Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

Dicamba

EC Number: 217-635-6
CAS Number: 1918-00-9

CLH-O-0000007132-84-01/F

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

Adopted

2 June 2022

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

Dicamba

Volume 1

Rapporteur Member State: Denmark
Co-Rapporteur Member State: Romania

Version History

When	What
2007/February	Initial DAR
2018/July	Draft RAR
2019/	Revised vol 1 according to ECHA accordance check
2020/December	Vol. 1 revised to include an ED assessment
2021/February	Revised vol 1 according to ECHA accordance check

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Level 1

Dicamba

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 THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

Two dossiers were submitted for the renewal of approval of the active substance dicamba at EU level in accordance with the requirements of Regulation (EC) No 1107/2009 and Commission Implementing Regulation (EU) 844/2012. The two submitters were Syngenta Crop Protection AG and Rotam Agrochemical Europe Limited.

This RAR reviews new data generated since the first approval of dicamba. In addition, already EU review data are summarised for the sake of completeness.

Proposal for MRL setting was included.

A proposal for Classification and Labelling is included within Vol. 1.

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

Denmark acting as Rapporteur Member State (RMS) has evaluated all sections of the dossier. The draft Renewal Assessment Report (dRAR) was subjected to quality assurance by the Co-RMS Romania.

1.1.3 EU Regulatory history for use in Plant Protection Products

Dicamba is an existing active substance, the renewal of which is part of the AIR III renewal programme.

Dicamba (CAS No 1918-00-9) was first included on Annex I of 91/414/EEC on 01/01/09 under Inclusion Directive 2008/69/CE. Denmark was the Rapporteur Member State (RMS). The date of expiration of approval is 31/12/2018 according to the Commission Implementing Regulation 540/2011/CE. The first notifier was Syngenta Crop Protection AG. Notifiers for the renewal are Syngenta Crop Protection AG and Rotam Agrochemical Europe Limited.

The following documents of the previous evaluation process resulting in the first approval of dicamba are considered to provide relevant review information on already accepted data or a reference to where such information and data can be found:

- Draft Assessment Report on dicamba prepared by Denmark, 2007 (DAR)
- DAR including its addendum (compiled version of November 2010 containing all individually submitted addenda (Denmark, 2010))
- European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance dicamba. EFSA Journal 2011;9(1):1965. [52 pp.] doi:10.2903/j.efsa.2011.1965. Available online: www.efsa.europa.eu/efsajournal.htm (EFSA review)
- SANCO review report on dicamba SANCO/829/08 – rev. 2 of 7th March 2008 (on 27 September 2011 the Standing Committee on Food Chain and Animal Health has taken note of the amendments of chapter 1, 3, 5, 6 and 7 and appendix II based on the EFSA Conclusion on the peer review of the pesticide risk assessment of the active substance dicamba. EFSA Journal 2011; 9(1): 1965.)
- Commission directive (EC) 2008/69/CE and Commission Implementing Regulations 1100/2011 and 540/2011
- On 12 July 2016 the Standing Committee on Plant, Animals, Food and Feed took note of the revision of this review report after the assessment of the confirmatory data. This assessment has been carried out in line with the Guidance document on the procedures for submission and assessment of confirmatory data following inclusion of an active substance in Annex to Regulation (EC) No 541/20117.

MRL

Commission Regulation (EU) No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annex I thereto.

Commission Regulation (EU) No 441/2012 of 24 May 2012 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for bifenazate, bifenthrin, boscalid, cadusafos, chlorantraniliprole, chlorothalonil, clothianidin, cyproconazole, deltamethrin, dicamba, difenoconazole, dinocap, etoxazole, fenpyroximate, flubendiamide, fludioxonil, glyphosate, metalaxyl-M, meptyldinocap, novaluron, thiamethoxam, and triazophos in or on certain products.

Commission Regulation (EU) No 737/2014 of 24 June 2014 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2-phenylphenol, chlormequat, cyflufenamid, cyfluthrin, dicamba, fluopicolide, flutriafol, fosetyl, indoxacarb, isoprothiolane, mandipropamid, metaldehyde, metconazole, phosmet, picloram, propyzamide, pyriproxyfen, saflufenacil, spinosad and trifloxystrobin in or on certain products.

Commission Regulation (EU) 2015/401 of 25 February 2015 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for acetamiprid, chromafenozide, cyazofamid, dicamba, difenoconazole, fenpyrazamine, fluazinam, formetanate, nicotine, penconazole, pymetrozine, pyraclostrobin, tau-fluvalinate and tebuconazole in or on certain products.

Commission Regulation (EU) 2015/845 of 27 May 2015: amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for azoxystrobin, chlorantraniliprole, cyantraniliprole, dicamba, difenoconazole, fenpyroximate, fludioxonil, glufosinate-ammonium, imazapic, imazapyr, indoxacarb, isoxaflutole, mandipropamid, penthiopyrad, propiconazole, pyrimethanil, spirotetramat and trinexapac in or on certain products.

1.1.4 Evaluations carried out under other regulatory contexts

There is a JMPR evaluation published of dicamba from 2010. There is a FAO specification from 2016.

1.2 APPLICANT INFORMATION

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

Syngenta Crop Protection AG
Schwarzwaldallee 215
P.O. Box
CH-4002 Basel
Switzerland

Rotam Agrochemical Europe Limited
Hamilton House
Mabledon Place
London WCIH 9BB
United Kingdom

1.2.2 Producer or producers of the active substance

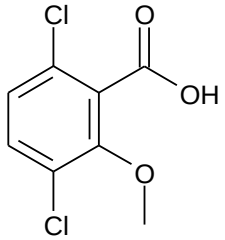
Syngenta Crop Protection AG
Schwarzwaldallee 215
P.O. Box
CH-4002 Basel
Switzerland

Rotam Agrochemical Co. Ltd.
7/F Cheung Tat Centre
No. 18, Cheung Lee Street
ChaiWan, Hong Kong
R.P. China

1.2.3 Information relating to the collective provision of dossiers

No Task Force was formed.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Dicamba
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	3,6-dichloro-2-methoxybenzoic acid
CA	Benzoic acid, 3,6-dichloro-2-methoxy-
1.3.3 Producer's development code number	Syngenta: SAN 837 Rotam: RC1176
1.3.4 CAS, EEC and CIPAC numbers	
CAS	1918-00-9
EC	217-635-6
CIPAC	85
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_8H_6Cl_2O_3$
Structural formula	
Molecular mass	221 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	Confidential. Please refer to Volume 4.
1.3.7 Specification of purity of the active substance in g/kg	Confidential. Please refer to Volume 4.
1.3.8 Identity and content of additives (such as stabilisers) and impurities	

1.3.8.1 Additives	Confidential. Please refer to Volume 4.
1.3.8.2 Significant impurities	Confidential. Please refer to Volume 4.
1.3.8.3 Relevant impurities	Please refer to Volume 4.
1.3.9 Analytical profile of batches	Confidential. Please refer to Volume 4.

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1	**Applicant**	Name: Syngenta Crop Protection AG Adress : Schwarzwaldallee 215 P.O. Box CH-4002 Basel; Switzerland Contact: Ewelina Sobczak Telephone number: +41 (61) 323 30 83 Fax number: +41 (61) 323 61 55 E-mail: ewelina.sobczak@syngenta.com
1.4.2	**Producer of the plant protection product**	Name: Syngenta Crop Protection AG Address: CH 4002 – Basel Switzerland Contact: Simon Baker Syngenta Jealott's Hill, UK Telephone number: +44 (0) 1344 414803 Fax number: +44 (0) 1344 416687 E-mail: simon.baker@syngenta.com
1.4.3	**Trade name or proposed trade name and producer's development code number of the plant protection product**	Trade name: Banvel Code number: A7254B
1.4.4	**Detailed quantitative and qualitative information on the composition of the plant protection product**	

<table>
<tr><td>1.4.4.1 Composition of the plant protection product</td><td>
Pure dicamba in A7254B
<table>
<tr><td>content of pure active substance:</td><td>480 g/L</td><td>41.0 % w/w</td></tr>
<tr><td>limits :</td><td>456 - 504 g/L</td><td>39.0 - 43.1 % w/w</td></tr>
</table>
Technical dicamba in A7254B
<table>
<tr><td colspan="3">at a minimum purity of the technical active substance of 88 % w/w.</td></tr>
<tr><td>content of technical active substance:</td><td>545 g/L</td><td>46.6 % w/w</td></tr>
<tr><td>limits :</td><td>520 - 570 g/L</td><td>44.4 – 48.7 % w/w</td></tr>
</table>
<table>
<tr><td colspan="3">at a typical purity of the technical active substance of 95 % w/w.</td></tr>
<tr><td>content of technical active substance:</td><td>505 g/L</td><td>43.2 % w/w</td></tr>
<tr><td>limits :</td><td>480 - 530 g/L</td><td>41.0 – 45.3 % w/w</td></tr>
</table>
</td></tr>
<tr><td>1.4.4.2 Information on the active substances</td><td>ISO common name: Dicamba
CAS No: 1918-00-9
EC No: 217-635-6
CIPAC No: 85
Salt, ester anion or cation present: None</td></tr>
<tr><td>1.4.4.3 Information on safeners, synergists and co-formulants</td><td>Confidential. Please refer to Volume 4.</td></tr>
<tr><td>1.4.5 Type and code of the plant protection product</td><td>State: Liquid
Type: Soluble concentrate
Code: SL</td></tr>
<tr><td>1.4.6 Function</td><td>Herbicide</td></tr>
<tr><td>1.4.7 Field of use envisaged</td><td>Field crops</td></tr>
<tr><td>1.4.8 Effects on harmful organisms</td><td>Systemic effect on a range of broadleaved weeds.</td></tr>
</table>

1.4.9	**Applicant**	Name: Rotam Agrochemical Europe Limited Address: Hamilton House Mabledon Place London WCIH 9BB United Kingdom Contact: Maria Delgado Cartay EU Regulatory Affairs Project Manager Address: Rotam Agrochemical Europe 75 cours Albert Thomas 6e avenue/Batiment D 69003 Lyon - FRANCE Phone No.: +34 697 845 408 Fax. No.: +33 4 27 02 73 41 E-mail: mariadelgado@rotam.com
1.4.10	**Producer of the plant protection product**	Name: Rotam Agrochemical Co. Ltd. Address: 26/F., E-Trade Plaza, 24 Lee Chung Street Chai Wan, Hong Kong Contact: Prabhakar Kumar Director, R&D, Regulatory & Business Development and Regional Head, South Asia & Taiwan Phone No.: +852 2505 3798 Fax. No.: +86 512 577 11523 E-mail: prabhakark@rotam.com
1.4.11	**Trade name or proposed trade name and producer's development code number of the plant protection product**	Trade names: OCEAL VERMEIL Code number: FH-048
1.4.12	**Detailed quantitative and qualitative information on the composition of the plant protection product**	

<table>
<tr><td>1.4.12.1 Composition of the plant protection product</td><td>Pure active substance

<table><tr><td>content of pure active substance :</td><td>700 g/kg</td><td>70.0 % w/w</td></tr><tr><td>limits :</td><td>675 - 725 g/kg</td><td>67.5 – 72.5 % w/w</td></tr></table>
Technical active substance
The active substance is with a minimum purity of 980.0 g/kg (98.0% w/w) on dry matter.

<table><tr><td>content of technical active substance :</td><td>714 g/kg</td><td>71.4 % w/w</td></tr><tr><td>limits :</td><td>689 - 740 g/kg</td><td>68.9 – 74.0 % w/w</td></tr></table></td></tr>
<tr><td>1.4.12.2 Information on the active substances</td><td>ISO common name: Dicamba
CAS No: 1918-00-9
EC No: 217-635-6
CIPAC No: 85
Salt, ester anion or cation present: Sodium salt</td></tr>
<tr><td>1.4.12.3 Information on safeners, synergists and co-formulants</td><td>Confidential. Please refer to Volume 4 for Rotam.</td></tr>
<tr><td>1.4.13 Type and code of the plant protection product</td><td>Type: Water soluble granules
Code: SG</td></tr>
<tr><td>1.4.14 Function</td><td>Herbicide</td></tr>
<tr><td>1.4.15 Field of use envisaged</td><td>Field crops</td></tr>
<tr><td>1.4.16 Effects on harmful organisms</td><td>Systemic effect on a range of broadleaved weeds.</td></tr>
</table>

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

Summary of representative uses evaluated for Syngenta, for which all risk assessments needed to be completed (*name of active substance or the respective variant*)

(Regulation (EU) N° 284/2013, Annex Part A, points 3, 4)

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. (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)		
Maize	Northern EU Central EU Southern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 12-19	1	-	-	200-500	0.288	N/A	PHI determined by growth stage at application and time to harvestable crop
Sorghum	Central EU Southern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 12-18	1	-	-	20-400	0.210	N/A	PHI determined by growth stage at application and time to harvestable crop
Oat	Northern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 21-29	1	-	-	200-400	0.096	N/A	PHI determined by growth stage at application and time to harvestable crop
Wheat	Northern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 21-29	1	-	-	200-400	0.096	N/A	PHI determined by growth stage at application and time to harvestable crop
Wheat	Southern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 10-32	1	-	-	200-400	0.120	N/A	PHI determined by growth stage at application and time to harvestable crop
Triticale	Northern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 21-29	1	-	-	200-400	0.096	N/A	PHI determined by growth stage at application and time to harvestable crop

Barley	Northern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 21-29	1	-	-	200-400	0.096	N/A	PHI determined by growth stage at application and time to harvestable crop
Rye	Northern EU	A7254B	F	Dicot and monocot weed plants	SC	480 g/L	Foliar spray	BBCH 21-29	1	-	-	200-400	0.096	N/A	PHI determined by growth stage at application and time to harvestable crop

(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

Summary of representative uses evaluated for Rotam, for which all risk assessments needed to be completed (*dicamba*)

(Regulation (EU) N° 284/2013, Annex Part A, points 3, 4)

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. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg, L product / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min-max	kg a.s./ha min-max (l)		
Maize	CZ, HU, PL, RO, SK	OCEAL/ FH-048	F	Dicot and monocot weed plants	SG	700 g/kg	Over-all spray-ing	BBCH 12-18	1	-	a) 0.4 b) 0.4	200-400	a) 280 b) 280	60	
Maize	ES, GR, IT	OCEAL/ FH-048	F	Dicot and monocot weed plants	SG	700 g/kg	Over-all spray-ing	BBCH 12-18	1	-	a) 0.4 b) 0.4	200-400	a) 280 b) 280	60	
Maize	FR	OCEAL/ FH-048	F	Dicot and monocot weed plants	SG	700 g/kg	Over-all spray-ing	BBCH 12-18	1	-	a) 0.4 b) 0.4	200-400	a) 280 b) 280	60	

(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

For the Annex I renewal of dicamba, the representative uses are in maize (FH-048) and maize, sorghum and small grain cereals (A7245B) for the control of annual and perennial broadleaved weeds.

