12	Dose range finding reproduction study (dietary) Rat (Albino Crl:CD Sprague Dawley) 8/sex/group	GLP EPA 83-4 Major deviations from OECD 416 (2001): - treatment initiated one week before mating rather than 10 weeks before mating - only one generation, F0 dams and F1 pups terminated on lactation day 7 - low number of females (8), GL recommends use of sufficient number of animals to yield preferably not less than 20 pregnant females at or near parturition. - oestrous cycle length and normality not investigated - testis and epididymis weight not investigated - sperm motility and sperm morphology not analysed	Not applicable. Pilot study	[redacted](1986) Report No.: S-2758 Vol. 3, B.6.6.1/01

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
		- total number of homogenisation-resistant testicular spermatids and cauda epididymal sperm not enumerated - physical development of the offspring not investigated - haematological and clinical parameters not investigated, organ weights not recorded, histopathological investigations not made - less number of observation points		
13	Two-generation reproductive study, dietary Rat (Crl: COBS/CD Sprague-Dawley) 30/sex/group (F0 and F1 generation)	GLP EPA 83-4 <u>Deviations from OECD 416 (2001):</u> - no analysis of sperm parameters - developmental and functional observations of pups were not performed - weighing of adrenals, brain, liver, pituitary gland, spleen, thyroids were not performed - histopathology of the vagina was not performed - dosing before mating period seems to be 9 weeks (the guideline recommends dosing to be continued for at least 10 weeks before the mating period)	Yes - no analysis of sperm parameters - developmental and functional observations of pups were not performed - weight of adrenals, brain, liver, pituitary gland, spleen, and thyroid were not measured - histopathology of the vagina was not performed	[redacted] (1987) Report No.: S-2778 Vol. 3, B.6.6.1/02
Report No. S-2763*	Five-Week Sub-chronic Feeding Study of High Purity RE-45601 (SX-1718) and RE-45601 Process Neutrals (SX-1717) in Rats Sprague-Dawley® Crl:CD® (SD) BR 10 rats/sex/group Supportive	No guideline followed. <u>Observations in study:</u> -Viability (daily) -Signs of toxicity (weekly and on the last day of the study) -Pupil responses (day 35) -Body weight (weekly, starting on day 0) -Food consumption (new food was provided twice weekly, at which time the remaining food was weighed to estimate consumption) -Test material intake (calculated weekly) -Haematology (platelet count, erythrocyte count, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, reticulocyte count, total and differential leukocyte morphology.) -Serum chemistry (sodium, potassium, chloride, calcium, phosphorus, glucose, triglycerides, cholesterol, total, direct and indirect bilirubin, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase activity, uric acid, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, total protein, albumin, globulin and albumin/globulin ratio)	Not applicable. No guideline study	[redacted] 1987 Report no. S-2763 Vol. 3. B.6.8.2/03

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
		-Gross necropsy (external surface of the body, all orifices and the cranial, thoracic, abdominal, and pelvic cavities with their associated organs and tissues) -Organ weights (brain, liver, adrenals, kidneys, and testes or ovaries) Histopathological examination (adrenals, brain, epididymis, kidneys, liver, lungs, ovaries, sciatic nerve, spinal cord, spleen, testes, urinary bladder, uterus and all gross lesions)		
***In vivo* Mechanistic Studies**				
14	Uterotrophic Assay, gavage Rat (Sprague Dawley)	OECD Guideline 440 <u>Deviations from OECD Guideline 440 (2007):</u> None	No	[redacted] (2020a) Report No.: 00155006 Vol. 3, B.6.8.3/03
15	Hershberger Assay, gavage Rat (Sprague Dawley)	OECD Guideline 441 <u>Deviations from OECD Guideline 441 (2009):</u> -a full necropsy was not carried out	No	[redacted] (2020b) Report No.: 00155007 Vol. 3, B.6.8.3/04
***In vitro* Mechanistic Studies**				
16	In vitro Aromatase Inhibition using Human Recombinant Microsomes	OPPTS 890.1200	-	Rijk J.C.W. (2020a) Report No.: 20221185 Vol. 3, B.6.8.3/01
17	Steroidogenesis assay using the Human H295R Adreno- carcinoma Cell Line	OECD Guideline 456 <u>Deviation from OPPTS) guideline 890.1200 (2019):</u> The microsomes should not be stored longer than 12 months according to the guideline, in this study they were stored “for a maximum of 2 years”	-	Rijk J.C.W. (2020b) Report No.: 20221184 Vol. 3, B.6.8.3/02
***In silico* mechanistic data**				
18	ER -ATG_ERa_ TRANS_up -ATG, ERE_CIS_up -ATG_Era_TRANS_dn -ATG_ERE_CIS_dn	-	-	Cloke et al (2020) Report No.: 1602215.UK0 – 9208

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
	AR -ATG, AR_TRANS_up -ATG_AR-TRANS-dn -TOX21_AR_LUC_ MDAKB2_Agonist_3u M_Nilutamide -TOX21_AR_LUC_ MDAKB2_Antagonist_0 .5nM_R1881 TR ATG_THRa1_TRANS_ up ATG_THRa1_TRANS_ dn			Vol. 3, B.6.8.3.5/01
Non-target Organisms Repeat Dose Toxicity Studies				
19	RE-45601 Technical: A One-Generation Reproduction Study with the Bobwhite (*Colinus virginianus*)	US EPA 71-4 (1982)/ASTM Draft Number 8 (1983)	Yes. The test method was not designed for evaluation of endocrine properties, but includes endpoints that are 'sensitive to but not diagnostic of' endocrine disruption according to Echa/Efsa GD.	[redacted] (1988a) (ID S-2836)
20	RE-45601 Technical: A One-generation Reproduction Study with the Mallard (*Anas platyrhynchos*)	US EPA 71-4 (1982)/ASTM Number 8 (1983) Draft	Yes. The test method was not designed for evaluation of endocrine properties, but includes endpoints that are 'sensitive to but not diagnostic of' endocrine disruption according to Echa/Efsa GD.	[redacted] (1988b) (ID S-2837)
21	RE-45601 Technical: A Pilot Reproduction Study with the Bobwhite (*Colinus virginianus*)	US EPA 71-4 (1982)/ASTM Draft Number 8 (1983)	Not applicable. Pilot study	[redacted] (1987a) (ID S-2833)
22	RE-45601 Technical: a pilot reproduction study with the mallard (*Anas platyrhynchos*)	US EPA 71-4 (1982)/ASTM Draft Number 8 (1983)	Not applicable. Pilot study	[redacted] (1987b) (ID S-2834)

Matrix Study ID	Study type	Guidance	ED endpoints missing	Reference
23	Clethodim: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*)	US EPA OPPTS 850.1000 (1996) US EPA OPPTS 850.1400 (1996) ASTM Standard E1241-05	Yes. The test method was not designed for evaluation of endocrine properties, but includes endpoints that are 'sensitive to but not diagnostic of' endocrine disruption according to Echa/Efsa GD.	[redacted] (2011) VP-37752
24	Clethodim: Fish short-term reproduction assays with the fathead minnow (*Pimephales promelas*)	U.S. EPA OPPTS 890.1350 ECD 229 (2012)	No	[redacted] (2020)
25	Clethodim: Amphibian metamorphosis assay with the African clawed frog (*Xenopus laevis*)	OECD 231 (2009)	No	[redacted] (2021)

* Effects on the liver, adrenal, red blood cell parameters and body weight grow were observed in this study. The effect on the adrenal (reduced weight) was included in the Lines of evidence for adverse effects and endocrine activity. For brain, testes, ovary, epididymis, sciatic nerves, there were no changes in organ weights or histopathology. The study is supportive data only.

2.10.2 ED assessment for humans

The lines of evidence for adverse effects and endocrine activity are detailed separately for the T modality (section 2.10.2.1) and then for the EAS modalities (section 2.10.2.2).

2.10.2.1 ED assessment for T-modality

	Sufficiently investigated
T-mediated parameters	Yes, based on the availability of the following studies: ID 1, 3, 4, 5, 6, and 7

According to the EFSA guidance document ("Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009" adopted 5 June 2018), a sufficient data set to support a conclusion on absence of thyroid related endocrine activity and adversity includes "the thyroid parameters foreseen to be investigated in the following studies OECD test guidelines 407, 408, 409 (and/or the one-year dog study, if available), 416 (or 443 if available) and 451-3". The parameters and the studies in which they were assessed in this data package are summarized in the table below.

Thyroid parameters foreseen to be investigated in OECD test guidelines 407, 408, 409, 416, 443, and 451-3

Parameter	**Investigated?**	**Study ID**	**Species**	**Study length**
T3 and/or T4 level	No	-	-	-
Thyroid stimulating hormone (TSH) level	No	-	-	-
Thyroid weight	Yes	4 and 5	Dog	13 w, 52 w
Thyroid histopathology	Yes	1, 3, 4, 5, 6, and 7	Rat (1, 3, 6) Dog (4, 5) Mouse (7)	5 w, 13 w, 2 y 13 w, 52 w 52 w

In addition to the parameters above, liver weight (measured in several studies, affected in most) and HDL/LDL ratio (not measured) can be considered T-mediated when a change is observed in combination with other thyroid-related endpoints.

2.10.2.1 Lines of evidence for adverse effects and endocrine activity related to T-modality, for parameters which may be ´Sensitive to-but-not -diagnostic-of EATS´, and for systemic/target organ toxicity in mammals following clethodim exposure.

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
18	In vitro mechanistic	Thyroid receptor	human liver cell line	24	Hours	Uptake from the medium (in vitro)	0	µM	No effect	No effect 0 (No TR agonist activity)	No TR-mediated activity	Thyroid weight was not measured in rodents but no remarks on thyroid histology were made. An indication of thyroid activity was observed in male dogs because of the higher thyroid weight in exposed animals (appears to be a dose response but only statistically significant in highest dose), but the number of individuals in dog studies are low, no hormone measurements were performed, and no remarks were made on the histology of the

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
												gland. Overall, thyroid related effects cannot be excluded nor confirmed.
15	In vivo mechanistic	Liver weight (Hershberger considered T-mediated only in combination with other thyroid endpoints)	Rat	10	Days	Oral	>200	mg/kg bw/day	No effect	No effect >200 mg/kg bw/day		
1	EATS-mediated	Thyroid histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect	No effects on thyroid histology were observed in any of the studies.	The increase in thyroid weight in male dogs (ID: 5) in the high dose group was large (~100% increase) and appeared dose	
3	EATS-mediated	Thyroid histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Thyroid histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	EATS-mediated	Thyroid histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Thyroid histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
7	EATS-mediated	Thyroid histo-pathology	Mouse	52	Weeks	Oral	>3000	ppm	No effect		dependent but no histo-pathological correlated changes were observed. No thyroid weight changes have been observed in females	
4	EATS-mediated	Thyroid weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect	A dose dependent increase in thyroid/parathyroid weight was observed in males (22, 45, and 91% increase in absolute weight; 33, 33, and 100% increase in relative weight) but not females in dogs exposed for 52 weeks. No effects were observed in either sex in the 13-week		
5	EATS-mediated	Thyroid weight	Dog	52	Weeks	Oral	300	mg/kg bw/d	Absolute and relative thyroid/parathyroid weight was sig-nificantly increased in males (183 and 200% of controls, respect-tively).			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										study, but it is noted that the highest dose was lower in that study.		
1	Sensitive to but not diagnostic of EATS	Adrenals histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect	No effects were observed on adrenal histopathology	Adrenal weight was clearly affected in one out of 8 studies, but no remarks have been made in the histo-pathological assessment. The animals in the study with reduced adrenal weight also	Evidence of effects on parameters sensitive to but not diagnostic of T-mediated adversity (Litter/pup weight, Foetal development, Post implantation loss, incidence of external malformations) at dose levels causing maternal toxicity. Effects may be secondary to maternal toxicity.
3	Sensitive to but not diagnostic of EATS	Adrenals histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Sensitive to but not diagnostic of EATS	Adrenals histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to but not diagnostic of EATS	Adrenals histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
6	Sensitive to but not diagnostic of EATS	Adrenals histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect		had a reduced body weight and increased liver weight. In another study, adrenals weights of both males and females were affected at 53 weeks but not 79 weeks: decrease in males and increase in females. The result in the latter study	
7	Sensitive to but not diagnostic of EATS	Adrenals histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Sensitive to but not diagnostic of EATS	Adrenals weight	Rat	5	Weeks	Oral	>8000	ppm	No effect	Lower adrenal weight (absolute and relative to brain weight) was observed in both males and females in a 5-week dietary study on rats, but no effect was observed in other rat studies (5w,		
2	Sensitive to but not diagnostic of EATS	Adrenals weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Sensitive to but not diagnostic of EATS	Adrenals weight	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Sensitive to but not	Adrenals weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of EATS									13w, and 2y. The rats that died during the acute oral study had enlarged adrenals along with several other clinical signs. In the 2y mouse study decreased adrenal weight was observed for all treated male groups and for low- and mid-dose group females when compared to respective control values.	is of unclear relevance.	
5	Sensitive to but not diagnostic of EATS	Adrenals weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Sensitive to but not diagnostic of EATS	Adrenals weight	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to but not diagnostic of EATS	Adrenals weight	mouse	52	Weeks	Oral	>3000	ppm	Change			
S-2763	Sensitive to but not diagnostic of EATS	Adrenals weight	Rat	5	Weeks	Oral	6800	ppm	Decrease			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										However, due to the inconsistency of the change between sexes, the lack of dose response and no differences at Week 79, this finding was not considered to be treatment related.		
1	Sensitive to but not diagnostic of EATS	Brain histo-pathology examination	Rat	5	Weeks	Oral	>8000	ppm	No effect	No effects have been observed on brain histology	No clear treatment related effects on the brain weight or histo-pathology	
3	Sensitive to but not diagnostic of EATS	Brain histo-pathology examination	Rat	13	Weeks	Oral	>5000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
4	Sensitive to but not diagnostic of EATS	Brain histo-pathology examination	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to but not diagnostic of EATS	Brain histo-pathology examination	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Sensitive to but not diagnostic of EATS	Brain histo-pathology examination	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to but not diagnostic of EATS	Brain histo-pathology examination	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Sensitive to but not diagnostic of EATS	Brain weight	Rat	5	Weeks	Oral	>8000	ppm	No effect	Relative brain weight was increased in two studies on rats (ID 1 and 3); however,		
2	Sensitive to but not	Brain weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of EATS									these increases are likely a result of reduced BW. No effects on absolute brain weight have been observed.		
3	Sensitive to but not diagnostic of EATS	Brain weight	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Sensitive to but not diagnostic of EATS	Brain weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to but not diagnostic of EATS	Brain weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Sensitive to but not diagnostic o, EATS	Brain weight	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to but not diagnostic of EATS	Brain weight	mouse	52	Weeks	Oral	>3000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
13	Sensitive to but not diagnostic of EATS	Dystocia	rat	28	weeks	Oral	>2500	ppm	No effect		No effects on dystocia	
13	Sensitive to but not diagnostic of EATS	Fertility (mammals)	rat	28	weeks	Oral	>2500	ppm	No effect		No effects on fertility	
13	Sensitive to but not diagnostic of EATS	Gestation length	rat	28	weeks	Oral	>2500	ppm	No effect		No effect on gestation length	
12	Sensitive to but not diagnostic of EATS	Litter size	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect		No effect on litter size	
13	Sensitive to but not diagnostic of EATS	Litter size	rat	28	weeks	Oral	>2500	ppm	No effect			
8	Sensitive to but not	Litter/pup weight	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Decrease	Mean foetal weights were	Reduction in foetal body	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of EATS									reduced in the animals of the top dose group (-10.6% for the composite foetal weight data). A tendency was observed already at 300 mg/kg bw/day (7% reduction, not statistically significant). Maternal effects at the top dose included: excess salivation and reduced body weight gain	weight were observed in three studies (rat and rabbit) but at doses causing maternal toxicity, including reductions in body weight and food consump-tion. In rats, reductions in combined pup weight (day 7) and pup weight gain (day 0-7), but not birth weight,	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
9	Sensitive to but not diagnostic of EATS	Litter/pup weight	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Decrease	Reduction in foetal body weight was observed in dose groups receiving 350 and 700 mg/kg bw/day. Maternal effects at those doses included: clinical signs and reduced body weight gain	were observed at all dose levels (500, 2000, and 5000 ppm) in the 5-week study. Maternal toxicity was only noted in the highest dose group. This effect on postnatal growth was not observed in either generation of the 2-generation study (doses used: 5, 20,	
10	Sensitive to but not diagnostic of EATS	Litter/pup weight	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Decrease	Reduction in foetal body weight was observed in dose groups receiving 300 and 500 mg/kg bw/day.		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										Maternal effects at those doses included reduced food consumption, reduced body weight gain and body weight, dried faeces, and mortality	500, and 2500 ppm).	
11	Sensitive to but not diagnostic of EATS	Litter/pup weight	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
12	Sensitive to but not diagnostic of EATS	Litter/pup weight	rat	5 to 6	Weeks	Oral	5000	ppm	Decrease	There was a significant decrease in combined pup weight (male and female) at day 7 and a decrease in combined pup		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										weight gain between days 0 and 7 for all three dose levels. Maternal food consumption and body weight was reduced in the highest but not in the lower dose groups.		
13	Sensitive to but not diagnostic of EATS	Litter/pup weight	rat	28	weeks	Oral	>2500	ppm	No effect			
8	Sensitive to but not diagnostic of EATS	Number of implantations, corpora lutea	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	No effect	Number of implantation sites was reduced (87 vs 126) - not statistically significant.	No clear indications that the number of implant-tation sites or corpora	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										Note that there were indications of SDA viral infections in some dams at gestation day 20.	lutea were affected. The pilot study in which a reduction in the number of implant-tation sites was observed was of limited reliability due to potential SDA infections in some individuals.	
9	Sensitive to but not diagnostic of EATS	Number of implantations, corpora lutea	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect			
10	Sensitive to but not diagnostic of EATS	Number of implantations, corpora lutea	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Sensitive to but not diagnostic of EATS	Number of implantations, corpora lutea	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
13	Sensitive to but not	Number of implantations,	rat	28	weeks	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of EATS	corpora lutea										
12	Sensitive to but not diagnostic of EATS	Number of live births	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect		The one effect on the number of stillborn were in the F0 generation (F0--> F1), something that was not evident in the second generation of the study.	
13	Sensitive to but not diagnostic of EATS	Number of live births	rat	28	weeks	Oral	>2500	ppm	No effect	Number of stillborn were increased in the F1 generation (F1 pups) but not in the F1 generation (F2 pups)		
8	Sensitive to but not diagnostic of EATS	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	No effect	Number of viable foetuses was reduced (86 vs 122) but not statistically significant.	The effect on foetal viability in the rabbit at high doses is likely related	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
9	Sensitive to but not diagnostic of EATS	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect		to maternal toxicity.	
10	Sensitive to but not diagnostic of EATS	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	Change	Four of the seven pregnant 500 mg/kg/day dosage group rabbits aborted during the study. All abortions occurred after completion of the dosage period. Three of the seven aborted 3 foetuses each and 1 rabbit aborted 2. One of the seven rabbits had 1		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										early resorption and 2 rabbits had 2 late resorptions. Clear signs of maternal toxicity were observed in the study: reduced food consumption (≥50 mg/kg bw/day), reduced bw gain (≥150 mg/kg bw/day), increased LW (≥150 mg/kg bw/day), and death (≥150 mg/kg bw/day)		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
11	Sensitive to but not diagnostic of EATS	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
1	Sensitive to but not diagnostic of EATS	Pituitary histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect	No treatment related microscopic lesions were observed in the pituitary	No effects on the pituitary weight or histo-pathology	
3	Sensitive to but not diagnostic of EATS	Pituitary histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Sensitive to but not diagnostic of EATS	Pituitary histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to but not diagnostic of EATS	Pituitary histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
6	Sensitive to but not diagnostic of EATS	Pituitary histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to but not diagnostic of EATS	Pituitary histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
4	Sensitive to but not diagnostic of EATS	Pituitary weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect	No effects on pituitary weight were observed		
5	Sensitive to but not diagnostic of EATS	Pituitary weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
9	Sensitive to but not diagnostic of EATS	Post implantation loss	rat	10 (GD 6-15)	Days	Oral	700	mg/kg bw/d	Increase	Increased post-implantation loss at 700 mg/kg bw/day. This in part was driven by	Increase in post implant-tation loss in the rabbit at high doses, considered secondary to	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										a single female with 15/16 foetal resorptions, with this female excluded the mean resorption rate was 1.1 which is slightly higher than concurrent control (0.8) and within HCD.	maternal toxicity.	
10	Sensitive to but not diagnostic of EATS	Post implantation loss	rabbit	13 (DG 7-19)	Days	Oral	500	mg/kg bw/d	Increase	Four of the seven pregnant 500 mg/kg/day dosage group rabbits aborted during the study. One of these rabbits		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										died following abortion. All abortions occurred after completion of the dosage period. Clear signs of maternal toxicity were observed in the study: reduced food consumption (≥50 mg/kg bw/day), reduced bw gain (≥150 mg/kg bw/day), increased LW (≥150 mg/kg bw/day), and death (≥150		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										mg/kg bw/day)		
11	Sensitive to but not diagnostic of EATS	Post implantation loss	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
13	Sensitive to but not diagnostic of EATS	Post implantation loss	rat	28	weeks	Oral	>2500	ppm	No effect			
8	Sensitive to but not diagnostic of EATS	Pre-implantation loss	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Increase	The mean pre-implantation loss ratio at 500 mg/kg bw/day was higher than the control.	Increase in pre-implan-tation loss in the rat at the highest dose. Note that the dosing commenced during the implant-	
9	Sensitive to but not	Pre-implantation loss	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of EATS										tation phase (GD 6) and therefore the pre-implan-tation losses may be unrelated to treatment.	
10	Sensitive to but not diagnostic of EATS	Pre-implantation loss	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Sensitive to but not diagnostic of EATS	Pre-implantation loss	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
9	Sensitive to but not diagnostic of EATS	Presence of anomalies (External, visceral, skeletal)	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Change	At 700 mg/kg there was an increased incidence of external malformation on a foetal (3.6%; 8/221 foetuses) and a litter (33.3%; 6/18 litters) basis. There were no	Increase in foetal malfor-mations and altered ossification processes occurred in two studies. No effects were observed at doses	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										external malformations in controls. The increased incidences (7 foetuses from 6 litters) of tail defects (absence of tail, short tail or filamentous tail) among foetuses of the high dose group were attributed to severe signs of maternal toxicity. Skeletal ossification variation data indicated retarded	without maternal toxicity and may thus be secondary to those effects.	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										ossification at 350 and 700 mg/kg bw/day.		
10	Sensitive to but not diagnostic of EATS	Presence of anomalies (External, visceral, skeletal	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Sensitive to but not diagnostic of EATS	Presence of anomalies (External, visceral, skeletal	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	No effect	Misaligned sutures (3.6% vs 0% in control): nasal irregular ossification (6.3% vs 2.2% in the control and 0.24% in HCD): angulation of hyoid alae (6.3% vs 1.4% in control and 1.29% in		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										HCD). Overall incidences of foetal alterations were 18.7%, 19.3%, 23.9%, and 23.4% in the control, low, mid, and high dose groups, respectively. Maternal toxicity at this dose included reduced food consumption and body weight gain, clinical signs (red substance in pan and dried faeces), and reduced		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										uterine weight (10%, n.s.)		
12	Sensitive to but not diagnostic of EATS	Presence of anomalies (External, visceral, skeletal	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Sensitive to but not diagnostic of EATS	Presence of anomalies (external, visceral, skeletal	rat	28	weeks	Oral	>2500	ppm	No effect			
12	Sensitive to but not diagnostic of EATS	Pup survival index	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect		No effect on pup survival index	
13	Sensitive to but not diagnostic of EATS	Pup survival index	rat	28	weeks	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
9	Sensitive to but not diagnostic of EATS	Sex ratio	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect		No effect on sex ratio	
10	Sensitive to but not diagnostic of EATS	Sex ratio	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Sensitive to but not diagnostic of EATS	Sex ratio	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
12	Sensitive to but not diagnostic o, EATS	Sex ratio	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Sensitive to but not diagnostic of EATS	Sex ratio	rat	28	weeks	Oral	>2500	ppm	No effect			
13	Sensitive to but not	Time to mating	rat	28	weeks	Oral	>2500	ppm	No effect		No effect on mating time	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of EATS											
1	Target organ toxicity	Aorta histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effect on aorta histo-pathology in the rat	Overall evidence of target organ toxicity (liver, blood) with the rat, mouse and dog, and systemic toxicity (lower body weight/body weight gain and food consumption, and in some cases clinical signs and mortality) across the tested species.
1	Target organ toxicity	Bone histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effect on bone histo-pathology in the rat mouse and dog	
4	Target organ toxicity	Bone histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Bone histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Bone histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Bone histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
4	Target organ toxicity	Bone marrow histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect		Hyperplasia of the sternum marrow in 1-year study	
5	Target organ toxicity	Bone marrow histopathology	Dog	52	Weeks	Oral	75	mg/kg bw/d	Change	Hyperplasia of the sternum marrow was		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										observed in one male and one female given 75 mg/kg bw/day and all animals given 300 mg/kg bw/day	with the dog but no effects in a short-term study. No effects with the mouse.	
7	Target organ toxicity	Bone marrow histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Eyes histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effect on the eyes histo-pathology	
1	Target organ toxicity	Heart histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on the heart weight or histo-pathology	
3	Target organ toxicity	Heart histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Heart histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Heart histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
6	Target organ toxicity	Heart histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Heart histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
4	Target organ toxicity	Heart weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Heart weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
1	Target organ toxicity	Kidney histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on the kidney weight or histo-pathology	
2	Target organ toxicity	Kidney histopathology	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Target organ toxicity	Kidney histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Kidney histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Kidney histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Kidney histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
7	Target organ toxicity	Kidney histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Kidney weight	Rat	5	Weeks	Oral	>8000	ppm	No effect			
2	Target organ toxicity	Kidney weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Target organ toxicity	Kidney weight	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Kidney weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Kidney weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Kidney weight	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Kidney weight	mouse	52	Weeks	Oral	>3000	ppm	No effect			
15	Target organ toxicity	Kidney weight	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			
1	Target organ toxicity	Liver histopathology	Rat	5	Weeks	Oral	1000	ppm	Change	Trace to mild centrilobular hypertrophy	Increased liver weights and histo-	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										was observed in males in the 1000, 4000 and 8000 ppm groups (30%, 60%, 80%, respectively) and in females in the 4000 and 8000 ppm groups (10%, 40%, respectively)	pathological changes (centri-lobular hypertrophy and in some cases vacuolation and pig-mentation) in the liver were induced in the short-term and long-term studies across the tested species.	
2	Target organ toxicity	Liver histopathology	mouse	4	Weeks	Oral	4000	ppm	Change	Increased hypertrophy of centrilobular hepatocytes was noted in all male mice (minimal to moderate) at 4000 ppm and eight of ten female mice		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										(minimal to slight)		
3	Target organ toxicity	Liver histopathology	Rat	13	Weeks	Oral	2500	ppm	Change	Increased incidence of centrilobular hypertrophy of the liver in males and females at 2500 (67%, 17%, respectively) and 5000 ppm (83%, 58%, respectively)		
4	Target organ toxicity	Liver histopathology	Dog	90	Days	Oral	125	mg/kg bw/d	Change	Vesiculation/vacuolation in the cytoplasm of centrilobular hepatocytes in all males at all levels including the		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										control group and in all treated females and 3/4 control females but increased in severity at 125 mg/kg bw/day.		
5	Target organ toxicity	Liver histopathology	Dog	52	Weeks	Oral	300	mg/kg bw/d	Change	Centrilobular to mid-zonal hepatocellular hypertrophy in five out of six males and four out of six females given 300 mg/kg bw/day. Increased pigmentation of the liver was observed in one male		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										given 75 mg/kg bw/day and all animals given 300 mg/kg bw/day		
6	Target organ toxicity	Liver histopathology	Rat	2	Years	Oral	2500	ppm	Change	Hypertrophy was observed in the highest dose group (2500 ppm). Females offered 2500 ppm had a slightly greater (12%) incidence of binucleated cells in the liver than the controls (2%), but the effect was of uncertain		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										toxicological significance.		
7	Target organ toxicity	Liver histopathology	mouse	52	Weeks	Oral	1000	ppm	Change	Centrilobular hypertrophy of the liver was observed in males given 1000 ppm (42%) and in males and females given 3000 ppm (100%). Pigment, described as morphologically compatible with haemosiderin and bile, was noted in males given 3000 ppm (69%).		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
1	Target organ toxicity	Liver weight	Rat	5	Weeks	Oral	1000	ppm	Increase	Absolute liver weight was significantly increased in males at 1000, 4000 and 8000 ppm (+12%, +13% and +15%, respectively) and in females at 8000 ppm (+12.5%). Relative liver weight was significantly increased in males and females at 4000 (+19.4% and +18.2% respectively) and 8000 ppm (+32% and		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										+33%, respectively).		
2	Target organ toxicity	Liver weight	mouse	4	Weeks	Oral	1500	ppm	Increase	The absolute liver with gallbladder weight was significantly increased in males at 1500 (+13%) and in males and females at 4000 ppm (+42% and +16%, respectively). The liver/body weight ratio was significantly increased for the same groups of animals		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										(+14%, +42% and +23%, respectively).		
3	Target organ toxicity	Liver weight	Rat	13	Weeks	Oral	2500	ppm	Increase	Absolute liver weight was significantly increased for females given 5000 ppm (114% of control), relative liver weight was significantly increased in both sexes given 2500 ppm (+12% in males and females) and 5000 ppm (+ 26% in males; + 28% in females) with		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										a dose-related trend.		
4	Target organ toxicity	Liver weight	Dog	90	Days	Oral	75	mg/kg bw/d	Increase	Absolute liver weight was increased in animals given 75 and 125 mg/kg bw/day (in males 116 and 134% of controls and in females 115 and 130% of controls)		
5	Target organ toxicity	Liver weight	Dog	52	Weeks	Oral	75	mg/kg bw/d	Increase	Absolute and relative liver weight was significantly increased in both sexes given 300 mg/kg bw/day (156 and 160% of		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										controls for males, respectively, and 170 and 168% of controls for females, respectively), and in females given 75 mg/kg bw/day (134 and 158% of controls, respectively). In males given 75 mg/kg bw/day absolute liver weight was increased (127%) and relative liver weight was		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										statistically significantly increased (116%).		
6	Target organ toxicity	Liver weight	Rat	2	Years	Oral	500	ppm	Increase	Absolute liver weights and liver weights adjusted for brain weight of females given 2500 ppm were statistically significantly increased (+24% and +23%, respectively) and absolute liver weight/ liver weight relative to BW in males were increased		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										21/18% (not statistically significant) while liver weight relative to brain weight was increased 24% (significant) in the 500 ppm group		
7	Target organ toxicity	Liver weight	mouse	52	Weeks	Oral	1000	ppm	Increase	At week 53, in males liver weights (absolute, body weight ratio and brain weight ratio) were statistically significantly increased at 2000/3000 ppm (+16%,		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										+31% and +21%, respectively), and liver weight ratio with brain weight was statistically significantly increased at 1000 mg/kg (+14%). At week 53 in female liver weight (body weight ratio and brain weight ratio) were statistically significantly increased at 2000/3000 ppm (+28%		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										and +18%, respectively). At week 79, only female liver weights (body weight ratio and brain weight ratio) were significantly increased at 3000 ppm (+14% and +16%, respectively).		
10	Target organ toxicity	Liver weight	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Increase	Increased absolute and relative liver weight in does given 300 and 500 mg/kg bw/day		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
15	Target organ toxicity	Liver weight	rat	10	Days	Oral	>200	mg/kg bw/d	No effect	No effect >200 mg/kg bw/day		
1	Target organ toxicity	Lung histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to lung histo-pathology except for foci of amphophilic alveolar macrophages observed in males and females of the two highest dose groups in the chronic oral oncogenicity study in mice. These findings were observed in	
2	Target organ toxicity	Lung histopathology	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Target organ toxicity	Lung histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Lung histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Lung histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Lung histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Lung histopathology	mouse	52	Weeks	Oral	>1000	ppm	Change			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
											mice that died/were sacrificed due to a moribund state during the study. Another finding in the highest dose group animals was systemic amyloidosis	
1	Target organ toxicity	Lymph nodes histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to lymph node histo-pathology	
3	Target organ toxicity	Lymph nodes histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Lymph nodes histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Lymph nodes histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
6	Target organ toxicity	Lymph nodes histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Lymph nodes histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Oesophagus histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to oesophagus histo-pathology	
4	Target organ toxicity	Oesophagus histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
1	Target organ toxicity	Pancreas histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to pancreas histo-pathology	
3	Target organ toxicity	Pancreas histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Pancreas histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Pancreas histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Pancreas histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Pancreas histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
1	Target organ toxicity	Salivary glands histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to salivary glands histo-pathology	
3	Target organ toxicity	Salivary glands histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Salivary glands histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Salivary glands histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Salivary glands histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Salivary glands histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Skeletal muscle histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to skeletal muscle	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
3	Target organ toxicity	Skeletal muscle histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect		histo-pathology	
4	Target organ toxicity	Skeletal muscle histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Skeletal muscle histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Skeletal muscle histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Skeletal muscle histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Skin histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to skin histo-pathology	
3	Target organ toxicity	Skin histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Skin histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
5	Target organ toxicity	Skin histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Skin histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Skin histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Small and large intestines histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to small and large intestine histo-pathology	
3	Target organ toxicity	Small and large intestines histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Small and large intestines histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Small and large intestines histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Small and large intestines histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
7	Target organ toxicity	Small and large intestines histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Spinal cord histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to spinal cord histo-pathology	
3	Target organ toxicity	Spleen histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect		No effects on spleen weight or histo-pathology	
4	Target organ toxicity	Spleen histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
7	Target organ toxicity	Spleen histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
5	Target organ toxicity	Spleen weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
1	Target organ toxicity	Stomach histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to stomach histo-pathology	
3	Target organ toxicity	Stomach histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Stomach histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
5	Target organ toxicity	Stomach histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Stomach histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Stomach histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Thymus histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to thymus histo-pathology	
3	Target organ toxicity	Thymus histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Thymus histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Thymus histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Thymus histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Thymus histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Trachea histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to trachea	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
3	Target organ toxicity	Trachea histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect		histo-pathology	
4	Target organ toxicity	Trachea histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
1	Target organ toxicity	Urinary bladder histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to urinary bladder histo-pathology	
3	Target organ toxicity	Urinary bladder histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Urinary bladder histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Urinary bladder histopathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Urinary bladder histopathology	Rat	2	Years	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
7	Target organ toxicity	Urinary bladder histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Systemic toxicity	Body weight	Rat	5	Weeks	Oral	4000	ppm	Decrease	Statistically significantly reduced bw in M at 8000 ppm (-13%) and in females at 4000 ppm (-8%) and 8000 ppm (-16%). Statistically significantly reduced bwg in males and females at 4000 ppm (-11% and -25%, respectively), and 8000 ppm (-28% and -	Body weight and/or body weight gain were affected in most studies. In several, food consum-ption was reduced as well and there may have been a palatability issue rather than a toxicol-ogical issue	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										44%, respectively)		
2	Systemic toxicity	Body weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Systemic toxicity	Body weight	Rat	13	Weeks	Oral	2500	ppm	Decrease	Body weight and body weight gain were significantly reduced in males given 2500 ppm (-7 and -10% when compared to control). Body weight was reduced in both sexes given 5000 ppm (-11% for both sexes, compared to		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										control). Body weight gain was reduced in both sexes at 5000 ppm (-18% and -24% of control for males and females, respectively).		
4	Systemic toxicity	Body weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Body weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Body weight	Rat	2	Years	Oral	2500	ppm	Decrease	Body weight and body weight gain was decreased in animals of each sex during the first year of treatment with		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										2500 ppm. Body weight was reduced after 2 years but this difference was not statistically significant (8% in males, and 13% in females.		
7	Systemic toxicity	Body weight	mouse	52	Weeks	Oral	>3000	ppm	No effect			
8	Systemic toxicity	Body weight	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Decrease	Reduction in body weight gain was observed in the dams receiving 500 mg/kg bw/day (-38.8%)		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
9	Systemic toxicity	Body weight	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Decrease	Reduction in body weight gain was observed in the dams at 350 and 700 mg/kg bw/day during the treatment period (-14.9% and -40.4%, respectively) and post treatment (GD 16-20; -17.1% for each group). Mean corrected body weight on gestation day 20 was reduced in dams at 750		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										mg/kg bw/day (-6.1%)		
10	Systemic toxicity	Body weight	rabbit	13 (DG 7-19)	Days	Oral	150	mg/kg bw/d	Decrease	Reduction in body weight gain day 13-20 from 150 mg/kg bw with a tendency already at 50 mg/kg bw/day. Reduction in body weight and body weight gain was observed in the dams receiving 300 and 500 mg/kg bw/day		
11	Systemic toxicity	Body weight	rabbit	13 (DG 7-19)	Days	Oral	100	mg/kg bw/d	Decrease	Administration of the 100 and 300 mg/kg/day dosages of the test substance		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										resulted in dosage-dependent, significant inhibition of average maternal body weight during the dosage period. Average maternal body weight change for days 7-20 of gestation (the dosage period) was +0.18. +0.13. +0.05($p<0.05$) and -0.10 kg ($p<0.01$) for control. low. middle and high dosage		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										group dose, respectively.		
12	Systemic toxicity	Body weight	rat	5 to 6	Weeks	Oral	5000	ppm	Decrease	A significant reduction in body weight was observed in parents receiving 5000 ppm. Body weight was stat sig reduced in males in weeks 1 and 2 of the study (-2% and -1.6%) and overall BWG was reduced weeks 0-3 (-18%). Female body weight and BWG was stat sig reduced at		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										end of pre-mating period of (-12.6% and 62.5%, respectively), BW was stat sig reduced at end of gestation (-13.4%), and on day 7 of lactation period (-16%).		
13	Systemic toxicity	Body weight	rat	28	weeks	Oral	2500	ppm	Decrease	Mean body weights were significantly reduced for both F0 and F1a adult males at 2500 ppm throughout the study. Body weights for F0		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										adult females were similar to those of control animals during the premating, gestation and lactation periods. Body weights for F1a adult females were significantly reduced during the pre-mating and gestation periods up through Day 14 of lactation. While body weights were reduced for F1a females, body weight		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										gain during gestation was not affected by treatment.		
14	Systemic toxicity	Body weight	rat	3	Days	Oral	135	mg/kg bw/d	Decrease	Mean body weight losses at 450 mg/kg/day group (Study Days 0–3); mean absolute body weight that was 14.0% lower than controls on Study Day 3. At 45 and 135 mg/kg/day, mean body weight losses were noted Study Days 0–1, resulting in		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										lower mean body weight gains when the overall treatment period (Study Days 0–3) was evaluated. However, mean absolute body weights at 45 and 135 mg/kg/day were within 3.3% of the control group value on Study Day 3.		
15	Systemic toxicity	Body weight	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
1	Systemic toxicity	Clinical chemistry and haematology	Rat	5	Weeks	Oral	1000	ppm	Change	The mean haemoglobin values for males were significantly decreased in the 1000, 4000 and 8000 ppm groups, and haematocrits were decreased for the 4000 and 8000 ppm groups. For females, mean haemoglobin values and erythrocyte counts were significantly reduced in the 5, 1000 and 8000 ppm	Obser-vations in blood/serum included reduced haematocrit, reduced haemo-globin, reduced erythrocyte count, increased reticulo-cytes, increased platelets, increased cholesterol and triglycerides, increased total protein, reduced	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										groups; the 200 and 4000 ppm females showed decreases in these parameters that were not statistically significant.	albumin/globulin ratio, alterations in alkaline phosphatase, increased chloride levels (fm), reduced BUN/creatinine ratio, and increased ALT.	
2	Systemic toxicity	Clinical chemistry and haematology	mouse	4	Weeks	Oral	625	ppm	Decrease	Red blood cell counts were significantly decreased in males at 1500 and 4000 ppm and in females at 1500 ppm. Haemoglobin was significantly decreased in males at 625, 1500 and 4000		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										ppm and in females at 1500 ppm. Haematocrit was significantly reduced in males at 4000 ppm.		
3	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	5000	ppm	Increase	Clinical chemistry showed significantly higher cholesterol, total protein and globulin values at 5000 ppm in males (131%, 165% and 109% of control values, respectively). A significant		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										decrease in blood urea nitrogen/creatine ratio was observed in females at 2500 ppm but not at 5000 ppm - this was considered to be unrelated to treatment.		
4	Systemic toxicity	Clinical chemistry and haematology	Dog	90	Days	Oral	125	mg/kg bw/d	Increase	Mean alkaline phosphatase (ALP) activity progressively increased in males and females given 125 mg/kg bw/day. Mean cholesterol levels in females given		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										125 mg/kg bw/day progressively increased these differences from control were statistically significant after 1 and 2 months. Albumin/globulin ratio was reduced in males at 125 mg/kg bw/day. Chloride levels were reduced in females at 75 and 125 mg/kg bw/day.		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
5	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	75	mg/kg bw/d	Change	Erythrocyte count was statistically significantly decreased in males given 300 mg/kg bw/day on days 270 and 360 and in females given 300 mg/kg bw/day on days 180, 270 and 360. Haemoglobin and haematocrit were statistically significantly decreased in females given 300 mg/kg		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										bw/day on days 180, 270 and 360. In males on day 360 the haemoglobin and haematocrit values were 8% lower than those of the control group (not statistically significant). Platelet count was statistically significantly increased in both sexes given 300 mg/kg bw/day during the		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										whole exposure period and a similar trend was observed in the 75 mg/kg bw group (20% higher than the control on 360) although not statistically significant. The white blood cell count was statistically significantly increased in females given 300 mg/kg bw/day on days 90, 180,		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										270 and 360 and in females given 75 mg/kg bw/day at day 90 only. Segmented neutrophils were significantly higher in females given 300 mg/kg bw/day on days 31, 90 and 270. The albumin/globulin ratio was decreased in females given 300 mg/kg bw/day at day 270 and 360. The glucose level was		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										significantly decreased in males given 300 mg/kg bw/day on day 270, in females given 300 mg/kg bw/day on days 180 and 360, and in females given 75 mg/kg bw/day at day 360. Alkaline phosphatase was significantly higher than the control group's from day 90 onwards at 75 and 300 mg/kg bw/day in both		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										sexes, although only statistically significant at the highest dose level. ALP had a decreasing trend over time in all groups but the highest dose group, in which it increased over time. ALT was statistically significantly increased at 300 mg/kg bw/day in both sexes from day 180 onwards. Cholesterol		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										was statistically significantly increased in males given 300 mg/kg bw/day at day 360 only and in females throughout the study. Triglycerides were significantly increased in both sexes given 300 mg/kg bw/day at day 360, and not statistically significantly increased (29-		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										41%) at 75 mg/kg bw/day.		
6	Systemic toxicity	Clinical chemistry and haematology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Systemic toxicity	Clinical chemistry and haematology	mouse	52	Weeks	Oral	2000/3000	ppm	Decrease	Red blood cell count was statistically significantly decreased in males given 2000/3000 ppm in week 27 and 79 and in females given 2000/3000 ppm in week 27. Haemoglobin and haematocrit were statistically		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										significantly decreased in males given 2000/3000 ppm in week 27.		
1	Systemic toxicity	Clinical signs	Rat	5	Weeks	Oral	>8000	ppm	No effect		Clinical signs occurred in some studies and included excess salivation, excess lacrimation, staining of the fur/skin in the anogenital area, red substance in	
2	Systemic toxicity	Clinical signs	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Systemic toxicity	Clinical signs	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Systemic toxicity	Clinical signs	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Clinical signs	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Clinical signs	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Systemic toxicity	Clinical signs	mouse	52	Weeks	Oral	>3000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
8	Systemic toxicity	Clinical signs	rat	10 (GD 6-15)	Days	Oral	300	mg/kg bw/d	Change	Excess salivation was seen with increased frequency in at 300 and 500 mg/kg bw/day	the pan, and dried faeces.	
9	Systemic toxicity	Clinical signs	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Change	Excess salivation was seen was seen at least once in 11/25 females at 350 mg/kg bw/day and 19/25 females at 700 mg/kg bw/day. Excessive lacrimation in 12/25 and staining of fur/skin in the anogenital area of 7/25		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										females at 700 mg/kg bw/day.		
10	Systemic toxicity	Clinical signs	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Systemic toxicity	Clinical signs	rabbit	13 (DG 7-19)	Days	Oral	100	mg/kg bw/d	Change	Signs of maternal toxicity were noted in females treated at 100 or 300 mg/kg bw/day and comprised clinical signs (red substance in the pan and dried faeces		
12	Systemic toxicity	Clinical signs	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Systemic toxicity	Clinical signs	rat	28	weeks	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
14	Systemic toxicity	Clinical signs	rat	3	Days	Oral	>450	mg/kg bw/d	No effect			
15	Systemic toxicity	Clinical signs	rat	10	Days	Oral	>200	mg/kg bw/da	No effect			
1	Systemic toxicity	Food consumption	Rat	5	Weeks	Oral	4000	ppm	Decrease	Statistically significantly reduced in males and females at 4000 ppm (-8% males; -10% females) and 8000 ppm (-22% males; -26% females)	Food consum-ption was affected in 8 studies. In five of them the test substance was incorp-orated in the diet and palatability may influence the outcome. However, oral gavage was used to administer	
2	Systemic toxicity	Food consumption	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Systemic toxicity	Food consumption	Rat	13	Weeks	Oral	2500	ppm	Decrease	Food consumption was significantly reduced in males and		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										females during treatment at 5000 ppm. Decreases were also noted at sporadic intervals for males in the 500 and 2500 ppm groups.	the substance in the three other studies which eliminates the palatability issue. Of the four studies without an observed effect on food consump-tion, oral gavage was used in three of them and administration via the diet was performed in	
4	Systemic toxicity	Food consumption	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Food consumption	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Food consumption	Rat	2	Years	Oral	2500	ppm	Decrease	Food consumption was significantly decreased in animals of each sex during the first		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										year to treatment with 2500 ppm.	one (4-week, mice).	
7	Systemic toxicity	Food consumption	mouse	52	Weeks	Oral	>3000	ppm	No effect			
8	Systemic toxicity	Food consumption	rat	10 (GD 6-15)	Days	Oral	>500	mg/kg bw/d	No effect			
9	Systemic toxicity	Food consumption	rat	10 (GD 6-15)	Days	Oral	700	mg/kg bw/d	Decrease	Reduction in food consumption was observed in the dams receiving 350 (Day 7 of gestation) and 700 mg/kg bw/day (Days 7-10 of gestion), but only the prolonged reduction in food		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										consumption at 700 mg/kg bw/day considered related to treatment.		
10	Systemic toxicity	Food consumption	rabbit	13 (DG 7-19)	Days	Oral	50	mg/kg bw/d	Decrease	Reduced feed consumption during the dosage period (≥50 mg/kg/day – statistically significant only at highest dose) with a post dosage increase in food consumption compared with the control (≥150 mg/kg/day, not		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										statistically significant),		
11	Systemic toxicity	Food consumption	rabbit	13 (DG 7-19)	Days	Oral	100	mg/kg bw/d	Decrease	Administration of the 100 and 300 mg/kg/day dosages of the test substance resulted in dosage-dependent, significant inhibition of average maternal food consumption during the dosage period		
12	Systemic toxicity	Food consumption	rat	5 to 6	Weeks	Oral	5000	ppm	Decrease	Food consumption was consistently lower for treated males		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										and females in all dose groups. However, the difference was statistically significant in high-dose males only.		
13	Systemic toxicity	Food consumption	rat	28	weeks	Oral	2500	ppm	Decrease	In general, food consumption of F0 adults was not affected by treatment except for decreases for animals at 2500 ppm during the initial exposure period. Food		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										consumption for F1a adult males was decreased on many of the intervals measured throughout the study. Food consumption for F1a adult females, however, was not affected during the pre-mating period but was reduced on Days 0-2, 2-5, and 9-12 of 1estation. Food consumption for F1a		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										females was not affected during lactation.		
1	Systemic toxicity	Mortality	Rat	5	Weeks	Oral	>8000	ppm	No effect		Mortality occurred in three studies: at a dose of 238/357 mg/kg bw/day in a 2-year study in non-pregnant mice, and at 300-700 mg/kg bw/day in pregnant rats and rabbits.	
2	Systemic toxicity	Mortality	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Systemic toxicity	Mortality	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Systemic toxicity	Mortality	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Mortality	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Mortality	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Systemic toxicity	Mortality	mouse	52	Weeks	Oral	2000/3000	ppm	Change	Survival for males and females of the 3000-ppm		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										group through Week 78 was significantly lower than control, and there was a significant negative trend, i.e. treatment-related decrease, in survival for both males and females.		
8	Systemic toxicity	Mortality	rat	10 (GD 6-15)	Days	Oral	>500	mg/kg bw/d	No effect			
9	Systemic toxicity	Mortality	rat	10 (GD 6-15)	Days	Oral	700	mg/kg bw/d	Increase	At 700 mg/kg bw/day maternal toxicity was evident as mortality		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										increased by a 20% (5/25).		
10	Systemic toxicity	Mortality	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Increase	Two of seven pregnant 300 mg/kg/day dosage group rabbits died, and one of seven pregnant 500 mg/kg/day dosage group rabbits aborted and died.		
11	Systemic toxicity	Mortality	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
12	Systemic toxicity	Mortality	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Systemic toxicity	Mortality	rat	28	weeks	Oral	>2500	ppm	No effect			
14	Systemic toxicity	Mortality	rat	3	Days	Oral	>450	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
15	Systemic toxicity	Mortality	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			

2.10.2.1.1.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Table 2.10.2.1.1.1-1. WoE for T-mediated adversity.

The effect of clethodim on thyroid weight was assessed in two dog studies of different lengths. In the shorter 13-week study, no effect was observed in the tested dose span of 1-125 mg/kg bw/day (ID 4). In the 1-year study (ID 5), males administered 1, 75, and 300 mg/kg bw /day had absolute thyroid weights that were 22%, 45%, and 91% higher, respectively, than that of the control group (only the difference between the control and the highest dose was statistically significant). No microscopic lesion or similar weight increase was noted in females, or in the other dog study weighing thyroids. Nor were there any microscopic changes in any of the other studies in rats or mice.
No exposure related remarks were noted in the microscopic assessment of thyroids from rats (ID 1, 3, and 6), mice (ID 7), or dogs (ID 4 and 5), including the male dogs with increased thyroid weight.
The male dogs exposed to 300 mg/kg bw /day (ID 5) with a 91% larger absolute thyroid weight also had increased liver weights, hepatocellular enlargement (often with cytoplasmic clearing), increased hepatic pigment, reduced A/G Ratio, increased ALK and ALT, and increased levels of cholesterol and triglycerides. Hyperplasia of the bone marrow was also observed, along with reduced erythrocyte and haemoglobin levels and increases in white blood cells and segmented neutrophils. The male dogs exposed to 75 mg/kg bw/day that had a non-statistically significant increase in thyroid weight (45%) had increased liver weights, increased platelet counts, increased cholesterol levels, and 1/6 individuals had hyperplasia in the bone marrow. The dogs in the lowest exposure group (1 mg/kg bw/day) had no significant effects (thyroid weight was 22% higher than that of the control group but this was not statistically significant). There were no effects on body weights and no clinical signs related to the exposure were observed in the study.
Additional effects that are not necessarily related to ED described in the data package include signs of anaemia, liver effects, effects on foetal development (generally occurring at doses with maternal toxicity), and reductions in body weight (although palatability issues may confound these results in some studies).
Clethodim was found to cause effects on the liver in all short-term studies (rat, mouse, and dog; ID 1, 2, 4, 5). These effects included increased liver weight, hypertrophy, vacuolisation of hepatocytes, increased pigmentation, effects on clinical chemistry parameters associated with liver damage and/or affected fat metabolism. Besides hepatotoxicity, several haematological effects were observed, most prominently anaemia. In addition, increases of the numbers of platelets and leukocytes were found in a 5-week rat study and the one-year dog study (ID 1, 5). The 1-year dog study also revealed bone-marrow hyperplasia in the sternum at the highest dose level. Effects observed during the long-term/carcinogenicity studies with rats and mice (ID 6, 7) included reduced body weight, increases in hepatic volume (liver weight and hypertrophy) and anaemia (reduction in the number of erythrocytes, haemoglobin, and haematocrit). These effects are consistent with those obtained in the short-term studies. Hepatotoxicity is considered the critical effect in the sense that it always (co-) determines the NOAEL of each study (ID: 1, 2, 4, 5, 6, 7, 10).
Foetal/pup growth was impeded in several studies (as indicated by reduced foetal or pup weight). In most of these studies maternal toxicity was evident and body weight/food consumption in the dams were lower than in the control group. The exception to this dose range finding reproduction study in the rat (ID 12). In that study, pups born to dams exposed to 500, 2000, and 5000 ppm of clethodim during gestation had a lower body weight at PND 7, but not birth weight, and a reduced body weight gain between PND 0 and 7.
No changes in absolute organ weights and no histopathological lesions in the kidneys, brain, or pituitary were observed in the rat, mouse, or dog. Relative brain and kidney weights were increased in two studies, likely a result of reduced body weight (ID 1 and 3).
Adrenal weights (absolute and relative to brain weight) were reduced in rats exposed to 597 (males) or 667 (females) mg/kg bw/day for females for 5 weeks (Report No.: S-2763). Adrenal weights were increased in females and decreased in male mice at the interim but not the terminal sacrifice (week 53 and 79, respectively (ID 7). Due to the inconsistency of the change between sexes, the lack of a dose -response and no differences at Week 79 this finding was not considered to be treatment related. Adrenal weight was unaffected in the other six studies (ID 1-6). No microscopic lesions in the adrenals have been reported.

In conclusion, there are no clear indications of T-mediated adversity in the available data package. While the increased thyroid weight in male dogs is substantial, no remarks were made on the thyroid histopathology of these dogs (or in any other study) and no such effect was observed in females. The increase is considered noteworthy but not enough to conclude that T-mediated adversity is observed. The biological significance of the effect is questioned.

Table 2.10.2.1.1.1-2. WoE for T-mediated endocrine activity.

Clethodim was not identified as a THRA agonist in the available ToxCast assay (ATG_THRa1_TRANS_up) (ID 18).
Measurements of T3, T4, and TSH were not included in the data package. However, no exposure related remarks were noted in the microscopic assessment of thyroids from rats (ID 1, 3, and 6), mice (ID 7), or dogs (ID 4 and 5).

In conclusion, there are some thyroid parameters missing from data package i.e., measurements of hormone levels. However, the thyroid parameters included in the test guidelines at the time when the studies in the data package were performed were assessed: thyroid weight (dog, but not in rat or mouse) and histopathology assessments (rat, dog, and mouse). For e.g., OECD 407 (ver. 1995 and 2008) and OECD 408 (ver. 1998), weighing thyroid is not mandatory; but histopathology must be performed. Furthermore, the ToxCast assay showed no thyroid receptor activity. Thus, the available data does not indicate T-mediated activity.

2.10.2.1.2 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

Table 2.10.2.1.2-1. Selection of relevant scenario.

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no "**T-mediated**" adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **T-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.2.1.3 MoA analysis for T-modality

Not applicable

2.10.2.1.4 Conclusion on the assessment of T-modality

The data package was slightly limited in terms of T-mediated parameters: thyroid weight was only measured in the dog and hormone measurements of the thyroid gland were not performed. However, histopathological assessments performed in the thyroid gland of dogs, rats, and mice and no effects were observed. Thyroid gland weight was increased in a dose dependent manner in male- but not female dogs exposed to clethodim for a year, however no microscopic treatment related changes were observed. While the weight increase of the thyroid in male dogs was substantial in the highest dose group (~100%), it is not considered enough to indicate T-mediated adversity due to the lack of effect in females and the absence of histopathological effects, also, no effects on thyroid weight was observed in the 90-day dog study.

There was some evidence of disruption of reproductive parameters sensitive to but not diagnostic of T-mediated adversity (litter/pup weight, foetal development, post implantation loss, incidence of external malformations) but these generally occurred at dose levels inducing maternal toxicity. The most common effects observed in the data package include liver effects (weight and hypertrophy) and accompanied alterations in serum measurements, and effects on the blood system (generally indicating anaemia). Body weights were commonly reduced, usually in combination with reduced food intake. Reduced food intake may in several cases be caused by palatability issues, but toxicity cannot be ruled out.

Overall, apart from the increased thyroid weight in male dogs, there is not much cause for concern regarding thyroid disruption in the observed effects. It is concluded that no T-mediated adversity was observed (Scenario 1a). Clethodim does not meet the criteria for T-mediated endocrine disruption.

2.10.2.2 ED assessment for EAS-modality

	Sufficiently investigated
EAS-mediated parameters	Yes, based on availability of the following studies: OECD TG 416: ID 13 OECD TG 440: ID 14 ToxCast ER Bioactivity Model: ID 18 OECD TG 441: ID 15 OECD TG 456: ID 17 OPPTS 890.1200: ID 16

All studies required for sufficiently assessing EAS-related activity are available and acceptable. According to the EFSA guidance document ("Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009" adopted 5 June 2018), all the 'EAS-mediated' parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (EOGRTS; OECD TG 443; with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation (OECD, 2012b)) or a two-generation reproductive toxicity study (OECD TG 416; test protocol according to latest version of January 2001 (OECD, 2001)) is needed for the EAS-related endocrine adversity to be sufficiently assessed. A two-generation study is available in the data package on clethodim (ID 13), but some EAS-mediated parameters were not assessed.

EAS-mediated parameters not investigated in the toxicological database for clethodim in the 2-generation study:

- Age at preputial separation of offspring

-Age at vaginal opening of offspring

-Ano-genital distance of foetuses and offspring

-Primordial follicles count

-Histopathology of cervix, coagulating gland and vagina

-Organ weights of adrenal glands, brain, epididymides, kidneys, liver, ovaries, pituitary, prostate, spleen, seminal vesicles/coagulating gland, thyroids, and uterus

-Sperm analysis

Some of the parameters not investigated in the 2-generation reproduction study were, however, measured in other repeated-dose studies. These include vaginal histopathology in the dog (ID: 4), weights of the epididymides in the mouse (ID: 2, 7), ovaries in the rat, mouse and dog (ID: 1, 2, 4, 5, 6, 7) and uterus in the mouse (ID: 7).

2.10.2.2.1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities.