Following normal harvest of an autumn or spring treated crop no restrictions apply. Waiting period for replacement crops in case of failure of a crop treated with dicamba may apply but will depend on dose, timing and succeeding crops. Recommendations for succeeding crops will be available on national labels.

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

Rotam: There are no other uses for dicamba than maize in the registrations of Rotam.

Syngenta: Plese refer to the table under 1.5.4.

1.5.4 Overview on authorisations in EU Member States

Rotam:

COUNTRY	PRODUCT NAME	CROP	TARGET PEST	REGISTRATION NUMBER
Central EU				
Czech Republic	OCEAL, PONANT	Maize	Weeds	5166-0; 5166-1
Germany	OCEAL	Maize	Weeds	007481-00
Hungary	OCEAL, MINERVE, PONANT	Maize	Weeds	04.2/1131-1/2014
Poland	OCEAL 700SG, VERMEIL 700SG	Maize	Weeds	R-44/2014, R-175/2014
Romania	OCEAL	Maize	Weeds	077PC
Slovakia	OCEAL	Maize	Weeds	11-11-1463
UK	OCEAL	Maize	Weeds	15618
North EU				
-				
South EU				
France	OCEAL, MINERVE, VERMEIL	Maize	Weeds	2130066
Greece	OCEAL, MINERVE, PONANT	Maize	Weeds	70099, 70100, 70126
Italy	OCEAL, MINERVE	Maize	Weeds	15288, 16232
Spain	OCEAL	Maize	Weeds	25-813

Since the AIR dossier submission, Rotam got registration in Austria (registration n°3835), Croatia (registration n°UP/I-320-20/16-03/196) and Portugal (registration n° 00848), still on maize at the same application dose rate.

Syngenta:

BANVEL 480 SL (A7254B)

A7254B is an SL formulation containing 480 g/L dicamba

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
1	France	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1/2*	14-30	0.6	0.288	200-500		*FR Split rate
2	France	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1/2*	14-30	0.6	0.288	200-500		*FR Split rate
3	Greece	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
4	Italy	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
5	Portugal	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
6	Spain	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
7	Italy	Sorghum	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-18	1	n/a	0.44	0.21	200-400		PHI determined by growth stage at application and time to harvestable crop

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
8	Spain	Wheat (inc durum wheat)	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 10-32	1	n/a	0.25	0.12	200-400		PHI determined by growth stage at application and time to harvestable crop
9	Italy	Wheat (inc durum wheat)	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 10-32	1	n/a	0.25	0.12	200-400		PHI determined by growth stage at application and time to harvestable crop
10	France	Fallow land (intercrops, Set aside)	F	*Dicot and monocot weed plants*	Foliar spray	Spring /Summer	1	n/a	0.3-0.6	0.144-0.288	100-400		PHI determined by growth stage at application and time to harvestable crop
11	France	Fallow land (intercrops, Set aside)	F	*Dicot and monocot weed plants*	Foliar spray	Spring /Summer	1	n/a	0.3-0.6	0.144-0.288	100-400		PHI determined by growth stage at application and time to harvestable crop
12	France	Stubbles	F	*Dicot and monocot weed plants*	Foliar spray	Post harvest	1	n/a	0.6	0.288	200-400		no restriction on rotation. Possibility to apply every year. Up to end October
13	France	Stubbles	F	*Dicot and monocot weed plants*	Foliar spray	Post harvest	1	n/a	0.6	0.288	200-400		no restriction on rotation. Possibility to apply every year. Up to end October
14	Italy	Stubbles	F	*Dicot and monocot weed plants*	Foliar spray	Post harvest	1	n/a	0.6	0.288	200-400		no restriction on rotation. Possibility to apply every year. Up to end October
15	France	Pasture, Grassland	F	*Rumex sp*	Foliar spray	Spring / Summer	2	42	1	0.48	200-400	14	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
16	France	Pasture, Grassland	F	*Rumex sp*	Foliar spray	Spring / Summer	2	42	1	0.48	200-400	14	
17	France	Rye grass	F	*Dicot and monocot weed plants*	Foliar spray	Spring /Summer	1	n/a	1	0.48	100-400	14	
18	France	Rye grass	F	*Dicot and monocot weed plants*	Foliar spray	Spring /Summer	1	n/a	1	0.48	100-400	14	
19	Belgium	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1		0.6	0.288	200-500		
20	Czech Republic	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
21	Slovakia	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
22	Hungary	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
23	Netherlands	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
24	Romania	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.4	0.192	200-500		To be used with a graminicide. PHI determined by growth stage at application and time to harvestable crop
25	Slovenia	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				Application rate				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
26	Romania	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
27	Hungary	Sorghum	F	Dicot and monocot weed plants	Foliar spray	BBCH 12-18	1	n/a	0.44	0.21	200-400		PHI determined by growth stage at application and time to harvestable crop
28	Slovenia	Sorghum	F	Dicot and monocot weed plants	Foliar spray	BBCH 12-18	1	n/a	0.44	0.21	200-400		PHI determined by growth stage at application and time to harvestable crop
29	Belgium	Fallow land (intercrops, Set aside)	F	Dicot and monocot weed plants	Foliar spray	Spring / Summer	1	n/a	1	0.48	100-400		PHI determined by growth stage at application and time to harvestable crop
30	Czech Republic	Fallow land (intercrops, Set aside)	F	Dicot and monocot weed plants	Foliar spray	Spring / Summer	1	n/a	1	0.48	100-400		PHI determined by growth stage at application and time to harvestable crop
31	Hungary	Total Weed control (non crop land)	F	Dicot and monocot weed plants	Foliar spray	Spring / Summer	1	n/a	0.75	0.36	200-400	n/a	15-25 cm growth stage of the weeds. It means that 50-60 % of the soil is covered.
32	Slovenia	Total Weed control (non crop land)	F	Dicot and monocot weed plants	Foliar spray	Spring / Summer	1	n/a	0.75	0.36	200-400	n/a	15-25 cm growth stage of the weeds. It means that 50-60 % of the soil is covered.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
33	Hungary	Stubbles	F	Dicot and monocot weed plants	Foliar spray	Post harvest	1	n/a	0.75	0.36	200-400	n/a	It means that once in every 3 years the stubble use is possible, only.
34	Slovenia	Stubbles	F	Dicot and monocot weed plants	Foliar spray	Post harvest	1	n/a	0.75	0.36	200-400	n/a	no restriction on rotation. Possibility to apply every year.
35	Belgium	Pasture, Grassland	F	Dicot and monocot weed plants	Foliar spray	Spring / Summer	2	n/a	1	0.48	200-400	14	
36	UK	Pasture, Grassland	F	Dicot and monocot weed plants	Foliar spray	Spring / Summer	2	n/a	1	0.48	200-400	14	
37	Estonia	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
38	Latvia	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
37	Lithuania	Maize	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.6	0.288	200-500		PHI determined by growth stage at application and time to harvestable crop
38	Estonia	Oat	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
39	Latvia	Oat	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
40	Lithuania	Oat	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
41	Estonia	Barley	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
42	Latvia	Barley	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
43	Lithuania	Barley	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
44	Estonia	Wheat (inc durum wheat)	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
45	Latvia	Wheat (inc durum wheat)	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
46	Lithuania	Wheat (inc durum wheat)	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
47	Estonia	Rye	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
48	Latvia	Rye	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
49	Lithuania	Rye	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
50	Estonia	Triticale	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
51	Latvia	Triticale	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop
52	Lithuania	Triticale	F	*Dicot and monocot weed plants*	Foliar spray	BBCH 21-29	1	n/a	0.2	0.096	200-400		PHI determined by growth stage at application and time to harvestable crop

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L **A7254B** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
53	Latvia	Fallow land (intercrops, Set aside)	F	*Dicot and monocot weed plants*	Foliar spray	Spring / Summer	1	n/a	1	0.48	100-400		PHI determined by growth stage at application and time to harvestable crop
54	Estonia	Total Weed control (non crop land)	F	*Dicot and monocot weed plants*	Foliar spray	Spring / Summer	1	n/a	0.75	0.36	200-400	n/a	15-25 cm growth stage of the weeds. It means that 50-60 % of the soil is covered.
55	Estonia	Stubbles	F	*Dicot and monocot weed plants*	Foliar spray	Post harvest	1	n/a	0.75	36	200-400	n/a	no restriction on rotation. Possibility to apply every year.

MONDAK 240 SL (A10037A)

A10037A is an SL formulation containing 240 g/L dicamba

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L A10037A / ha a) max. rate per appl. b) max. total rate per crop/season	kg dicamba / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
1	Italy	Maize	F	Dicot and monocot weed plants	Foliar spray	BBCH 12 –18	1/2*	N/A	1.2	0.288	200-500	N/A	* FR split dose option (192+96) PHI determined by growth stage at application and time to harvestable crop
2	Italy	Sorghum	F	Dicot and monocot weed plants	Foliar spray	BBCH 12-18	1	N/A	0.75	0.18	200-400	N/A	PHI determined by growth stage at application and time to harvestable crop
3	Italy	Stubble	F	Dicot and monocot weed plants	Foliar spray	Spring/summer Post harvest	1	N/A	1.2	0.288	200-400	N/A	No restriction on rotation. Possibility to apply every year
4	Italy	Total weeds control	F	Dicot and monocot weed plants	Foliar spray	Spring/summer	1	N/A	0.6	0.144	200-400	N/A	Intercrop No restriction on rotation. Possibility to apply every year

CADENCE 70 WG (A9781A)

A9781A is a WG formulation containing 700 g/kg dicamba

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	kg **A9781A** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
1	France	Maize	F	*Dicot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.41	0.288	200-400		PHI determined by growth stage at application and time to harvestable crop
2	France	Maize	F	*Dicot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.41	0.288	200-400		PHI determined by growth stage at application and time to harvestable crop
3	Austria	Maize (inc sweetcorn)	F	*Dicot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.41	0.288	200-400		PHI determined by growth stage at application and time to harvestable crop
4	Czech Republic	Maize	F	*Dicot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.41	0.288	200-400		PHI determined by growth stage at application and time to harvestable crop
5	Hungary	Maize	F	*Dicot weed plants*	Foliar spray	BBCH 12-19	1	n/a	0.41	0.288	200-400		PHI determined by growth stage at application and time to harvestable crop
6	France	Fallow land (intercrops, Set aside)	F	*Dicot weed plants*	Foliar spray	Spring /Summer	1	n/a	0.2-0.4	0.140-0.280	100-400		PHI determined by growth stage at application and time to harvestable crop

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
					Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	kg **A9781A** / ha a) max. rate per appl. b) max. total rate per crop/season	kg **Dicamba** / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max		
7	France	Fallow land (intercrops, Set aside)	F	*Dicot weed plants*	Foliar spray	Spring /Summer	1	n/a	0.2-0.4	0.140-0.280	100-400		PHI determined by growth stage at application and time to harvestable crop
8	Austria	Sorghum	F	*Dicot weed plants*	Foliar spray	BBCH 12-18	1	n/a	0.3	0.21	200-400		PHI determined by growth stage at application and time to harvestable crop
9	Hungary	Sorghum	F	*Dicot weed plants*	Foliar spray	BBCH 12-18	1	n/a	0.3	0.21	200-400		PHI determined by growth stage at application and time to harvestable crop
10	France	Stubbles	F	*Dicot weed plants*	Foliar spray	Post harvest	1	n/a	0.4	0.28	200-400	n/a	no restriction on rotation. Possibility to apply every year..
11	France	Stubbles	F	*Dicot weed plants*	Foliar spray	Post harvest	1	n/a	0.4	0.28	200-400	n/a	no restriction on rotation. Possibility to apply every year.

SPANDIS/DINIRO (A18385B)

A18385 is a WG formulation containing 400 g/kg dicamba + 40 g/kg prosulfuron + 100 g/kg nicosulfuron

1	2	3	4	5	6	7	8	9	10	11	11	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**					**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha [f]
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg A18385B/ ha a) max. rate per appl. b) max. total rate per crop/season	g prosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g dicamba/ ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Zonal uses (field or outdoor uses, certain types of protected crops)															
1	AT	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	BE	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	CZ	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	HU	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	HU	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed

1	2	3	4	5	6	7	8	9	10	11	11	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**					**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha [f]
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg A18385B/ ha a) max. rate per appl. b) max. total rate per crop/season	g prosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g dicamba/ ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
															(e.g Adigor@ 1.0-1.5L/ha)
1	NL	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	RO	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	RO	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	SI	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	SK	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8	9	10	11	11	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**					**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha [f]
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg A18385B/ ha a) max. rate per appl. b) max. total rate per crop/season	g prosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g dicamba/ ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	SK	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	UK	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	BG	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	BG	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	FR	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	FR	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based

1	2	3	4	5	6	7	8	9	10	11	11	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**					**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha [f]
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg A18385B/ ha a) max. rate per appl. b) max. total rate per crop/season	g prosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g dicamba/ ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
							3rd year)								adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	GR	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	GR	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	IT	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	IT	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	HR	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8	9	10	11	11	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**					**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg A18385B/ ha a) max. rate per appl. b) max. total rate per crop/season	g prosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g dicamba/ ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. g safener/synergist per ha [f]
1	HR	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	MT	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	MT	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	PT	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	PT	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)
1	ES	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every	N/A	a) 0.5 b) 0.5	a) 20 b) 20	a) 50 b) 50	a) 200 b) 200	200-400	n.s.	tank-mixed oil-based adjuvant needed (e.g

1	2	3	4	5	6	7	8	9	10	11	11	11	12	13	14
Use-No. [e]	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**					**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg A18385B/ ha a) max. rate per appl. b) max. total rate per crop/season	g prosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g dicamba/ ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. g safener/synergist per ha [f]
							3rd year)								Adigor@ 1.0-1.5L/ha)
1	ES	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	a) 16 b) 16	a) 40 b) 40	a) 160 b) 160	200-400	n.s.	proportional mitigation; tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)

CALLISTO TURBO (A18032E)

A18032E is a WG formulation containing 312.5 g/kg dicamba + 150 g/kg mesotrione + 100 g/kg nicosulfuron