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
18	In vitro mechanistic	Androgen receptor	human liver cell line	24	Hours	Uptake from the medium (in vitro)	0	µM	No effect		No AR agonist or antagonist activity in the ToxCast assays	No in vitro or in vivo mechanistic data indicate EAS-mediated activity
18	In vitro mechanistic	Androgen receptor	human breast cell line	24	Hours	Uptake from the medium (in vitro)	0	µM	No effect			
16	In vitro mechanistic	CYP19	human	15	Minutes	Uptake from the medium (in vitro)	>1 mM	mM	No effect		No inhibition of aromatase activity	
17	In vitro mechanistic	Estradiol synthesis	human	48	Hours	Uptake from the medium (in vitro)	>31.6 µM		No effect		No effect on estradiol release by H295R cells	
18	In vitro mechanistic	Estrogen receptor	human liver cell line	24	Hours	Uptake from the medium (in vitro)	0	µM	No effect		No ER agonist activity in the ToxCast assays	
18	In vitro mechanistic	Estrogen receptor	human liver cell line	24	Hours	Uptake from the medium (in vitro)	0	µM	No effect			
17	In vitro mechanistic	Testosterone synthesis	human	48	Hours	Uptake from the medium (in vitro)	>31.6 µM		No effect		No effect on testosterone release by H295R cells	
15	In vivo mechanistic	Adrenals weight (Hershberger)	rat	10	Days	Oral	>200	mg/kg bw/	No effect		No treatment related weight changes were observed in androgen	
15	In vivo mechanistic	Cowpers glands weight (Hershberger)	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
15	In vivo mechanistic	Glans penis weight (Hershberger)	rat	10	Days	Oral	>200	mg/kg bw/d	No effect		responsive organs.	
15	In vivo mechanistic	LABC weight (Hershberger)	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			
15	In vivo mechanistic	Prostate weight (Hershberger)	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			
15	In vivo mechanistic	Seminal vesicles weight (Hershberger)	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			
14	In vivo mechanistic	Uterus histo-pathology (UT assay)	rat	3	Days	Oral	>450	mg/kg bw/d	No effect		There were no observed effects on uterus weight or histo-pathology	
14	In vivo mechanistic	Uterus weight (UT assay)	rat	3	Days	Oral	>450	mg/kg bw/d	No effect			
14	In vivo mechanistic	Uterus weight (UT assay)	rat	3	Days	Oral	>450	mg/kg bw/d	No effect			
1	EATS-mediated	Epididymis histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects have been observed on epididymis histology (rat, dog, mouse) or epididymis weight (rat)	EATS-mediated parameters were unaffected in most studies. Relative testis weight was increased in one study, but this was likely a result of the reduced body weight rather than a hormonal effect. Reduced absolute ovary weight was observed in the 90-d studies (dog
3	EATS-mediated	Epididymis histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Epididymis histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	EATS-mediated	Epididymis histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Epididymis histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	EATS-mediated	Epididymis histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
13	EATS-mediated	Epididymis histo-pathology	rat	28	weeks	Oral	>2500	ppm	No effect			and rat) but the relevance is unclear, and no such effects were observed in the long-term studies. Absolute uterus weights were reduced in dams following gestational exposure in rats and rabbits. Oestrus cyclicity was not mentioned in most studies which limits the possibility of interpreting the differences in ovary and uterus weights. In males, absolute but not relative seminal vesicles and prostate weights were reduced in rats. Overall, there were some organ weight changes that may indicate EAS-mediated effects but there are no clear trends in the data package.
13	EATS-mediated	Epididymis weight	rat	28	weeks	Oral	>2500	ppm	No effect			
13	EATS-mediated	Estrus cyclicity	rat	28	weeks	Oral	>2500	ppm	No effect		No effect on oestrus cyclicity in the rat	
1	EATS-mediated	Mammary gland histo-pathology (female)	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on female mammary gland histology (rat, dog, mouse)	
3	EATS-mediated	Mammary gland histo-pathology (female)	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Mammary gland histo-pathology (female)	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	EATS-mediated	Mammary gland histo-pathology (female)	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Mammary gland histo-pathology (female)	Rat	2	Years	Oral	>2500	ppm	No effect			
7	EATS-mediated	Mammary gland histo-pathology (female)	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	EATS-mediated	Ovary histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on female ovary histology (rat, dog, mouse)	
3	EATS-mediated	Ovary histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Ovary histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
5	EATS-mediated	Ovary histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Ovary histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	EATS-mediated	Ovary histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
13	EATS-mediated	Ovary histo-pathology	rat	28	weeks	Oral	>2500	ppm	No effect			
1	EATS-mediated	Ovary weight	Rat	5	Weeks	Oral	>8000	ppm	No effect		The differences in ovary weights observed in the 90-d studies (dog and rat) are of unclear relevance. Oestrus cyclicity not mentioned. Differences in ovary weights were not observed in the long- term studies in dog and rat.	
3	EATS-mediated	Ovary weight	Rat	13	Weeks	Oral	>5000	ppm	No effect	All relative and absolute weights were lower than the control, but no dose response was apparent.		
4	EATS-mediated	Ovary weight	Dog	90	Days	Oral	75	mg/kg bw/d	Change	The mean ovary weight of dogs administered 75 mg/kg bw/d was lower and those of dogs administered 125mg/kg bw/day were higher than the mean ovary weight of the control group. The differences were not significant but rather large (30-		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										82%). Both relative and absolute weights were affected		
5	EATS-mediated	Ovary weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Ovary weight	Rat	2	Years	Oral	>2500	ppm	No effect			
13	EATS-mediated	Ovary weight	rat	28	weeks	Oral	>2500	ppm	No effect			
1	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on prostate or seminal vesicle histology (rat, dog, mouse)	
3	EATS-mediated	Prostate histo-pathology (with seminal vesicles and coagulating glands)	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Prostate histo-pathology (with seminal vesicles and coagulating glands)	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	EATS-mediated	Prostate histo-pathology (with seminal vesicles and coagulating glands)	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Prostate histo-pathology (with seminal	Rat	2	Years	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
		vesicles and coagulating glands)										
7	EATS-mediated	Prostate histo-pathology (with seminal vesicles and coagulating glands)	mouse	52	Weeks	Oral	>3000	ppm	No effect			
13	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	28	weeks	Oral	>2500	ppm	No effect			
1	EATS-mediated	Seminal vesicles histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect			
7	EATS-mediated	Seminal vesicles histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
13	EATS-mediated	Seminal vesicles histo-pathology	rat	28	weeks	Oral	>2500	ppm	No effect			
13	EATS-mediated	Seminal vesicles weight	rat	28	weeks	Oral	2500	ppm	Decrease		There was a reduced absolute but not relative seminal vesicles weight in the F1 generation of the 2500 ppm group (food intake and BW also reduced)	
13	EATS-mediated	Prostate weight	rat	28	weeks	Oral	2500	ppm	Decrease		There was a reduced absolute but not relative prostate weight	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
											in the F1 generation of the 2500 ppm group (food intake and BW also reduced)	
1	EATS-mediated	Testis histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on testis histology (rat, dog, mouse)	
3	EATS-mediated	Testis histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Testis histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	EATS-mediated	Testis histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Testis histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	EATS-mediated	Testis histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
13	EATS-mediated	Testis histo-pathology	rat	28	weeks	Oral	>2500	ppm	No effect			
1	EATS-mediated	Testis weight	Rat	5	Weeks	Oral	>8000	ppm	No effect	Relative testes weight was increased at 8000 ppm, likely a result of the reduced body weight	Relative testis weight was increased in two studies on rats; however, these increases are likely a result of reduced BW. No effects on absolute testis weight have been observed.	
2	EATS-mediated	Testis weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	EATS-mediated	Testis weight	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Testis weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	EATS-mediated	Testis weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Testis weight	Rat	2	Years	Oral	>2500	ppm	No effect			
7	EATS-mediated	Testis weight	mouse	52	Weeks	Oral	>3000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
13	EATS-mediated	Testis weight	rat	28	weeks	Oral	>2500	ppm	No effect	Relative testes weight was increased in the F0 males, likely a result of the reduced body weight		
1	EATS-mediated	Uterus histo-pathology (with cervix)	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on uterus histology (rat, dog, mouse)	
3	EATS-mediated	Uterus histo-pathology (with cervix)	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	EATS-mediated	Uterus histo-pathology (with cervix)	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	EATS-mediated	Uterus histo-pathology (with cervix)	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	EATS-mediated	Uterus histo-pathology (with cervix)	Rat	2	Years	Oral	>2500	ppm	No effect			
7	EATS-mediated	Uterus histo-pathology (with cervix)	mouse	52	Weeks	Oral	>3000	ppm	No effect			
13	EATS-mediated	Uterus histo-pathology (with cervix)	rat	28	weeks	Oral	>2500	ppm	No effect			
2	EATS-mediated	Uterus weight (with cervix)	mouse	4	Weeks	Oral	>4000	ppm	No effect		Uterus weights were reduced in two studies after exposure during gestational days 6-15 in rat and 7-19 in rabbit. BW was reduced in both studies. Food consump-	
7	EATS-mediated	Uterus weight (with cervix)	mouse	52	Weeks	Oral	>3000	ppm	No effect			
9	EATS-mediated	Uterus weight (with cervix)	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Decrease	7% reduction in the 100 mg/kg bw/day group, 10 % in the 350		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										mg/kg bw/day group, and 27% in the 700 mg/kg bw/day group (only the top dose was statistically significant).	tion was reduced in the rabbit study. Uterus weight was not affected in non-pregnant rats or mice, nor in the pilot with rabbits.	
10	EATS-mediated	Uterus weight (with cervix)	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	EATS-mediated	Uterus weight (with cervix)	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Decrease	Not statistically significant 10% decrease in absolute uterus weight in the 300 mg/kg bw/day dose group when compared to controls.		
13	EATS-mediated	Uterus weight (with cervix)	rat	28	weeks	Oral	>2500	ppm	No effect			
13	EATS-mediated	Vagina histo-pathology	rat	28	weeks	Oral	>2500	ppm	No effect		No effect on vagina histo-pathology	
1	Sensitive to, but not diagnostic of, EATS	Adrenals histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on adrenals histology (rat, dog, mouse)	Effects were observed on parameters sensitive to but not diagnostic of T-mediated adversity (Litter/pup
3	Sensitive to, but not diagnostic of, EATS	Adrenals histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
4	Sensitive to, but not diagnostic of, EATS	Adrenals histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			weight, Foetal development, Post implantation loss, incidence of external malformations, and altered adrenals weight). The effects on offspring/ reproduction mainly occurred at dose levels causing maternal toxicity and may thus be secondary to maternal toxicity.
5	Sensitive to, but not diagnostic of, EATS	Adrenals histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Sensitive to, but not diagnostic of, EATS	Adrenals histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to, but not diagnostic of, EATS	Adrenals histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	5	Weeks	Oral	>8000	ppm	No effect		Adrenal weight was clearly affected in one out of 8 studies (S-2763) but no remarks have been made in the histopathological assessments. The rats in the study with reduced adrenal weight also shad a reduced body weight and increased liver weight. In another study, adrenals weights of both male and female mice were affected at	
2	Sensitive to, but not diagnostic of, EATS	Adrenals weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Sensitive to, but not	Adrenals weight	Rat	2	Years	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of, EATS										53 weeks but not 79 weeks: decrease in males and increase in females. The results in the latter study are of unclear relevance.	
7	Sensitive to, but not diagnostic of, EATS	Adrenals weight	mouse	52	Weeks	Oral	>3000	ppm	Change	Adrenals weight (absolute and relative to both BW and brain weight) was reduced in males of all exposure groups at the interim sacrifice, i.e. week 53 (34-55% reduction, no dose response) but not at the terminal sacrifice, i.e. week 79. In females, adrenals weight (absolute and relative to both BW and brain weight) was increased in all exposure groups at the interim sacrifice (10-41% increase, no dose response) but not at the		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										terminal sacrifice. Unclear relevance.		
S-2763	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	5	Weeks	Oral	6800	ppm	Decrease			
1	Sensitive to, but not diagnostic of, EATS	Brain histo-pathology examination	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on brain histology (rat, dog, mouse)	
3	Sensitive to, but not diagnostic of, EATS	Brain histo-pathology examination	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Sensitive to, but not diagnostic of, EATS	Brain histo-pathology examination	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to, but not diagnostic of, EATS	Brain histo-pathology examination	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Sensitive to, but not diagnostic of, EATS	Brain histo-pathology examination	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to, but not diagnostic of, EATS	Brain histo-pathology examination	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	5	Weeks	Oral	>8000	ppm	No effect	Relative brain weight was increased at 8000 ppm, likely a result of the reduced body weight	Relative brain weight was increased in two studies on rats; however, these increases are likely a result of	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
2	Sensitive to, but not diagnostic of, EATS	Brain weight	mouse	4	Weeks	Oral	>4000	ppm	No effect		reduced BW. No effects on absolute brain weight have been observed.	
3	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	13	Weeks	Oral	>5000	ppm	No effect	Relative brain weight was increased in both sexes at 5000 ppm, likely a result of the reduced body weights		
4	Sensitive to, but not diagnostic of, EATS	Brain weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to, but not diagnostic of, EATS	Brain weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to, but not diagnostic of, EATS	Brain weight	mouse	52	Weeks	Oral	>3000	ppm	No effect			
13	Sensitive to, but not diagnostic of, EATS	Dystocia	rat	28	weeks	Oral	>2500	ppm	No effect		No effects on dystocia	
13	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	rat	28	weeks	Oral	>2500	ppm	No effect		No effects on fertility	
13	Sensitive to, but not	Gestation length	rat	28	weeks	Oral	>2500	ppm	No effect		No effect on gestation length	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of, EATS											
12	Sensitive to, but not diagnostic of, EATS	Litter size	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect		No effect on litter size	
13	Sensitive to, but not diagnostic of, EATS	Litter size	rat	28	weeks	Oral	>2500	ppm	No effect			
8	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Decrease	Mean foetal weights were reduced in the animals of the top dose group (-10.6% for the composite foetal weight data). A tendency was observed already at 300 mg/kg bw/day (7% reduction, not statistically significant). Maternal effects at the top dose included: excess salivation and reduced body weight and body weight gain	Reduction in foetal body weight were observed three studies (rat and rabbit) but at doses causing maternal toxicity, including reductions in body weight and food consump-tion. In rats, reductions in combined pup weight (day 7) and pup weight gain (day 0-7), but not birth weight, were observed at all dose levels (500, 2000, and 5000 ppm) in the 5-week study. Maternal toxicity was only noted	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
9	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Decrease	Reduction in foetal body weight was observed in dose groups receiving 350 and 700 mg/kg bw/day. Maternal effects at those doses included: clinical signs and reduced body weight gain	in the highest dose group. This effect on postnatal growth was not observed in either generation of the 2-generation study (doses used: 5, 20, 500, and 2500 ppm).	
10	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Decrease	Reduction in foetal body weight was observed in dose groups receiving 300 and 500 mg/kg bw/day. Maternal effects at those doses included reduced food consumption, reduced body weight gain and body weight, dried faeces, and mortality		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
11	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
12	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	5 to 6	Weeks	Oral	500	ppm	Decrease	There was a significant decrease in combined pup weight (male and female) at day 7 and a decrease in combined pup weight gain between days 0 and 7 for all three dose levels (500-5000 ppm). Maternal food consumption and body weight was reduced in the highest but not the lower dose groups.		
13	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	rat	28	weeks	Oral	>2500	ppm	No effect			
8	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Decrease	Number of implantation sites was reduced (87 vs 126) - not statistically significant	No clear indications that the number of implantation sites or corpora lutea were affected. The	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
9	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect		pilot study in which a reduction in the number of implantation sites was observed was of limited reliability due to potential SDA infections in some individuals.	
10	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
13	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	rat	28	weeks	Oral	>2500	ppm	No effect			
12	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect		The one potential effect on the number of stillborn were in the F0 generation (F0--> F1), something that was not evident in the second generation of the study.	
13	Sensitive to, but not diagnostic of, EATS	Number of live births	rat	28	weeks	Oral	>2500	ppm	No effect	Number of stillborn were increased in the F1 generation (F1 pups) but not in the F1 generation (F2 pups)		
8	Sensitive to, but not diagnostic of, EATS	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Decrease	Number of viable foetuses was reduced (86 vs 122) but not statistically significant. Indications of maternal	The reduction in foetal viability observed in two studies is likely related to maternal toxicity.	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										SDA infections were present in some dams (all groups)		
9	Sensitive to, but not diagnostic of, EATS	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect			
10	Sensitive to, but not diagnostic of, EATS	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 (DG 7-19)	Days	Oral	500	mg/kg bw/d	Change	Four of the seven pregnant 500 mg/kg/day dosage group rabbits aborted during the study. All abortions occurred after completion of the dosage period. Three of the seven aborted 3 foetuses each and 1 rabbit aborted 2. One of the seven rabbits had 1 early resorption and 2 rabbits had 2 late resorptions. Clear signs of maternal toxicity were		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										observed in the study: reduced food consumption (≥50 mg/kg bw/day), reduced bw gain (≥150 mg/kg bw/day), increased LW (≥150 mg/kg bw/day), and death (≥150 mg/kg bw/day)		
11	Sensitive to, but not diagnostic of, EATS	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
1	Sensitive to, but not diagnostic of, EATS	Pituitary histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on the pituitary weight or histo-pathology	
3	Sensitive to, but not diagnostic of, EATS	Pituitary histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Sensitive to, but not diagnostic of, EATS	Pituitary histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to, but not diagnostic of, EATS	Pituitary histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
6	Sensitive to, but not diagnostic of, EATS	Pituitary histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Sensitive to, but not diagnostic of, EATS	Pituitary histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
4	Sensitive to, but not diagnostic of, EATS	Pituitary weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Sensitive to, but not diagnostic of, EATS	Pituitary weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
9	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rat	10 (GD 6-15)	Days	Oral	700	mg/kg bw/d	Increase	Increased post-implantation loss at 700 mg/kg bw/day. This in part was driven by a single female with 15/16 foetal resorptions, with this female excluded the mean resorption rate was 1.1 which is slightly higher than concurrent control (0.8)	Increase in post implantation loss in the rabbit at high doses, considered secondary to maternal toxicity.	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										and within HCD.		
10	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Increase	Four of the seven pregnant 500 mg/kg/day dosage group rabbits aborted during the study. One of these rabbits died following abortion. All abortions occurred after completion of the dosage period. Clear signs of maternal toxicity were observed in the study: reduced food consumption (≥50 mg/kg bw/day), reduced bw gain (≥150 mg/kg bw/day), increased LW (≥150 mg/kg bw/day), and death (≥150 mg/kg bw/day)		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
11	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
13	Sensitive to, but not diagnostic of, EATS	Post implantation loss	rat	28	weeks	Oral	>2500	ppm	No effect			
8	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Increase	The mean pre-implantation loss ratio at 500 mg/kg bw/day was higher than the control.	Increase in pre-implantation loss in the rat at the highest dose. Note that the dosing commenced during the implantation phase (GD 6) and therefore the pre-implantation losses may be unrelated to treatment.	
9	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect			
10	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
9	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Increase	At 700 mg/kg there was an increased incidence of external malformation on a foetal (3.6%; 8/221 foetuses) a litter (33.3%; 6/18 litters)	Increase foetal malformations and altered ossification processes occurred in two studies. No effects were observed at doses without maternal toxicity	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										basis. There were no external malformations in controls. The increased incidences (7 foetuses) of tail defects (absence of tail, short tail or filamentous tail) among foetuses of the high dose group were attributed to severe signs of maternal toxicity. Skeletal ossification variation data indicated retarded ossification at 350 and 700 mg/kg bw/day, considered to represent a fetotoxic response.	and may thus be secondary to those effects.	
10	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
11	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Increase	Misaligned sutures (3.6% vs 0% in control): nasal irregular ossification (6.3% vs 2.2% in the control and 0.24% in HCD): angulation of hyoid alae (6.3% vs 1.4% in control and 1.29% in HCD). Overall incidences of foetal alterations were 18.7%, 19.3%, 23.9%, and 23.4% in the control, low, mid, and high dose groups, respectively.		
12	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Sensitive to, but not	Presence of anomalies (external,	rat	28	weeks	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
	diagnostic of, EATS	visceral, skeletal										
12	Sensitive to, but not diagnostic of, EATS	Pup survival index	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect		No effect on pup survival index	
13	Sensitive to, but not diagnostic of, EATS	Pup survival index	rat	28	weeks	Oral	>2500	ppm	No effect			
9	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	10 (GD 6-15)	Days	Oral	>700	mg/kg bw/d	No effect		No effect on sex ratio	
10	Sensitive to, but not diagnostic of, EATS	Sex ratio	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Sensitive to, but not diagnostic of, EATS	Sex ratio	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
12	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Sensitive to, but not diagnostic of, EATS	Sex ratio	rat	28	weeks	Oral	>2500	ppm	No effect			
13	Sensitive to, but not diagnostic of, EATS	Time to mating	rat	28	weeks	Oral	>2500	ppm	No effect		No effect on time to mating	
1	Target organ toxicity	Aorta histopathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effect on aorta histo-pathology in the rat	Overall evidence of target organ toxicity (liver, blood) with the