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	C-EU CZ, SK, SL, HU, RO SEU – FR, PT, ES, BG, HR	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
2	CZ	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
3	CZ	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
4	SK	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
5	SK	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
6	SK	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
7	SL	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
8	SL	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
9	SL	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
10	HU	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
11	HU	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
12	HU	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
13	RO	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
14	RO	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
15	RO	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
16	FR	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
17	FR	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
18	FR	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
19	PT	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
20	PT	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
21	PT	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
22	ES	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
23	ES	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
24	ES	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
25	BG	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
26	BG	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
27	BG	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	Kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
28	HR	Maize	F	*Annual/perennial BLW & grasses*	Foliar Spray	BBCH 12-19	1	n/a	0.6	187.5 g dicamba 90 g mesotrione 60 g nicosulfuron	80-400	nr	annually where soil clay content >10 %; split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
29	HR	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	annually where soil clay content <10 %; proportional mitigation measures split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)
30	HR	Maize	F	*Annual/perennial BLW & grasses narrower spectrum*	Foliar Spray	BBCH 12-19	1	n/a	0.4	125 g dicamba 60 g mesotrione 40 g nicosulfuron	80-400	nr	Annually regardless of soil clay content; proportional mitigation measures (less than those for 0.6 kg/ha); split rate application possible tank-mixed oil-based adjuvant needed (e.g Adigor @ 1.0-1.5L/ha)

MILAGRO PLUS (A19658H)

A19658H is an OD formulation containing 220 g/kg dicamba + 50 g/kg nicosulfuron

1	2	3	4	5	6	7	8	9	10	11		12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**				**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg dicamba/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Zonal uses (field or outdoor uses, certain types of protected crops)														
1	Hungary	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	
2	Hungary	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	0.8	40	176	100-400	soil clay content <10 %	
3	Hungary	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	
4	Romania	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	
5	Romania	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	0.8	40	176	100-400	soil clay content <10 %	
6	Romania	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	
7	Slovenia	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	
8	Slovenia	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	0.8	40	176	100-400	soil clay content <10 %	
9	Slovenia	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	

1	2	3	4	5	6	7	8	9	10	11		12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**				**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg dicamba/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
10	Greece	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	
11	Greece	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	0.8	40	176	100-400	soil clay content <10 %	
12	Greece	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	
13	Italy	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	
14	Italy	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	0.8	40	176	100-400	soil clay content <10 %	
15	Italy	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	
16	Spain	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	
17	Spain	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	0.8	40	176	100-400	soil clay content <10 %	
18	Spain	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	
19	Bulgaria	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No. (e)	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F, Fn, Fpn G, Gn, Gpn or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha (f)
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season	g or kg nicosulfuron/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg dicamba/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max	
20	Bulgaria	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	0.8	40	176	100-400	soil clay content <10 %	
21	Bulgaria	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	
22	Croatia	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	1.2	60	264	100-400	soil clay content >10 % recommendation from 0.8 - 1.2 L/ha	
23	Croatia	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	1	NA	0.8	40	176	100-400	soil clay content <10 %	
24	Croatia	Maize	F	*Dicot & Grass weeds*	foliar	BBCH 12-18	2	7-15	a) 0.8 b) 1.2	a) 40 b) 60	a) 176 b) 264	100-400	Split / soil clay content >10 %	

CALLISTO PLUS 170SC (A17072C)

A17072C is an SC formulation containing 120 g/L dicamba + 50 g/L mesotrione

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: develop-mental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	L product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	Czech Republic	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
2	Hungary	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
3	Romania	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
4	Slovakia	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
5	Bulgaria	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
6	France	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
7	France	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
8	Greece	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	

1	2	3	4	5	6	7	8		10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Minimum interval between applications (days)	L product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
9	Italy	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
10	Portugal	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	
11	Spain	maize	F	*Broad Leaved Weeds (annual/perennial)*	foliar	BBCH 12-19	1	n/a	2	100 g mesotrione 240 g dicamba	80/400	n/a	

CASPER 55 WG and PARSEC (A14031E)

A14031E is a WG formulation containing 500 g/kg dicamba + 50 g/kg prosulfuron

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	kg A14031E / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
1	Austria	maize	F	annual dicots + convolvolus	Foliar	BBCH 12-18	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	150-400		
2	Belgium	maize	F	annual and perennial dicots	Foliar	BBCH 12-19 (see remarks)	1 or 2	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	150-400	60d Sillage & 90d Grain	Existing registration; 1 app at 0.3kg/ha BBCH 12-16 or 2 appl: 1st at 0.1-0.2kg/ha BBCH 12-16 & 2nd 0.1-0.2kg/ha BBCH 18-19
3	Germany	maize	F	annual dicots + convolvolus	Foliar	BBCH 12-18	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	150-400		
4	Czech Republic	maize	F	annual dicots + convolvolus	Foliar	BBCH 12-18	1	na	a) 0.4 b) 0.4	20 g prosulfuron 200 g dicamba	150-400	60d Sillage & 90d Grain	
5	Netherlands	maize	F	annual and perennial dicots	Foliar	BBCH 12-19 (see remarks)	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	150-400	60d Sillage & 90d Grain	Existing registration; 1 app at 0.3kg/ha BBCH 12-16 or 2 appl: 1st at 0.1-0.2 kg/ha BBCH 12-16 & 2nd 0.1-0.2kg/ha BBCH 18-19
6	UK	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	150-400	60d Sillage & 90d Grain	Existing registration; not maize grown for seed production Use recommended with adjuvant: NIS
7	Poland	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	150-400		

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	kg A14031E / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
8	Hungary	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400	60d Sillage & 90d Grain	
9	Hungary	sorghum	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400		
10	Hungary	sweet corn	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400		
11	Romania	maize	F	annual and perennial dicots except Convolvolus & hibuscus	Foliar	BBCH 12-18 / 4-6 leaves	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400	60d Sillage & 90d Grain	
12	Romania	sorghum	F	annual and perennial dicots	Foliar	BBCH 12-18 / 4-6 leaves	1	NA	a) 0.4 b) 0.4	20 g prosulfuron 200 g dicamba	150-400		
13	Romania	barley	F	annual and perennial dicots except Convolvolus	Foliar	BBCH 12-18	1	NA	0.2	10 g prosulfuron 100 g dicamba	150-400	-	
14	Romania	wheat	F	annual and perennial dicots except Convolvolus, Viola and Delphinium	Foliar	BBCH 12-18	1	NA	0.25	10-12.5 g prosulfuron 100-125 g dicamba	150-400	-	
15	Slovakia	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.25-0.3 b) 0.25-0.3	12.5-15 g prosulfuron 125-150 g dicamba	150-400	60d Sillage & 90d Grain	Existing registration; Use recommended with adjuvant: +0.5% ATPLUS

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	kg A14031E / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
16	France - N	maize and seed production	F	annual and perennial dicots	Foliar	BBCH 12-19 (see remarks)	1 (-2)	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	60d Sillage & 90d Grain	Existing registration; 1 app at 0.3kg/ha BBCH 12-16 or 2 appl: 1st at 0.1-0.2kg/ha BBCH 12-16 & 2nd 0.1-0.2kg/ha BBCH 18-19;
17	France - N	Sorghum	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	60d	
18	France - N	Millet (hungarian & proso)	F	annual and perennial dicots	Foliar		1 (-2)	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	60d	
19	France - N	Sugarcane	F	annual and perennial dicots	Foliar	BBCH 12- 18	1 (-2)	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	180d	
20	France - S	maize and seed production	F	annual and perennial dicots	Foliar	BBCH 12-19 (see remarks)	1 (-2)	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	60d Sillage & 90d Grain	Existing registration; 1 app at 0.3kg/ha BBCH 12-16 or 2 appl: 1st at 0.1-0.2kg/ha BBCH 12-16 & 2nd 0.1-0.2kg/ha BBCH 18-19;
21	France - S	Sorghum	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	60d	
22	France - S	Millet (hungarian & proso)	F	annual and perennial dicots	Foliar		1 (-2)	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	60d	
23	France - S	Sugarcane	F	annual and perennial dicots	Foliar	BBCH 12-18	1 (-2)	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	80-400	180d	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	kg A14031E / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
24	France - N	Industrial sites including railways and parks and garden pathways, cemeteries, alleys	F	annual and perennial dicots	Foliar	Not applicable	1	NA	a) 0.3 b) 0.3	a) 15 b) 15	a) 150 b) 150	80-400	
25	France - S	Industrial sites including railways and parks and garden pathways, cemeteries, alleys	F	annual and perennial dicots	Foliar	Not applicable	1	NA	a) 0.3 b) 0.3	a) 15 b) 15	a) 150 b) 150	80-400	
26	Italy	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400	60d Sillage & 90d Grain	Existing registration; Use recommended with adjuvant: NIS
27	Italy	sorghum	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400		Existing registration; Use recommended with adjuvant: NIS
28	Spain	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400	60d Sillage & 90d Grain	Existing registration; Use recommended with adjuvant: +0.2L/ha wetter/adjuvant
29	Spain	sorghum	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400		
30	Portugal	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400	60d Sillage & 90d Grain	

1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Application				**Application rate**				
Use No.	**Member state(s)**	**Crop and/or situation** (crop destination/ purpose of crop)	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	Method / Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	kg A14031E / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	**PHI** (days)	**Remarks:** e.g. safener/synergist per ha
31	Bulgaria	maize	F	annual dicots	Foliar	BBCH 12-18	1	NA	a) 0.3 b) 0.3	15 g prosulfuron 150 g dicamba	150-400	14 d Sillage	
32	Croatia	maize	F	annual and perennial dicots	Foliar	BBCH 12-18	1	NA	a) 0.3-0.4 b) 0.3-0.4	15-20 g prosulfuron 150-200 g dicamba	150-400	56 d Sillage & Grain	Existing registration (current GAP registered is BBCH 13-15; 0.3-0.5 kg/ha; 200-400 L water /ha; 56 d PHI sillage and grain); recommend use with adjuvant: NIS rate of 300-350 g/ha product, with the addition of 300 ml/ha of non-ionic wetting agent.

Level 2

Dicamba

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

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

Rotam:
Literature review has been performed according to:

- EFSA (2011). Guidance of EFSA, 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.
- AGES (2013). External scientific report, Case studies for the application of the Guidance of EFSA on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, using substances for which dossiers are submitted under Regulation (EU) No 1141/2010, EFSA supporting publication 2013:EN-511.

Syngenta:
A summary of the methodology employed is given below.

1. A very broad search was conducted in 18 scientific source databases for dicamba and its metabolites using the search terms listed in CA 9.5.1.
2. Duplicates titles from between the data bases were automatically removed from the output.
3. A rapid assessment of the titles was conducted to remove any additional duplicates and any obviously irrelevant titles (where enough information was available from the title alone).
4. A further rapid assessment was conducted using summary abstracts and any clearly irrelevant titles were removed.
5. A detailed assessment of the full-text documents for the remaining titles was conducted using the criteria developed for study relevance.
6. Any relevant papers were highlighted and assessed for reliability.

2.1 IDENTITY

2.1.1 Summary or identity

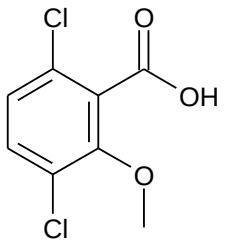
Dicamba is a systemic herbicide for the control of annual and perennial broadleaf dicotyledonous weed species.

Chemical name (IUPAC): 3,6-dichloro-2-methoxybenzoic acid

Molecular formula: $C_8H_6Cl_2O_3$

Mass: 221 g/mol

Structure formula:



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	Solid	Widlak A., 1993b Widlak A., 1993c Daum A., 2015 Chambers J., 2010	Visual
Melting/freezing point	114-116°C	Widlak A., 1993a	Measured
Boiling point	Thermal decomposition starts at about 230°C before the boiling point is reached	Das, 1999	Measured
Relative density	Not a requirement according to 283/2013		
Vapour pressure	$1.67 \cdot 10^{-3}$ Pa (25°C)	Chen, 1994	Vapour pressure curve based on eight measurements (95-111°C) Extrapolated vapour pressure at 25°C : $1.25 \cdot 10^{-5}$ mm Hg ≡ $1.67 \cdot 10^{-3}$ Pa
Surface tension	66.9 – 72.2 mN/m	O'Connor B., 2015 Chambers J., 2010	Measured
Water solubility	Syngenta: Temperature: 25°C. Purity: 99.6% Pure water pH 1.8 6.6 g/L Buffer solution pH 4.1 >250 g/L Buffer solution pH 6.8 >250 g/L Buffer solution pH 8.2 >250 g/L Rotam: Temperature: 25°C. Purity: 99.7% Pure water pH 1.98 7.3 g/L	Kettner, 1999a Chambers J., 2010	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
	Buffer solution pH 4 >3560 g/L Buffer solution pH 7 >3560 g/L Buffer solution pH 8 >3560 g/L		
Partition coefficient n-octanol/water	Syngenta: Temperature: 25°C. Purity: 99.6% pH 5.0: log P_{OW} = - 0.55, P_{OW} = 0.28 pH 6.8: log P_{OW} = - 1.8, P_{OW} = 0.017 pH 8.9: log P_{OW} = - 1.9, P_{OW} = 0.012 Rotam: Temperature: 25°C. Purity: 99.72% pH 5.1: log P_{OW} = - 0.78; P_{OW} = 0.1661 pH 7.0: log P_{OW} = - 2.30; P_{OW} = 0.0051 pH 9.1: log P_{OW} = - 2.42; P_{OW} = 0.0039	Kettner, 1999b Chambers J., 2010	Measured
Henry's law constant	H = 5.06 x 10^{-5} Pa m^3 mol^{-1} (25°C) (Based on a water solubility of 7.3 g/L) H' = 1.0 x 10^{-4} Pa m^3 mol^{-1} (25°C) (Based on a water solubility of 6.6 g/L recalculated to include only the neutral form of dissolved a.i.: 3565 mg/L)	Burkhard, 1999a Chambers J., 2010	Calculated
Flash point	Not determined. Not needed as the melting point is > 40°C	Angly, 1999a	
Flammability	Not highly flammable	Angly, 1999a	Tested
Explosive properties	No explosive properties under effect of thermal -, shock – or friction.	Angly, 1999c	Tested
Self-ignition temperature	Not self-igniting	Angly, 1999b	Tested
Oxidising properties	Not considered an oxidising substance	Angly, 1999d	Tested
Granulometry	Not a requirement according to 283/2013		
Solubility in organic solvents and identity of relevant degradation products	Syngenta: Temperature: 25°C. Purity: 89.5% Acetone >500 g/L Ethyl acetate >500 g/L Methanol >500 g/L Octanol 490 g/L Dichloromethane 340 g/L Toluene 180 g/L Hexane 2.8 g/L Rotam: Temperature: 25°C. Purity: 98.85%	Das, 2001b Chambers J., 2010	Measured

Dicamba **Volume 1 – Level 2**

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td></td><td>Acetone >250 g/L
Ethyl acetate 200-250 g/L
Methanol >250 g/L
Octanol >250 g/L
Dichloromethane 340 g/L
Toluene 80-100 g/L
Hexane < 10 g/L</td><td></td><td></td></tr>
<tr><td>Dissociation constant</td><td>Syngenta:
pKa = 1.87 (Purity: 99.2%)

Rotam:
pKa = 2.10 (Purity: 99.7%)</td><td>Bebel, 1993

Burkhard, 1999b

Chambers J., 2010</td><td>Measured</td></tr>
<tr><td>Viscosity</td><td>Not a requirement according to Regulation 283/2013</td><td></td><td></td></tr>
<tr><td>Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td><u>UV/VIS</u>
Solutions:
Neutral: methanole
Acidic: methanole / HCl
Basic : methanole / NaOH

<table>
<tr><th>Solution</th><th>Wavelength [nm]</th><th>Molar extinction coefficient [L / mol cm]</th></tr>
<tr><td>neutral
neutral</td><td>228
280</td><td>10130
737</td></tr>
<tr><td>acidic
acidic</td><td>228
280</td><td>10119
1028</td></tr>
<tr><td>basic
basic</td><td>228
280</td><td>10522
343</td></tr>
</table>
The absorption is tailing from 280 nm to 310nm. No absorption maximum between 310 nm and 750 nm was observed.

<u>IR</u>
Absorption peaks:

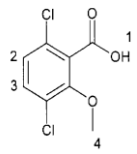
<table>
<tr><th>Wavenumber (cm^{-1})</th><th>Assignment</th></tr>
<tr><td>3300-2500</td><td>COO-H stretch</td></tr>
<tr><td>1714</td><td>C=O stretch</td></tr>
<tr><td>1581, 1461</td><td>ar C-C</td></tr>
<tr><td>1288</td><td>ar C-OCH_3 stretch assymetric</td></tr>
</table></td><td>Oggenfuss, 1999</td><td>Measured</td></tr>
</table>

Property	Value	Reference	Comment (e.g. measured or estimated)

1005	ar C-OCH_3 stretch symmetric

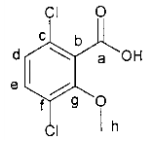
NMR

^{1}H-NMR



Chemical shift (ppm)	Assignment
4.0	4
7.2, 7.4	2, 3
7.3	Solvent
Not detected	1

^{13}C-NMR



Chemical shift (ppm)	Assignment
62	h
125-133	b,c,d,e,f
154	g
170	a

MS

Type of analyser: Quadropole

Ionization mode: Electron impact

Ionization energy: 70 eV

Mass spectrum interpretation:

m/z	Fragment ion
220	Molecular ion, M^+ (with typical isotope-pattern at m/z 222 and m/z 224 for CL-atoms)

Property	Value	Reference	Comment (e.g. measured or esti-mated)
	203 M^+-OH 191 M^+-NMR 175 m/z 203-CO 173 m/z 203-OCH_2 160 m/z 191-OCH_3 45 COOH		

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

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A.14	The substance is not considered an explosive	Purity: 89.8%	Angly, 1999a

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

Dicamba is not considered an explosive, as concluded from the test results on thermal sensitivity (effect of flame) and mechanical sensitivity (shock and friction)

2.2.1.1.1.2 Comparison with the CLP criteria

Not explosive according to the CLP criteria.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Dicamba does not meet the criteria for classification as an explosive.

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Not applicable.

2.2.1.1.3 Oxidising gases *[equivalent to section 8.3 of the CLH report template]*

Not applicable.

2.2.1.1.4 Gases under pressure *[equivalent to section 8.4 of the CLH report template]*

Not applicable.

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Not applicable.

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 3: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A.10	Not highly flammable	Purity: 89.8%	Angly, 1999a

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

A flame of a gas burner resulted in melting of the substance. Dicamba did not catch fire, neither unmelted nor melted. Dicamba is therefore not considered highly flammable.

2.2.1.1.6.2 Comparison with the CLP criteria

Not flammable according to the CLP criteria.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids
Dicamba does not meet the criteria for classification as flammable.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*
Not tested/Not relevant

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*
Not applicable

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*
Not tested/Not relevant

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 4: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A.16	Not self-heating	Purity: 89.8%	Angly, 1999b

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances
Dicamba was placed in an oven at room temperature; the temperature-time curve relating to conditions in the centre of the sample was recorded while the temperature of the oven was increased at a rate of 0.5°C/min. There was no significant observation on the temperature-time curve between room temperature and the melting point. Dicamba is therefore not considered self-heating or self-igniting.

2.2.1.1.10.2 Comparison with the CLP criteria
Not self-heating according to the CLP criteria.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances
Dicamba does not meet the criteria for classification as self-heating.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*
Not applicable

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*
Not applicable

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 5: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A.17	Not considered an oxidising substance	Purity: 89.8%	Angly, 1999d

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids
Dicamba and cellulose was mixed in different ratios and ignited. No evidence of oxidizing properties was observed.

2.2.1.1.13.2 Comparison with the CLP criteria
Not an oxidising solid according to the CLP criteria.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids
Dicamba does not meet the criteria for classification as an oxidising substance.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Not applicable

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

Not tested

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

A7254B (Dicamba 480 g/L SL)
The formulation A7254B is a light yellow liquid with a weak amine like odour. It is neither explosive nor oxidising. It is autoflammable at 465°C. The formulation has a pH of 8.3 while the pH of a 1% dilution of it is 7.5. The density is 1.170 g/cm^3 at 20°C. The results of storage stability tests indicate that the formulation has a shelf life of at least 2 years.

OCEAL (FH-048)
The formulation OCEAL is a light brown uniform granule with a vanilla type odour. The granules has a size of 500 – 1000 µm (99.95% of granules > 500 µm and 98.66 % of granules < 1000 µm). The formulation is not explosive, not highly flammable and not highly oxidising. It is autoflammable at 246°C. pH of a 1% dilution of the formulation is 7.33. The pour density is 0.60 g/mL and the tap density is 0.625 g/mL. The formulation is considered to be dust free.The results of storage stability tests indicate that the formulation has a shelf life of at least 2 years.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS proposed no classification for the following physical hazards based on the available data:

Explosives

An EEC A.14 test for testing explosiveness showing as dicamba does not meet the criteria for classification as an explosive.

Flammable solids

An EEC A.10 test for testing flammability showing as dicamba does not meet the criteria for classification as flammable.

Self-heating substances

An EEC A.16 test for testing self-heating properties showing as dicamba does not meet the criteria for classification as self-heating.

Oxidising solids

An EEC A.17 test for testing oxidizing properties showing as dicamba does not meet the criteria for classification as an oxidising substance.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

Explosives

According to the CLP criteria a substance or mixture is not classified as explosive when "*there are no chemical groups associated with explosive properties present in the molecule*".

RAC notes that no such potentially explosive groups were present in the chemical structure of dicamba. Overall, RAC supports the DS's proposal for **no classification for explosivity.**

Flammable solids

A flame of a gas burner resulted in melting of the substance and dicamba did not catch fire, whether melted or not. Overall, RAC supports the DS's proposal for **no classification of dicamba for flammability.**

Self-heating substances

According to the Guidance on the Application of the CLP Criteria, substances or mixtures with a low melting point (< 160 °C) should not be considered for classification since the melting process is endothermic and the substance-air surface is drastically reduced. RAC notes that the melting point of dicamba is 115 °C and therefore the criteria for classification of dicamba as self-heating solid is not met and **no classification is warranted.**

Oxidising solids

According to the Guidance on the Application of the CLP Criteria the classification procedure as oxidizing solid need not be applied if the substance contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen. Dicamba meets this requirement and therefore RAC supports the DS's proposal for **no classification for oxidizing solid.**

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

Dicamba can be applied post-emergence to a range of monocotyledonous crops such as maize, barley and wheat. Timing of application and maximum dose vary between crops. Dicamba controls a wide range of annual and perennial broadleaved weeds. Key target of dicamba are broadleaf weeds belonging to the families: *Amaranthaceae*, *Chenopodiaceae*, *Asteracea*, *Convolvulaceae*, *Solanaceae*, *Polygonaceae* and *Brassicacea.*

2.3.2 Summary of information on the development of resistance

According to the HRAC classification dicamba belongs to the Group O herbicides. Resistance to this group of herbicides is very rare and there are only a few restricted occurrences of confirmed resistance to dicamba and none in Europe. However where resistance to other members of this mode of action group has been confirmed there may also be cross-resistance to dicamba. As resistance to dicamba is very rare, dicamba can be a useful component of resistance management strategy used, e.g., in mixture with herbicides with a higher incidence of resistance such as the sulfonylureas.

2.3.3 Summary of adverse effects on treated crops

Maize can form fascinated or fused abnormal brace roots. Stems can become brittle and break and they can also become weakened and formed a curved, or 'goose-neck', shape. Dicamba can cause normally tolerant monocot species to lay flat for a time just after treatment but these symptoms often disappears within hours or days after treatment.

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

Not relevant.

2.4 FURTHER INFORMATION

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

Handling

Dicamba
Avoid contact with skin, eyes and clothing. Avoid inhalation of fog and vapours. Do not eat, drink or smoke while working.

A7254B (Dicamba 480 g/L SL)
Avoid contact with skin and eyes. When using do not eat, drink or smoke.

OCEAL (FH-048)
Do not eat, drink or smoke when using this product. Wash skin with mild soap and water.

Storage

Dicamba
Store the product in closed original containers. Protect from light and humidity. Keep out of the reach of children. Keep away from food, drink and animal feedingstuffs.

A7254B (Dicamba 480 g/L SL)
Keep containers tightly closed in a dry, cool and well-ventilated place. Keep out of the reach of children. Keep away from food, drink and animal feeding stuffs.

OCEAL (FH-048)
Keep container closed when not in use. Store in a well-ventilated place. Keep container tightly closed.

Transport

Dicamba
Use unbreakable containers, make sure they cannot fall, and label in accordance with regulations.
Rail / Road (RID / ADR): Not classified as dangerous good
Sea (IMDG-Code): Not classified as dangerous good
Air (ICAO / IATA): Not classified as dangerous good

A7254B (Dicamba 480 g/L SL)

UN number:	3082
Transport document description (ADR):	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (DICAMBA-DIMETHYLAMMONIUM), 9, III
Transport document description (IMDG):	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (DICAMBA-DIMETHYLAMMONIUM), 9, III, MARINE POLLUTANT

Transport document description (IATA-DGR):	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (DICAMBA-DIMETHYLAMMONIUM), 9, III
Transport hazard class (UN):	9
Packaging group:	III

OCEAL (FH-048)

UN number:	3077
Proper Shipping Name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (Dicamba)
Transport document description (ADR):	UN 3077 ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S.(Dicamba), 9, III, (E)
Transport document description (IMDG):	UN 3077 ENVIRONMENTALLY HAZARDOUSSUBSTANCE, SOLID, N.O.S.(Dicamba), 9, III, MARINE POLLUTANT
Transport hazard class (UN):	9
Packaging group:	III

Fire-fighting measures

Dicamba

Combustibility:	This product is combustible at elevated temperatures.
Suitable Extinguishing Media:	Dry chemical extinguisher, foam, carbon dioxide or waterspray (do not use direct jet of water).
Special Hazards during Fire Fighting:	Combustion products are toxic and/or irritant. Measures have to be taken to prevent the contaminated extinguishing agent from seeping into the ground or from spreading uncontrollably.
Hazardous Combustion Products:	Carbon dioxide; carbon monoxide; nitrogen oxides; hydrogen chloride
Protective Equipment for Fire Fighting:	Use self-contained breathing apparatus. Wear protective equipment.

A7254B (Dicamba 480 g/L SL)
Suitable extinguishing media:
Extinguishing media - small fires: Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Extinguishing media - large fires: Use alcohol-resistant foam or water spray.
Extinguishing media which shall not be used for safety reasons: Do not use a solid water stream as it may scatter and spread fire.

Specific hazards during fire fighting: As the product contains combustible organic components, fire will produce dense black smoke containing hazardous products of combustion. Exposure to decomposition products may be a hazard to health.

Special protective equipment for firefighters: Wear full protective clothing and self-contained breathing apparatus.

Further information to minimise the hazards arising: Do not allow run-off from fire fighting to enter drains or water courses. Cool closed containers exposed to fire with water spray.

Hazardous decomposition products likely to be generated in the event of fire: Combustion or thermal decomposition will evolve toxic and irritant vapours.

OCEAL (FH-048)

Suitable extinguishing media:	Dry chemical powder, alcohol-resistant foam, carbon dioxide (CO2). Do not use a heavy water stream as it may extend the fire
Firefighting instructions	Use water spray or fog for cooling exposed containers. Exercise caution when fighting any chemical fire. Do not fight fire when fire reaches explosives
Protection during firefighting	Do not enter fire area without proper protective equipment, including respiratory protection
Fire hazard:	Hazardous decomposition products may be released during prolonged heating like smokes, carbon monoxide and dioxide, nitrogen oxides (NOx).
Explosion hazard:	Product is not explosive
Reactivity:	The product is stable at normal handling- and storage conditions

2.4.2 Summary of procedures for destruction or decontamination

2.4.2.1 *Controlled incineration:*

The active substance dicamba (SAN 837), can be disposed of safely by incineration in a modern incinerator, licensed to treat special contaminated waste, which fulfils the following conditions: temperature > 800°C, minimum residence time within the incinerator: 2 seconds, equipped with a washing unit for flue gases. The ashes have to be disposed of at a suitable, approved waste disposal site. Wash water has to be disposed of via a suitable wastewater treatment plant.

A temporary formation of polyhalogenated dibenzo-p-dioxins and dibenzo-furans during incineration cannot be fully excluded. It should be noted that the halogen content with 32% is well below 60% and therefore not critical, and also that the reaction products are completely destroyed at temperatures above 800°C.

2.4.2.2 *Procedures for the Decontamination of Water in the Case of an Accident:*

Fire fighting water has to be contained, concentrated and decontaminated by filtration using charcoal. The water can be disposed of at a suitable sewage treatment plant or incinerated. The charcoal can be disposed of in a suitable waste incineration plant in accordance with the official regulations.

A7254B (Dicamba 480 g/L SL)

Spilled liquid formulation should first be adsorbed onto a solid, such as sand, inert clay filler, saw dust or soil, before being swept up into a safe container to await disposal.

As the halogen content of A7254B is below the 60% trigger value, high temperature incineration is the preferred means of disposal for the active substances, formulated products, contaminated materials or contaminated packaging. Directive 96/47/EEC defines the controlled conditions for incineration. Incineration should be carried out in a licensed incinerator operating at a temperature above 800°C and with a minimum gas phase residence time of two seconds.