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
1	Target organ toxicity	Bone histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effect on bone histo-pathology in the rat mouse and dog	rat, mouse and dog, and systemic toxicity (lower body weight/body weight gain and food consumption, and in some cases clinical signs and mortality) across the tested species.
4	Target organ toxicity	Bone histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Bone histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Bone histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Bone histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
4	Target organ toxicity	Bone marrow histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect		Hyperplasia of the sternum marrow in 1-year study with the dog but no effects in a short-term study. No effects with the mouse.	
5	Target organ toxicity	Bone marrow histo-pathology	Dog	52	Weeks	Oral	75	mg/kg bw/d	Change	Hyperplasia of the sternum marrow was observed in one male and one female given 75 mg/kg bw/day and all animals given 300 mg/kg bw/day		
7	Target organ toxicity	Bone marrow histopathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Eyes histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effect on eye histo-pathology in the rat	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
1	Target organ toxicity	Heart histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on the heart histo-pathology	
3	Target organ toxicity	Heart histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Heart histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Heart histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Heart histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Heart histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
4	Target organ toxicity	Heart weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect		No effects on the heart weight	
5	Target organ toxicity	Heart weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
1	Target organ toxicity	Kidney histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on the kidney histo-pathology	
2	Target organ toxicity	Kidney histo-pathology	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Target organ toxicity	Kidney histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Kidney histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Kidney histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
6	Target organ toxicity	Kidney histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Kidney histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Kidney weight	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects on the kidney weight	
2	Target organ toxicity	Kidney weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Target organ toxicity	Kidney weight	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Kidney weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Kidney weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Kidney weight	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Kidney weight	mouse	52	Weeks	Oral	>3000	ppm	No effect			
15	Target organ toxicity	Kidney weight	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			
1	Target organ toxicity	Liver histo-pathology	Rat	5	Weeks	Oral	1000	ppm	Change	Trace to mild centrilobular hypertrophy was observed in males in the 1000, 4000 and 8000 ppm groups (30%,	Increased liver weights and histo-pathological changes (centri-lobular hypertrophy and in some cases vacuolation and	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										60%, 80%, respectively) and in females in the 4000 and 8000 ppm groups (10%, 40%, respectively)	pigmen-tation) in the liver were induced in the short-term and long-term studies across the tested species.	
2	Target organ toxicity	Liver histo-pathology	mouse	4	Weeks	Oral	4000	ppm	Change	Increased hypertrophy of centrilobular hepatocytes was noted in all male mice (minimal to moderate) at 4000 ppm and eight of ten female mice (minimal to slight)		
3	Target organ toxicity	Liver histo-pathology	Rat	13	Weeks	Oral	2500	ppm	Change	Increased incidence of centrilobular hypertrophy of the liver in males and females at 2500 (67%, 17%, respectively) and 5000 ppm (83%, 58%, respectively)		
4	Target organ toxicity	Liver histo-pathology	Dog	90	Days	Oral	125	mg/kg bw/d	Change	Vesiculation/ vacuolation in the cytoplasm		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										of centrilobular hepatocytes in all males at all levels including the control group and in all treated females and 3/4 control females but increased in severity at 125 mg/kg bw/day.		
5	Target organ toxicity	Liver histo-pathology	Dog	52	Weeks	Oral	300	mg/kg bw/d	Change	Centrilobular to mid-zonal hepatocellular hypertrophy in five out of six males and four out of six females given 300 mg/kg bw/day. Increased pigmentation of the liver was observed in one male given 75 mg/kg bw/day and all animals given 300 mg/kg bw/day		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
6	Target organ toxicity	Liver histo-pathology	Rat	2	Years	Oral	2500	ppm	Change	Hypertrophy was observed in the highest dose group (2500 ppm). Females offered 2500 ppm had a slightly greater (12%) incidence of binucleated cells in the liver than the controls (2%), but the effect was of uncertain toxicological significance.		
7	Target organ toxicity	Liver histo-pathology	mouse	52	Weeks	Oral	1000	ppm	Change	Centrilobular hypertrophy of the liver was observed in males given 1000 ppm (42%) and in males and females given 3000 ppm (100%). Pigment, described as morpho-logically compatible with haemo-siderin and bile, was noted in		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										males given 3000 ppm (69%).		
1	Target organ toxicity	Liver weight	Rat	5	Weeks	Oral	1000	ppm	Increase	Absolute liver weight was significantly increased in males at 1000, 4000 and 8000 ppm (+12%, +13% and +15%, respectively) and in females at 8000 ppm (+12.5%). Relative liver weight was significantly increased in males and females at 4000 (+19.4% and +18.2% respectively) and 8000 ppm (+32% and +33%, respectively).		
2	Target organ toxicity	Liver weight	mouse	4	Weeks	Oral	1500	ppm	Increase	The absolute liver with gallbladder weight was significantly increased in males at 1500 (+13%) and		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										in males and females at 4000 ppm (+42% and +16%, respectively). The liver/body weight ratio was significantly increased for the same groups of animals (+14%, +42% and +23%, respectively).		
3	Target organ toxicity	Liver weight	Rat	13	Weeks	Oral	2500	ppm	Increase	Absolute liver weight was significantly increased for females given 5000 ppm (114% of control), relative liver weight was significantly increased in both sexes given 2500 ppm (+12% in males and females) and 5000 ppm (+ 26% in males; + 28% in females) with		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										a dose-related trend.		
4	Target organ toxicity	Liver weight	Dog	90	Days	Oral	75	mg/kg bw/d	Increase	Absolute liver weight was increased in animals given 75 and 125 mg/kg bw/day (in males 116 and 134% of controls and in females 115 and 130% of controls)		
5	Target organ toxicity	Liver weight	Dog	52	Weeks	Oral	75	mg/kg bw/d	Increase	Absolute and relative liver weight was significantly increased in both sexes given 300 mg/kg bw/day (156 and 160% of controls for males, respectively, and 170 and 168% of controls for females, respectively), and in females given 75 mg/kg bw/day (134 and 158% of		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										controls, respectively). In males given 75 mg/kg bw/day absolute liver weight was increased (127%) and relative liver weight was statistically significantly increased (116%).		
6	Target organ toxicity	Liver weight	Rat	2	Years	Oral	500	ppm	Increase	Absolute liver weight and liver weight adjusted for brain weight of females given 2500 ppm were statistically significantly increased (+24% and +23%, respectively) and absolute liver weight/ liver weight relative to BW in males were increased 21/18% (not statistically significant)		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										while liver weight relative to brain weight was increased 24% (significant) in the 500 ppm group		
7	Target organ toxicity	Liver weight	mouse	52	Weeks	Oral	1000	ppm	Increase	At week 53, in males liver weights (absolute, body weight ratio and brain weight ratio) were statistically significantly increased at 2000/3000 ppm (+16%, +31% and +21%, respectively), and liver weight ratio with brain weight was statistically significantly increased at 1000 mg/kg (+14%). At week 53 in females, liver weight (body weight ratio and brain weight ratio)		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										was statistically significantly increased at 2000/3000 ppm (+28% and +18%, respectively). At week 79, only female liver weights (body weight ratio and brain weight ratio) were significantly increased at 3000 ppm (+14% and +16%, respectively).		
10	Target organ toxicity	Liver weight	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Increase	Increased absolute and relative liver weight in doses given 300 and 500 mg/kg bw/day		
1	Target organ toxicity	Lung histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to lung histopathology	
2	Target organ toxicity	Lung histo-pathology	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Target organ toxicity	Lung histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
4	Target organ toxicity	Lung histopathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Lung histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Lung histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Lung histo-pathology	mouse	52	Weeks	Oral	1000	ppm	Increase			
1	Target organ toxicity	Lymph nodes histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to lymph node histopathology	
3	Target organ toxicity	Lymph nodes histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Lymph nodes histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Lymph nodes histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Lymph nodes histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Lymph nodes histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Oesophagus histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to oesophagus histo-pathology	
4	Target organ toxicity	Oesophagus histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
1	Target organ toxicity	Pancreas histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to pancreas histo-pathology	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
3	Target organ toxicity	Pancreas histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Pancreas histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Pancreas histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Pancreas histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Pancreas histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Salivary glands histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to salivary glands histo-pathology	
3	Target organ toxicity	Salivary glands histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Salivary glands histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Salivary glands histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Salivary glands histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Salivary glands histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Skeletal muscle histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to skeletal muscle histo-pathology	
3	Target organ toxicity	Skeletal muscle histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
4	Target organ toxicity	Skeletal muscle histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Skeletal muscle histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Skeletal muscle histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Skeletal muscle histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Skin histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to skin histo-pathology	
3	Target organ toxicity	Skin histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Skin histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Skin histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Skin histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Skin histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Small and large intestines histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to small and large intestine histo-pathology	
3	Target organ toxicity	Small and large intestines	Rat	13	Weeks	Oral	>5000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
		histo-pathology										
4	Target organ toxicity	Small and large intestines histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Small and large intestines histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Small and large intestines histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Small and large intestines histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Spinal cord histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to spinal cord histo-pathology	
3	Target organ toxicity	Spleen histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect		No effects on spleen weight or histo-pathology	
4	Target organ toxicity	Spleen histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
7	Target organ toxicity	Spleen histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
5	Target organ toxicity	Spleen weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
1	Target organ toxicity	Stomach histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to stomach histo-pathology	
3	Target organ toxicity	Stomach histopathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Stomach histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Stomach histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Stomach histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Stomach histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Thymus histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to thymus histo-pathology	
3	Target organ toxicity	Thymus histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Thymus histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Thymus histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Thymus histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Thymus histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Target organ toxicity	Trachea histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to trachea histo-pathology	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
3	Target organ toxicity	Trachea histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Trachea histopatholog y	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
1	Target organ toxicity	Urinary bladder histo-pathology	Rat	5	Weeks	Oral	>8000	ppm	No effect		No effects to urinary bladder histo-pathology	
3	Target organ toxicity	Urinary bladder histo-pathology	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Target organ toxicity	Urinary bladder histo-pathology	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Target organ toxicity	Urinary bladder histo-pathology	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Target organ toxicity	Urinary bladder histo-pathology	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Target organ toxicity	Urinary bladder histo-pathology	mouse	52	Weeks	Oral	>3000	ppm	No effect			
1	Systemic toxicity	Body weight	Rat	5	Weeks	Oral	4000	ppm	Decrease	Statistically significantly reduced bw in M at 8000 ppm (-13%) and in females at 4000 ppm (-8%) and 8000 ppm (-16%). Statistically significantly reduced bwg in males and females at	Body weight and/or body weight gain were affected in most studies. In several, food consump-tion was reduced as well and there may have been a palatability issue rather than a toxicol-ogical issue	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										4000 ppm (-11% and -24%, respectively) and 8000 ppm (-28% and -42%, respectively)		
2	Systemic toxicity	Body weight	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Systemic toxicity	Body weight	Rat	13	Weeks	Oral	2500	ppm	Decrease	Body weight and body weight gain were significantly reduced in males given 2500 ppm (-7 and -10% when compared to control). Body weight was reduced in both sexes given 5000 ppm (-11% for both sexes, compared to control). Body weight gain was reduced in both sexes at 5000 ppm (-18% and -24% of control for		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										males and females, respectively).		
4	Systemic toxicity	Body weight	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Body weight	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Body weight	Rat	2	Years	Oral	2500	ppm	Decrease	Body weight and body weight gain was decreased in animals of each sex during the first year of treatment with 2500 ppm. Body weight was reduced after 2 years but this difference was not statistically significant (8% in males, and 13% in females.		
7	Systemic toxicity	Body weight	mouse	52	Weeks	Oral	>3000	ppm	No effect			
8	Systemic toxicity	Body weight	rat	10 (GD 6-15)	Days	Oral	500	mg/kg bw/d	Decrease	Reduction in body weight gain was observed in the dams receiving 500 mg/kg		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										bw/day (-38.8%)		
9	Systemic toxicity	Body weight	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Decrease	Reduction in body weight gain was observed in the dams at 350 and 700 mg/kg bw/day during the treatment period (-14.9% and -40.4%, respectively) and post treatment (GD 16-20; -17.1% for each group). Mean corrected body weight on gestation day 20 was reduced in dams at 750 mg/kg bw/day (-6.1%)		
10	Systemic toxicity	Body weight	rabbit	13 (DG 7-19)	Days	Oral	150	mg/kg bw/d	Decrease	Reduction in body weight gain day 13-20 from 150 mg/kg bw with a tendency already at 50		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										mg/kg bw/day. Reduction in body weight and body weight gain was observed in the dams receiving 300 and 500 mg/kg bw/day		
11	Systemic toxicity	Body weight	rabbit	13 (DG 7-19)	Days	Oral	100	mg/kg bw/d	Decrease	Administ-ration of the 100 and 300 mg/kg/day dosages of the test substance resulted in dosage-dependent, significant inhibition of average maternal body weight during the dosage period. Average maternal body weight change for days 7-20 of gestation (the dosage period) was +0.18. +0.13. +0.05($p<0.05$) and -0.10 kg		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										($p<0.01$) for control. low. middle and high dosage group dose, respectively.		
12	Systemic toxicity	Body weight	rat	5 to 6	Weeks	Oral	5000	ppm	Decrease	A significant reduction in body weight was observed in parents receiving 5000 ppm. Body weight was stat sig reduced in males in weeks 1 and 2 of the study (-2% and -1.6%) and overall BWG was reduced weeks 0-3 (-18%). Female body weights and BWG was stat sig reduced at end of pre-mating period of (-12.6% and 62.5%, respectively), BW was stat sig reduced at end of gestation (-13.4%), and on day 7 of		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										lactation period (-16%).		
13	Systemic toxicity	Body weight	rat	28	weeks	Oral	2500	ppm	Decrease	Mean body weights were significantly reduced for both F0 and F1a adult males at 2500 ppm throughout the study. Body weights for F0 adult females were similar to those of control animals during the premating, gestation and lactation periods. Body weights for F1a adult females were significantly reduced during the pre-mating and gestation periods up through Day 14 of lactation. While body weights were reduced for		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										F1a females, body weight gain during gestation was not affected by treatment.		
14	Systemic toxicity	Body weight	rat	3	Days	Oral	45	mg/kg bw/d	Decrease	Mean body weight losses at 450 mg/kg/day group (Study Days 0–3); mean absolute body weight that was 14.0% lower than controls on Study Day 3. At 45 and 135 mg/kg/day, mean body weight losses were noted Study Days 0–1, resulting in lower mean body weight gains when the overall treatment period (Study Days 0–3) was evaluated. However, mean absolute body weights at 45 and 135		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										mg/kg/day were within 3.3% of the control group value on Study Day 3.		
15	Systemic toxicity	Body weight	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			
1	Systemic toxicity	Clinical chemistry and haematology	Rat	5	Weeks	Oral	1000	ppm	Change	The mean haemoglobin values for males were significantly decreased in the 1000, 4000 and 8000 ppm groups, and haematocrits were decreased for the 4000 and 8000 ppm groups. For females, mean haemoglobin values and erythrocyte counts were significantly reduced in the 5, 1000 and 8000 ppm groups; the 200 and 4000 ppm females showed decreases in	Obser-vations in blood/serum included reduced haematocrit, reduced haemo-globin, reduced erythrocyte count, increased reticulocytes, increased platelets, increased cholesterol and triglycerides, increased total protein, reduced albumin/ globulin ratio, alterations in alkaline phosphatase, increased chloride levels, reduced BUN/ creatinine ratio, and increased ALT.	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										these parameters that were not statistically significant.		
2	Systemic toxicity	Clinical chemistry and haematology	mouse	4	Weeks	Oral	625	ppm	Decrease	Red blood cell counts were significantly decreased in males at 1500 and 4000 ppm and in females at 1500 ppm. Haemoglobin was significantly decreased in males at 625, 1500 and 4000 ppm and in females at 1500 ppm. Haematocrit was significantly reduced in males at 4000 ppm.		
3	Systemic toxicity	Clinical chemistry and haematology	Rat	13	Weeks	Oral	5000	ppm	Increase	Clinical chemistry showed significantly higher cholesterol, total protein and globulin values at		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										5000 ppm in males (131%, 165% and 109% of control values, respectively). A significant decrease in blood urea nitrogen/creatine ratio was observed in females at 2500 ppm but not at 5000 ppm - this was considered to be unrelated to treatment.		
4	Systemic toxicity	Clinical chemistry and haematology	Dog	90	Days	Oral	125	mg/kg bw/d	Increase	Mean alkaline phosphatase (ALP) activity progressively increased in males and females given 125 mg/kg bw/day. Mean cholesterol levels in females given 125 mg/kg bw/day progressively increased these differences		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										from control were statistically significant after 1 and 2 months. Albumin/globulin ratio was reduced in males at 125 mg/kg bw/day. Chloride levels were reduced in females at 75 and 125 mg/kg bw/day.		
5	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	75	mg/kg bw/d	Change	Erythrocyte count was statistically significantly decreased in males given 300 mg/kg bw/day on days 270 and 360 and in females given 300 mg/kg bw/day on days 180, 270 and 360. Haemoglobin and haematocrit were statistically significantly		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										decreased in females given 300 mg/kg bw/day on days 180, 270 and 360. In males on day 360 the haemoglobin and haematocrit values were 8% lower than those of the control group (not statistically significant) Platelet count was statistically significantly increased in both sexes given 300 mg/kg bw/day during the whole exposure period and a similar trend was observed in the 75 mg/kg bw group (20% higher than the control on 360) although not		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										statistically significant. The white blood cell count was statistically significantly increased in females given 300 mg/kg bw/day on days 90, 180, 270 and 360 and in females given 75 mg/kg bw/day at day 90 only. Segmented neutrophils were significantly higher in females given 300 mg/kg bw/day on days 31, 90 and 270. The albumin/globulin ratio was decreased in females given 300 mg/kg bw/day at day 270 and 360. The glucose level was significantly decreased in males given		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										300 mg/kg bw/day on day 270, in females given 300 mg/kg bw/day on days 180 and 360, and in females given 75 mg/kg bw/day at day 360. Alkaline phosphatase was significantly higher than the control group's from day 90 onwards at 75 and 300 mg/kg bw/day in both sexes, although only statistically significant at the highest dose level. ALP had a decreasing trend over time in all groups but the highest dose group, in which it increased over time. ALT was		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										statistically significantly increased at 300 mg/kg bw/day in both sexes from day 180 onwards. Cholesterol was statistically significantly increased in males given 300 mg/kg bw/day at day 360 only and in females throughout the study. Triglycerides were significantly increased in both sexes given 300 mg/kg bw/day at day 360, and not statistically significantly increased (29-41%) at 75 mg/kg bw/day.		
6	Systemic toxicity	Clinical chemistry and haematology	Rat	2	Years	Oral	>2500	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
7	Systemic toxicity	Clinical chemistry and haematology	mouse	52	Weeks	Oral	2000/3000	ppm	Decrease	Red blood cell count was statistically significantly decreased in males given 2000/3000 ppm in week 27 and 79 and in females given 2000/3000 ppm in week 27. Haemoglobin and haematocrit were statistically significantly decreased in males given 2000/3000 ppm in week 27.		
1	Systemic toxicity	Clinical signs	Rat	5	Weeks	Oral	>8000	ppm	No effect		Clinical signs occurred in some studies and included excess salivation, excess lacrimation, staining of the fur/skin in the anogenital area, red substance in the pan, and dried faeces.	
2	Systemic toxicity	Clinical signs	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Systemic toxicity	Clinical signs	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Systemic toxicity	Clinical signs	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Clinical signs	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Clinical signs	Rat	2	Years	Oral	>2500	ppm	No effect			
7	Systemic toxicity	Clinical signs	mouse	52	Weeks	Oral	>3000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
8	Systemic toxicity	Clinical signs	rat	10 (GD 6-15)	Days	Oral	300	mg/kg bw/d	Change	Excess salivation was seen with increased frequency in at 300 and 500 mg/kg bw/day		
9	Systemic toxicity	Clinical signs	rat	10 (GD 6-15)	Days	Oral	350	mg/kg bw/d	Change	Excess salivation was seen was seen at least once in 11/25 females at 350 mg/kg bw/day and 19/25 females at 700 mg/kg bw/day. Excessive lacrimation in 12/25 and staining of fur/skin in the anogenital area of 7/25 females at 700 mg/kg bw/day.		
10	Systemic toxicity	Clinical signs	rabbit	13 (DG 7-19)	Days	Oral	>500	mg/kg bw/d	No effect			
11	Systemic toxicity	Clinical signs	rabbit	13 (DG 7-19)	Days	Oral	100	mg/kg bw/d	Change	Signs of maternal toxicity were noted in females treated at 100 or 300 mg/kg bw/day and		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										comprised clinical signs (red substance in the pan and dried faeces		
12	Systemic toxicity	Clinical signs	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Systemic toxicity	Clinical signs	rat	28	weeks	Oral	>2500	ppm	No effect			
14	Systemic toxicity	Clinical signs	rat	3	Days	Oral	>450	mg/kg bw/d	No effect			
15	Systemic toxicity	Clinical signs	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			
1	Systemic toxicity	Food consumption	Rat	5	Weeks	Oral	4000	ppm	Decrease	Statistically significantly reduced in males and females at 4000 ppm (-8% males; -10% females) and 8000 ppm (-22% males; -26% females). May be related to palatability	Food consump-tion was affected in 8 studies. In five of them the test substance was incorporated in the diet and palatability may influence the outcome. However, oral gavage was used to administer the substance in the three other studies which eliminates the palatability issue. Of the four studies without an observed effect on food consump-tion, oral gavage was used in three of	
2	Systemic toxicity	Food consumption	mouse	4	Weeks	Oral	>4000	ppm	No effect			
3	Systemic toxicity	Food consumption	Rat	13	Weeks	Oral	2500	ppm	Decrease	Food consumption was significantly reduced in males and females during		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										treatment at 5000 ppm. Decreases were also noted at sporadic intervals for males in the 500 and 2500 ppm groups. May be related to palatability	them and adminis-tration via the diet was performed in one (4-week, mice).	
4	Systemic toxicity	Food consumption	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Food consumption	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Food consumption	Rat	2	Years	Oral	2500	ppm	Decrease	Food consumption was significantly decreased in animals of each sex during the first year to treatment with 2500 ppm.		
7	Systemic toxicity	Food consumption	mouse	52	Weeks	Oral	>3000	ppm	No effect			
8	Systemic toxicity	Food consumption	rat	10 (GD 6-15)	Days	Oral	>500	mg/kg bw/d	No effect			
9	Systemic toxicity	Food consumption	rat	10 (GD 6-15)	Days	Oral	700	mg/kg bw/d	Decrease	Reduction in food consumption was observed in the dams receiving 350		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										(Day 7 of gestation) and 700 mg/kg bw/day (Days 7-10 of gestion), but only the prolonged reduction in food consumption at 700 mg/kg bw/day considered related to treatment.		
10	Systemic toxicity	Food consumption	rabbit	13 (DG 7-19)	Days	Oral	50	mg/kg bw/d	Decrease	Reduced feed consumption during the dosage period (≥50 mg/kg/day – statistically significant only at highest dose) with a post dosage increase in food consumption compared with the control (≥150 mg/kg/day, not statistically significant),		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
11	Systemic toxicity	Food consumption	rabbit	13 (DG 7-19)	Days	Oral	100	mg/kg bw/d	Decrease	Adminis-tration of the 100 and 300 mg/kg/day dosages of the test substance resulted in dosage-dependent, significant inhibition of average maternal food consumption during the dosage period		
12	Systemic toxicity	Food consumption	rat	5 to 6	Weeks	Oral	5000	ppm	Decrease	Food consumption was consistently lower for treated males and females in all dose groups. However, the difference was statistically significant in high-dose males only.		
13	Systemic toxicity	Food consumption	rat	28	weeks	Oral	2500	ppm	Decrease	In general, food consumption of F0 adults was not affected by treatment		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										except for decreases for animals at 2500 ppm during the initial exposure period. Food consumption for F1a adult males was decreased on many of the intervals measured throughout the study. Food consumption for F1a adult females, however, was not affected during the pre-mating period but was reduced on Days 0-2, 2-5, and 9-12 of gestation. Food consumption for F1a females was not affected during lactation.		
1	Systemic toxicity	Mortality	Rat	5	Weeks	Oral	>8000	ppm	No effect		Mortality occurred in three	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
2	Systemic toxicity	Mortality	mouse	4	Weeks	Oral	>4000	ppm	No effect		studies: at a dose of 238/357 mg/kg bw/day in a 2-year study in non-pregnant mice, and at 300-700 mg/kg bw/day in pregnant rats and rabbits.	
3	Systemic toxicity	Mortality	Rat	13	Weeks	Oral	>5000	ppm	No effect			
4	Systemic toxicity	Mortality	Dog	90	Days	Oral	>125	mg/kg bw/d	No effect			
5	Systemic toxicity	Mortality	Dog	52	Weeks	Oral	>300	mg/kg bw/d	No effect			
6	Systemic toxicity	Mortality	Rat	2	Years	Oral	2500	ppm	No effect			
7	Systemic toxicity	Mortality	mouse	52	Weeks	Oral	2000/3000	ppm	Change	Survival for males and females of the 3000-ppm group through Week 78 was significantly lower than control, and there was a significant negative trend, i.e. treatment-related decrease, in survival for both males and females.		
8	Systemic toxicity	Mortality	rat	10 (GD 6-15)	Days	Oral	>500	mg/kg bw/d	No effect			
9	Systemic toxicity	Mortality	rat	10 (GD 6-15)	Days	Oral	700	mg/kg bw/d	Increase	At 700 mg/kg bw/day maternal toxicity was evident as mortality increased by a 20% (5/25).		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
10	Systemic toxicity	Mortality	rabbit	13 (DG 7-19)	Days	Oral	300	mg/kg bw/d	Increase	Two of seven pregnant 300 mg/kg/day dosage group rabbits died, and one of seven pregnant 500 mg/kg/day dosage group rabbits aborted and died.		
11	Systemic toxicity	Mortality	rabbit	13 (DG 7-19)	Days	Oral	>300	mg/kg bw/d	No effect			
12	Systemic toxicity	Mortality	rat	5 to 6	Weeks	Oral	>5000	ppm	No effect			
13	Systemic toxicity	Mortality	rat	28	weeks	Oral	>2500	ppm	No effect			
14	Systemic toxicity	Mortality	rat	3	Days	Oral	>450	mg/kg bw/d	No effect			
15	Systemic toxicity	Mortality	rat	10	Days	Oral	>200	mg/kg bw/d	No effect			

2.10.2.2.1.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Table 2.10.2.2.1.1-1. WoE for EAS-mediated adversity.

Overall, no clear signs of EAS-mediated adversity have been observed at doses not causing overt toxicity. Observed effects on organ weights and reproduction occurred at dose levels producing effects like reduced food consumption and/or body weight, increased liver weight accompanied by microscopic alterations (hypertrophy and in some cases pigmentation and vacuolation), alteration in clinical chemistry parameters (e.g. cholesterol and ALK) and blood system effects (anaemia).
Increased left, right and combined testes weight/body weight ratios were observed in rats at 8000 ppm (equivalent to 515 mg/kg bw/day) group following 5-weeks exposure (ID 1) and in F0 male rats exposed to 163 mg/kg bw/day throughout pre-mating, mating, gestation, and lactation of the F1 pups (ID 13). These increases were due to the reduced body weights and the absolute weights were not affected. Testis weight was not affected in other studies assessing it (ID 2, 3, 4, 5, 6, 7) and no histopathological lesions were observed (ID 1, 2, 3, 4, 5, 6, 7).
The weights of the seminal vesicles and the prostate were not affected by treatment in the Hershberger assay (ID 15; five weeks exposure of male rats to 20 - 200 mg/kg bw/day) but absolute weights of the two organs were reduced in male rats of the F1-generation (11% and 25%, respectively) that were exposed to 151.2 mg/kg bw/day in the multigenerational reproductive toxicity study (ID 13). The F1 males in this study also displayed reduced food consumption and body weight. No effects have been observed in the histopathological assessments of the two organs (seminal vesicles: ID 1, 7, 13; prostate: ID 1, 3, 4, 5, 6, 7)
No treatment related weight changes of the Cowper's gland, glans penis, or LABC muscle group were observed in the Hershberger assay (ID 15). No effects have been observed on epididymis histology (rat, dog, mouse; ID 1, 3, 4, 5, 6, 7, 13). Relative epididymis weight was increased in male rats of the F1 generation (ID 13) but this was due to the reduced body weight. The absolute weight of the epididymis was unaffected in this study as well as in the Hershberger assay (ID 13, 15).
No microscopic lesions in the ovaries were detected in dog, rat, or mouse (ID 1, 3, 4, 5, 6, 7, 13) but in two studies differences in ovary weight were observed (ID 3, 4). In the 13-week rat study, relative and absolute ovary weights were lower in exposed groups than in the control, but no dose response was apparent. The mean relative and absolute ovary weights of dogs administered 75 mg/kg bw/day for 90 days were lower, and those of dogs administered 125 mg/kg bw/day were higher than those of the control group. The differences were not statistically significant but notable due to the size (30-82%). Oestrus cyclicity was not determined in these studies, and differences in ovary weights were not observed in the longer-term studies in dog and rat.
No effects have been observed on uterus histology (ID 1, 3, 4, 5, 6, 7, 13, 15). Uterus weights were reduced in two studies after exposure during gestational days 6-15 in rat and 7-19 in rabbit. In the teratology study in rats (ID 9), a 7% reduction in uterus weight compared with the control was observed in dams exposed to 100 mg/kg bw/day, a 10 % reduction in the 350 mg/kg bw/day group, and a 27% reduction in the 700 mg/kg bw/day group (only the top dose was statistically significant). In the teratology study in rabbits (ID 11), a statistically significant 10% decrease in absolute uterus weight was observed in the 300 mg/kg bw/day dose group when compared to controls.
No effect on the histology of the female mammary gland (rat, dog, mouse; ID 1, 3, 4, 5, 6, 7)
In the 2-generation reproduction toxicity study with the rat (ID: 13), no treatment related effect was observed on the vaginal cytology or the oestrous cycles of any F0 or F1a females. No changes in mating indices, pregnancy rates, or male fertility were observed in the F0 or F1a adults. No treatment related effects on litter size, sex ratio, or litter survival were evident. An increase in stillborn pups was observed in F1a litters of the highest dose group, but in light of historical control data and the lack of a similar response in F2a litters, this finding was not considered to be treatment related. Necropsy and histopathological observations did not reveal any adverse effects of treatment in F1a or F2a pups.
The number of implantation sites (87 vs 126) and as a result the number of viable foetuses (86 vs 122) were reduced, albeit not statistically significant, in the pilot teratology study in rats (ID 8); however, it is noted that there were signs of SDA

infections in some individuals. There was no such effect in the full teratology study in rats (ID 9) nor in the pilot or full teratology study in rabbits (ID 10, 11).
In the teratology study in rats (ID 9), there was an increase in post-implantation loss at 700 mg/kg bw/day. This in part was driven by a single female with 15/16 foetal resorptions, with this female excluded the mean resorption rate was 1.1 which was slightly higher than concurrent control (0.8) and within HCD. This dose induced severe maternal toxicity (mortality, reduced food consumption and body weight gain, clinical signs). In the pilot teratology study in rabbits (ID 10), four of the seven pregnant 500 mg/kg/day dosage group rabbits aborted after completion of the dosage period. One of these rabbits died following abortion. Clear signs of maternal toxicity were observed in the study: reduced food consumption (≥50 mg/kg bw/day), reduced bw gain (≥150 mg/kg bw/day), increased LW (≥150 mg/kg bw/day), and death (≥150 mg/kg bw/day). There was no increase in post-implantation loss in the teratology study in rabbits (ID 11).
Foetal sex ratios were unaffected by clethodim (ID 9, 10, 11, 12, 13).
Increase foetal malformations and altered ossification processes occurred in two studies (ID 9 and 11). In the teratology study in rats (ID 9), there was an increased incidence of external malformation on a foetal (3.6%; 8/221 foetuses) and litter (33.3%; 6/18 litters) basis, and an increase in incidence of tail defects (7 foetuses; absence of tail, short tail or filamentous tail) in the high dose group. Skeletal ossification variation data indicated retarded ossification at 350 and 700 mg/kg bw/day. Maternal effects at ≥350 mg/kg bw/day included reduced body weight gain, clinical signs (excessive salivation, red/mucoid nasal discharge, alopecia, staining of the anogenital area), and reduced uterine weight. Additional signs at 700 mg/kg bw/day included excessive lacrimation and mortality. In the developmental toxicity study in rabbits ID 11), there was an increased incidence of angulated hyoid alae, misaligned sutures (fontanelle), and nasal irregular ossification in the foetuses at 300 mg/kg bw/day; a dose level which caused reduced food consumption and body weight gain, clinical signs (red substance in pan, dried faeces), and reduced uterine weight in the dams. Overall incidences of fetal alterations were 18.7%, 19.3%, 23.9%, and 23.4% in the control, low, mid, and high dose groups, respectively (0, 25, 100 and 300 mg/kg bw/day). The dams exposed to 100 mg/kg bw/day had dried faeces and reduced food consumption and body weight gain.
Reduction in foetal body weight were observed three studies (rat and rabbit; ID 8, 9, 10) but at doses causing maternal toxicity, including reductions in body weight and food consumption. The effects on foetal body weight are therefore considered secondary to maternal toxicity.
In rats, reductions in combined pup weight (day 7) and pup weight gain (day 0-7), but not birth weight, were observed at all dose levels (500, 2000, and 5000 ppm) in the 5-week pilot study (ID 12). Maternal toxicity was only noted in the highest dose group and included reduced food consumption (possible palatability issue) and reduced body weight gain (only statistically significant in males). This effect on postnatal growth was not observed in either generation of the 2-generation study (doses used: 5, 20, 500, and 2500 ppm; ID 13).
No changes in absolute organ weights and no histopathological lesions in the kidneys, brain, or pituitary were observed in the rat, mouse, or dog. Relative brain and kidney weights were increased in two studies, likely a result of reduced body weight (ID 1 and 3).
Adrenal weights (absolute and relative to brain weight) were reduced in rats exposed to 597 (males) or 667 (females) mg/kg bw/day for females for 5 weeks (Report No.: S-2763) and increased in females and decreased in male mice at the interim but not the terminal sacrifice (week 53 and 79, respectively; unclear relevance; ID 7). Adrenal weight was unaffected in the other seven studies (ID 1-6, 15). No microscopic lesions in the adrenals have been reported (ID 1, 3, 4, 5, 6, 7).
Clethodim was found to cause effects on the liver in all short-term studies (rat, mouse, and dog; ID 1, 2, 4, 5). These effects included increased liver weight, hypertrophy, vacuolisation of hepatocytes, increased pigmentation, effects on clinical chemistry parameters associated with liver damage and/or affected fat metabolism. Besides hepatotoxicity, several haematological effects were observed, most prominently anaemia. In addition, increases of the numbers of platelets and leukocytes were found in a5 week rat study and the one-year dog study (ID 1, 5). The 1-year dog study also revealed bone-marrow hyperplasia in the sternum at the highest dose level. Effects observed during the long-term/carcinogenicity studies with rats and mice (ID 6, 7) included reduced body weight, increases in hepatic volume (liver weight and hypertrophy) and anaemia (reduction in the number of erythrocytes, haemoglobin, and haematocrit). These effects are consistent with those obtained in the short-term studies. Hepatotoxicity is considered the critical effect in the sense that it always (co-)determines the NOAEL of each study (ID: 1, 2, 4, 5, 6, 7, 10).

No EAS-mediated adversity was found at doses not causing overt toxicity, the overall assessment of the integrated lines of evidence indicates that there is no evidence to support an EAS-mediated ED identification.

Table 2.10.2.2.1.1-2. WoE for EAS-mediated endocrine activity.

The ToxCast assays available did not indicate any ER agonistic or AR agonistic/antagonistic activity: ATG_ERa_TRANS_up, ATG_ERE_CIS_up, ATG_AR_TRANS_up, TOX21_AR_LUC_MDAKB2_Agonist_3uM_Nilutamide, TOX21_AR_LUC_MDAKB2_ Antagonist_0.5nM_R1881 (ID 18). There are no ToxCast AUC model data for the estrogen or androgen receptor.
There were no changes in uterus weight or histopathology in the Uterotrophic assay (ID14)
There were no weight changes in androgen dependent organs in the Hershberger assay (ID 15)
No hormone levels have been measured in vivo but there was no effect on estradiol or testosterone release by H295R cells in vitro (ID 17)
Clethodim showed no aromatase inhibitory activity using human recombinant microsomes (ID 16).

EAS-related activity was sufficiently investigated in mechanistic studies (Hershberger-, Uterotrophic-, Aromatase- and Steroidogenesis assays) (refer to Vol. 3. section B.6.8.3 for details). These studies were negative. Thus, no EAS-mediated endocrine activity was observed.

2.10.2.2.2 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

Table 2.10.2.2.2-1. Selection of relevant scenario.

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "EAS-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	**Conclude: ED criteria not met because no EAS-mediated endocrine activity observed**	**X**
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

There are endpoints missing from the data package, namely *in vivo* serum hormonal levels, sperm analyses, anogenital distance in offspring, age at balano-preputial separation, and age at vaginal opening. However, since EAS-related activity was sufficiently investigated, no indications of such activity was found in the data set, and since no EAS-mediated adversity was found at doses not causing overt toxicity, the overall assessment of the integrated lines of evidence indicates that there is no evidence to support an EAS-mediated ED identification. The ED criteria for EAS-modalities are not met for Clethodim (scenario 2a (ii)).

2.10.2.2.3 MoA analysis for EAS-modalities

Not applicable

2.10.2.2.3.1 Postulate MoA

Not applicable

2.10.2.2.3.2 Further information to be generated to postulate MoA

Not applicable

2.10.2.2.3.3 Empirical support of the postulated MoA

Not applicable

2.10.2.2.3.4 Conclusion on MoA analysis

Not applicable

2.10.2.2.4 Conclusion of the assessment of EAS-modalities

There are some endpoints missing from the data package, namely *in vivo* serum hormonal levels, sperm analyses, anogenital distance in offspring, age at balano-preputial separation, and age at vaginal opening. However, since EAS-related activity was sufficiently investigated, no indications of such activity was found in the data set, and since no EAS-mediated adversity was found at doses not causing overt toxicity, the overall assessment of the integrated lines of evidence indicates that there is no evidence to support an EAS-mediated ED classification. The ED criteria for EAS-modalities are not met for Clethodim (scenario 2a (ii)).

2.10.2.3 Overall conclusion on the ED assessment for humans

Clethodim does not meet the criteria for endocrine disruption by the EATS-modalities.

2.10.3 ED assessment for non-target organisms

2.10.3.1 ED assessment for T-modality

Table 2.10.3-1. Data sufficiency for clethodim via the T-modality for non-target organisms

	Sufficiently investigated
T-mediated parameters	Yes T-mediated parameters have been investigated directly with an OECD CF level 3 amphibian metamorphosis test (AMA, OECD 231) which showed no T-mediated activity. The data package is supported by CF Level 4 data from a fish early life stage test (ELS) and two avian reproduction studies.