OCEAL (FH-048)

The spilled formulation should be swept or shovelled into a container before disposal.

High temperature incineration may be used for disposal for the product and/or contaminated materials or packaging. Incineration should take place in an authorised incinerator at temperature above 800°C.

2.4.3 Summary of emergency measures in case of an accident

Dicamba

Personal precautions:

Ensure suitable personal protection during removal of spillage (for details see safety data sheet).

Clean up methods:

Cover spillage with moist earth or sawdust. Transfer to a container for disposal. Wash the spillage area with water. Do not allow spilled product or wash solution to enter sewers, drains, dams, creeks or any other waterways.

Procedures for the decontamination of water in the case of an accident:

Contaminated water must be contained. It may be decontaminated by filtration using charcoal and then concentrated. The water should be incinerated. The charcoal can be disposed on in a suitable waste incineration plant in accordance with official regulations.

A7254B (Dicamba 480 g/L SL)

Decontamination of areas, vehicles and buildings:
Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal according to local / national regulations.
If the product contaminates rivers and lakes or drains inform respective authorities.
Do not contaminate ponds, waterways or ditches with chemical or used container.
Do not dispose of waste into sewer.

Disposal of damaged packaging, absorbents and other materials :
Empty remaining contents. Triple rinse containers. Empty containers should be taken to an approved waste handling site for recycling or disposal. Do not re-use empty containers.

First aid measures:
Inhalation: Immediately move to fresh air. If breathing is irregular or stopped, administer artificial respiration. Keep patient warm and at rest. Call a physician or Poison Control Centre immediately.
Skin contact: Take off all contaminated clothing immediately. Wash off immediately with plenty of water. If skin irritation persists, call a physician. Wash contaminated clothing before re-use.
Eye contact: Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Remove contact lenses. Immediate medical attention is required.
Ingestion: If swallowed, seek medical advice immediately and show this container or label. Do NOT induce vomiting.
Medical advice: There is no specific antidote available. Treat symptomatically.

OCEAL (FH-048)

Accidental release measures:
Personal precautions, protective equipment and emergency procedures:
Wear a self-contained breathing apparatus and appropriate personal protective equipment (PPE).
Evacuate unnecessary personnel. Avoid inhalation of vapour and spray mist
Environmental precautions:
Avoid release to the environment. Prevent entry to sewers and public waters.
Methods and material for containment and cleaning up:
Sweep or shovel spills into appropriate container for disposal according to local / national regulations

First aid measures:

General:	Call a physician or poison control center immediately
Inhalation:	When symptoms occur: go into open air and ventilate suspected area
Skin contact:	When symptoms occur: rinse immediately with plenty of water
Eye contact:	Rinse first with plenty of water and if necessary take medical advice
Ingestion:	Rinse mouth with plenty of water. DO NOT induce vomiting. Seek medical advice

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

Analysis of the active substance as manufactured
Adequate methodology exists for the determination of dicamba as manufactured. The methods fulfil the requirements of SANCO/3030/99 rev. 4.

Formulation analysis

Adequate methodology exists for the determination of dicamba in the preparations A7254B and FH-048. The methods fulfil the requirements of SANCO/3030/99 rev. 4.

Methods for Risk Assessment

Methods in support of environmental fate studies:

Adequate methodology exists. Details are provided in Vol.3 B.5.Full description is not required for studies conducted with radioactive labelled substance.

Methods in support of residue studies:

All the methods used for the generation of pre-authorisation data for dicamba in maize and cereals are validated according to SANCO/3029/99. The LOQ in all methods is 0.01 mg/kg or 0.05 mg/kg.

Methods in support of toxicological, ecotoxicolgical and phys/chem studies:

Adequate methodology exists. Details are provided in Vol.3 B.5.

2.5.2 Methods for post control and monitoring purposes

Food and feed of plant and animal origin:

Adequate methods are available to monitor the respective current residue definition in plant material and food of animal origin. The methods are sufficiently validated and independent validated according to Sanco/825/00 rev. 8.1. The LOQ is 0.01 mg/kg.

Soil and water:

Both applicants submitted adequate methodology for the determination of dicamba and DCSA in soil and for the determination of dicamba, DCSA and 5-OH in water. The methods fulfil the requirements of SANCO/825/00 rev. 8.1. Details are provided in vol 3 B.5.

Air:

Both applicants submitted adequate methodology for the determination of dicamba in air. The methods fulfil the requirements of SANCO/825/00 rev. 8.1. Details are provided in B.5.

Body fluids and tissues:

Adequate methods are available for the determination of dicamba in body fluids and tissues. The methods fulfil the requirements of SANCO/825/00 rev. 8.1. Details are provided in B.5.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

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

Table 6: Summary table of toxicokinetic studies

Type of study **TG/GLP**	**Dose levels** **Animal species, strain; sex**	**Substance** **Batch**	**Results**	**References**
Absorption, distribution, depletion and excretion in rats – oral single dose OECD 417 (1984)/GLP	0.5 and 200 mg/kg bw Wistar rats	[phenyl-U-^{14}C]dicamba Unlabelled: AMS 163/101 Radiolabelled: ILA-72.1	A fast and almost complete (98 – 99 % of administered dose) absorption was observed with peak blood concentrations measured 0.5 hours after dosing for both dose levels. A second maximum was observed 2-4 hours after administration indicating some enterohepatic circulation. Elimination was predominantly via urine and only to a small extent via faeces. Tissue concentrations were highest 4 hours after administration with rapid depletion thereafter.	Hassler, S. (2002) KCA 5.1.1/01
Absorption, distribution and excretion in rats – oral repeated dose No TG/GLP	75 – 800 mg/kg bw Wistar and Sprague-Dawley rats	[phenyl-U-^{14}C]dicamba Labelled: 037H9294 Unlabelled: 52103810	A fast absorption was observed with peak blood concentrations measured 0.5-1 hour after multiple dosing with 75 to 800 mg/kg bw. While absorption was independent of the dose level, elimination processes were saturated at the higher dose levels (≥ 150 and 250 mg/kg bw).	Leibold et al. (1998a) KCA 5.1./02
Absorption, distribution and excretion in rats – oral repeated dose OECD 417 (1984)/GLP	50 – 800 mg/kg bw Wistar rats	[phenyl-U-^{14}C]dicamba Labelled: 787-0102 Unlabelled: 52103810	Dicamba was readily absorbed into systemic circulation with peak blood concentrations of radioactivity measured 0.5-2 hours after multiple dosing with 50-800 mg/kg bw in rat. While absorption was independent of the dose level, elimination processes were saturated at the higher dose levels (> 100-200 mg/kg bw).	Beimborn, D.B. (2003) KCA 5.1.1/03
Absorption, distribution, metabolism and excretion in rats – oral single dose OECD 417 (1984)/GLP	10 mg/kg bw CD VAF /Plus rats	[phenyl-U-^{14}C]dicamba Labelled: Lot 911115 Unlabelled: RS-M36-020492	Dicamba was almost quantitatively absorbed and excreted rapidly but was metabolised only to very minor extent as most of the compound was excreted unchanged predominantly via urine (about 93% of applied dose within 24 hours). Metabolisation involved the demethylation of the methyl ether leading to the respective alcohol DCSA (NOA 414746) (about 0.6% of applied dose). Most of the absorbed dose was eliminated via urine; the remainder via faeces (~ 2% of absorbed dose). Absorption, excretion and the metabolic pathways in the rat were similar after application of dicamba and its amine salts (DMA-, IPA- and DGA-salts).	Ekdawi et al. (1994a) KCA 5.1.1/04
Determination of 5-hydroxy dicamba in rats	10 mg/kg bw CD VAF /Plus rats	[phenyl-U-^{14}C]dicamba	5-hydroxy dicamba is a minor metabolite in rats	Ekdawi et al. (1994b) KCA 5.1.1/05

Dicamba **Volume 1 – Level 2**

Type of study **TG/GLP**	**Dose levels** **Animal species, strain; sex**	**Substance** **Batch**	**Results**	**References**
OECD 417 (1984)/GLP		Labelled: Lot 911115 Unlabelled dicamba: RS-M36-020492		
Absorption, distribution, metabolism and excretion in mice, rats, rabbits and dogs – oral single dose OECD 417 (1984)/before GLP	89 (mice), 102 (rats), 100 (rabbits) and 88.2 (dogs) mg/kg bw Swiss albino mice Sprague-Dawley rats New Zealand white rabbits Beagle dogs – all females	[phenyl-U-^{14}C]dicamba No batch no. given	Dicamba was readily and extensively (> 85% of administered dose) absorbed into systemic circulation with peak blood concentrations of radioactivity measured 1 hour after dosing with 100 mg/kg bw for rats and dogs. Half-life times were slightly longer in dogs (2.1 h) than in rats (1.1 h). Elimination was predominantly via urine and only small extent via faeces (0.5-5.7% of applied dose). The elimination was uniformly in all species except mice with slightly higher faecal values (9.4% of applied dose). Independent of the species the administered radioactivity was excreted rapidly (≥ 85% within 48 hours) resulting in very low tissue residues. Uniformly in all species unchanged dicamba was the main component of excreta and tissues.	Atallah et al. (1980) KCA 5.1.1/06
Metabolism of dicamba – oral single dose in rats OECD 417 (1984)/GLP	0.5 and 200 mg/kg bw Wistar rats	[phenyl-U-^{14}C]dicamba Labelled: ILA-72.1 Unlabelled dicamba: AMS 163/101	An oral dose of dicamba was almost quantitatively absorbed but was metabolised only to very minor extent as most of the compound was excreted unchanged predominantly via urine. Metabolisation involved glucuronyl conjugation of the benzoic acid group resulting in metabolite M1 (about 0.5% of applied dose) and the demethylation of the methyl ether leading to the respective alcohol DCSA (NOA 414746) and/or its glucuronic acid conjugate M2 (totally about 0.2-0.3% of applied dose). A further minor metabolite derived from hydroxylation at position 5 of the phenyl ring resulting in 5-OH dicamba (NOA 405873). Most of the absorbed dose was eliminated via urine; the remainder via faeces (< 2% of absorbed dose). The metabolic pathways in the rat were not significantly influenced by dose and sex.	Briswalter, C. (2003) KCA 5.1.1/07
Absorption, distribution, excretion and metabolism in rat following oral administration OECD 417 (1984)/GLP	0.5 and 200 mg/kg b.w Wistar rats	[Ring-U-14C]-RC1176 RTM/DCMB/DCSA/090326 (DCSA) HHBT-049-00-1 (5OH-dicamba)	Dicamba is rapidly absorbed by gastro intestinal tract and rapidly excreted mainly via urine, independently of the dose or the sex. Unchanged dicamba was the main component of excreta. Cmax was reached at 0.5 hours. >90% of the dose excreted by day 7. Highest residue levels in tissues and organs at 0.5 h. and close or LOQ within 12-24 h after dosage. The highest radioactivity content was found in kidneys. 5OH-dicamba was detected in urine and feces. The presents of a minor glucuronide derivative of [RING-U-14C]-dicamba in urine was confirmed.	NEW Arevalo M. (2010a) KCA 5.1.1/08

Type of study **TG/GLP**	**Dose levels** **Animal species, strain; sex**	**Substance** **Batch**	**Results**	**References**
Toxicokinetic study in rat following repeated oral administration OECD 417 (1984)/GLP	200 mg/kg b.w. Wistar rats	[Ring-U-^{14}C]-RC1176 XVIII/2	Following repeated oral administration, dicamba is rapidly absorbed by gastrointestinal tract and undergoes an enterohepatic circulation. The (Day 7) C_{max} and $AUC_{0\rightarrow24}$ values are similar to those obtained after a single dose (Day 1), demonstrating an absence of accumulation potential. C_{max} was reached at 0.5 hours. At 4 hours after administration, a second maximum was observed, probably due to enterohepatic circulation. Plasma levels were close to or below LOQ after 24 hours.	NEW Arevalo M. (2010b) KCA 5.1.1/09
Dicamba – In Vitro Comparative Metabolism of [phenyl-U-^{14}C] Dicamba in Human and Rat Liver Microsomes No TG/GLP	Human and Rat Liver Microsomes	[phenyl-U-^{14}C] dicamba 9314SJR027-5	No Phase I NADPH-dependent metabolism of dicamba occurred in human liver microsomes and rat liver microsomes.	NEW Thibaut R. (2016) KCA 5.1.1/10

Dicamba was rapidly absorbed and then efficiently and rapidly eliminated mainly via urinary excretion independent of the species, the mode of administration (gavage, mixed with the diet), and the dosage used. No significant pharmacokinetic difference was observed between the species (rat, mouse, dog, rabbit). The maximum blood concentrations were reached within 1 hour and then declined very rapidly with a half-life time of 1.1 to 2.1 hours.

After a repeated oral exposure in rats (at 200 mg/kg b.w. for 7 days), dicamba is rapidly absorbed with a peak blood concentraton at 0.5 hours (Tmax). At 4 hours after administration, a second maximum was observed, probably due to enterohepatic circulation.

Distribution

The amount of total radiocarbon in the body was generally very low due to the fast elimination. Tissue levels were low (max. 4.5 ppm after 16 hours) and declined rapidly (max. 0.14 ppm after 96 hours). Kidneys contained the highest residue levels (which is in accordance with the urinary excretion of dicamba) followed by blood and liver. No accumulation of dicamba was observed. In the rat, T_{max} in blood was reached within 0.5 h after dosing of 0.5 and 200 mg/kg bw with maximum concentrations of 0.11-0.13 ppm (low dose) and 51-68 ppm (high dose). Plasma half-life time was 2 h at both concentrations.

Metabolism

Only a limited degree of parent dicamba was metabolised and represented the major radiocarbon fraction in urine, faces and examined tissues (86-98%). The metabolite 3,6-dichloro-2-hydroxybenzoic acid (DCSA, NOA 414746) was found in small quantities in the urine of all species. The glucuronide of dicamba was found in the urine of rats. The presence of 5-hydroxy dicamba (5-OH dicamba, NOA 405873 – an important plant metabolite) was confirmed in rat urine.