2.10.3.1.1 Lines of evidence for adverse effects and endocrine activity related to T-modality

Table 2.10.3-2. Lines of evidence for clethodim via the T-modality relevant for non-target organisms

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence
18	*In vitro* mechanistic	Thyroid receptor	Human liver cell line	24	Hours	Uptake from the medium (in vitro)	0 µM	No effect	No effect 0 (No TR agonist activity)	No T-mediated activity	Increased thyroid weight was observed in males in the 1-year dog study but not in females and not in the 90-day dog study. Thus, considered of unclear biological relevance.
15	*In vivo* mechanistic	Liver weight (Hershberger, considered T-mediated only in combination with other thyroid endpoints)	Rat	10	Days	Oral	>200 mg/kg bw/day	No effect	No effect		
25	EATS-mediated	Developmental stage	*Xenopus laevis*	21	Days	Uptake from water	24 mg/L	Decrease	Day 7 - Statistically significant reduction in development score at 24 mg/L, 53 compared with 54 in controls (P <0.05)	Lack of follicular cell hypertrophy and hyperplasia at the highest concentration consistent with the NF Stage Score 55. Therefore, effects seen not considered to be endocrine related.	A thyroid antagonist would be expected to cause delays in somatic growth, but would also tend to cause thyroid gland proliferation, which did not occur in this study. Likely onset of systemic toxicity.
25a		Developmental stage	*Xenopus laevis*	21	Days	Uptake from water	24 mg/L	Decrease	Day 21 - Statistically significant decrease in development stage at 24mg/L (55) compared with controls (57)		
25a		Hind limb length	*Xenopus laevis*	21	Days	Uptake from water	0.25 mg/L	Decrease	Day 21 - Statistically significant decrease at 0.25, 2.4 and 24 mg/L (0.41, 0.38 and 0.24 mm respectively) compared with control (0.45 mm)	Reduced growth indicative of onset of systemic toxicity.	
25		Thyroid histopathology (amphibian)	*Xenopus laevis*	21	Days	Uptake from water	24 mg/L	Decrease	Treatment related lack of thyroid follicular cell hypertrophy and hyperplasia in 24 mg/L group compared with controls.	Lack of follicular cell hypertrophy and hyperplasia at the highest concentration consistent with the NF Stage Score 55. Associated with delay in somatic growth seen in this group. Non-endocrine MoA.	
25		Hind limb length	*Xenopus laevis*	21	Days	Uptake from water	> 24.0 mg/L	No effect	Day 7	No effect	No effect
25	Sensitive to, but not diagnostic	Body weight (amphibian)	*Xenopus laevis*	21	Days	Uptake from water	2.4 mg/L	Decrease	Day 7 -Statistically significant reduction in	Delay in growth and development indicative	Onset of systemic toxicity

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence
	of, EATS								body weight at 2.4 and 24 mg/L (208 mg and 116 mg respectively) compared with 280 mg in controls (P < 0.05)	of onset of systemic toxicity. Thyroid histopathology concomitant with growth stage of tadpoles at highest concentration, Non-endocrine MoA.	
25a		Body weight (amphibian)	*Xenopus laevis*	21	Days	Uptake from water	2.4 mg/L	Decrease	Day 21 – Statistically significant reduction in body weight at 2.4 and 24 mg/L (773 mg and 225 mg, respectively) compared with 1053 mg in controls (P < 0.05)		
19		Body weight (bird)	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect	No effect	No effect	No effect
19		Body weight (bird)	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect	No effect	No effect	No effect
19			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000 ppm	Change	Incidental statistically significant variation in body weight during test, no effect on overall body weight change.		
22			Mallard	6	Weeks	Oral	>3000 ppm	Change	Variability on body weight throughout, slight reduction in overall body weight gain at 3000 ppm, not statistically significant		
23		Body weight (fish)	Sheepshead minnow	34	Days	Uptake from water	11 mg/L	Decrease	Significant reduction in wet weight at 11 mg a.s./L (71.8 mg) compared with solvent control (92.2 mg)	No T activity identified in mammalian data, therefore effects seen not "EATS"- mediated. Likely onset of systemic toxicity	Onset of systemic toxicity
23a		Body weight (fish)	Sheepshead minnow	34	Days	Uptake from water	11 mg/L	Decrease	Statistically significant reduction in dry weight at 11 mg a.s./L (16.9 mg) compared with solvent control (21.4 mg a.s./L)		
19		Cracked eggs	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect	No effect	No effect	No effect
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
19		Egg production	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19b			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence
19c			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20b			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20c			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000 ppm	No effect			
22			Mallard	6	Weeks	Oral	>3000 ppm	No effect			
19		Egg viability (% viable embryo of egg set)	Bobwhite quail	22	Weeks	Oral	1000 ppm	Decrease	Slight decrease at 1000 ppm of 72% compared with controls of 91%. Not statistically significant but NOEC lowered to reflect this	No T activity identified in mammalian data, therefore effects seen not "EATS"- mediated. Likely onset of systemic toxicity	Onset of systemic toxicity
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect	No effect	No effect	No effect
19		Eggshell thickness	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
19		Gross pathology (bird)	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000 ppm	No effect			
22			Mallard	6	Weeks	Oral	>3000 ppm	No effect			
19		Hatchability	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19b			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19c			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
23		Hatching success	Sheepshead minnow	34	Days	Uptake from water	>11 mg/L	No effect			
23		Embryo time-to-hatch	Sheepshead minnow	34	Days	Uptake from water	>11 mg/L	No effect			
23		Length (fish)	Sheepshead minnow	34	Days	Uptake from water	11 mg/L	Decrease	Significant reduction in length at 11 mg a.s./L (18.3 mm) compared with solvent control (19.8 mm)	No T activity identified in mammalian data, therefore effects seen not "EATS"- mediated. Likely onset of systemic toxicity.	Onset of systemic toxicity
25		Snout- vent length/growth	*Xenopus laevis*	21	Days	Uptake from water	2.4 mg/L	Decrease	Day 7 -Statistically significant reduction in SNV t at 2.4 and 24 mg/L (14.0 mm and 11.2 mm respectively) compared with 15.4 mm in controls ($P < 0.05$)	Associated with delay in growth and development as a result of exposure to clethodim. Lack of effects at the cellular level confirm non-	

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment of the integrated line of evidence
25a		Snout- vent length/growth	*Xenopus laevis*	21	Days	Uptake from water	2.4 mg/L	Decrease	Day 21 – Statistically significant reduction in SNV at 2.4 and 24 mg/L (21.7 mm and 13.7 mm respectively). Compared with 24.2 mm in controls ($P < 0.05$)	endocrine MoA.	
19		Viable embryos	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect	No effect	No effect	No effect
19a			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19b			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20b			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
19	Systemic toxicity	Mortality	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000 ppm	No effect			
22			Mallard	6	Weeks	Oral	>3000 ppm	No effect			
25		Mortality (amphibian)	*Xenopus laevis*	21	Days	Uptake from water	>24.0 mg/L	No effect			
25a		Mortality (amphibian)	*Xenopus laevis*	21	Days	Uptake from water	>24.0 mg/L	No effect			
19		Survival (bird)	Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000 ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20b			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
20c			Mallard	19	Weeks	Oral	>1000 ppm	No effect			
23		Survival (fish)	Sheepshead minnow	34	Day	Uptake from water	>11 mg/L	No effect			
19	Systemic toxicity	Feed consumption	Bobwhite quail	22	Weeks	Oral	>1000 ppm	Change	Incidental statistically significant variation in weekly feed consumption throughout trial. Not treatment related	No effect	No effect
20			Mallard	19	Weeks	Oral	>1000 ppm	Change	Incidental statistically significant variation in weekly feed consumption throughout trial. Not treatment related		
21			Bobwhite quail	6	Weeks	Oral	>3000 ppm	No effect	No effect	No effect	No effect
22			Mallard	6	Weeks	Oral	>3000 ppm	No effect	No effect	No effect	No effect

2.10.3.1.1.1 Assessment of the integrated lines of evidence and weight

Table 2.10.3-3. WoE for T mediated adversity/activity

Amphibians No T-mediated adversity evident in a valid AMA (tested over mean measured concentrations of 0.25, 2.4, 24 mg a.s./L clethodim). • A significant decrease in snout vent length, hind limb length and wet weight compared with controls followed a concentration response. Tadpole development delayed at the highest test concentration with a lower mean development stage at testtermination compared with the controls. • Complete lack of follicular cell hypertrophy and hyperplasia in thyroid tissue at the highest test concentration are in concordance with the NF Stage Score of 55 of the tadpoles. • No significant difference in thyroid histopathology compared with controls following exposure to 0.25 and 2.4 mg a.s./L clethodim. • No mortalities Therefore, the delay in somatic growth and development seen, particularly at the highest concentration, is driven by a non-endocrine mode of action. Effects are attributed to the onsetof systemic toxicity.
Birds No statistically significant adversity seen in four avian reproduction studies. Two full studies (exposure with bobwhite quail and mallard (exposure period 22 and 19 weeks respectively) over a dose range of 0, 120, 300 and 1000 ppm, and two pilot studies (exposure reduced to 6weeks) with bobwhite quail and mallard over a dose range of 0, 100, 300, 1000 and 3000 ppm.
Fish An ELS with sheepshead minnow tested over a concentration range 0, 0.26, 0.66, 1.6, 4.2 and11 mg/L (mean measured). • No effects on survival, time to hatch and hatching success. • Reduction in growth (length, wet and dry weight) at the highest test concentration,considered onset of systemic toxicity.
Mammals • No T mediated activity was observed in the ToxCast assay using human liver cells (ID: 24). • Thyroid hormones were not measured in any of the tested species. However, a lack of adversity in the thyroid weights or histopathology indicates no significant changes in hormonal balance. This is supported by a lack of effects in the pituitary gland. The available data meet guidance criteria for sufficiency.

From the mammalian dataset, no clear evidence of T-mediated adversity was identified *in vivo*.

Results from the AMA measured T-mediated effects directly *in vivo*. There was a clear treatment-related effect evident at the cellular level. In the 24 mg a.s./L group, there was no follicular cell hypertrophy and hyperplasia seen in the thyroid tissue. All other treatment groups showed thyroid histopathology comparable with the controls, where there was mild follicular cell hyperplasia and hypertrophy. Follicular cell hypertrophy and hyperplasia are stimulated by increased levels of circulating TSH, of which the highest level in *Xenopus laevis* occur between NF Stages 58 to 62. Development of larvae was delayed in the highest test group (NF stage 55 compared to 57 in the other groups), which could explain the differences in histopathology between the test groups. Exposure to T-agonists would reduce follicular cell proliferation as a consequence of inhibited TSH release via a negative feedback loop. However, this would be accompanied by a reduced time to metamorphosis and accelerated somatic growth. Accelerated growth was not seen, conversely, a statistically significant decrease in development stage compared with controls was observed at the highest test concentration (24 mg a.s./L) on day 7 and 21. Further, a statistically significant reduction in hind limb length was evident from 0.25 mg a.s./L compared with controls at day 21 only. The histopathological findings at the higher test concentration were in concordance with tadpoles of NF Stage Score of 55. It is possible that clethodim could interfere directly with THS secretion. However, it is more likely that general toxic effects, such as reduced food consumption would lead to the observed somatic effects combined with the thyroid histopathology observed. Therefore, the decrease in growth seen at the higher test concentration is attributed to non-endocrine effects. This is supported by ToxCast data that confirmed no T-activity, as there cannot be T-mediated adversity in the absence of T-activity.

The AMA is supported by two full avian reproduction studies with bobwhite quail and mallard and a fish ELS with sheepshead minnow. In the study with mallard, there was a slight reduction in egg viability (% of eggs set) at 1000 ppm, the highest test concentration. In the ELS, reduced growth was seen at the highest test concentration (11 mg a.s./L). In both cases, effects were considered to be due to the onset of systemic toxicity; this is in line with the findings from the AMA.

The available dataset for clethodim showed no T-mediated adversity in tadpoles up to the highest concentration tested. The decrease in growth of tadpoles seen is attributed to a non-endocrine mode of action likely due to the onset of systemic toxicity. No T-mediated activity was identified from ToxCast assays. The overall assessment of the integrated lines of evidence indicates that there is no T-mediated adversity in non-target organisms.

2.10.3.1.2 Initial analysis of the evidence and identification of the relevant scenario

Table 2.10.3-4. Selection of relevant scenario

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is **not "T-mediated"** adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because **no T-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate	

			missing “EATS-mediated” parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.3.1.3 MoA analysis for T-modality

Not applicable.

2.10.3.1.4 Conclusion on the ED assessment for T-modality

Overall, there is sufficient weight-of-evidence (WoE) to indicate that clethodim does not affect the T-modality.

2.10.3.2 ED assessment for EAS-modality

Table 2.10.3-5. Data sufficiency for clethodim via the EAS-modality for non-target organisms

	Sufficiently investigated
EAS-mediated parameters	Yes EAS-mediated parameters have been investigated directly with an OECD CF level 3 fish short-term reproduction test (FSTRA, OECD 229) which showed no EAS-mediated adversity or activity. The data package is supported by CF Level 4 data from a fish early life stage test (ELS) and two avian reproduction studies.

2.10.3.2.1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

Table 2.10.3-6. Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose		Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
16	*In vitro* mechanistic	Androgen receptor	Human (Aromatase Assay)	15	Minutes	Uptake from the medium (in vitro)	>1 mM	-	No effect	-	No ER- and AR-mediated agonistic and/or antagonistic activity. No effect on steroidogenesis.	Overall, no evidence of E-, A- S- mediated activity.
16				15	Minutes	Uptake from the medium (in vitro)	>1 mM	-	No effect	-		
18			Human liver cell line	24	Hours	Uptake from the medium (in vitro)	-	-	No effect	-		
18			Human breast cell line	24	Hours	Uptake from the medium (in vitro)	-	-	No effect	-		
17		Estradiol synthesis	Human (H295R steroidogenesis assay)	48	Hours	Uptake from the medium (in vitro)	>31.6	µM	No effect	-		
18		Estrogen receptor	Human liver cell line	24	Hours	Uptake from the medium (in vitro)	-	-	No effect	-		
18			Human liver cell line	24	Hours	Uptake from the medium (in vitro)	-	-	No effect	-		
17		Testosterone synthesis	Human (H295R steroidogenesis assay)	48	Hours	Uptake from the medium (in vitro)	>31.6	µM	No effect	-		
18		Thyroid receptor	Human liver cell line	24	Hours	Uptake from the medium (in vitro)	-	-	No effect	-		
15	*In vivo* mechanistic	Adrenals weight (Hershberger)	Rat	10	Days	Oral	>200	mg/kg bw/day	No effect	-		
15		Cowpers glands weight (Hershberger)		10	Days	Oral	>200	mg/kg bw/day	No effect	-		
15		Glans penis weight (Hershberger)		10	Days	Oral	>200	mg/kg bw/day	No effect	-		
15		LABC weight (Hershberger)		10	Days	Oral	>200	mg/kg bw/day	No effect	-		
15		Liver weight (Hershberger, considered T-mediated only in combination with other thyroid endpoints)		10	Days	Oral	>200	mg/kg bw/day	No effect	-		
15		Prostate weight (Hershberger)		10	Days	Oral	>200	mg/kg bw/day	No effect	-		
15		Seminal vesicles		10	Days	Oral	>200	mg/kg bw/day	No effect	-		

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose		Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
		weight (Hershberger)										
14		Uterus histopathology (UT assay)		3	Days	Oral	>450	mg/kg bw/day	No effect	-		
14		Uterus weight (UT assay)		3	Days	Oral	>450	mg/kg bw/day	No effect	-		
14		Uterus weight (UT assay)		3	Days	Oral	>450	mg/kg bw/day	No effect	-		
24a	*In vivo* mechanistic	Vitellogenin (VTG) in females	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect	No effect	No effect	No effect
24		Vitellogenin (VTG) in males	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect			
24	EATS-mediated	Histopathology (gonad, reproductive ducts)	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	Change	Slight increase in testicular stage scores at 10 mg a.s./L, driven by one individual and not considered to be biologically meaningful.	No effect	No effect
24		Male 2nd sex characteristics in females	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect	No effect		
24		Male 2nd sex characteristics in males	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect			
19	Sensitive to, but not diagnostic of, EATS	Body weight (bird)	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect	No effect	No effect	No effect
19			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000	ppm	Change	Incidental statistically significant variation in body weight during test, no effect on overall body weight change.		
22			Mallard	6	Weeks	Oral	>3000	ppm	Change	Variability on body weight throughout, slight reduction in overall body weight gain at 3000 ppm, not statistically significant		
23		Body weight (fish)	Sheepshead minnow	34	Days	Uptake from water	11	mg/L	Decrease	Significant reduction in wet weight at 11 mg a.s./L (71.8 mg) compared with solvent	No EAS activity identified in mammalian data, therefore effects seen	Onset of systemic toxicity

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose		Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
										control (92.2 mg)	not “EATS”-mediated. Likely onset of systemic toxicity	
23a		Body weight (fish)	Sheepshead minnow	34	Days	Uptake from water	11	mg/L	Decrease	Statistically significant reduction in dry weight at 11 mg a.s./L (16.9 mg) compared with solvent control (21.4 mg a.s./L)		
24		Body weight (fish)	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect	No effect	No effect	No effect
19		Cracked eggs	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
19		Egg production	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19b			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19c			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20b			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20c			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000	ppm	No effect			
22			Mallard	6	Weeks	Oral	>3000	ppm	No effect			
19		Egg viability (% viable embryo of egg set)	Bobwhite quail	22	Weeks	Oral	1000	ppm	Decrease	Slight decrease at 1000 ppm of 72% compared with controls of 91%. Not statistically significant but NOEC lowered to reflect this	No EAS activity identified in mammalian data, therefore effects seen not “EATS”-mediated. Likely onset of systemic toxicity	Onset of systemic toxicity
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect	No effect	No effects	No effects
19		Eggshell thickness	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
19		Gross pathology (bird)	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000	ppm	No effect			
22			Mallard	6	Weeks	Oral	>3000	ppm	No effect			
19		Hatchability	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19b			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19c			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose		Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
23		Hatching success	Sheepshead minnow	34	Days	Uptake from water	>11	mg/L	No effect			
23		Embryo time-to-hatch	Sheepshead minnow	34	Days	Uptake from water	>11	mg/L	No effect			
24		Gonado-somatic index	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect			
24a		Gonado-somatic index	Fathead minnow	22	Days	Uptake from water	> 10.0	mg/L	No effect			
23		Length (fish)	Sheepshead minnow	34	Days	Uptake from water	11	mg/L	Decrease	Significant reduction in length at 11 mg a.s./L (18.3 mm) compared with solvent control (19.8 mm)	No EAS activity identified in mammalian data, therefore effects seen not “EATS”-mediated. Likely onset of systemic toxicity	Onset of systemic toxicity
24		Length (fish)	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect	No effect	No effects	No effects
24		Reproduction (fecundity, fertility)	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	Mean cumulative no. eggs No effect			
24a		Reproduction (fecundity, fertility)	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	Mean no. eggs/female No effect			
24		Reproduction (fecundity, fertility)	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	Mean % fertility (no. fertile eggs) No effect			
19		Viable embryos	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19b			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20b			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
19	Systemic toxicity	Mortality	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
21			Bobwhite quail	6	Weeks	Oral	>3000	ppm	No effect			
22			Mallard	6	Weeks	Oral	>3000	ppm	No effect			
19		Survival (bird)	Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
19a			Bobwhite quail	22	Weeks	Oral	>1000	ppm	No effect			
20			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20a			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20b			Mallard	19	Weeks	Oral	>1000	ppm	No effect			
20c			Mallard	19	Weeks	Oral	>1000	ppm	No effect			

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose		Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence
23		Survival (fish)	Sheepshead minnow	34	Days	Uptake from water	>11	mg/L	No effect			
24		Survival (fish)	Fathead minnow	21	Days	Uptake from water	> 10.0	mg/L	No effect			
19	Systemic toxicity	Feed consumption	Bobwhite quail	22	Weeks	Oral	>1000	ppm	Change	Incidental statistically significant variation in weekly feed consumption throughout trial. Not treatment related	No effects	No effects
20			Mallard	19	Weeks	Oral	>1000	ppm	Change	Incidental statistically significant variation in weekly feed consumption throughout trial. Not treatment related		
21			Bobwhite quail	6	Weeks	Oral	>3000	ppm	No effect	No effect		
22			Mallard	6	Weeks	Oral	>3000	ppm	No effect	No effect		

2.10.3.2.1.1 Assessment of the integrated lines of evidence and weight of evidence

Table 2.10.3-7. WoE for T mediated adversity/activity

Fish (FSTRA) No evidence of EAS- mediated adversity evident in a valid FSTRA (tested over mean measured concentrations of 0.11, 1.1, 10 mg a.s./L clethodim). • No effect on growth (wet weight and length) of fish compared with controls. • No effect on VTG levels in either male or female fish compared with controls. • No effect on secondary sexual characteristics in either male or female fish. • No effect on gonadosomatic index in either male or female fish compared with controls. • No mortalities.
Fish (ELS) An ELS with sheepshead minnow tested over a concentration range 0, 0.26, 0.66, 1.6, 4.2 and 11mg/L (mean measured). • No effects on survival, time to hatch and hatching success. • Reduction in growth (length, wet and dry weight) at the highest test concentration, considered onset of systemic toxicity.
Birds No statistically significant adversity seen in four avian reproduction studies. Two full studies with bobwhite quail and mallard over a dose range of 0, 120, 300 and 1000 ppm, and two pilot studies (exposure reduced to 6 weeks) with bobwhite quail and mallard over a dose range of 0, 100, 300, 1000 and 3000 ppm.
Mammals • Clethodim showed no ER- or AR-mediated agonistic activity in a limited selection of ToxCast assays using human liver and breast cells (ID: 18). There are no ToxCast AUC model data for the estrogen or androgen receptor. However, other *in silico* models (COMPARA; CERAPP potency levels) showed no ER- or AR-mediated (ant)agonistic activity (US EPA, 2020a,b). • Clethodim showed no aromatase inhibitory activity using human recombinant microsomes (ID: 16). • Clethodim did not alter estradiol and testosterone release by H295R cells (ID: 17) • Clethodim showed no ER- or AR-mediated agonistic and/or antagonistic activities in the Hershberger assay and Uterotrophic assay (ID: 14, 15). There were no weight changes in the reproductive organs under the condition of the assays. • Serum hormonal levels were not measured in available *in vivo* studies for clethodim. There is also a lack of information on sexual maturation of offspring and sperm analysis. However, a lack of effects on *in vitro* and *in vivo* mechanistic studies support a lack of ER- or AR-mediated (ant)agonistic activities of clethodim.

There are some endpoints missing from the mammalian data package, namely *in vivo* serum hormonal levels, sperm analyses, anogenital distance in offspring, age at balanopreputial separation, and age at vaginal opening. However, since EAS-related activity was sufficiently investigated, no indications of such activity was found in the data set, and since no EAS-mediated adversity was found at doses not causing overt toxicity, the overall assessment of the integrated lines of evidence indicates that there is no evidence to support an EAS-mediated ED classification. The ED criteria for EAS-modalities are not met for Clethodim (scenario 2a (ii)).

Results from the FSTRA showed that vitellogenin levels in male and female fish were unaffected by exposure to clethodim up to the maximum concentration tested of 10 mg a.s./L clethodim; thus, confirming no *in vivo* activity via the E and S modality. Secondary sexual characteristics in males and females were unaffected, indicating no E and A-mediated effects. No adverse effects were observed in the gonadosomatic index in males and females. One of the males in the 10 mg a.s./L treatment group had a gonadosomatic index significantly higher than all other males in the group. This was identified as an outlier and was excluded from analysis. There was a slight increase in testicular stage scores in males at the highest test concentration, however this increase was heavily influenced by the single male with the abnormal GSI. Since there were no apical or endocrine mediated endpoints and the higher testicular stage scores are not considered to be abnormal, this slight elevation to the testicular stage score was not considered to be biologically meaningful.

The FSTRA is supported by two full avian reproduction studies with bobwhite quail and mallard and a fish ELS with sheepshead minnow. In the study with mallard, there was a slight reduction in egg viability (% of eggs set) at 1000 ppm, the highest test concentration. In the ELS, reduced growth was seen at the highest test concentration (11 mg a.s./L). In both cases, effects were considered to be due to the onset of systemic toxicity, supporting the findings from the FSTRA.

The available dataset for clethodim showed no EAS-mediated activity or adversity up to the highest concentrations tested in non-target organisms *in vivo*.

2.10.3.2.2 Initial analysis of the evidence and identification of the relevant scenario for the ED assessment of EAS-modality

Table 2.10.3-8. Selection of relevant scenario

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is **not "EAS- mediated"** adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because **no EAS-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS- mediated" parameters. Depending on the outcome move to corresponding scenario	

Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.3.2.3 MoA analysis for EAS-modalities

Not applicable.

2.10.3.2.4 Conclusion on the ED assessment for the EAS-modality

Overall, there is sufficient weight-of-evidence (WoE) to indicate that clethodim does not affect the EAS- modality.

2.10.3.3 Overall conclusion on the ED assessment for non-target organisms

The available dataset for clethodim for non-target vertebrates (other than mammals) consists of two CF Level 3 studies (FSTRA and AMA) and five CF level 4 studies, four with birds (avian reproduction studies) and one with fish (ELS study). There were significant effects on fish growth in the ELS study, and only a slight effect on body weight in one of the bird studies. The FSTRA study did not show any significant effects on EAS-mediated parameters, except for an increase in GSI which was driven by one data point. The AMA study showed significant effects on several parameters (developmental stage, wet weight, snout to vent length and normalised hind-limb length), but a potential action via the T-modality is not supported by the histopathological findings. Overall, clethodim meets the data sufficiency requirements for assessing endocrine activity effects via the EATS-modalities for non-target organisms. The available data do not indicate that clethodim meets the criteria for endocrine disruption in non-target organisms.

2.10.4 Overall conclusion on the ED assessment

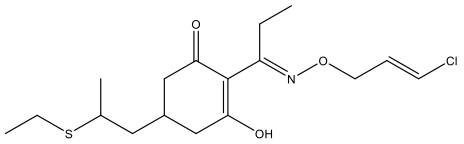
Clethodim does not meet the criteria for endocrine disruption by the EATS-modalities.

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA [SECTIONS 1-6 OF THE CLH REPORT]

2.11.1 Identity of the substance

2.11.1.1 Name and other identifiers of the substance

Table 75. 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)	(5*RS*)-2-{(1*EZ*)-1-[(2*E*)-3-chloroallyloxyimino]propyl}-5-[(2*RS*)-2-(ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one
Other names (usual name, trade name, abbreviation)	CA: 2-[1-[[[(2*E*)-3-chloro-2-propen-1-yl]oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one
ISO common name (if available and appropriate)	Clethodim
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	99129-21-2
Other identity code (if available)	CIPAC No. 508
Molecular formula	$C_{17}H_{26}ClNO_3S$
Structural formula	
SMILES notation (if available)	Canonical: CCC(=NOCC=CCl)C1=C(CC(CC1=O)CC(C)SCC)O Isomeric: CC/C(=N\OC/C=C/Cl)/C1=C(CC(CC1=O)CC(C)SCC)O
Molecular weight or molecular weight range	359.92 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Clethodim contains one chiral centre for which R and S optical forms exist. Where the R and S optical forms have been analysed separately the R:S isomer ratio remained at ca 1:1. Furthermore, there is *E*/Z-isomerism around the C=N bond. It has been demonstrated by the applicant and accepted by the RMS that the ratio of the *E/Z* isomers is an equilibrium that will depend on physical/chemical factors. It is not a fixed value.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not available
Degree of purity (%) (if relevant for the entry in Annex VI)	93%

2.11.1.2 Composition of the substance

Table 76. 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)**
Clethodim (CAS 99129-21-2)	Min. 93 %w/w	Aquatic chronic 3 H412 Acute tox. 4 H302 Skin sens. 1 H317	Aquatic chronic 3 H412 Acute tox. 4 H302 Skin sens. 1 H317
Toluene* (CAS 108-88-3)	Max 0.4 %w/w	Flam. Liq. 2 (H225)	Flam. Liq. 2 (H225)

		Skin Irrit. 2 (H315) Asp. Tox. 1 (H304) STOT SE 3 (H336) STOT RE 2 (H373) Repr. 2 (H361d)	Skin Irrit. 2 (H315) Asp. Tox. 1 (H304) STOT SE 3 (H336) STOT RE 2 (H373) Repr. 2 (H361d)

*Does not contribute to the classification at the specified level (max. 0.4 %w/w).

Table 77. 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
Impurities not considered relevant impurities due to (eco)toxicological or environmental concerns are confidential information and do not contribute to the classification and labelling. Toluene is considered a relevant impurity but does not contribute to the classification at the specified level (max. 0.4 %w/w).				

Table 78. 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
No additives that contribute to the classification are present.					

Table 79. Test substances (non-confidential information).

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Physical hazards				
Technical material, 92.4 % Batch Number: 6F57523000	92.4 %		Slightly below specification (min. 93 %).	Franke, 2005 (20050374.01)
93 % Batch Number: X-29014-170-01	93 %			Franke, 2006 (20050645.01)
98.5 % Lot Number: AS-70373	98.5 %			Butler & O'Connor, 2009 (2699/0001)
Technical material 95.98 % Batch 4478	95.98 %			Winkler, 2020 (PS20190380-1) Arif, 2022 (GLP3016010712R1/2022) Kuchta, 2022b (CSL-21-1644.01) Kuchta, 2022c (CSL-21-1644.02)
Technical material, 95.82 % Batch Number: 4478	95.82 %			Gledhill, 2022 (GLP3016011271R1/2022)

Toxicology and ecotoxicology		
Batch	**Study title**	**Reference**
RE-45601 Technical SX-1688 (83.3 %)	RE-45601 (SX-1688): An acute oral toxicity study with the bobwhite.	CA 8.1.1.1/01 [redacted] 1986a 162-165

	RE-45601 Technical (SX-1688): A dietary LC50 study with the bobwhite	CA 8.1.1.2/01 [redacted] 1986b 162-166
	RE-45601 Technical (SX-1688): A Dietary LC50 Study with the Mallard	CA 8.1.1.2/02 [redacted] 1986 162-167
	RE-45601 Technical: A One-Generation Reproduction Study with the Bobwhite (*Colinus virginianus*) RE-45601 Technical: A Pilot Reproduction Study with the Bobwhite (*Colinus virginianus*)	CA 8.1.1.3/02 (main study) and CA 8.1.1.3/01 (pilot study) [redacted] (main study) [redacted] (pilot study) 1988a (main study); 1987a (pilot study) 162-183 (main study); 162-176 (pilot study)
	RE-45601 Technical: A One-generation Reproduction Study with the Mallard (*Anas platyrhynchos*) RE-45601 Technical: a pilot reproduction study with the mallard (*Anas platyrhynchos*)	CA 8.1.1.3/04 (main study) and CA 8.1.1.3/03 (pilot study) [redacted] (main study) [redacted] (pilot study) 1988b (main study); 1987b (pilot study) 162-177 (pilot study), 162-184 (main study)
	Acute Toxicity of CHEVRON RE-45601 Technical to Rainbow Trout in a Static Test System	CA 8.2.1/01 [redacted] 1986a 34968
	Acute Toxicity of CHEVRON RE-45601 Technical to Bluegill Sunfish in a Static Test System	CA 8.2.1/02 [redacted] 1986b 34967
	Uptake, Depuration and Bioconcentration of [Allyl-2 ^{14}C] and [Cyclohexene-1-one-4, 6 ^{14}C] RE-45601 to Bluegill Sunfish (*Lepomis macrochirus*)	CA 8.2.2.3/01 [redacted] 1987 35636
	Characterization of ^{14}C residues in bluegill sunfish treated with [Allyl-2 ^{14}C]-clethodim or [Cyclohexene-1-one-4, 6 14C]-clethodim	CA 8.2.2.3/02 [redacted] 1988 MEF-0020
	Acute Toxicity of Chevron RE-45601 Technical to *Daphnia magna* in a Static Test System	CA 8.2.4.1/01 Forbis, A.D. 1986 34969
	The Phytotoxicity of RE-45601 Technical with Duckweed (*Lemna gibba* G3) in a Static System	CA 8.2.7/01 Rhodes, J.E. and Hughes, J.S. 1991 65-01-1
	The acute oral toxicity of RE-45601 technical (SX-1688) in adult male and female rats.	CA 5.2.1/01 [redacted] 1986 S 2498
	Acute Oral Toxicity Study in Mice with Cheveron RE-45601 Technical	CA 5.2.1/02 [redacted] 1986 2107-143
	The Acute Dermal Toxicity of RE-45601 Technical (SX-1688) in Adult Male and Female Rabbits	CA 5.2.2/01 [redacted] 1986 CEHB 2510
	The Acute Inhalation Toxicity of RE-45601 Technical (SX-1688) in Rats	CA 5.2.3/01 [redacted] 1986 CEHB 2513

	The Acute Eye Irritation Potential of RE-45601 Technical (SX-1688)	CA 5.2.5/0.1 [redacted] 1986 CEHB 2511
	Four-Week Subchronic Oral Toxicity Study in Mice Chevron RE-45601 Technical Final Report	CA 5.3.1/02 [redacted] 1986 S-2733
	13-Week Oral Toxicity Study in Rats with RE-45601 Technical (SX-1688)	CA 5.3.2/01 [redacted] 1986 S-2765
	A Ninety-Day Subchronic Oral Toxicity Study in Dogs with Chevron RE-45601 Technical	CA 5.3.2/02 [redacted] 1987 S-2759
	One-Year Oral Toxicity Study in Dogs with Chevron RE-45601 Technical (SX-1688)	CA 5.3.2/03 [redacted] 1988 S-2964
	Four-Week Repeated-Dose Dermal Toxicity Study in Rats With RE-45601 Technical (SX-1688)	CA 5.3.3/01 [redacted] 1987 S-2848
	Microbial / Mammalian Microsome Mutagenicity Plate Incorporation Assay with RE-45601 (83% Purity, SX-1688)	CA 5.4.1/02 Machado M.L. 1986a S-2760
	Microbial / Mammalian Microsome Mutagenicity Plate Incorporation Assay with RE-45601 Technical (83.3% Purity, SX-1688)	CA 5.4.1/03 Machado M.L. 1986b S-2859
	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells with Chevron RE-45601 Technical	CA 5.4.1/06 Putman D.L. 1986 S-2761
	Cytogenetic Assay in Bone Marrow Cells of Rats Following Acute Oral Exposure to RE-45601 Technical	CA 5.4.2/01 [redacted] 1987 S-2864
	In Vivo – In Vitro Hepatocyte DNA Repair Assay: In Vitro Evaluation of Unscheduled DNA Synthesis (UDS) Following Oral Administration of Chevron RE-45601 Technical to B6C3F1 Mice	CA 5.4.2/02 [redacted] 1986 S-2762
	Chronic Oral Oncogenicity Study in Mice with Chevron RE-45601 Technical (SX-1688)	CA 5.5/02 [redacted] 1988 S-2867
	Combined Chronic Oral Toxicity/ Oncogenicity Study in Rats with RE-45601 Technical (SX-1688)	CA 5.5/01 [redacted] 1988 S-2766
	Two Generation (One Litter) Reproduction Study in Rats with RE-45601 Technical	CA 5.6.1/02 [redacted] 1987 S-2778
	Pilot Rat Reproduction Study with Cheveron RE-45601 Technical	CA 5.6.1/01 [redacted] (1986) 1986 S-2758
	Pilot Teratology Study in Rats with Chevron RE-45601 Technical	CA 5.6.2/01 [redacted] 1986 S-2807

	Teratology Study in Rats with Chevron RE-45601 Technical	CA 5.6.2/02 [redacted] 1987 S-2808
	Pilot Teratology Study in Rabbits with Chevron RE-45601 Technical	CA 5.6.2/03 [redacted] 1986 S-2734
	Argus Research Laboratories, Inc. Protocol 303-007 Teratology Study in Rabbits with Chevron-45601 (Chevron Protocol No. S-2869)	CA 5.6.2/04 [redacted] 1987 S-2869
	The Comparative Acute Oral Toxicity of RE-51228 (SX-1796) and RE-45601 Technical (SX-1688) in Adults Female Rats	CA 5.8.1.2/01 [redacted] 1988 S-3159
	The Potential of RE-45601 Technical (SX-1688) To Induce Cytochrome P-450 Following 21-Day Oral Administration in Male Rats	CA 5.8.2/02 [redacted] 1989 S-3055
AS 506r (95.4 %)	Clethodim: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*)	CA 8.2.2.1/01 [redacted] 2011 263A-127
	An Oral (Gavage) Acute Neurotoxicity Study of Clethodim in Rats	CA 5.7.1/01 [redacted] 2012 WIL-194041
	A 28-Day Dietary Dose Range-Finding Neurotoxicity Study of Clethodim in Rats	CA 5.7.1/02 [redacted] 2012 WIL-194039
	A 90-Day Oral Dietary Neurotoxicity Study of Clethodim in Rats	CA 5.7.1/03 [redacted] 2012 WIL-194040
	A 28-Day Oral (Dietary) Dose Range-Finding Immunotoxicity Study of Clethodim in Female B6C3F1 Mice	CA 5.8/01 [redacted] 2012 WIL-194037
	A 28-Day Oral (Dietary) Immunotoxicity Study of Clethodim in Female B6C3F1 Mice	CA 5.8/02 [redacted] 2012 WIL-194038
40716 (92.5 %)	Assessment of Toxic Effects of Clethodim Technical on *Daphnia magna* using the 21 Days Reproduction Test	CA 8.2.5.1/01 Knoch, M. 1995 95027/01-ARDm.
SX-1845 (91.1 %)	Clethodim Technical (SX-1845): A 14-day Toxicity Test with Duckweed (*Lemna gibba* G3)	CA 8.2.7/02 Grimstead, S.R., Holmes, C.M. and Peters, G.T. 1991 162A-115A
4478 (95.98 %)	Clethodim: Fish short-term reproduction assay with the fathead minnow (*Pimephales promelas*)	CA 8.2.3/01 [redacted] 2020 443A-166A
	Clethodim: Amphibian metamorphosis assay with the African clawed frog (*Xenopus laevis*)	CA 8.2.3/02 [redacted] 2021 443A-165

	Clethodim Technical: Toxicity to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test	CA 8.2.6.1/01 Siche, O. and Mollandin G. 2020a 140061210
	Clethodim Technical: Toxicity to *Navicula pelliculosa* in an Algal Growth Inhibition Test	CA 8.2.6.2/01 Siche, O. and Mollandin G. 2020b 140061218
	Clethodim Technical: Toxicity to the Aquatic Plant *Glyceria maxima* in a Semi-Static Growth Inhibition Test	CA 8.2.7/03 Armbruster, H. 2020 136151245
	Clethodim technical: Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory	CA 8.3.1.1.1/01 and CA 8.3.1.1.2/01 Berg, C. 2020 140061035
	Clethodim Technical: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil	CA 8.4.1/01 Straube, D. 2020a 140061022
	Clethodim Technical: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil	CA 8.4.2/01 Straube, D. 2020b 140061016
	Clethodim Technical: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil	CA 8.4.2/02 Straube, D. 2020c 140061089
	Clethodim Technical and Clethodim Oxazole Sulfoxide: Effects on Terrestrial Plants: Vegetative Vigour Test	CA 8.6.2/06 Bützler, R. and Kowalczyk, F.2020 53191087
	Interspecies Comparison of In Vitro Metabolism of [^{14}C] Clethodim in Rat, Dog and Human Hepatocytes	CA 5.1.2/01 Krebbers, S. 2020 20182210
	Evaluation of in vitro Phototoxicity of Clethodim Technical in 3T3 Fibroblasts using the Neutral Red Uptake Assay	CA 5.2.7/01 Gijsbrechts J.J.A. 2020 20182211
	Evaluation of the Mutagenic Activity of Clethodim Technical in the Salmonella typhimurium Reverse Mutation Assay and the Escherichia coli Reverse Mutation Assay (Plate Incorporation and Pre-Incubation Methods)	CA 5.4.1/01 Groot A.P. 2020 20182212
	An in vitro Micronucleus Assay with Clethodim Technical in Cultured Peripheral Human Lymphocytes	CA 5.4.1/04 De Jong B.G. 2021 2020-33038
	In Vitro Aromatase Inhibition using Human Recombinant Microsomes	CA 5.8.3/01 Rijk J.C.W. 2020a 20221185
	Screening Clethodim Technical for Modulation of Steroidogenesis using the Human H295R Adreno-carcinoma Cell Line	CA 5.8.3/02 Rijk J.C.W. 2020b 20221184
	A Uterotrophic Assay of Clethodim Technical Administered Orally in Young Adult Ovariectomized Rats	CA 5.8.3/03 [redacted] 2020a 00155006
	A Hershberger Assay of Clethodim Technical Administered Orally in Peripubertal Orchidoepididymectomized Rats	CA 5.8.3/04 [redacted] 2020b 00155007

1209 (96.12 %)	Clethodim: Toxicity Effects to Adult Worker Honey Bees (*Apis mellifera* L.) after Chronic Oral Exposure under Laboratory Conditions	CA 8.3.1.2/01 Kimmel, S. 2016 20160123
	Clethodim: Toxicity to Honey Bee (*Apis mellifera* L.) Larvae after Single Exposure under In Vitro Laboratory Conditions	CA 8.3.1.3/01 Kimmel, S. 2016 2016030
6F50568000 (93.4 %)	Effects of Clethodim Technical on the Activity of the Soil Microflora in the Laboratory	CA 8.5/01 Reis, K-H. 2005 24991080
	Acute Dermal Irritation in Rabbits	CA 5.2.4/01 [redacted] 2005 29389 TAL
6F10972000 (93.7 %)	Clethodim: A Laboratory Study to Evaluate Bioaccumulation in Earthworms	CA 8.1.3/01 Schöbinger, U. 2012 S11-03866
10773 (94.6%)	Acute Toxicity Testing of Clethodim Technical on Activated Sludge with the Respiration Inhibition Test	CA 8.8/01 Dengler, D. 2002 20011424/01-AAHT
6F57523000 (93.5 %)	Clethodim Technical: Contact Hypersensitivity in Albino Guinea Pigs, Maximization-Test	CA 5.2.6/01 [redacted] 2006 A42210
SX-1653 (83.4 %)	Five-Week Pilot Feeding Study in Rats with RE-45601 Technical (SX-1653)	CA 5.3.1/01 [redacted] 1986 S-2720
10195-36 (92.7%)	Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay In Vitro	CA 5.4.1/05 Lehn H. 1990 T6033343
SX-1718 (96.1%)	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells with Purified Chevron RE-45601	CA 5.4.1/07 Putman D.L. 1986b S-2865
M021400MLR	A Toxicity Test to Compare the Effect of Clethodim and its Metabolite Clethodim sulfoxide on Vegetative Vigour of Selected Species (Lolium perenne and Echinochloa crus-galli)	CA 8.6.2/03 Balluff, M. 2003a 20033008/S1-FGVV
	A Toxicity Test to Compare the Effect of Clethodim and its Metabolite Clethodim sulfone on Vegetative Vigour of Selected Species (Lolium perenne and Echinochloa crus-galli)	CA 8.6.2/04 Balluff, M. 2003b 20033009/S1-FGVV
	A Toxicity Test to Compare the Effect of Clethodim and its Metabolite Clethodim Oxazole Sulfone on Vegetative Vigour of Selected Species (Lolium perenne and Echinochloa crus-galli)	CA 8.6.2/05 Balluff, M. 2003c 20033010/S1-FGVV

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 80. Proposed harmonised classification and labelling according to the CLP criteria.

	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	606-150-00-9	Clethodim (ISO); (5*RS*)-2-{(1*EZ*)-1-[(2*E*)-3-chloroallyloxyimino]propyl}-5-[(2*RS*)-2-(ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one	-	99129-21-2	Acute Tox. 4 Skin Sens. 1 Aquatic Chronic 3	H302 H317 H412	GHS07 Wng	H302 H317 H412	EUH066		
Dossier submitters proposal	606-150-00-9	Clethodim (ISO); (5*RS*)-2-{(1*EZ*)-1-[(2*E*)-3-chloroallyloxyimino]propyl}-5-[(2*RS*)-2-(ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one	-	99129-21-2	**Retain** Acute Tox. 4 Skin Sens. 1 **Add** Self-react. G STOT-RE 2 **Modify** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H302 H317 **Add** H373 **Modify** H400 H410	**Retain** GHS07 Wng **Add** GHS08 GHS09	**Retain** H302 H317 **Add** H373 **Modify** H410	**Retain** EUH066	**Add** ATE = 1133 mg/kg bw M = 10 M = 10	
Resulting Annex VI entry if agreed by RAC and COM	606-150-00-9	clethodim (ISO); (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-(ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one	-	99129-21-2	Self-react. G Acute Tox. 4 Skin Sens. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H373 H400 H410	GHS07 GHS08 GHS09 Wng	H302 H317 H373 H410	EUH066	ATE = 1133 mg/kg bw M = 10 M = 10	

2.11.2.2 Additional hazard statements / labelling

Table 81. Reason for not proposing harmonised classification and status under CLH public consultation.

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
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	Data conclusive but not sufficient for classification	Yes
Flammable solids	Hazard class not applicable	Yes
Self-reactive substances	Harmonised classification proposed	Yes
Pyrophoric liquids	Data conclusive but not sufficient for classification	Yes
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Data conclusive but not sufficient for classification	Yes
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data 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	Data conclusive but not sufficient for classification.	Yes

Additional hazard statement:

EUH066 (“Repeated exposure may cause skin dryness or cracking”)

2.11.3 History of the previous classification and labelling

The previous harmonised classification and labelling was discussed and concluded in 2015.

2.11.4 Identified uses

Only used as herbicide.

2.11.5 Data sources

Please see RAR Vol 3, B.2.15, B.6.10, B.8.5 and B.9.11.

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

2.12.1 STEP 1: Exclusion of degradation products of no concern

None of the metabolites are degradation products of no concern and hence all progress to Step 2.

2.12.2 STEP 2: Quantification of potential groundwater contamination

Based on the current PECgw calculations (see 2.8.6.2), predict concentrations of clethodim sulfoxide (max. 0.198 µg/L), clethodim sulfone (max. 1.778 µg/L) and clethodim oxazole sulfone (max. 0.684 µg/L) are expected to exceed the parametric drinking water limit of 0.1 µg/L for at least one scenario considering the worst-case use in sugar beets (300 g a.s./ha). Metabolite clethodim oxazole sulfoxide was calculated to be present in groundwater at a maximum concentration of 0.1 µg/L.

According to Sanco/221/2000 –rev.10- final, based on the current PECgw values three metabolites are identified at step 2 requiring further assessment, as metabolite clethodim oxazole sulfoxide does not exceed the trigger. Clethodim oxazole sulfoxide was still included in the further steps of the relevance assessment.

However, new PECgw calculations have been set as a data gap due to proposed changes to the input parameters and the relevance assessment may need to be updated following the submission of such data.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.12.3.1 STEP 3, Stage 1: screening for biological activity

Clethodim is an acetyl CoA carboxylase (ACCase) inhibitor (HRAC Group A), which acts on the plant meristem, interacting with the enzyme responsible for the biosynthesis of lipids. As the cell membranes are composed of phospholipids, clethodim stops new cell growth leading to the gradual death of the plant.

Four clethodim metabolites were predicted to reach groundwater concentrations of 0.1 µg/L or above (see 2.12.2) for the 300 g a.s./ha application in sugar beet, which triggers the screening for biological activity. Vegetative vigour studies on terrestrial plants are available for each of these metabolites, tested side-by-side with the parent compound clethodim. The studies were performed on two surrogate species for target weeds, i.e., *Lolium perenne* (rye grass) and *Echinochloa crus-galli* (cockspur grass) according to OECD 208 and are summarised in detail in Vol 3CA, B.9.6.

Clethodim sulfoxide had a lower herbicidal activity than clethodim, *i.e.*, by a factor of 3.7 for *L. perenne* and 4.9 for *E. crus-galli*, based on ER_{50} for biomass.

Clethodim sulfone had a lower herbicidal activity than clethodim, *i.e.*, by a factor of 3.5 for *L. perenne* and 3.7 for *E. crus-galli*, based on ER_{50} for biomass.

Clethodim oxazole sulfone had a considerably lower herbicidal activity than clethodim, *i.e.*, by a factor of 49 for *L. perenne* and 95 for *E. crus-galli*, based on ER_{50} for biomass.

Clethodim oxazole sulfoxide had a considerably lower herbicidal activity than clethodim, *i.e.*, by a factor of 15 for *L. perenne* and 33 for *E. crus-galli,* based on ER_{50} for biomass.

The SANCO guidance 221/2000-rev.10-final (25 February 2003) 'Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC' (later Council Regulation EC 1107/2009) describes a metabolite to be comparable to the parent compound (and thus not relevant from a biological activity viewpoint) when "clearly less than 50% of the activity of the parent" is observed. As the biological activity of the four metabolites screened was lower by more than a factor 2 compared to the parent compound, no metabolites as considered relevant at this stage.

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

Based on the available information, the parent compound clethodim is considered non-genotoxic.

RE-45924 (Clethodim sulfoxide)

Clethodim sulfoxide is a major metabolite in urine and faeces, representing 46-61% and 2-5% of the administered dose in urine and faeces, respectively. Therefore, clethodim sulfoxide can be considered to have been assessed by the toxicology studies with the parent, thus it is considered to be non-genotoxic.

Clethodim oxazole sulfoxide (RE-47796)

The genotoxic potential of clethodim oxazole sulfoxide has been investigated in three *in vitro* studies (see below) which are assessed in detail in Volume 3 section B.6.8.1.5 and summarised in Volume 1 section 2.6.8.1.5.

Table 2.12.3.2-1. Studies on the genotoxic potential of clethodim oxazole sulfoxide

Study	Species	Purity (%)	Results	Reference
Ames test	*S. typhimurium, E. coli*	98.5	Negative	Groot, 2020
In vitro mammalian gene mutation	Mouse lymphoma L5178Y cells	98.5	Negative	Groot, 2021
In vitro micronucleus	Peripheral human lymphocytes	98.5	Negative	De Jong, 2021

All submitted genotoxicity studies with clethodim oxazole sulfoxide are considered acceptable and showed negative results. Clethodim oxazole sulfoxide is not considered genotoxic.

RE-47253 (Clethodim sulfone)

The genotoxic potential of clethodim sulfone has been investigated in a number of studies (see below) which are assessed in detail in Volume 3 section B.6.8.1.4 and summarised in Volume 1 section 2.6.8.1.4.

Table 2.12.3.2-2. Studies on the genotoxic potential of clethodim sulfone

Study	Species	Purity (%)	Results	Reference
Ames test	*S. typhimurium, E. coli*	99.2	Positive without activation in TA100 & TA1535 Negative with and without activation in with TA 1537 and TA 98 or with WP2*uvr*A.	Stevenson, 2004a
Ames test	*S. typhimurium* (TA100 & TA 1535)	99.86	Not mutagenic	Williams, 2008

Study	Species	Purity (%)	Results	Reference
In vitro chromosomal aberration	Chinese hamster ovary cells (CHO)	99.2	Negative without activation Positive with activation	Innes, 2005
In vitro chromosomal aberration	Chinese hamster ovary cells (CHO)	99.86	Negative with activation	Lloyd, 2009
In vitro mammalian gene mutation	Mouse lymphoma L5178Y cells	99.2	Equivocal without activation Positive with activation	Riach, 2003a
In vitro mammalian gene mutation	Mouse lymphoma L5178Y cells	99.9	Negative without activation	Stone, 2009
In vivo mouse micronucleus	Mouse, Crl:CD-1 (ICR)	99.3	Equivocal	[redacted]2007a
In vivo mouse micronucleus	Mouse, CRL:NMRI	99.1	Negative	[redacted]2021
In vivo/in vitro unscheduled DNA synthesis	Mouse, Crl:CD-1 (ICR)	99.3	Negative	[redacted]2007b

One of the two Ames test showed that clethodim sulfone was mutagenic to *Salmonella typhimurium* TA 1535 and TA 100 in the absence of S9 when tested in DMSO up to a predetermined maximum concentration of 5000 µg per plate. Because of this a conclusion of non-mutagenicity cannot be reached. A **data gap** is suggested by the RMS.

Clethodim sulfone gave an equivocal/inconclusive response when tested for mutagenic activity in mouse lymphoma L5178Y cells in the absence of S9 mix but was shown to be mutagenic in the presence of S9. A second MLA was performed that showed that the test item was negative without rat liver S9 (no conclusions could be drawn with S9 because of precipitation in the cell medium). **A data gap** is suggested by the RMS.

One of the two *in vitro* chromosomal aberration tests showed that clethodim sulfone was clastogenic in CHO cells in the presence of S9 mix. Two *in vivo* micronucleus studies were performed. The first one gave equivocal results as a small increase in micronuclei in the polychromatic erythrocytes of the bone marrow was observed but the increase (1.5-5 MN PCE/2000 PCE scored) remained within the historical control range (5 MN PCE/2000 PCE scored). A second *in vivo* micronucleus study was performed in which it was concluded that clethodim sulfone was not clastogenic or aneugenic. The study followed OECD TG 474 (2016) without deviations and plasma levels confirmed sufficient systemic exposure. Overall, the RMS considers this information sufficient and concludes that clethodim sulfone is not clastogenic or aneugenic. In addition, clethodim sulfone was negative in the *in vivo/in vitro* unscheduled DNA synthesis in mouse primary hepatocyte cultures.

In summary, genotoxic properties of clethodim sulfone cannot be excluded. Data gaps for gene mutations (follow up data for positive Ames and MLA) were identified.

RE-47797 (Clethodim oxazole sulfone)

The genotoxic potential of clethodim oxazole sulfone has been investigated in three *in vitro* studies and one *in vivo* study (see below) which are assessed in detail in Volume 3 section B.6.8.1.3 and summarised in Volume 1 section 2.6.8.1.3.

Table 2.12.3.2-3. Studies on the genotoxic potential of clethodim oxazole sulfone

Study	Species	Purity (%)	Results	Reference
Ames test	*S. typhimurium, E. coli*	98.9	Negative with and without activation in all strains	Stevenson, 2004b

Study	Species	Purity (%)	Results	Reference
In vitro chromosomal aberration	Chinese hamster ovary cells (CHO)	98.9	Negative without activation Positive with activation	Hart & Stevenson, 2005
In vitro mammalian gene mutation	Mouse lymphoma L5178Y cells	98.9	Negative without activation Equivocal with activation (not biological relevant)	Riach, 2009b
In vivo mouse micronucleus	Mouse, Crl:CD-1 (ICR)	99.5	Inconclusive	[redacted] 2007c

Clethodim oxazole sulfone was not mutagenic an Ames test with *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (WP2*uvr*A).

The clethodim oxazole sulfone (RE-47797) gave statistical significance, when tested for mutagenic activity in mouse lymphoma L5178Y cells, in the presence of S9-mix, at concentrations extending into the toxic range. However, taking the Global Evaluation Factor (GEF) into account for the microwell version of $12x\ 10^{-6}$, shows that the results are not biological relevant since all mutation fraction values were below the GEF. Clethodim oxazole sulfone was not mutagenic in the absence of S9-mix when tested to the predetermined maximum concentration of 5000 µg/mL (4 h exposure) and at concentrations extending into the toxic range (24 h exposure).

The *in vitro* CHO chromosomal aberration test was positive with, but negative without, S9 activation and thus an *in vivo* mouse micronucleus (MN) test was performed. The MN study indicated that clethodim oxazole sulfone was not clastogenic after treatment of mice with 2000 mg/kg bw for two days. However, no evidence that the item induced toxicity to the bone marrow was presented and thus no conclusions can be drawn. This is considered **a data gap.**

In summary, genotoxic properties of clethodim oxazole sulfone cannot be excluded. Data gaps for genotoxicity (follow up data for lack of evidence for bone marrow exposure) was identified.

QSAR

The *in silico* assessment of clethodim groundwater metabolites (Derek Nexus v.6.1.0 and OECD Toolbox v4.4) predicts that clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide or clethodim oxazole sulfone can be considered to be of no greater genotoxicity concern than the parent, although it should be noted that the oxazole metabolites were out of domain for the leadscope genotoxicity predictions (Report No. 1602214.UKO – 7878). However, this does not cover the data gaps indicated in the experimental data package on the metabolites

Conclusion: Step 3, stage 2

Clethodim sulfoxide can be considered to have been assessed by the toxicology studies with the parent, thus it is considered to be non-genotoxic. Clethodim oxazole sulfoxide was assessed in a fully accepted data package that gave negative results and is also considered non-genotoxic. Neither of these two metabolites are therefore considered "relevant" in this regulatory context.

No conclusion on relevance can be drawn for clethodim sulfone and clethodim oxazole sulfone because of data gaps in the genotoxicity data package. Thus, these two metabolites are considered as "potentially relevant" in the absence of further information.

2.12.3.3 STEP 3, Stage 3: screening for toxicity

Clethodim, the parent compound, has the following harmonized classifications: Acute Tox. 4. H302: Harmful if swallowed and Skin Sens. 1. H317: May cause an allergic skin reaction.

RE-45924 (Clethodim sulfoxide)

Clethodim sulfoxide is a major metabolite in urine and faeces, representing 46-61% and 2-5% of the administered dose in urine and faeces, respectively. Its toxicity is considered covered by the toxicity studies with the parent compound. The classification of the parent, clethodim, does not indicate any need for further assessment of clethodim sulfoxide. Thus, it is considered a "non-relevant" metabolite under regulatory aspects.

Clethodim oxazole sulfoxide

The toxicity of clethodim oxazole sulfoxide has been investigated in two studies (see below) which are assessed in detail in Volume 3 section B.6.8.1.5 and summarised in Volume 1 section 2.6.8.1.5.

Table 2.12.3.2-5. Studies on the toxicity of clethodim oxazole sulfoxide

Study	Dose levels	NOAEL/ LOAEL	Effects
14-day dose range-finding study Dietary exposure Rat, Han Wistar Crl:WI (Han), [redacted] (2020c)	0, 50, 500 or 2500 ppm (equal to 0, 5.5, 56.3 and 270.9 mg/kg bw per day for ♂, 0, 5.3, 56.1 and 246.5 mg/kg bw per day for ♀)	NOAEL: 2500 ppm (270.9 mg/kg bw ♂; 246.5 mg/kg bw ♀) LOAEL: -	No test item related effects observed.
28-day toxicity study Dietary exposure Rat, Han Wistar Crl:WI (Han) [redacted] (2021)	0, 50, 500 or 2500 ppm (equal to 0, 4.3, 41.2 and 211.7 mg/kg bw per day for ♂, 0, 4.5, 46.3 and 221.9 mg/kg bw per day for ♀)	NOAEL: 2500 ppm (211.7 mg/kg bw ♂; 221.9 mg/kg bw ♀) LOAEL: -	Mean pituitary gland weight was higher in males at 2500 ppm (18% higher for absolute weight), mean adrenal gland weight was higher in females at 2500 ppm (17% higher for absolute weight) and mean uterus weight was higher in females at 500 and 2500 ppm (50% and 16% higher, respectively, for absolute weight). However, there was no histological correlate to these findings.

The effects observed in the 28-day toxicity study does not indicate that clethodim oxazole sulfoxide falls under the criteria for a STOT-RE classification. NOAEL in the 28-day toxicity study conducted with clethodim oxazole sulfoxide (NOAEL: 211.7 mg/kg bw/day) was higher than the NOAEL obtained in the 28-day toxicity study conducted with the parent compound clethodim (NOAEL: 12.5 mg/kg bw/day). Thus, the metabolite oxazole sulfoxide is considered a "non-relevant" metabolite under regulatory aspects.

RE-47253 (Clethodim sulfone)

No assessment is done for clethodim sulfone since this metabolite did not pass step 3 stage 2 (data gaps for genotoxicity) (see section 2.12.3.2).

RE-47797 (Clethodim oxazole sulfone)

The metabolite clethodim oxazole sulfone did not pass step 3 stage 2 (see section 2.12.3.2).

No studies on the toxicity of clethodim oxazole sulfone has been provided but an *in silico* model predicted that it was of no greater concern than the parent compound.

As a conclusion on general toxicology, clethodim oxazole sulfone (RE-47797) was not likely to be of greater toxicological concern than the parent compound based on the QSAR prediction (no unique alerts identified).

QSAR

Screening for the toxicity of clethodim sulfone, clethodim oxazole sulfoxide and clethodim oxazole sulfone has been carried out using various in silico toxicology models including, Derek Nexus, the OECD QSAR Toolbox and Leadscope. The model outputs for these metabolites were compared to those of the parent, clethodim.

According to both Derek Nexus and the OECD QSAR Toolbox, no unique alerts were identified for the metabolites of unknown toxicity when compared to the parent (clethodim) and major rat metabolite (clethodim sulfoxide) (Report No.: 1602214.UK0 – 4078)

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

The threshold of concern approach uses a toxicological threshold of concern of 0.02 µg/kg bw/day, which when assuming a consumption of 2 litres of water per day, all of which comes from the upper soil layer, relates to an acceptable estimated upper limit for the concentration of a metabolite of 0.75 µg/L. If exposure from other sources can be expected, e.g., crops, these must be taken into account as well.

Clethodim sulfoxide is found in levels below 0.01 mg/kg bw in onions and sugar beet roots in all residue trials. The same is true for garlic, based on extrapolation from trials in onions. Clethodim oxazole sulfoxide has only been found in rotational crops, and in edible parts no residues above 0.01 mg/kg are expected. The exposure to these two metabolites via food is therefore negligible. The PECgw values for clethodim sulfoxide and clethodim oxazole sulfoxide do not exceed the threshold of concern.

No exposure assessment is done for clethodim sulfone and clethodim oxazole sulfone at this step since these metabolites did not pass step 3 stage 2 (see section 2.12.3.2).

2.12.5 STEP 5: Refined risk assessment

No refined risk assessment is needed for clethodim sulfoxide and clethodim oxazole sulfoxide since sufficient information was available for these metabolites to do a satisfactory assessment at step 4. The current PECgw values for clethodim sulfoxide and clethodim oxazole sulfoxide do not exceed the threshold of concern. However, new PECgw calculations have been set as a data gap due to proposed changes to the input parameters and new PECgw may trigger an assessment of these metabolites at step 5.

No assessment was done for the metabolites clethodim sulfone and clethodim oxazole sulfone since these metabolites did not pass step 3 stage 2 (see section 2.12.3.2).

RMS proposal - metabolite clethodim sulfone:

The metabolite clethodim sulfone (RE-47253) did not pass step 3 stage 2 (data gap for genotoxicity) (see section 2.12.3.2). Thus, this metabolite is considered as “potentially relevant” in the absence of further data. With regard to general toxicology, RMS will consider this metabolite as “relevant” since the 28-day oral toxicity study conducted with clethodim sulfone indicated effects on male reproductive organs (germ cell degeneration in the testis and

cellular debris and decreased sperm in the epididymis) (for details on study results see Vol. 3, B.6.8.1.4/02). No reproductive toxicity study is however available. The guidance document states that "if there is reason to expect that a certain degradation product may have toxicological hazards of concern, a targeted testing may be necessary". Another option could be to apply an additional safety factor of 10 in the risk assessment provided that the metabolite clethodim sulfone is not shown to be genotoxic. The NOAEL in the 28-day oral toxicity study conducted with clethodim sulfone was 4.1 mg/kg bw/day and application of a safety factor for inter- and intraspecies differences of 100, and an additional safety factor of 10 would result in an ADI/AOEL of 0.004 mg/kg bw/day. The magnitude of additional safety factor of 10 is considered sufficient for an extrapolation of study duration (subacute to chronic exposure) and the lack of data for reproductive toxicity.

RMS proposal: the concern for genotoxicity and reproductive toxicity and need for an additional safety factor in the risk assessment to be discussed at expert meeting.