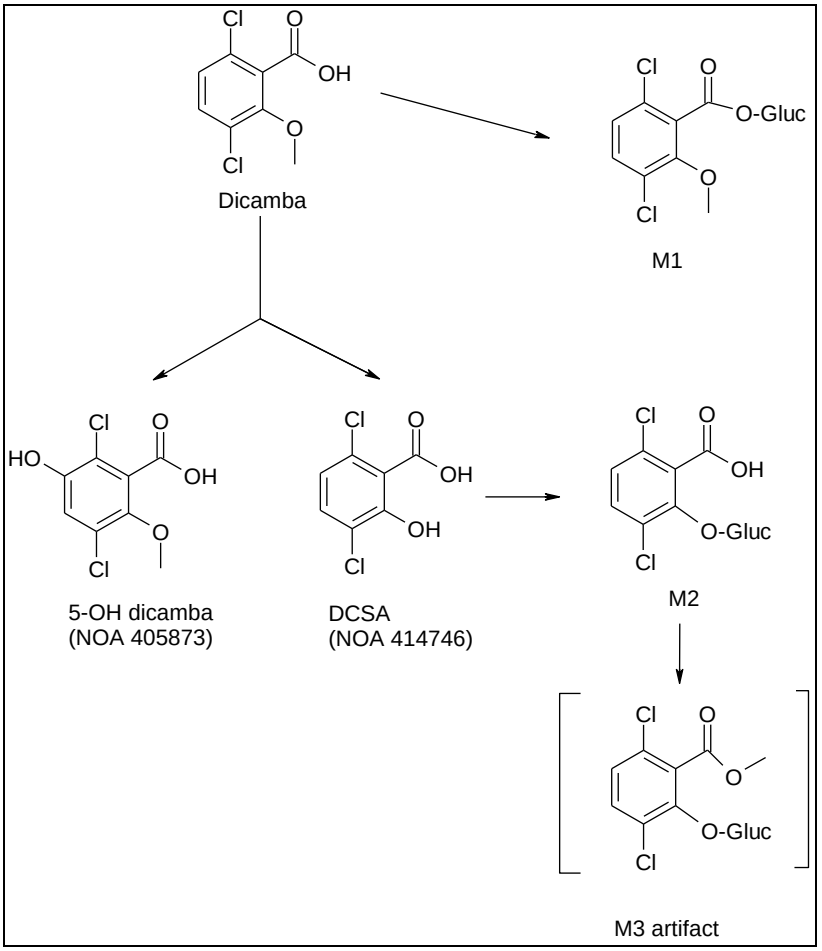
Dicamba metabolic pathway in rodents is summarised in the figure below.

Absorption/Elimination

Independent of the species and the dose level, dicamba was rapidly absorbed and eliminated. The elimination of dicamba has been shown to be rapidly and almost completely excreted *via* urine (85-98% of applied dose within 24 hours) in several rat studies. The percentage of dicamba elimination via faces was low (1 to 5 % of applied dose). One study showed that the elimination was uniformly in all species (rats, rabbits and dogs) except mice with slightly higher faecal values (9.4% of applied dose). Elimination via urine in mice was 72.76 % after 24 hours and 83.8% after 48 hours. A recent pharmacokinetic study in rats revealed that the renal excretion is saturated at higher dose levels (> 100 - 200 mg/kg bw).

Based on the results from an *in vitro* comparative metabolism of dicamba in human and rat liver microsome, it is confirmed that dicamba is poorly metabolized by Phase I oxidative processes in the liver. These results are identical in rats and humans.

Dicamba metabolism in the rat:



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

The toxicokinetic information is considered acceptable and adequate.

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 7: Summary table of animal studies on acute oral toxicity

Study type **TG/GLP**	**Animal sex, species and strain**	**Substance** **Batch**	**Dose levels, duration of exposure**	**Value** **LD_{50}**	**Reference**
Acute oral toxicity ~OECD 401 (1987)/before GLP	Spartan rats 6 groups of 5 females and 5 males	Dicamba (technical), Purity 85.8% (presumed)	500, 794, 1250, 1984, 3150 or 5000 mg/kg body weight	Calculated LD_{50}: Females 1581 mg dicamba/kg bw.	Wazeter and Goldenthal, 1974 KCA 5.2.1/01

(Data from original CLP proposal)		Batch not reported		Males 1879 mg dicamba/kg bw. Corrected for purity: Females 1356 mg dicamba/kg bw. Males 1612 mg dicamba/kg bw.	(study acceptable)

Table 8: Summary table of human data on acute oral toxicity

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Incident report, accidental exposure	1% Banvel M spray (340g MCPA, 30g dicamba/L)	Farmer sprayed a wheat field using knapsack sprayer for 30 minutes. When spraying against the wind face and arms were contaminated	Symptoms were transient glucosuria, ataxia, and weakening of tendon reflexes. Nausea, bloating, loss of appetite and palpitations occurred the day following exposure. At six day had vomiting and abdominal pain. At eight days gastroscopy revealed hemorrhagic gastro-duodenitis which had resolved at follow up 5 weeks later.	Huepp and Hesselmann (1979)
Prospective study from patients notified to the Poisons Unit following acute poisoning	12 patients had ingested dicamba formulations containing more than one herbicide in most cases.	The study examined the relation between blood herbicide concentration and the effect of alkaline diuresis on outcome of patients following acute poisoning. Blood and urine sample from all patients was examined (HPLC with limit of sensitivity of 10 mg/L for dicamba).	Plasma dicamba concentration was 0.02 g/L or less in 4 patients. There was no indication that dicamba had contributed to toxicity in any patient.	Flanagan *et al* (1990)
A retrospective observational case series of 14 patients	14 patients (5 female, 9 male) admitted to hospital after consuming dicamba containing product. There is no clear indication of the exact level of exposure.	The study reported information on clinical manifestation, patient management and final outcome after intentional ingestion of dicamba containing products.	Acute symptoms comprised transient clinical signs (depressed mental state, irritability or confusion, nausea, vomiting, or anorexia), changes in EKG (prolonged QTc intervals followed by sinus tachycardia) and/or increased lactate, leucocytosis, elevated creatinine kinase and metabolic acidosis. All findings were rapidly reversible with no or supportive treatment (hydration, sodium bicarbonate).	Moon and Chun (2014)
A retrospective observational case series of patients that ingested herbicides.	Twelve patients had consumed 50 – 300 mL of dicamba product (40% dicamba; dicamba as dimethylamine salt).	Information on clinical manifestation (APACHE II scores), patient management and final outcome are provided.	None of the patients that ingested dicamba died. There was no significant relationship between amount of dicamba ingested and clinical outcome or APACHE II scores. Most patients were discharged within 1 week after admission to the hospital except for 4 patients	Park *et al* (2011)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			needing longer treatment due to pre-existing health conditions or hospital-infection, which are considered unrelated to dicamba exposure.	

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

The acute oral toxicity study of dicamba in rats, performed prior to GLP and OECD guidelines, was conducted with minor deviations not considered to compromise the scientific validity of the study. The study was also the basis for the existing classification for acute oral toxicity. **However, the purity was only presumed in this study and not directly measured.** No clinical observations were reported but body weight gain was normal, by day 14 post dose, in surviving animals. There was no examination post mortem.

The calculated LD_{50} was 1581 mg dicamba/kg bw in females and 1879 mg dicamba/kg bw in males, which were the basis for the existing minimum classification. The LD_{50} corrected for purity was 1612 mg dicamba/kg bw for males and 1356 mg/kg bw for females.

Limited human data are available but there is no evidence of significant acute systemic toxicity in humans following acute exposures.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

LD_{50} values of 1879 mg dicamba/kg bw for males and 1581 mg/kg bw for females were found in an acute oral study.

According to CLP, classification is based on the lowest acute toxicity estimate (ATE) value available i.e. the lowest ATE in the most sensitive appropriate species tested.

Dicamba meets the criteria for classification in acute oral toxicity category 4 (300 mg/kg bw < ATE ≤ 2000 mg/kg bw). The lowest LD_{50} value of 1581 mg/kg bw shall be used as the Acute Toxicity Estimate (ATE).

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox. 4, harmful if swallowed (H302) ATE = 1581 mg/kg bw according to Regulation (EC) No 1272/2008.

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

Table 9: Summary table of animal studies on acute dermal toxicity

Study type TG/GLP	Animal sex, species and strain	Substance Batch	Dose levels duration of exposure	Results	Reference
Acute dermal toxicity OECD 402 (1987)/GLP	Alpk:AP_fSF (Wistar-derived) rats 1 group of 5 females and 5 males	Dicamba tech. (SAN 837 tech.), Purity 90.4% B2826511	2000 (1808 pure dicamba) mg/kg bw, 24 hours exposure	LD_{50} > 2000 mg dicamba/kg bw for males and females Corrected for purity: LD_{50} > 1808 mg dicamba/kg bw for males and females	Johnson, 2002 KCA 5.2.2/01 (study acceptable)

Acute dermal toxicity OECD 402 (1987)/GLP	CRL:(WI)BR Wistar rats 1 group of 5 males and 5 females	dicamba (RC1176) Purity 98.85% RTM/DCMB/03/20090612	2000 mg/kg bw, 24 hours exposure	LD_{50} > 2000 mg dicamba/kg bw for males and females	NEW Zelenák V. 2010a KCA 5.2.2/02 (study acceptable)

Table 10: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Incident report, accidental exposure	1% Banvel M spray (340g MCPA, 30g dicamba/L)	Farmer sprayed a wheat field using knapsack sprayer for 30 minutes. When spraying against the wind face and arms were contaminated	Symptoms were transient glucosuria, ataxia, and weakening of tendon reflexes. Nausea, bloating, loss of appetite and palpitations occurred the day following exposure. At six days had vomiting and abdominal pain. At eight days gastroscopy revealed hemorrhagic gastro-duodenitis which had resolved at follow up 5 weeks later.	Huepp and Hesselmann (1979)

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

The LD_{50} values of dicamba were provided in two acute dermal toxicity studies performed in accordance with OECD 402 (1987) and GLP. In Wistar rats, neither cutaneous reactions nor systemic clinical signs related to the administration of the test item were observed. LD_{50} was found to be >2000. In Alpk:AP_fSF rats, none of the animals died and there were no signs of systemic toxicity. Three males and all the females showed signs of slight skin irritation, and scabs were still apparent on the skin of one female at the end of the study. Apart from scabs in this one animal, there were no macroscopic abnormalities at examination post mortem. LD_{50} was determined to be >2000 mg dicamba/kg bw (>1808 mg/kg purity corrected) when applied once to the shaved intact skin of male and female rats.

Huep W.W., Hesselmann J., 1979, Severe acute erosive-hemorrhagic gastroduodenitis following to spraying of the herbicide Banvel M. Deutsche medizinische Wochenschrift, 104(14), 525
A farmer sprayed a wheat field with a 1% Banvel M spray broth using a knapsack sprayer for half an hour (Banvel M contains 340g MCPA and 30g Dicamba per litre concentrate). When he was spraying against the wind his face and arms were contaminated. The following day he suffered from nausea, bloating, loss of appetite and palpitation of the heart. Six days later the symptoms were vomiting and abdominal pain. The family doctor prescribed Metoclopramid (Paspertin®). Eight days after the exposure a gastrocopy revealed hemorrhagic gastro-duodenitis which had resolved at follow up five weeks later. No laboratory confirmation of exposure to the two herbicides was performed.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

Based on the results (no deaths in rats >2000 mg dicamba/kg bw), no classification for acute dermal toxicity is warranted for dicamba according to Regulation (EC) No 1272/2008.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification for acute dermal toxicity is warranted for dicamba according to Regulation (EC) No 1272/2008.

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

Table 11: Summary table of animal studies on acute inhalation toxicity

Study type TG/GLP	**Animal sex, species and strain**	**Substance Batch**	**Dose levels duration of exposure**	**Results**	**Reference**
Acute inhalation toxicity OECD 403 (2009)/GLP (study acceptable)	Sprague-Dawley derived, albino rats 1 group of 5 males and 5 females	Dicamba Purity: 97.8% w/w 201410375	5.14 mg/L(nose-only) for 4 h.	LC_{50} > 5.14 mg dicamba/L for males and females Corrected for purity: LC_{50} > 5.03 mg dicamba/L for males and females	NEW Durnado J 2015 KCA 5.2.3/01
Acute inhalation toxicity OECD 403 (1981)/GLP (study acceptable)	Alpk:AP_fSD (Wistar-derived) rats 3 groups of 5 females and 5 males	Dicamba Tech. (SAN 837 Tech.) Purity 91.2% B2826511	Target concentrations : of 1, 2.5 and 5 mg/L air (males only at 1 and 2.5 mg/l). Analysed conc.: 1.011, 2.373 and 4.591 mg/L Achieved gravimetric concentration 1.182, 2.676 and 5.191 mg/L (nose-only) for 4 h.	Inhalation LC_{50} (males): 4.46 mg dicamba/L Inhalation LC_{50} (females): >5.19 mg dicamba/L Corrected for purity: LC_{50} females > 4.73 mg dicamba/L LC_{50} males 4.07 mg dicamba/L	Kilgour, 2001 KCA 5.2.3/02
Acute inhalation toxicity OECD 403 (1981)/GLP (study acceptable)	CRL:(WI)BR Wistar rats 3 groups of 5 males and 1 group of 5 females	Dicamba (RC1176) Purity: 98.85% RTM/DCMB/03/20090612	Mean achieved doses: 5.01, 3.98, 4.50 mg/L, (nose-only) for 4 h.	LC_{50} females > 5.01 mg dicamba/L LC_{50} males 5.11 mg dicamba/L (technical material)	NEW Nagy K. 2010 KCA 5.2.3/03

No relevant human data are available.

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

In an acute, nose-only inhalation toxicity study in Alpk:APfSD rats (Kilgour, 2001), a group of 5 males and 5 females were exposed to aerosolised dicamba for 4 hours, at a particulate concentration of 5.19 mg/L. No deaths were observed during exposure, but 3/5 males and 1/5 females were found dead shortly after the cessation of exposure. Further groups of 5 males (only) were then exposed to particulate concentrations of 2.68 or 1.18 mg/L dicamba. There was one death shortly after exposure to 2.68 mg/L but no deaths following exposure to 1.18 mg/L.
The principal clinical signs were respiratory tract irritation (laboured breathing, changes in breathing depth and/or rate, abnormal respiratory noise). These signs were seen at all three dose levels. These effecs are discussed further in relation to specific target organ toxicity, single exposure in section 2.6.2.10. At 2.68 mg/kg wet fur (all animals) and stains around the nose (1/5) were observed. All animals displayed changes indicative of mild toxicity: decreased activity and salivation. Signs of moderate or mild toxicity (hunched posture, piloerection, salivation, decreased activity, coldness to touch, reduced foot withdrawal reflex, reduced response to sound) were present at 5.19 mg/L. At 5.19 mg/L, one male was prostrate and all females had muscular rigidity. The respiratory effects, seen during and immediately after exposure in all animals exposed to 5.19 mg/L and 2.68 mg/L, were transient and most animals had recovered by day 3, although abnormal respiratory noise persisted in some animals exposed to 2.68 mg/L until day 4. All animals were symptom free from day 5.
At 5.19 mg/L, surviving males lost weight over the first 3 days after exposure while females lost weight on day 2 but all animals then gained weight.
Necropsy findings in animals which died prematurely included: partially deflated / mottled lungs in 2/3 males exposed to 5.19 mg/L; dark spots in the lung in 1/1 male exposed to 2.68 mg/L; dark liver in 2/3 males exposed to 5.19 mg/L. There were no other treatment related macroscopic changes.
Acute inhalation 4 hour LC_{50} values of 4.46 mg/L (90% CL 2.80–40.5 mg/L) for males and >5.19 mg/L for females were derived. LC_{50} values corrected for purity were 4.07 mg/L for males and F >4.73 mg/L for females.

In a second study (Durnado, 2015) a group of 5 male and 5 female Sprague-Dawley rats were exposed via inhalation (nose-only exposure) to 5.14 mg/L of dicamba for 4 hours. None of the rats died and all gained body weight during the study. Following exposure all rats exhibited irregular respiration and hypoactivity. Additionally, two males had anogenital staining. All animals had recovered by day 3. There were no gross abnormalities at necropsy. The acute inhalation LC_{50} was > 5.14 mg/L in male and female rats in this study.

In the third study (Nagy K. 2010) groups of Wistar rats were exposed to an atmosphere of the test material for a single period of four hours (nose only). A target concentration of 5.0 mg/L was used for the first exposure group (5 males and 5 females). Subsequent targets were based on the results of the preceding exposures in order to produce a range of mortality rates (2 other groups of 5 males each).

Clinical observations revealed wet fur and fur staining were commonly recorded on the day of the exposure and several days after exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be biologically significant.

In surviving animals, significant clinical signs commonly noted on the day of exposure and continuing during the observation period included laboured, noisy, gasping respiration and sneezing. In addition, ataxia, lethargy, hunched posture; tiptoe gait, eye partially closed and emaciation were noted in some survivors during the first week of the observation period. No clinical signs were noted from Day 7 of the observation period. A single four hour nose-only exposure of dicamba to the Wistar rat resulted in death of 2 males at 5.01 mg/L and 1 male at 4.50 mg/L on Day 0 or 1. At necropsy, no specific cause of death was determined for these animals. No test item-related macroscopic findings were noted at any dose level following a 14-day observation period. LC_{50} for females was > 5.01 mg dicamba/L and LC_{50} for males was 5.11 mg dicamba/L.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

A LC_{50} value of 4.46 mg dicamba/L for males were found in the Kilgour (2001) study.

According to CLP, classification is based on the lowest acute toxicity estimate (ATE) value available i.e. the lowest ATE in the most sensitive appropriate species tested.

Dicamba meets the criteria for classification in acute inhalation toxicity category 4 (1.0 mg/L < ATE ≤ 5.0 mg/L). The lowest LC_{50} value of 4.46 mg/Lin males shall be used as the Acute Toxicity Estimate (ATE).

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Dicamba should be classified as Acute Tox. 4, harmful if inhaled (H332), ATE = 4.46 mg/L according to Regulation (EC) No 1272/2008.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of dicamba as Acute Tox. 4 H302 (harmful if swallowed) with an ATE = 1581 mg/kg bw based on the lowest estimated LD_{50} reported in an acute oral toxicity study with rats.

The DS proposed classification of dicamba as Acute Tox. 4 H332 (harmful if inhaled) with an ATE = 4.46 mg/L based on the lowest estimated LC_{50} reported in an acute inhalation toxicity study in rats.

No classification of dicamba for acute toxicity via dermal exposure was proposed by the DS based on lack of mortality at the limit dose, as reported in two independent acute dermal toxicity studies in rats.

Comments received during consultation

One Member State Competent Authority (MSCA) supported the proposed classification of dicamba as Acute Tox. 4 H302 (harmful if swallowed) and Acute Tox. 4 H332 (harmful if inhaled).

Assessment and comparison with the classification criteria

The table below summarises the results of the acute toxicity studies with animals.

Table: *Summary of animal studies on acute toxicity with dicamba*

Study	Dose level	Results			Reference
Acute oral toxicity Assimilated to OECD 401 No GLP Spartan rats 5 animals/sex/dose	Dicamba (technical) Vehicle: corn oil Presumed purity: 85.8% Batch not reported 500, 794, 1250, 1984, 3150 and 5000 mg/kg body weight	MALES			KCA 5.2.1/01
		Dose (mg/kg)	Day	Dead/5 animals	
		500	14	0	
		794	1	1	
			14	1	
		1250	1	2	
			14	2	
		1984	1	1	
			14	1	
		3150	1	5	
			14	5	
		5000	1	4	
			2	1	
			14	5	
		FEMALES			
		Dose (mg/kg)	Day	Dead/5 animals	
		500	14	0	
		794	1	0	
			14	0	
		1250	1	2	
			14	2	
		1984	1	3	
			14	3	
		3150	1	5	

		5000 14 5 1 5 2 5 14 5 Calculated LD_{50}: Females 1581 mg dicamba/kg bw Males 1879 mg dicamba/kg bw Calculated LD_{50} corrected for purity: Females 1356 mg dicamba/kg bw Males 1612 mg dicamba/kg bw	
Acute dermal toxicity OECD 402 GLP Alpk:APfSF (Wistar-derived) rats 5 animals/sex	Dicamba (technical) Purity: 90.4% 2000 mg/kg bw Vehicle: water 24 hours exposure	None of the animals died and there were no signs of systemic toxicity All showed an overall bodyweight gain during the study Three males and all the females showed signs of slight skin irritation Scabs were still apparent on the skin of one female at the end of the study No other macroscopic abnormalities at post-mortem examination LD_{50} > 2000 mg dicamba/kg bw for both males and females LD_{50} corrected for purity > 1808 mg dicamba/kg bw for both males and females	KCA 5.2.2/01
Acute dermal toxicity OECD 402 GLP CRL:(WI)BR Wistar rats 5 animals/sex	Dicamba Purity: 98.85% No vehicle: test item administered as supplied (powder/off-white) 2000 mg/kg bw 24 hours exposure	No mortality occurred No clinical signs No local dermal signs No test-related effects on body weight and body weight gain No test-related macroscopic findings LD_{50} > 2000 mg dicamba/kg bw for both males and females	KCA 5.2.2/02
Acute inhalation toxicity OECD 403 GLP	Dicamba Purity: 97.8% (w/w) No vehicle	All animals survived Following exposure all rats exhibited irregular respiration and hypo activity 2 males had ano-genital staining	KCA 5.2.3/01

<table>
<tr><td>Sprague-Dawley derived albino rats

5 animals/sex</td><td>5.14 mg/L

Nose-only

4 h

MMAD = 2.05 µm

GSD = 2.27 µm</td><td>All animals recovered by day 3

All rats exhibited gained body weight

No gross abnormalities were seen in necropsy

LC_{50} > 5.14 mg dicamba/L for both males and females

LC_{50} corrected for purity > 5.03 mg dicamba/L for both males and females</td><td></td></tr>
<tr><td>Acute inhalation toxicity

OECD 403

GLP

Alpk:APfSD Wistar-derived rats

5 animals/sex/dose</td><td>Dicamba (technical)

Purity: 91.2%

No vehicle

Achieved gravimetric concentration: 1.18, 2.68 and 5.19 mg/L

MMAD (µm) = 3.56, 4.81 and 4.63 for 1.18, 2.68 and 5.19 mg/L; respectively

GSD (µm) = 2.10, 1.75 and 2.18 for 1.18, 2.68 and 5.19 mg/L; respectively

Nose-only

4 h</td><td>Respiratory tract irritation (laboured breathing, changes in breathing depth and/or rate, abnormal respiratory noise) at all three dose levels

At 2.68 mg/kg wet fur (all animals) and stains around the nose (1/5) were observed

All animals had decreased activity and exhibited salivation

Hunched posture, piloerection, salivation, decreased activity, coldness to touch, reduced foot withdrawal reflex, reduced response to sound at 5.19 mg/L

Respiratory effects were transient and most animals had recovered by day 3

All animals were symptom free from day 5

<table><tr><th>Gravimetric concentration (mg/L)</th><th>Dead/ Treated</th><th>Latency (days)</th></tr><tr><td>5.19 ± 0.85</td><td>3/5 M, 1/5 F</td><td>1</td></tr><tr><td>2.68 ± 0.41</td><td>1/5 M</td><td>1</td></tr><tr><td>1.18 ± 0.39</td><td>0/5</td><td>-</td></tr></table>
LC_{50} (males): 4.46 mg dicamba/L
LC_{50} (females): >5.19 mg dicamba/L

Corrected for purity:

LC_{50} males 4.07 mg dicamba/L
LC_{50} (females) > 4.73 mg dicamba/L</td><td>KCA 5.2.3/02</td></tr>
<tr><td>Acute inhalation toxicity

OECD 403

GLP

CRL:(WI)BR Wistar rats</td><td>Dicamba

Purity: 98.85%

No vehicle

Mean achieved doses: 5.01, 3.98, 4.50 mg/L</td><td>On the day of exposure and continuing during the observation period: laboured, noisy, gasping respiration and sneezing

Ataxia, lethargy, hunched posture, tiptoe gait, eye partially closed and emaciation in some survivors during the first week of the observation period

No clinical signs were noted from day 7</td><td>KCA 5.2.3/03</td></tr>
</table>

3 groups of 5 males and 1 group of 5 females	MMAD (µm) = 2.88, 3.26 and 3.56 for 5.01, 3.98 and 4.50 mg/L; respectively GSD (µm) = 2.07, 1.82 and 2.03 for 5.01, 3.98 and 4.50 mg/L Nose-only 4 h	Normal bodyweight gain during the observation period No test item-related macroscopic findings were noted at any dose Males dead: 2, 0, 1 for 5.01, 3.98 and 4.50 mg/L; respectively Females dead at 5.01 mg/L: 0 LC_{50} females > 5.01 mg dicamba/L LC_{50} males = 5.11 mg dicamba/L

Comparison with the criteria

Acute oral toxicity

In addition to the animal studies summarised in the Table above, the CLH report also contains human data coming from accidental exposures, prospective studies from patients notified from poisons units and retrospective observational studies. These studies described an array of clinical symptoms related to the nervous system (ataxia, depressed mental state, irritability, etc.), to the gastric system (vomiting, abdominal pain, haemorrhagic gastro-duodenitis, etc.) and others. In general, none of these studies provides information relevant for classification.

The only available animal studies reported LD_{50}s (after correction for purity) of 1612 and 1356 mg/kg bw for male and females, respectively (Table above). Therefore, dicamba meets the criteria for classification in acute oral toxicity category 4 (300 mg/kg bw < ATE ≤ 2000 mg/kg bw). RAC notes that in the acute inhalation toxicity studies there are no reasons to consider the apparent differences in sensitivity between sexes to be significant. Therefore, RAC proposes to consider as the ATE the geometric mean of the LD_{50}s for males and females (1484 mg/kg bw) rounded to 1500 mg/kg bw.

Overall, **RAC supports the DS's proposal for classification for dicamba as Acute Tox. 4 with the hazard statement H302 (harmful if swallowed) with an ATE of 1500 mg/kg bw.**

Acute dermal toxicity

Two animal studies showed as dicamba, at limit dose of 2000 mg/kg bw caused no mortalities (Table above). The CLH-report also provides information from a report describing an accidental dermal exposure showing clinical symptoms quite similar to those described above for oral exposures and these data were not considered relevant for classification. Therefore, based on the reported results, **RAC supports the DS's proposal for no classification of dicamba for dermal acute toxicity.**

Acute inhalation toxicity

There were three different acceptable studies with animals where the lowest reported LC_{50} was 4.07 mg/L (after purity correction) (Table above). RAC notes that in some of the acute inhalation toxicity studies an MMAD above 4 µm was reported (Table above); this increases the concern that the real toxicity of dicamba may indeed be greater with particles with an MMAD below 4 µm. Therefore, dicamba meets the criteria for classification for acute inhalation toxicity in category 4 (1.0 mg/L < ATE ≤ 5.0 mg/L).

Overall, **RAC supports the DS's proposal for classification of dicamba as Acute Tox. 4 with the hazard statement H332 (harmful if inhaled) with an ATE of 4.0 mg/L.**

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

Table 12: Summary table of animal studies on skin corrosion/irritation

Study type **TG/GLP**	**Animal sex, species and strain**	**Substance** **Batch**	**Dose levels duration of exposure**	**Results**	**Reference**
Skin irritation OECD 404 (1992)/GLP, occulusive dressing was used instead of semi-occusive dressing (study acceptable)	New Zealand White rabbits, 3 animals, one male and two females	Dicamba tech. (SAN 837 Tech.), Purity 91.0% Y01040/007 (milled Y01040/005)	0.5 g, 4-hour	No skin reaction in 2/3 animals. Signs of skin irritation present in 1/3 animals for 7 days, all resolved by 14 days. Mean scores for that animal at 24, 48 and 72 hours: Erythema: 1.7, 0, 0 Oedema: 0.7, 0, 0	Johnson, 2002 KCA 5.2.4/01
Skin irritation OECD 404 (2002)/GLP (study acceptable)	New Zealand White rabbits, 3 males	Dicamba (RC1176) Purity: 98.85% RTM/DCMB/03/20090612	0.5 g, 4-hour	The individual mean scores (at 24, 48 and 72 hours) for erythema and oedema were 0.00, 0.00 and 0.00 respectively.	NEW Zelenák V. 2010b KCA 5.2.4/02

Table 13: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Incident report, accidental exposure	1% Banvel M spray (340g MCPA, 30g dicamba/L)	Farmer sprayed a wheat field using knapsack sprayer for 30 minutes. When spraying against the wind face and arms were contaminated	Nausea, bloating, loss of appetite and palpitations occurred the day following exposure. At six days had vomiting and abdominal pain. At eight days gastroscopy revealed hemorrhagic gastro-duodenitis which had resolved at follow up 5 weeks later.	Huepp and Hesselmann (1979)
Incident report, accidental exposure		1976 one employee developed a contact dermatitis working in the technical flake operation. One of his arms became inflamed during the hot months when he was wearing short sleeve shirt.	He was seen by a doctor and given topical steroid and released. The second case occurred around 1977 and was also a contact dermatitis from technical flake. Treatment was the same and employee improved in response to this treatment. These cases prompted a Policy change to require long sleeve shirts. No further episodes have occurred since this change in policy.	The information is from a questionnaire that was obtained from BASF 2003, which reports on cases of adverse health incidences in production workers since 1973 (up to 2003).