2.12.6 Overall conclusion

The metabolites clethodim sulfoxide and clethodim oxazole sulfoxide are not relevant metabolites in groundwater in accordance with SANCO/221/2000 - rev.11, Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances regulated under Regulation (EC) No 1107/2009. At the currently predicted maximal concentrations in groundwater of <0.75 µg/L, these metabolites passed the relevance assessment.

The metabolites clethodim sulfone and clethodim oxazole sulfone are considered "potentially relevant" in the absence of further data (data gaps for genotoxicity). These metabolites did not pass Step 3, stage 2.

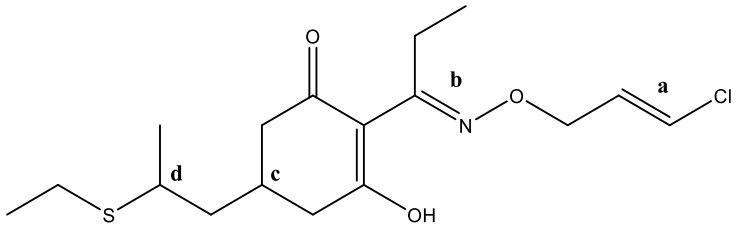
However, new PECgw calculations have been set as a data gap due to proposed changes to the input parameters and the section on the relevance of metabolites in groundwater will need to be updated following the submission of such data.

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

The chemical structure of clethodim (see below) contains two double bonds (a – alkyl group and b – oxime ether), for each of which *E* and *Z* geometric isomers theoretically exist. However, the manufacturing process for technical clethodim gives rise to E geometry with respect to the allyl group and a mixture of E and Z isomers with respect to the oxime ether. Thus, technical clethodim as manufactured is considered to be a mixture of the (*E*, *E*) and (*Z*, *E*) geometric isomers. Generally, these isomers can be separated with conventional HPLC columns. The *E* and *Z* isomers at the oxime ether will exist in equilibrium and thus analytical techniques will show the resolved isomers in varying ratios depending on the environment (polarity, pH, temperature etc.). In general, the *E* (trans) form is the major isomer.

Clethodim also contains one chiral centre (d), for which *R* and *S* optical isomers exist. Conventional chromatographic techniques do not separate optical isomers and their relative proportions have only been examined in one study (Irmer, 2020, Report number S17-08723). The metabolites clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, clethodim imine and clethodim imine sulfoxide will also retain the chiral centre. Although the carbon at position c may appear to exhibit chirality, there is rapid keto-enol

tautomerism present which renders both sides of the cyclohexane ring as equivalent, thus position c is not a chiral centre.



The isomeric composition was also discussed in the EFSA conclusion at the Annex I inclusion of clethodim (EFSA Journal 2011;9(10):2417). Even though the individual properties (toxicity, behaviour in the environment) of the *R* and *S* isomers were not explicitly determined, additional information was not requested. Since then, new extensive guidance has been published on the matter of stereoisomeric active substances (EFSA Journal 2019;17(8):5804). However, this guidance is not applicable for the clethodim renewal, and the RMS' opinion is that further information should not be requested when the data requirements have not changed, and no new applicable guidance is available. More detailed information on how the isomeric composition is addressed in the different sections of the dossier is available under the subheadings below.

2.13.1 Identity and physical chemical properties

The manufacturing process is not considered to favour any of the *R* and *S* isomers, and is considered to produce a racemic mixture with regards to the *R*/*S* isomerism (see Volume 4 for further details). At position b, clethodim is a mixture of *E* and *Z* isomers and only the *E* isomer is present at position a. The exact isomer ratio with regard to the oxime ether (*E*, *E*: *Z*, *E*) of clethodim is dependent on the conditions of the test system. It should be noted that the applicant has not been able to confirm the exact manufacturing process used for production of the batches used in the phys/chem studies but has stated that all of the batches used for testing are considered to be racemic mixtures with regards to the *R*/*S* isomerism. It should however be noted that this statement has not been confirmed by any analytical results or other documentation.

2.13.2 Methods of analysis

Methods of analysis for clethodim, and its structurally similar metabolites, determine the content of *E, E* and *Z, E* isomers. On occasion this results in two peaks in the chromatograms. On these occasions the quantity of the analyte is determined by the sum of the two peaks, or by consistently using only the major peak. The ratio of the size of these two peaks will be affected by the analytical conditions (solvent, temperature etc.), since the Z/E isomerism at the oxime ether is not fixed.

The R and S isomers cannot be separated by conventional chromatography columns, and it is thus generally not known what ratio of these two isomers that is measured and tested throughout the studies in the dossier. One method by Irmer (2020), Report number S17-08723, has been submitted for the environmental fate section, using radiolabelled material, where the relative composition of the *R* and *S* isomers is measured by chiral HPLC coupled

with MS and radiodetection to investigate the degradation in surface water. No other analytical methods capable of separation the *R* and *S* isomers were supplied by the applicant.

2.13.3 Mammalian toxicity

There is currently no information on the difference in toxicity between the *S* and *R* isomers. Similarly, there is no specific information as to whether these isomers are absorbed and metabolised differently. Nonetheless, the testing of the racemic mixture means that the individual enantiomers have been tested and the boundary condition is that each isomer would have to have toxicity/absorption etc. within a factor of 2 of the racemate. Currently, the preferential degradation of the R and S isomers of clethodim has only been investigated in surface water (Irmer 2020). Those results indicate that there is no preferential degradation of the *R* and *S* isomers for the *E, E* isomer. From these results is not possible to conclude on the absence of preferential degradation in other matrices, and it cannot be excluded that further information on the difference in toxicity of the *S* and *R* isomers could be of relevance.

Sine the ratio of the *E* and *Z* isomers at the oxime ether is dependent on the physicochemical parameters, they cannot be isolated and the relevant isomeric composition in this aspect can be considered to have been tested in the toxicological studies.

2.13.4 Operator, Worker, Bystander and Resident exposure

No information available.

2.13.5 Residues and Consumer risk assessment

The following information was supplied by the applicant:

No chiral analysis occurred in any of the metabolism studies or residue studies and hence there is no specific information regarding differential metabolism of the *R* and *S* isomers.

Two separate residue definitions for risk assessment are proposed: the first (RA1) is for clethodim, clethodim sulfoxide, clethodim sulfone, M14R/M15R, M16R/M17R and M18R/M19R and the second (RA2) is for 3-chloroallyl alcohol glucoside (M14A/M15A).

Of the residue definition for risk assessment 1 (RA1), the metabolites clethodim sulfoxide and clethodim sulfone retain the two stereo-isomeric centres, whereas in the pentanedioic acid metabolites M14R/M15R, M16R/M17R and M18R/M19R, both E and Z isomeric regions of the oxime ether and the allyl group were removed. The stereo-isomeric centre (c) is also removed in the pentanedioic acid metabolites M14R/M15R, M16R/M17R and M18R/M19R. Thus, all the components of the residue definition for risk assessment 1 (RA1) contain one or more stereo-isomeric centres. The 3-chloroallyl alcohol glucoside is expected to retain its E form after cleavage from the precursor based on plant metabolism and high-temperature hydrolysis data.

In relation to the representative uses, only sugar beet roots and onion bulbs are commodities used for human consumption. In sugar beet roots, all individual compounds of the residue definitions RA1 and RA2 were found to be below the respective LOQ of the method. Therefore, an investigation of the isomeric composition is neither

required nor possible. In onion bulb, single positive residues were found at or slightly above the LOQ, with levels up to 0.009 mg/kg for clethodim sulfoxide, 0.010 mg/kg for clethodim sulfone and 0.02 mg/kg for M16R/M17R. These residues are too low to allow reliable assessment of their isomeric composition. It is noteworthy that the maximum consumer intake as calculated for RA1 using the EFSA PRIMo is only 0.1% ADI (for the Netherlands child). The maximum consumer intake calculated for RA2 is only 1% ADI (again for the Netherlands child) and is unaffected by isomer considerations since 3-chloroallyl alcohol glucoside contains no stereocentre. Therefore, the isomeric composition of the low residues in sugar beet root and onion bulb are considered to have no impact on the consumer risk assessments.

More generally the consumer risk assessment for clethodim when accounting for all uses, in addition to those that are representative uses in this renewal dossier, also has an adequate margin of safety to account for any potential changes in the *R*:*S* isomer ratio for consumed crops, in combination with any variations in the toxicity of either of the individual stereoisomers.

Further information added by the RMS:

At the annex I inclusion it was determined that no further information concerning the isomer ratio in treated crop residues was required having regard to the very low consumer exposure resulting from the representative use (EFSA Journal 2011;9(10):2417).

2.13.6 Environmental fate

The degradation of clethodim in soil is very rapid, and the main metabolites (clethodim sulfoxide and clethodim sulfone) degrade rather quickly; see section 2.8.1.2. No separate assessment of the proportions of the *R* and *S* isomers was undertaken for clethodim or any of the metabolites. Given the rapid overall degradation, the degradation rate for the racemate is considered to provide a suitable worst case value for risk assessment in soil and groundwater.

In a modern aerobic mineralisation study in surface water (Irmer, 2020; Report number S17-08723; CA 7.2.2.2/01) conducted over 68 days, the clethodim *R*:*S* isomer ratio remained at ca 1:1 during the study for the major isomeric form (E). For the Z-form, the analysis was less conclusive, due to the overall low concentration of the Z-isomer in the samples. Nevertheless, the analysis indicated that there is no preferential degradation of clethodim in aquatic systems.

Since clethodim is a racemic mixture of the *R* and *S* isomers the degradation kinetics account for both isomers. No impact on the risk assessment is expected.

2.13.7 Ecotoxicology

Environmental fate data confirm that clethodim and the major metabolites degrade rapidly in soil, there is however no information on the possible preferential degradation of the *R* and *S* isomers in soil, and no information has been provided on the ecotoxicology of the isolated isomers. The previously mentioned study by Irmer (2020), Report number S17-08723, does however indicate that there is no preferential degradation of the *R* and *S* isomers in aquatic environments, at least for the *E*,*E* isomer.

All ecotoxicology testing occurred with the racemic mixture of *R* and *S* isomers (based on the manufacturing process not favouring any isomer; see Volume 4) and hence there is no specific information as to whether the *R* isomer is more or less toxic than the *S* isomer.

2.14 RESIDUE DEFINITION

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin:

RA1: Sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R, and M18R/M19R, expressed as clethodim (provisional, pending conclusion on toxicological profile of metabolites)

RA2: M14A/M15A

Food of animal origin:

Sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim (provisional, pending conclusion no toxicological profile of clethodim sulfone).

Soil:

Clethodim, clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, CBA and CAA

Groundwater:

Clethodim, clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, CBA and CAA

Surface water:

Clethodim, clethodim sulfoxide, clethodim sulfone, clethodim oxazole sulfoxide, clethodim oxazole sulfone, CBA, CAA, clethodim imine, clethodim imine sulfoxide, DME sulfoxide, clethodim imine ketone, 3-chloro-propenal, unknown metabolite M20

Sediment:

Clethodim, clethodim sulfoxide, clethodim sulfone, clethodim imine, clethodim imine sulfoxide, unknown metabolite M20

Air:

Clethodim

2.14.2 Definition of residues for monitoring

Food of plant origin:

Sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim (provisional, pending conclusion no toxicological profile of clethodim sulfone).

Food of animal origin:

Sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim (provisional, pending conclusion no toxicological profile of clethodim sulfone).

Soil:

Clethodim

Groundwater:

Clethodim, clethodim sulfone, clethodim oxazole sulfone

Surface water:

Clethodim

Sediment:

Clethodim

Air:

Clethodim

2.15 EFFECT OF WATER TREATMENT PROCESSES ON THE NATURE OF RESIDUES PRESENT IN SURFACE WATER

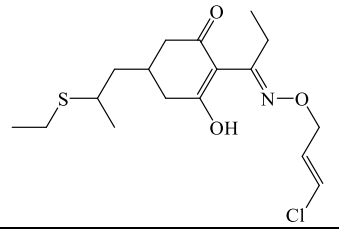
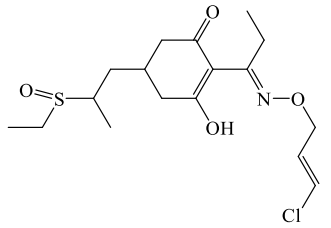
The applicant provided a short position paper (Jarvis & Tallentire, 2020, 1602214.UK0-1829) addressing the potential for the formation of hazardous transformation products from the reaction of clethodim and its metabolites in water treatment processes. It contains a theoretical discussion and a reference to experimental data in a published paper (Sandín-España, Magrans & García-Baudín, 2005) which is evaluated in detail in Volume 3, Annex B.8.2.4 (AS). The theoretical discussion points at the low probability of clethodim and its metabolites to reach drinking water due to the high absorption, rapid biotic and photolytical degradation in soil.

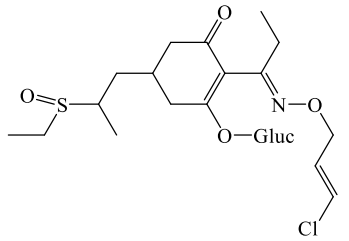
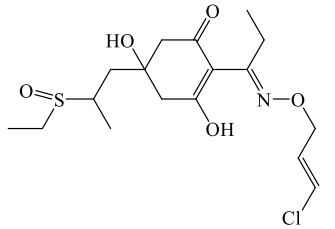
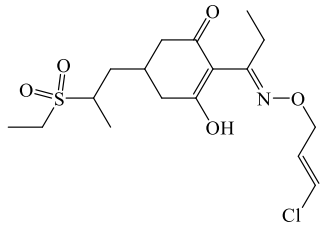
The experimental data indicates that clethodim degrades very rapidly (DT50= < 1 s) and rapidly (DT50=8 min) in the reaction with sodium hypochlorite and monochloramine, respectively. The major metabolite formed was in both cases clethodim sulfoxide with an estimated DT50 (decline from max) of 4.4 s and 9.3 h for the two reactions, respectively. The further degradation products were clethodim sulfone and other minor identified metabolites (hydroxylated in the cyclohexanodione ring) and unidentified metabolites as analysed by HPLC-MS. The fragmentation pattern and isotope abundance indicated that none of the unidentified metabolites contained additional chlorine.

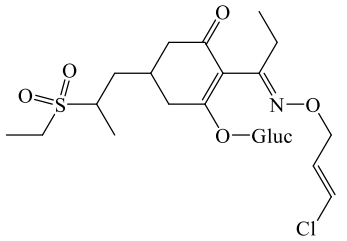
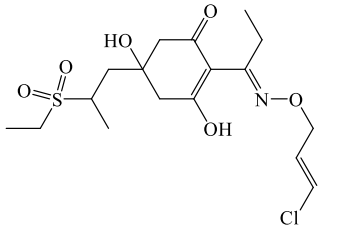
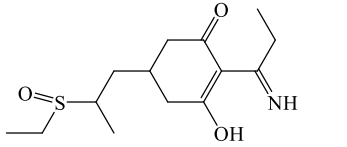
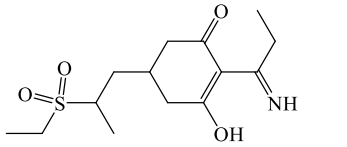
In conclusion, with reference to the preliminary PECsw and PECgw, clethodim and at least some of its metabolites may have a potential to reach levels >0.1 µg/L in drinking water which means that the effect of water treatment processes on the nature of the residues of clethodim needs to be further studied. The RMS considers that the provided experimental data only in part addresses this as LOQ was not reported meaning that the levels of the unidentified

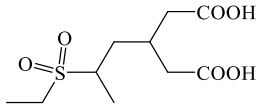
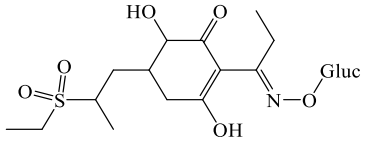
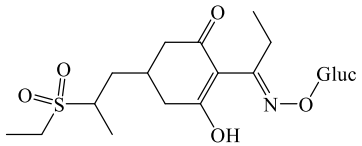
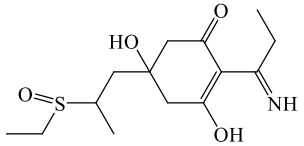
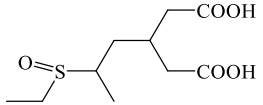
minor metabolites remained unknown. Further testing with other commonly used water treatment agents may also be required. Consequently, a data gap for further data is set.

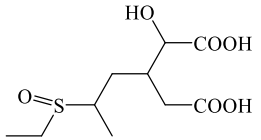
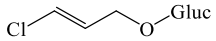
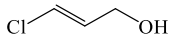
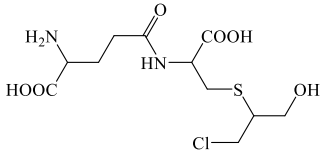
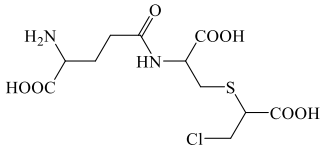
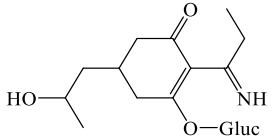
2.16 SUBSTANCES AND METABOLITES; STRUCTURES, CODES, SYNONYMS

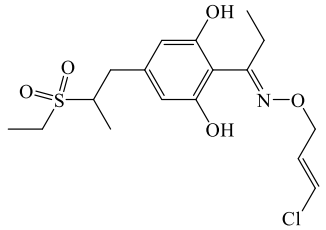
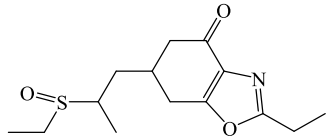
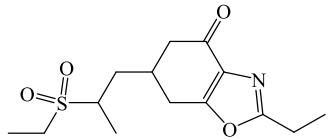
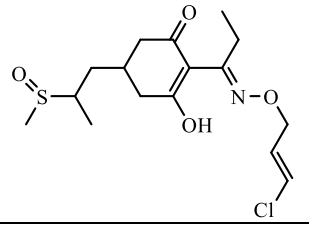
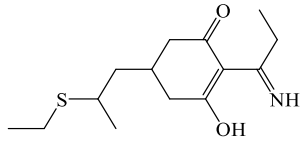
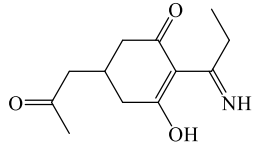
Code Number (Synonyms)	Description	Compound found in	Structure
Name: Clethodim Code: RE-45601	IUPAC: (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-ethylthio)propyl]-3-hydroxycyclohex-2-en-1-one SMILES: CC/C(C1=C(O)CC(CC(C)SCC)CC1=O)=N\OC/C=C/Cl		
Name: Clethodim sulfoxide Code: RE-45924	IUPAC: (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-ethylsulfinyl)propyl]-3-hydroxycyclohex-2-en-1-one SMILES: CC/C(C1=C(O)CC(CC(C)S(CC)=O)CC1=O)=N\OC/C=C/Cl	Spinach (foliage 2.8-6.8% TRR) Carrot (roots 16-34% TRR) Carrot (foliage 11-22% TRR) Cotton (seeds 3.1-4.3% TRR) Cotton (foliage 4.1-5.3% TRR) Soya (seeds 32% TRR) Soya (foliage 4.5-5.9% TRR) Tomato (foliage 25-29% TRR) Hen (kidney 40-43% TRR, liver 31-33% TRR, skin 48-57% TRR, heart 37-48% TRR, fat 15-41% TRR, muscle 37-51% TRR, egg yolk 25-37% TRR, egg white 26-82% TRR) Goat (milk 15-29% TRR, liver 33% TRR, kidney 37% TRR, heart 43% TRR, muscle 41-52% TRR, fat 47% TRR) Soil (73.4%) Water (61.5%) Rat (61% urine) Dog (hepatocytes) Human (hepatocytes)	

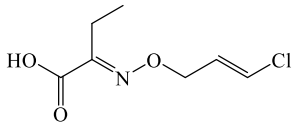
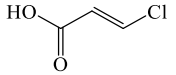
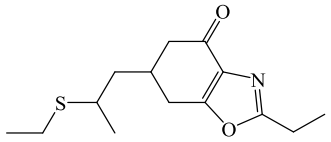
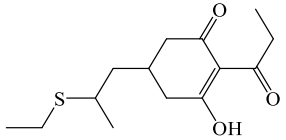
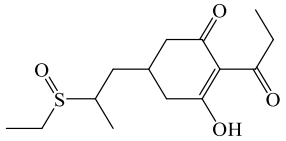
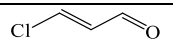
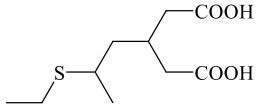
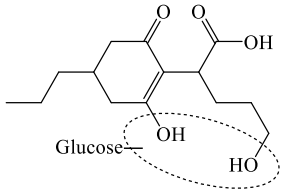
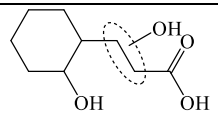
Name: Clethodim sulfoxide glucoside Code: RE-45924 glucoside	IUPAC: (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-ethylsulfinyl)propyl]-3-hydroxycyclohex-2-en-1-one glucoside SMILES: CC/C(C1=C(O[C@H](O[C@@H]2CO)[C@H](O)[C@H]([C@@H]2O)O)CC(CC(C)S(CC)=O)CC1=O)=N\OC/C=C/Cl	Carrot (roots 5.9-9.9% TRR) Carrot (foliage 2.9-15% TRR) Cotton (foliage 2.7-10% TRR) Soya (seeds 8.5-12% TRR) Soya (foliage 25-27% TRR)	
Name: Clethodim 5-hydroxy sulfoxide Code: RE-51229	IUPAC: (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-ethylsulfinyl)propyl]-3,5-dihydroxycyclohex-2-en-1-one SMILES: CC/C(C1=C(O)CC(CC(C)S(CC)=O)(O)CC1=O)=N\OC/C=C/Cl	Carrot (roots 6.4-7.3% TRR) Carrot (foliage 1.0-1.6% TRR) Cotton (seeds 0.4-0.6% TRR) Cotton (foliage 1.1-1.4% TRR) Soya (seeds 4.0-7.1% TRR) Soya (foliage 1.4% TRR) Rat (4% urine)	
Name: Clethodim sulfone Code: RE-47253	IUPAC: (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-ethylsulfonyl)propyl]-3-hydroxycyclohex-2-en-1-one SMILES: CC/C(C1=C(O)CC(CC(C)S(CC)(=O)=O)CC1=O)=N\OC/C=C/Cl	Spinach (foliage 0.3-0.6% TRR) Carrot (roots 3.4-4.6% TRR) Carrot (foliage 0.4-6.1% TRR) Cotton (seeds 0.4-2.8% TRR) Cotton (foliage 0.6-1.8% TRR) Soya (seeds 4.6-5.1% TRR) Soya (foliage 0.9% TRR) Tomato (foliage 7.3-9.7% TRR) Hen (kidney 25-28% TRR, liver 21-27% TRR, skin 17-28% TRR, heart 22-28% TRR, fat 10-16% TRR, muscle 27-34% TRR, egg yolk 11-29% TRR, egg white 9.9-38% TRR) Goat (liver 3.2% TRR) Soil (42.2%)* Water (13.5%) Rat (1% urine)	

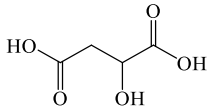
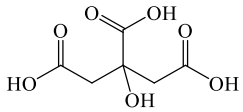
Name: Clethodim sulfone glucoside Code: RE-47253 glucoside	IUPAC: (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-ethylsulfonyl)propyl]-3-hydroxycyclohex-2-en-1-one glucoside SMILES: CC/C(C1=C(O[C@H](O[C@@H]2CO)[C@H](O)[C@H]([C@@H]2O)O)CC(CC(C)S(CC)(=O)=O)CC1=O)=N\OC/C=C/Cl	Carrot (roots 0.5-4.3% TRR) Carrot (foliage 0.5-4.3% TRR) Cotton (foliage 1.3-5.0% TRR) Soya (seeds 1.3-2.5% TRR) Soya (foliage 2.0-12% TRR)	
Name: Clethodim 5-hydroxy sulfone Code: RE-51228	IUPAC: (5RS)-2-{(1EZ)-1-[(2E)-3-chloroallyloxyimino]propyl}-5-[(2RS)-2-ethylsulfonyl)propyl]-3,5-dihydroxycyclohex-2-en-1-one2 SMILES: CC/C(C1=C(O)CC(CC(C)S(CC)(=O)=O)(O)CC1=O)=N\OC/C=C/Cl	Carrot (roots 7.6-10% TRR) Carrot (foliage 1.7-1.9% TRR) Cotton (seeds 0.6-1.6% TRR) Cotton (foliage 0.4-0.6% TRR) Soya (seeds 10-11% TRR) Soya (foliage 2.2-3.1% TRR) Rat (1% urine)	
Name: Clethodim imine sulfoxide Code: RE-47718 M21R	IUPAC: (5RS)-5-[(2RS)-2-ethylsulfinyl)propyl]-3-hydroxy-2-(1-iminopropyl)cyclohex-2-en-1-one SMILES: N=C(CC)C1=C(O)CC(CC(C)S(CC)=O)CC1=O	Spinach (immature 14% TRR) Carrot (roots 9.9% TRR) Carrot (foliage 13-22% TRR) Cotton (foliage 18% TRR) Cotton (seeds 6.0% TRR) Soya (seeds 7.8% TRR) Soya (foliage 14% TRR) Tomato (foliage 3.5% TRR) Tomato (fruit 4.5% TRR) Goat (liver 1.5% TRR, kidney 4.1% TRR, fat 4.7% TRR) Water (21.7%) Rat (7% urine)	
Name: Clethodim imine sulfone Code: RE-47719 M23R, M24R	IUPAC: (5RS)-5-[(2RS)-2-ethylsulfonyl)propyl]-3-hydroxy-2-(1-iminopropyl)cyclohex-2-en-1-one SMILES: N=C(CC)C1=C(O)CC(CC(C)S(CC)(=O)=O)CC1=O	Spinach (foliage 6.3-7.5% TRR) Carrot (root 8.6% TRR) Carrot (foliage 5.9-7.4% TRR) Cotton (seeds 2.3% TRR) Cotton (foliage 4.1% TRR) Soya (seeds 8.1% TRR) Soya (foliage 8.7% TRR) Tomato (foliage 2.9% TRR)	

Name: 3-[(2-Ethylsulfonyl) propyl]-pentanedioic acid Code: M18R, M19R DME sulfone acid	IUPAC: 3-((2RS)-2-(ethylsulfonyl)propyl) pentanedioic acid SMILES: CC(S(CC)(=O)=O)CC(CC(O)=O)CC(O)=O	Spinach (foliage 9.7-13% TRR) Carrot (roots 8.8-13% TRR) Carrot (foliage 0-9.2% TRR) Tomato (foliage 1.8-1.9% TRR) Tomato (fruit 2.1% TRR)	
Name: Hydroxy clethodim imine sulfone glucoside Code: M20R(a)	IUPAC: (5RS)-5-((2RS)-2-(ethylsulfonyl)propyl)-3,6-dihydroxy-2-((EZ)-1-(hydroxyimino)propyl)cyclohex-2-en-1-one glucoside SMILES: CC/C(C1=C(O)CC(CC(C)S(CC)(=O)=O)C(O)C1=O)=N\O[C@H](O[C@@H]2CO)[C@H](O)[C@H]([C@@H]2O)O	Spinach (foliage 9.7-13% TRR as sum of M20R(a+b))	
Name: Clethodim imine sulfone glucoside Code: M20R(b)	IUPAC: (5*RS*)-5-((2RS)2-(ethylsulfonyl)propyl)-3-hydroxy-2-((EZ)-1-(hydroxyimino)propyl)cyclohex-2-en-1-one glucoside SMILES: CC/C(C1=C(O)CC(CC(C)S(CC)(=O)=O)CC1=O)=N\O[C@H](O[C@@H]2CO)[C@H](O)[C@H]([C@@H]2O)O	Spinach (foliage 9.7-13% TRR as sum of M20R(a+b))	
Name: 5-Hydroxy imine sulfoxide Code: M22R	IUPAC: (5RS)-5-((2RS)-2-(ethylsulfinyl)propyl)-3,5-dihydroxy-2-(1-iminopropyl)cyclohex-2-en-1-one SMILES: N=C(CC)C1=C(O)CC(CC(C)S(CC)=O)(O)CC1=O	Carrot (foliage 13% TRR as sum with clethodim imine sulfoxide)	
Name: 3-[(2-Ethylsulfinyl) propyl]-pentanedioic acid Code: M16R, M17R DME sulfoxide acid	IUPAC: 3-[((2RS)-2-ethylsulfinyl)propyl]-pentanedioic acid SMILES: CC(S(CC)=O)CC(CC(O)=O)CC(O)=O	Spinach (foliage 33-35% TRR) Carrot (roots 13-14% TRR) Carrot (foliage 8.9-9.2% TRR) Water (48.9%) Tomato (foliage 2.6% TRR)	

Name: Hydroxy 3-[(2-Ethylsulfinyl) propyl]-pentanedioic acid Code: M14R, M15R	IUPAC: 3-((2RS)-2-(ethylsulfinyl)propyl)-2-hydroxypentanedioic acid SMILES: CC(S(CC)=O)CC(C(O)C(O)=O)CC(O)=O	Spinach (foliage 13-14% TRR) Carrot (roots 7.7-12% TRR) Carrot (foliage 3.6-11% TRR) Tomato (foliage 4.6-19% TRR) Tomato (fruit 18% TRR)	
Name: 3-Chloroallyl alcohol glucoside Code: M14A, M15A	IUPAC: (E)-3-chloroallyl alcohol glucoside SMILES: Cl/C=C/CO[C@H](O[C@@H]1CO)[C@H](O)[C@H]([C@@H]1O)O	Spinach (foliage 21-23% TRR) Carrot (roots 3.1-6.5% TRR) Carrot (foliage 3.6-4.8% TRR)	
Name: 3-Chloroallyl alcohol	IUPAC: (E)-3-chloroallyl alcohol SMILES: Cl/C=C/CO	Residues (high temperature hydrolysis 99-101% of AR)	
Name: 2-(Glutamyl-cysteinyl)-3-chloropropanol Code: M19A	IUPAC: N^5-(1-carboxy-2-((1-chloro-3-hydroxypropan-2-yl)thio)ethyl) glutamine SMILES: NC(C(O)=O)CCC(NC(C(O)=O)CSC(CCl)CO)=O	Spinach (leaves 6.8-9.5% TRR)	
Name: 2-(glutamyl-cysteinyl)-3-chloroacrylic acid Code: M22A	IUPAC: N^5-(1-carboxy-2-((1-carboxy-2-chloroethyl)thio)ethyl)glutamine SMILES: NC(C(O)=O)CCC(NC(C(O)=O)CSC(CCl)C(O)=O)=O	Carrot (foliage 7.3% TRR)	
Name: 3-hydroxy-5-(2-hydroxypropyl)-2-(1-iminopropyl)cyclohex-2-en-1-one glucose conjugate Code: M19R	IUPAC: (5RS)-5-((2RS)-2-hydroxypropyl)-2-(1-iminopropyl)-3-(((3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)cyclohex-2-en-1-one SMILES: N=C(CC)C1=C(O[C@H](O[C@@H]2CO)[C@H](O)[C@H]([C@@H]2O)O)CC(CC(C)O)CC1=O	Carrot (foliage 11-14% TRR)	

Name: Aromatic sulfone Code: RE-50419	IUPAC: (*EZ*)-1-(4-((2RS)-2-(ethylsulfonyl)propyl)-2,6-dihydroxyphenyl)propan-1-one *O*-((*E*)-3-chloroallyl) oxime SMILES: CC/C(C1=C(O)C=C(CC(C)S(CC)(=O)=O)C=C1O)=N\OC/C=C/Cl	Carrot (roots 0.8-1.4% TRR) Carrot (foliage 0.3-0.6% TRR) Cotton (foliage 0.4-0.5% TRR) Soya (seeds 1.5-1.9% TRR) Soya (foliage 0.4-0.5% TRR) Rat (0.5% urine)	
Name: Clethodim oxazole sulfoxide Code: RE-47796	IUPAC: 2-ethyl-(6RS)-6-((2RS)-2-(ethylsulfinyl)propyl)-6,7-dihydrobenzo[*d*]oxazol-4(5*H*)-one SMILES: O=C1C2=C(OC(CC)=N2)CC(CC(C)S(CC)=O)C1	Tomato (foliage 3.7-5.6% TRR) Tomato (fruit 14% TRR) Soil (6.0%) Water (0.8%) rotational carrot (3.2% TRR), lettuce (3.6% TRR), wheat straw (3.6% TRR) Rat (3% urine) (high temperature hydrolysis 89-91% of AR)	
Name: Clethodim oxazole sulfone Code: RE-47797, M4	IUPAC: 2-ethyl-(6RS)-6-((2RS)-2-(ethylsulfonyl)propyl)-6,7-dihydrobenzo[*d*]oxazol-4(5*H*)-one SMILES: O=C1C2=C(OC(CC)=N2)CC(CC(C)S(CC)(=O)=O)C1	Tomato (foliage 7.2% TRR) Soil (10.0%) Water (2.8%) rotational carrot (1.7-1.8% TRR), lettuce (7.1% TRR), wheat straw (3.3-6.7% TRR)	
Name: S-methyl sulfoxide Code: RE-47507	IUPAC: 2-((*E*)-1-((((*E*)-3-chloroallyl)oxy)imino)propyl)-3-hydroxy-5-(2-(methylsulfinyl)propyl)cyclohex-2-en-1-one SMILES: CC/C(C1=C(O)CC(CC(C)S(C)=O)CC1=O)=N\OC/C=C/Cl	Goat (milk 4.3-11% TRR, liver 6.2% TRR, kidney 31% TRR, heart 37% TRR, muscle 29-32% TRR, fat 29% TRR) Rat (8% urine)	
Clethodim imine	IUPAC: 5-(2-(ethylthio)propyl)-3-hydroxy-2-(1-iminopropyl)cyclohex-2-en-1-one SMILES: N=C(CC)C1=C(O)CC(CC(C)SCC)CC1=O	Water (36.4%)*	
Clethodim imine ketone	IUPAC: 3-hydroxy-2-(1-iminopropyl)-5-(2-oxopropyl)cyclohex-2-en-1-one SMILES: N=C(CC)C1=C(O)CC(CC(C)=O)CC1=O	Water (11.8%)*	

2-[3-chloroallyloxyimino]butanoic acid (CBA)	IUPAC: (E)-2-((((E)-3-chloroallyl)oxy)imino)butanoic acid SMILES: OC(/C(CC)=N/OC/C=C/Cl)=O	Soil (18.7%)	
Trans-3-chloroacrylic acid (CAA)	IUPAC: (E)-3-chloroacrylic acid SMILES: OC(/C=C/Cl)=O	Soil (18.1%)	
Clethodim oxazole	IUPAC: 2-ethyl-6-(2-(ethylthio)propyl)-6,7-dihydrobenzo[d]oxazol-4(5H)-one SMILES: O=C1C2=C(OC(CC)=N2)CC(CC(C)SCC)C1	Residues (high temperature hydrolysis 12-97% of AR) water: (7.7%)*	
Clethodim-trione	IUPAC: 5-(2-(ethylthio)propyl)-3-hydroxy-2-propionylcyclohex-2-en-1-one SMILES: O=C(CC)C1=C(O)CC(CC(C)SCC)CC1=O	Residues (high temperature hydrolysis 3.0-5.3% of AR)	
Clethodim-trione sulfoxide Code: RE-47386	IUPAC: 5-(2-(ethylsulfinyl)propyl)-3-hydroxy-2-propionylcyclohex-2-en-1-one SMILES: O=C(CC)C1=C(O)CC(CC(C)S(CC)=O)CC1=O	Rat (1% urine) Residues (high temperature hydrolysis 2.5-7.1% of AR)	
Trans-3-chloropropenal	IUPAC: (*E*)-3-chloroacrylaldehyde SMILES: Cl/C=C/C=O	Water (21.8%)*	
Deoxy-M17R	IUPAC: 3-(2-(ethylthio)propyl)pentanedioic acid SMILES: CC(SCC)CC(CC(O)=O)CC(O)=O	Goat when dosed with M17R (kidney 68% TRR, liver 19% TRR)	
Hydroxy pentanoic acid glucoside (RT26)	IUPAC: 5-hydroxy-2-(2-hydroxy-6-oxo-4-propylcyclohex-1-en-1-yl)pentanoic acid glucoside SMILES: O=C(O)C(CCCO)C1=C(O)CC(CCC)CC1=O	Tomato (foliage 6.1-30% TRR) Tomato (fruit 27% TRR)	
Hydroxy propanoic acid	IUPAC: 2-hydroxy-3-(2-hydroxycyclohexyl)propanoic acid or 3-hydroxy-3-(2-hydroxycyclohexyl)propanoic acid	Tomato (foliage 1.1-6.2% TRR) Tomato (fruit 4.9% TRR)	

	SMILES: OC1CCCCC1CC(O)C(O)=O or OC1CCCCC1C(O)CC(O)=O		
Malic acid	IUPAC: 2-hydroxysuccinic acid SMILES: OC(CC(O)C(O)=O)=O	Tomato (foliage 0.8-17% TRR)	
Citric acid	IUPAC: 2-hydroxypropane-1,2,3-tricarboxylic acid SMILES: OC(CC(C(O)=O)(O)CC(O)=O)=O	Tomato (foliage 3.1% TRR)	
Unknown metabolite M20	unknown	Water/sediment (8.8%)*	unknown

*Value amended to align with the RMS' assessment (e.g., due to rounding differences or including values that were omitted in the applicant's summary table).

LEVEL 3

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the approval criteria – Article 4 and Annex II of Regulation (EC) No 1107/2009

3.1.1.1 Article 4		**Yes**	**No**	
	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically, the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		Pending additional data and further discussion during the peer review of the substance (see below).
3.1.1.2 Submission of further information (Annex II 2.2)		**Yes**	**No**	
i)	It is considered that a complete dossier has been submitted	X		Regarding the data gaps identified during the evaluation, please refer to section 3.1.4.
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			Not relevant.
3.1.1.3 Restrictions on approval (Annex II 2.3)		**Yes**	**No**	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.	X		The following conditions for approval are proposed: a) the minimum degree of purity of the active substance is 930 g/kg i) the need to impose risk mitigation measures for non-target terrestrial plants.
3.1.1.4 Criteria for the approval of an active substance (Annex II 3)				
Dossier (Annex II 3.1)		**Yes**	**No**	

i)	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		See Vol 1, Level 2. sections 2.6.10.1 (ADI), 2.6.10.2 (ARfD). 2.6.10.3 (AOEL) and 2.6.10.4 (AAOEL)
ii)	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		a) The metabolism in plants was investigated in three crop groups (root and tuber vegetables, leafy vegetables and fruit crops) with acceptable studies and in pulses and oilseeds with supportive studies. Clethodim is extensively metabolised and the same metabolic pathways were identified, although the quantity of the metabolites differed between crops. Photolytic degradation occurs, and therefore the major metabolites formed in outdoor conditions (pentanedioic acids) were not identified in studies performed indoors. For enforcement, the residue definition in plants is proposed as: Sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim. For risk assessment, a general residue definition in plant commodities is proposed as sum of clethodim, clethodim sulfoxide, clethodim sulfone and metabolites M14R/M15R, M16R/M17R, M18R/M19R and M14A/M15A, expressed as clethodim. However, the RMS identified a data gap for genotoxicity for M17R, M14R/15R and M18R/M19R. The final residue definition is therefore pending the outcome of this genotoxicity assessment. For processed commodities, it could not be concluded based on available data and the representative uses if a separate residue definition would be necessary. Clethodim, clethodim sulfoxide and clethodim sulfone were extensively degraded to oxazole, and 3-chloroallyl alcohol in high-temperature hydrolysis studies. The metabolism in rotational crops was similar to the one in primary crops, but would need to be investigated in a field trial, simulating more realistic outdoor conditions. It is tentatively concluded that a separate residue definition is not necessary. Livestock metabolism of clethodim was investigated in old studies in poultry (laying hen) and in lactating ruminants (goat). These studies were considered supportive only, and the results are less relevant, since the parent clethodim is not expected in the feed. The metabolism of the pentanedioic acid metabolite M17R was investigated in goat. Major metabolites were parent M17R and deoxy-M17R. Based on the calculated dietary burden for the pentanedioic acid metabolites at the representative uses, the residues of M14R/M15R, M16R/M17R and M18R/M19R and the proposed deoxy-M17R metabolite are predicted to be well below 0.01 mg/kg (i.e. non-detectable or < LOQ) in any livestock commodity. Therefore, these metabolites are considered to be not relevant for inclusion in the residue definitions for monitoring or risk assessment purposes in animal commodities. The residue definition for enforcement and risk assessment of animal commodities is proposed as sum of clethodim, clethodim sulfoxide and clethodim sulfone, expressed as clethodim. b) Sufficient supervised residue trials are available in support of the intended uses in sugar beet, onion and garlic. The calculated dietary burdens of livestock were found to exceed the trigger value of 0.004 mg/kg bw for all groups except swine. Feeding studies in poultry and lactating cows are available, indicating that no residues above the LOQ are expected in any animal commodity at the estimated intake levels.

				Based on metabolism studies in rotational crop, residues of clethodim and all relevant metabolites are expected to be below the LOQ (<0.01 mg/kg) in food items when clethodim is applied according to the intended cGAPs. However, residue levels above the LOQ may be present in cereal feed items. See detailed evaluation in Volume 3, B7. c) Not relevant, residues below 0.1 mg/kg and exposure less than 10% of the ADI. d) Yes, MRLs are proposed for representative crops. No residues above the LOQ are expected in animal commodities and honey. Appropriate methods are available to determine the residues. See detailed evaluation in Volume 3, B5 and B7. e) Not relevant. See data gaps under 3.1.4.7.
iii)	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		Data gaps have been identified regarding the environmental fate and exposure of clethodim and its metabolites (PECsoil, PECgw and PECsw/sed, see 3.1.4).
Efficacy (Annex II 3.2)		**Yes**	**No**	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		No new data available, and not required (renewal).
Relevance of metabolites (Annex II 3.3)		**Yes**	**No**	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.		X	The dossier did not permit establishment of the toxicological relevance of following metabolites: clethodim imine sulfone, clethodim 5-OH sulfone, clethodim oxazole sulfone, clethodim sulfone, DME sulfoxide acid (M17), deoxy-M17R, M18R/M19R, M14R/M15R, DME sulfone acid (M18R). Data gaps have been identified for these metabolites as metabolites in crop/ groundwater (see Level 2 sections 2.6.8 and 2.12)
Composition (Annex II 3.4)		**Yes**	**No**	
i)	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.		X	Pending submission of further data to assess the (eco)toxicological relevance of impurities (see section 2.1 and section 3.1.4 and Volume 4 for further details).
ii)	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	X		Declared minimum clethodim content according to 2017 FAO evaluation report (930 g/kg).
iii)	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted.		X	-
Methods of analysis (Annex II 3.5)		**Yes**	**No**	
i)	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or	X		-

	synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.			
ii)	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	Data gap identified for analytical methods for monitoring of the metabolites clethodim sulfone and clethodim oxazole sulfone in drinking/groundwater.
iii)	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health (Annex II 3.6)				
Impact on human health - ADI, AOEL, ARfD (Annex II 3.6.1)		**Yes**	**No**	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		ADI: 0.16 mg/kg bw/day AOEL: 0.2 mg/kg bw/day ARfD: not necessary AAOEL: not necessary See Level 2, section 2.6.10 for explanation
Impact on human health – proposed genotoxicity classification (Annex II 3.6.2)		**Yes**	**No**	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, the substance SHOULD BE classified or proposed for classification, in accordance with the provisions of Regulation (EC) No 1272/2008, as mutagen category 1A or 1B.		X	See Level 2, section 2.6.4 Based on the available *in vitro* and *in vivo* genotoxicity studies, no classification for germ cell mutagenicity is warranted.
Impact on human health – proposed carcinogenicity classification (Annex II 3.6.3)		**Yes**	**No**	
i)	**It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, the substance SHOULD BE classified or proposed for classification, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogen category 1A or 1B.**		X	See Level 2, section 2.6.5 Based on the available long-term studies, no classification for carcinogenicity is warranted.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable.

Impact on human health – proposed reproductive toxicity classification (Annex II 3.6.4)		**Yes**	**No**	
i)	**It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, the substance SHOULD BE classified or proposed for classification, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 1A or 1B.**		X	See Level 2, section 2.6.6 Based on the available reproductive toxicity studies, no classification for reproductive toxicity is warranted.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable
Impact on human health – proposed endocrine disrupting properties classification (Annex II 3.6.5)		**Yes**	**No**	
i)	It is considered that the substance SHOULD BE identified as having endocrine disrupting properties in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009.		X	See Level 2, section B.2.10. Clethodim does not meet the criteria for endocrine disruption by the EATS-modalities.
ii)	Linked to above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.		X	
Fate and behaviour in the environment				
Persistent organic pollutant (POP) (Annex II 3.7.1)		**Yes**	**No**	
	It is considered that the active substance FULFILS the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	The criteria for bioaccumulation or long-range transport are not fulfilled. The criterion for persistence in soil is not fulfilled. The RMS concludes that clethodim does not fulfil the criteria of a POP. See discussion under separate sub-headings in Vol 1, Level 2, sections 2.8.1.3, 2.8.2.3 and 2.9.2.1
Persistent, bioaccumulative and toxic substance (PBT) (Annex II 3.7.2)		**Yes**	**No**	

	It is considered that the active substance FULFILS the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	The toxicity criterion (T) is fulfilled. (E_rC_{10} <0.01 mg /L for *Glyceria maxima*). The criterion for bioaccumulation is not fulfilled. The criterion for persistence is not fulfilled. The RMS concludes that clethodim does not fulfil the criteria of a PBT. See discussion under separate sub-headings in Vol 1, Level 2, sections 2.6.3-2.6.6, 2.8.1.3, 2.8.2.3 and 2.9.2.1.
Very persistent and very bioaccumulative substance (vPvB) (Annex II 3.7.3)		**Yes**	**No**	
	It is considered that the active substance FULFILS the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	The criterion for bioaccumulation is not fulfilled. The criterion for persistence in soil is not fulfilled. The RMS concludes that clethodim does not fulfil the criteria of a vPvB. See discussion under separate sub-headings in Vol 1, Level 2, 2.8.1.3, 2.8.2.3 and 2.9.2.1
Ecotoxicology (Annex II 3.8)		**Yes**	**No**	
i)	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		See 2.9.9.3 in Vol 1. The acute risk to honeybees as per SANCO 2002 is acceptable. However, in line with EFSA 2013, risk assessment for honeybees requires further refinement, and data are needed for bumble bees and solitary bees.
ii)	**It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance HAS endocrine disrupting properties that may cause adverse effects on non-target organisms.**		X	According to the EFSA/ECHA guidance (2019) for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, no further data are needed for non-target organisms.
iii)	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			Not applicable.
iv)	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		The proposed conditions of use of plant protection products containing Clethodim are expected to have no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour, based on SANCO 2002. Based on EFSA 2013 (a guidance that has not yet been noted by the Standing Committee on Plants, Animals, Food and Feed) further refinement is needed for honeybees. Moreover, no data have been provided for bumble bees and solitary bees.
Residue definition (Annex II 3.9)		**Yes**	**No**	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.		X	The residue definition for enforcement purposes is pending the finalization of the relevance assessment of metabolites in groundwater (see section 2.12).

				For risk assessment of residues in plants and animal commodities: reference is made to 2.14
Fate and behaviour concerning groundwater (Annex II 3.10)		**Yes**	**No**	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		Calculations of PEC in groundwater were available for the parent and all metabolites for which an assessment is necessary. Based on this PECgw, three metabolites were identified that are likely to exceed the parametric drinking water limit for most representative uses. For two of these metabolites the assessment of relevance could not be finalised (see 2.12). For at least one use (i.e. 120 g/ha in onions), current FOCUS PEARL and PELMO PECgw of clethodim and its metabolites did not exceed 0.1 µg/l in two scenarios (Porto and Thiva), indicating a safe use. However, the substance specific input parameters and pathway considered in the modelling are not in line with the RMS proposal. New PECgw calculations are thus required and may change this conclusion.

3.1.2 Proposal - Candidate for substitution

Candidate for substitution		**Yes**	**No**	
	It is considered that the active substance shall be approved as a candidate for substitution		X	The RMS concludes that clethodim does not fulfil any of the criteria for identification as candidate for substitution.

3.1.3 Proposal – Low risk active substance

Low-risk active substances		**Yes**	**No**	
	It is considered that the active substance shall be considered of low risk. **In particular it is considered that the substance should NOT be classified or proposed for classification in accordance with Regulation (EC) No 1272/2008 as at least one of the following:** — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. **In addition it is considered that the substance is NOT:** — persistent (half-life in soil more than 60 days),		X	The active substance clethodim is a highly potent herbicide which cannot be identified as a low risk substance. Clethodim is currently classified for skin sensitisation in Cat 1 (H317). The RMS is of the opinion that classification should be retained, therefore the substance cannot be considered of low risk.

	— has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.			

3.1.4 List of studies to be generated, still ongoing or available but not evaluated

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation(s)				
Information on the toxicological, ecotoxicological and environmental relevance of impurities (please refer to Volume 4 for further details3.1).	All representative uses	X		
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation(s)				
Spectra (UV/VIS, IR, NMR, MS) for the impurity that is proposed to be considered a relevant impurity for the renewal (please refer to Volume 4 for further details).	All representative uses	X		
3.1.4.3 Data on uses and efficacy				
No data gaps identified.				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
No data gaps identified.				
3.1.4.5 Methods of analysis				
Analytical method for quantification of the impurity that is proposed to be considered a relevant impurity for the renewal in the formulation (please refer to Volume 4 for further details).	All representative uses	X		
Analytical method for monitoring of the metabolites clethodim sulfone and clethodim oxazole sulfone in drinking/groundwater.	All representative uses	X		
Analytical validation methods in the 3-CAA genotoxicity study (*i.e.*, reports by Zachary and Andy 2021 and Jutson and Evers 2021) have been submitted by the Applicant, but due to time limitations, they have not been assessed by the RMS, nor included in the current version of the RAR.	All representative uses			X

3.1.4.6 Toxicology and metabolism				
Vol 3, B.6.8.1.3/04 **metabolite clethodim oxazole sulfone (RE-47797)):** data gap for genotoxicity (follow-up data for lack of evidence for bone marrow exposure in the mouse micronucleus test)	All representative uses	X		
Vol 3, B.6.8.1.4/03 and B.6.8.1.4/07 **-metabolite clethodim sulfone (RE-47253):** data gap for gene mutations (follow-up data for positive responses in Ames test and MLA)	All representative uses	X		
Clethodim imine sulfone (RE-47719): data gap (genotox, aneuploidy)	All representative uses	X		
Clethodim 5-OH sulfone (RE-51228): data gap (genotox, aneuploidy)	All representative uses	X		
DME sulfoxide acid (M17R): data gap (genotox, aneuploidy)				
Deoxy-M17R: data gap (genotox)				
3-[(2-ethylsulfonyl)propyl]pentanedioic acid (M18R/M19R): data gap (genotox, aneuploidy)				
Hydroxy-3-[(2-Ethylsulfinyl)]-propyl]-pentanedioic acid (M14R/M15R): data gap (genotox, aneuploidy)				
DME sulfone acid (M18R): data gap (genotox, aneuploidy)				
3.1.4.7 Residue data				
Storage stability study in animal commodities	Sugar beet	X		
Metabolism in rotational crops (study performed outdoors)	All representative uses	X		
Metabolism study in poultry investigating relevant metabolites present in feed	Sugar beet	X		
3.1.4.8 Environmental fate and behaviour				
Field dissipation studies for metabolites clethodim oxazole sulfoxide and clethodim oxazole sulfone.	All representative uses	X		
Further data to address the effect of water treatment processes on the nature of residues present in surface water/ground water.	All representative uses	X		
Further characterisation or identification of the unknown metabolite M20 detected in 2x >5% of applied radioactivity in the pond test system of a water/sediment study.	All representative uses	X		
PECgw for a.s. and all metabolites listed in the definition of residues for risk assessment with input parameters and pathway agreed upon during peer-review.	All representative uses	X		
PECsw/sed for a.s. with input parameters agreed upon during peer-review.	All representative uses	X		
PECsoil for a.s. and all metabolites with input parameters agreed upon during peer-review.	All representative uses	X		
3.1.4.9 Ecotoxicology				

Revised risk assessments in line with the updated PEC values (see 3.1.4.8 above).	All representative uses	X		
Risk assessment for all representative uses besides the worst case, for a.s. metabolites and representative formulated product, for all non-target organisms.	Onions and garlic (all) Sugar beet (120 g a.s./ha)	X		
Discussion on the single exposure study currently included in the RAR for chronic toxicity to honeybee larvae vs repeated exposure studies, which are recommended in EFSA 2013.	All representative uses	X		
Refinement of the risk assessment for honeybees, as per EFSA 2013.	All representative uses	X		
Further consideration on risks to bumble bees and solitary bees.	All representative uses	X		

3.1.5 Issues that could not be finalised

Area of the risk assessment that could not be finalised on the basis of the available data[1)]		Relevance in relation to representative use(s)
1.	Groundwater risk assessment for metabolites	all representative uses
2.	Consumer risk assessment	Onion and garlic
3.	Risk assessment for bumblebees and solitary bees	all representative uses

1) An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

3.1.6 Critical areas of concern

Critical area of concern identified[1)]		Relevance in relation to representative use(s)
1.	-	
2.	-	
3.	-	

1) An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.
An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

3.1.7 Overview table of the concerns identified for each representative use considered

Note: If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.

Representative use:		Sugar beet		Onion/garlic	
		120 g/ha	300 g/ha	120 g/ha	240 g/ha
Operator risk	Risk identified				
	Assessment not finalised				
Worker risk	Risk identified				
	Assessment not finalised				
Bystander risk	Risk identified				
	Assessment not finalised				
Consumer risk	Risk identified				
	Assessment not finalised			X(2)	X(2)
Risk to wild non target terrestrial vertebrates	Risk identified				
	Assessment not finalised				
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified				
	Assessment not finalised		X (3)		
Risk to aquatic organisms	Risk identified				
	Assessment not finalised				
Groundwater exposure active substance	Legal parametric value breached				

Representative use:		Sugar beet		Onion/garlic	
		120 g/ha	300 g/ha	120 g/ha	240 g/ha
	Assessment not finalised				
Groundwater exposure metabolites	Legal parametric value breached				
	Parametric value of 10 µg/L[(a)] breached				
	Assessment not finalised	X [(1)]	X [(1)]	X [(1)]	X [(1)]
Comments/Remarks					

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a) Value for non-relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification
Assessment of metabolite clethodim sulfone. The concerns for genotoxicity and reproductive toxicity, and need for an additional safety factor in the risk assessment need to be discussed at expert meeting.	There are data gaps identified for genotoxicity (follow-up data for positive responses in Ames test and MLA). With regards to general toxicology, the 28-day oral toxicity study conducted with clethodim sulfone indicated effects on male reproductive organs (germ cell degeneration in the testis and cellular debris and decreased sperm in the epididymis) (for details on study results see Vol. 3, B.6.8.1.4/02). No reproductive toxicity study is however available. RMS proposes to apply an additional safety factor of 10 in the risk assessment to fill in the missing data, provided that the metabolite clethodim sulfone is not shown to be genotoxic. The NOAEL in the 28-day oral toxicity study conducted with clethodim sulfone was 4.1 mg/kg bw/day and application of a safety factor for inter- and intraspecies differences of 100, and an additional safety factor of 10 would result in an ADI/AOEL of 0.004 mg/kg bw/day. The magnitude of additional safety factor of 10 is considered sufficient for an extrapolation of study duration (subacute to chronic exposure) and the lack of data for reproductive toxicity.
Assessment of metabolite clethodim oxazole sulfone. The concern for genotoxicity needs to be discussed at expert meeting.	A data gap is identified for genotoxicity. Follow-up data for lack of evidence for bone marrow exposure in the mouse micronucleus test is needed according to RMS

3.1.9 Critical issues on which the Co-RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision-making process are listed.

No disagreements have been noted between the RMS and the Co-RMS (Lithuania).

3.2 PROPOSED DECISION

The RMS cannot conclude at this stage on approval of Clethodim under Regulation (EC) No 1107/2009. The proposal of a decision by the RMS is pending results from additional data addressing i) the toxicological relevance assessment of the metabolites clethodim imine sulfone, clethodim 5-OH sulfone, clethodim oxazole sulfone, clethodim sulfone, DME sulfoxide acid (M17), deoxy-M17R, M18R/M19R, M14R/M15R, DME sulfone acid (M18R) and ii) the risk assessment to groundwater and consumers, for at least one representative use.

It is considered that the following is specified in Part A of the Commission Implementing Regulation for the approval of the active substance:

N/A.

It is considered that the following be specified in Part B of the Commission Implementing Regulation as areas requiring particular attention from Member States when evaluating applications for product authorisation(s):

Risk mitigation for terrestrial non-target higher plants.
Potential contamination of groundwater by metabolites of clethodim, when the active substance is applied in regions with vulnerable soil and/or with vulnerable climatic conditions.

It is proposed that the Member States concerned shall request the submission of confirmatory information:
(a) where new data requirements are established during the evaluation process, or
(b) as a result of new scientific and technical knowledge, or
(c) to increase confidence in the decision.

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH ANY APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
Drift mitigation measures are needed to protect terrestrial plants (see Vol 1, 2.9.9.5)	All representative uses

APPENDICES

APPENDIX 1 GUIDANCE DOCUMENTS USED IN THIS ASSESSMENT

General

SANCO/2012/11251 rev. 5 [Guidance Document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012 (the Renewal Regulation)]

EFSA (European Food Safety Authority), 2019. Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances, EFSA supporting publication 2019:EN-1612. 49 pp. doi:10.2903/sp.efsa.2019.EN-1612

Section Identity, Physical chemical properties and Analytical methods

Section Identity

None.

Section Physical chemical properties

Manual on development and used of FAO and WHO specifications for pesticides, First Edition – third revision-March 2016

Guidance on the application of the CLP Criteria, version 5.0, July 2017

Section Analytical methods

Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013., Guidance document SANCO/3030/99 rev.5

Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods, SANTE 2017/10632 Rev. 3

Guidance document on pesticide residue analytical methods, SANCO/825/00 rev. 8.1

Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4

Section Data on application and efficacy

None.

Section Toxicology

ECHA (European Chemicals Agency), 2017. Guidance on the Application of the CLP Criteria; Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0, July 2017. Reference: ECHA-17-G-21-EN; ISBN: 978-92-9020-050-5

ECHA and EFSA (European Chemicals Agency and European Food Safety Authority), with the technical support of the Joint Research Centre (JRC), 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311, 135 pp.

EFSA (European Food Safety Authority), 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092, 49 pp.

EFSA (European Food Safety Authority), 2012, Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579

EFSA (European Food Safety Authority), 2014c. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55pp.

EFSA (European Food Safety Authority), 2017, Guidance on dermal absorption. EFSA Journal 2017;15(6):4873.

European Commission, 2012. Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003-rev. 10.1, 13 July 2012.

SANCO/221/2000 - rev.11, Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances regulated under Regulation (EC) No 1107/2009.

Section Residues and consumer risk assessment

EFSA (European Food Safety Authority), 2016. Guidance on the establishment of the residue definition for dietary risk assessment. EFSA Journal 2016;14(12):4549

EFSA (European Food Safety Authority), 2017. Pesticides MRL guidelines animal model 2017.

European Commission, 2019. Technical guidelines on data requirements for setting maximum residue levels, comparability of residue trials and extrapolation of residue data on products from plant and animal origin. SANTE/2019/12752.

OECD (Organisation for Economic Co-operation and Development), 2009. Guidance document on overview of residue chemistry studies. ENV/JM/MONO(2009)31, 28 July 2009.

OECD (Organisation for Economic Co-operation and Development), 2020. OECD MRL calculator: spreadsheet for single data set and spreadsheet for multiple data set, 23 April 2020. In: Agricultural pesticides and biocides. Available online: www.oecd.org

JMPR (Joint Meeting on Pesticide Residues), 2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Rome, Italy, 20–29 September 2004, 383 pp.

JMPR (Joint Meeting on Pesticide Residues), 2007. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Geneva, Switzerland, 18–27 September 2007, 164 pp.

Section Fate and behaviour in environment

European Commission (2014) - Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU" Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 3, 613 pp.

EFSA (European Food Safety Authority), 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp., doi:10.2903/j.efsa.2014.3662

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APPENDIX 2 REFERENCE LIST

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None.

Section data on application and efficacy

None.

Section toxicology

None.

Section residue and consumer risk assessment

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None.

ANNEX

Annex 1 **Excel file reporting Livestock Overview Dietary Burden**

Annex 2 **Excel file reporting PRIMo**

Annex 3 **Excel file reporting the available information relevant for ED assessment**

See separate Excel-files.