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

The skin irritation potential of dicamba technical was investigated in a standard guideline study in rabbits (Johnson, 2002). No signs of systemic toxicity were seen in any animal over the course of the study. Signs of mild skin irritation (erythema, oedema, scabbing, thickening and wrinkling) were seen in 1/3 rabbits between 1-7 days after decontamination but these had all resolved within 14 days of application. Dicamba is, therefore, not a skin irritant to the rabbit.

In a second study in rabbits (Zelenák,2010b), at observation one, 24, 48 and 72 hours after patch removal, there were no observed clinical signs noted on the skin of the treated animals. As no clinical signs were observed up to 72 hours after patch removal, the study was terminated after the 72 hours observation. The individual mean scores (considering readings at 24, 48 and 72 hours after patch removal) for erythema and oedema were 0.00, 0.00 and 0.00 respectively.

In humans, there have been two cases of adverse health effects following dermal exposure during manufacture. These occurred in 1976 and 1977 and resulted in skin rashes which resolved with treatment with topical steroids. Subsequently handling advice was changed to include wearing of long sleeves. No further cases of skin effects resulting from the handling of dicamba have been reported.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

The results of the rabbit skin irritation study do not meet the criteria for classification.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Dicamba does not meet the criteria for classification as a skin irritant.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of dicamba for skin irritation/corrosion based on two studies with rabbits showing lack of effects.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

The table below summarises the animal studies for skin irritation/corrosion with dicamba

***Table**: Summary of the animal studies on skin corrosion/irritation with dicamba*

Study	Dose level	Results	Reference
Skin irritation OECD 404 GLP New Zealand White rabbits Occlusive dressing 1 male and 2 females	Dicamba technical Vehicle: water Purity: 91.0% 0.5 g 4-hour	No skin reaction in 2/3 animals Signs of skin irritation present in 1/3 animals for 7 days, all resolved by 14 days Mean scores for that animal at 24, 48 and 72 hours: Erythema: 1.7, 0, 0 Oedema: 0.7, 0, 0	KCA 5.2.4/01
Skin irritation OECD 404 GLP New Zealand White rabbits 3 males	Dicamba Purity: 98.85% No vehicle: test item grounded as supplied 0.5 g 4-hour	No reported erythema or oedema (mean score were 0.00 at 24, 48 and 72 hours)	KCA 5.2.4/02

Comparison with the criteria

In addition to the animal studies summarised in the table above, the CLH report also contains human data coming from two cases of adverse health effects following dermal exposure during manufacture. These occurred in 1976 and 1977 and resulted in skin

rashes which resolved after treatment with topical steroids. Subsequently, handling advice was changed to include wearing of clothing with long sleeves. No further cases of skin effects resulting from the handling of dicamba have been reported. Moreover, in another accidental exposure, nausea, bloating, loss of appetite and palpitations occurred the day following exposure, together with vomiting and abdominal pain by day 6 and haemorrhagic gastro-duodenitis by day 8 resolved 5 weeks later.

The results reported for animal studies do not meet the criteria for classification. Thus, RAC supports the DS's proposal for **no classification of dicamba as a skin irritant.**

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

Table 14: Summary table of animal studies on serious eye damage/eye irritation

Study type **TG/GLP**	**Animal sex, species and strain**	**Substance** **Batch**	**Results**	**Reference**
Eye irritation ~OECD 405/before GLP and OECD guideline (study acceptable)	Male and female New Zealand White rabbits 2 groups: group I (5 minutes then wash) – 5 rabbits, group II (24 hours then wash) – 3 rabbits	Dicamba (technical), Purity 85.8% Batch not reported	Serious eye damage to the rabbit eye	Wazeter and Goldenthal, 1974 KCA 5.2.5/01

Table 15: Summary table of human data on eye corrosion/irritation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Incident report, accidental exposure		A contractor was installing a pipe bracket on a nitrogen line below the second floor grating in the Dicamba Flaking area on October 2, 2001.	The activity caused dust from the Flaker to fall through the grating into the left eye of the contractor. Eye irritation resulted as reported by nurse's report, and was treated by flushing and irrigating on site and over the counter Advil by the contractor's physician.	The information on are from a questionnaire that was obtained from BASF 2003, which reports on cases of adverse health incidences in production workers since 1973 (up to 2003).

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

Dicamba **Volume 1 – Level 2**

irritation

The eye irritation potential was investigated in a pre-guideline study in rabbits (Wazeter and Goldenthal, 1974). Eyes of rabbits were exposed to the test compound for 5 minutes (Group 1) or 24 hours (Group 2). Following exposure, the eyes were gently washed with water.

In both goups, there was evidence of severe ocular irritation. Corneal opacity was observed from 1 hour post instillation and persisted until 21 days after instillation in some rabbits. A mean corneal opacity score of ≥ 3 was observed in all animals at 48 and 72 hours. Iridial irritation was observed from 1 hour post instillation and was present in all animals at 24 and 48 hours and persisted in some rabbits for 7 days. Conjunctival redness and swelling (chemosis) was also seen in all rabbits, generally from 1 hour post instillation. Other signs of severe ocular irritation included blanching, purulent ocular discharge, fluorescein staining and pannus and in some animals these were present 21 days following instillation.

Eye irritation of Dicamba tech. to rabbits. Group I – 5 minutes exposure.

	Corneal opacity					**Iris lesion**		**Conjunctival redness**			**Conjunctival chemosis**				
Grade	0	1	2	3	4	0	1	0	1	2	0	1	2	3	4
1 h	1/5	2/5	2/5			1/5	4/5	4/5	1/5				3/5	2/5	
24 h		2/5	1/5	2/5			5/5		2/5	3/5				4/5	1/5
48 h			2/5	2/5	1/5		5/5		2/5	3/5			3/5	1/5	1/5
72 h					5/5	1/5	4/5		2/5	3/5			1/5	2/5	2/5
24-72h mean	*3.1*					*0.9*		*1.6*			*3.0*				
7 d			3/5	1/5	1/5	1/5	4/5		4/5	1/5		1/5	2/5	2/5	
14 d	2/5	2/5	1/5			5/5		3/5	2/5			1/5	3/5	1/5	
21 d		2/3	1/3			3/3		2/3	1/3			1/3	2/3		

Eye irritation of Dicamba tech. to rabbits. Group II – 24 hours exposure.

	Corneal opacity					**Iris lesion**			**Conjunctival redness**			**Conjunctival chemosis**				
Grade	0	1	2	3	4	0	1	2	0	1	2	0	1	2	3	4
1 h		1/3	1/3		1/3		3/3		1/3	1/3	1/3			1/3	2/3	
24 h			1/3	1/3	1/3		3/3		1/3	1/3	1/3					3/3
48 h				2/3	1/3		3/3			1/3	2/3				3/3	
72 h					3/3		2/3	1/3			3/3				1/3	2/3
24-72h mean	*3.4*					*1.1*			*1.6*			*3.6*				
7 d		1/3	1/3		1/3		2/3	1/3		1/3	2/3			2/3		1/3
14 d	1/3	1/3			1/3	2/3	1/3		1/3	2/3			1/3	1/3		1/3
21 d		1/2			1/2	1/2	1/2		2/2				1/2			1/2

Even if the study was conducted prior to Guideline 405, it is considered acceptable for evaluation of the potential serious eye damage/eye irritation of dicamba.

A single incident of eye exposure during manufacture has been recorded. In 2001 a contractor working below the dicamba flaking area disturbed some dust from the flaker, which fell through the grating into his eye resulting in irritation. Local first aid involved irrigation of the affected eye and the contractor's physician also recommended the taking of ibuprofen.

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

21 days after installation, effects on cornea and conjunctiva were still observed in the eyes of some rabbits indicating

possible irreversibility. Furthermore, the mean scores in at least 3/5 (Group 1) and 3/3 (Group 2) animals for corneal opacity were ≥ 3 (mean scores at 24, 48 and 72 hours). These data exceed the criterion for classification of irreversible effects. The study results warrant a classification of dicamba as Eye Dam. 1, Causes serious eye damage (H318) according to Regulation (EC) No 1272/2008.

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

Dicamba should be classified as Eye Dam. 1, Causes serious eye damage (H318) according to Regulation (EC) No 1272/2008.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification of dicamba as Eye Dam.1 based on a study with rabbits showing non-reversible serious eye damage both after 5 minutes and 24 hours of exposure.

Comments received during consultation

One MSCA supported the proposed classification of dicamba as Eye Dam. 1 H318 (causes serious eye damage).

Assessment and comparison with the classification criteria

The table below summarises the results of the animal study for serious eye damage with dicamba.

***Table**: Summary of the animal study on serious eye damage/irritation with dicamba*

Study	Dose level	Results	Reference
Eye irritation Similar to OECD 405 No GLP Male and female New Zealand White rabbits	Dicamba (technical) Purity: 85.8% No vehicle: substance placed conjunctival sac of the right eye Group I: 5 minutes exposure and wash, 5 rabbits Group II: 24 hours exposure and wash,	24-72 h mean Exposure / Opacity / Iris / Redness / Chemosis 5 min: 3.1 / 0.9 / 1.6 / 3.0 24 h: 3.4 / 1.1 / 1.6 / 3.6 Corneal opacity was observed from 1 hour post instillation and persisted until 21 days after instillation in some rabbits Iridial irritation was observed from 1 hour post instillation and was present in all animals at 24 and 48 hours and persisted in some rabbits for 7 days Conjunctival redness and swelling (chemosis) was also seen in all rabbits, generally from 1 hour post instillation Other signs of severe ocular irritation included blanching, purulent ocular discharge, fluorescein staining and pannus and in some animals these were present 21 days following instillation	KCA 5.2.5/01

3 rabbits

Comparison with the criteria

In addition to the animal study summarised in the table above, the CLH report also contained human data. A single incidence of eye exposure during manufacture has been recorded. In 2001 a contractor working below the dicamba flaking area disturbed some dust from the flaker, which fell through the grating into his eye resulting in irritation. Local first aid involved irrigation of the affected eye and the contractor's physician also recommended taking ibuprofen.

In the study in rabbits, 21 days after installation, effects on cornea and conjunctiva were still observed in the eyes of some rabbits indicating possible irreversibility. Furthermore, the mean scores in 3/5 (group 1) and 3/3 (group 2) animals for corneal opacity were ≥ 3 (mean scores at 24, 48 and 72 hours). These data exceed the criterion for classification of irreversible effects. Overall, RAC supports the DS's proposal for **classification of dicamba as Eye Dam. 1, H318 (causes serious eye damage).**

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

No information available.

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

No information available.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

No information available.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

No information available.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The CLH report does not contain information about respiratory sensitisation and this hazard class was not open for comments during the consultation of the CLH report.

Comments received during consultation

One Company-Downstream user commented that based on inhalation toxicity and skin sensitisation data, the statement "No evidence of respiratory sensitisation" would be appropriate.

Assessment and comparison with the classification criteria

RAC proposes no **classification of dicamba for respiratory sensitisation due to lack of data.**

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

Table 16: 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
Maximisation study OECD 406 GLP	Guinea pig Ibm:GOHI (Himalayan spotted) 30 females (20 test, 10 controls)	Dicamba (Technical material; batch 52625110; purity 86.3%) Vehicle: ethanol / Vaseline	Induction: Intradermal: 5% in ethanol, 5% in a 50:50 mixture of FCA / physiological saline (1:1) and ethanol, FCA and physiological saline (50:50). Topical: 25% in vaselinum album under an occlusive dressing for 48 hours. Challenge: 10% in vaselinum album.	Induction: skin responses (erythema and oedema) observed in some animals from days 2-7. Challenge: Challenge sites assessed at 24 and 48 hours. No dermal reaction following challenge in test or control animals. % positive reactions at 24 and 48 hours Control group : Dicamba 0%,0% Vehicle 0%, 0% Test group : Dicamba 0%,0% Vehicle 0%, 0% **Sensitisation rate = 0%.**	Ullmann *et al* (1991)

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

No clinical signs of systemic toxicity and no effects on body weight development were noted.
The application area around the injection sites 1 to 3 of control and test group animals was found to show erythema and edema from day 2 to 7, necroses from day 8 to 13, encrustation from day 13 to 17 and exfoliation from day 18 to 25 (termination of the test). The epidermal induction in test group females resulted in grade 1 edema in 1/20 and erythema in 8/20 animals at the 24 and/or 48 hour readings. There were no signs of irritation or oedema in any of the test or control group animals after challenge application.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

Dicamba does not meet the criteria for classification as a skin sensitiser.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Dicamba does not meet the criteria for classification as a skin sensitiser.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of dicamba for skin sensitisation based on the lack of effects noted in a single maximisation study with Guinea pigs.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

The table below summarises the results of the animal study for skin sensitisation with dicamba.

***Table**: Summary of the animal studies on skin sensitisation with dicamba*

Study	Dose level	Results	Reference
Maximisation study OECD 406 GLP Guinea pig Ibm:GOHI (Himalayan spotted) 30 females (20 test, 10 controls)	Dicamba (technical material) Purity: 86.3% <u>Induction</u> Intradermal: 5% in ethanol Topical: 25% in vaselinum album under an occlusive dressing for 48 hours <u>Challenge</u> 10% in vaselinum album	Induction: erythema and oedema observed in some animals from days 2-7 Challenge: No dermal reaction following challenge in test or control animals after 24 and 48 h	KCA 5.2.6 / 01

Comparison with the criteria

There were no signs of irritation or oedema in any of the test control or control group animals after challenge application, thus, dicamba does not meet the criteria for classification. Overall, RAC supports the DS's proposal for **no classification of dicamba as skin sensitiser.**

2.6.2.8 Phototoxicity

Table 17: Summary table of studies on phototoxicity

Study type TG/GLP	Animal sex, species and strain	Substance Batch	Results	Reference
In vitro phototoxicity test OECD 432 (2004)/GLP	Mouse fibroblast cell line Balb/3T3, clone A31	Dicamba technical Purity 90.1% P.MG2726410	No phototoxic effects observed. The study was performed with UVA 315-400 nm.	NEW Gehrke H, 2015 K-CA 5.2.7/01
In vitro phototoxicity test OECD 432 (2004)/GLP Deviation from TG 432: UV/vis absorption spectrum of the test	Mouse embryo fibroblasts from the Balb/c 3T3 clone 31 (ATCC - CCL163)	Dicamba technical Purity 98.9% 20140901136	No phototoxic effects observed. The study was performed with UVA 320-400 nm.	NEW Ostinet D., 2016 KCA 5.2.7/02

Study type TG/GLP	Animal sex, species and strain	Substance Batch	Results	Reference
substance according to OECD TG 101 was not determined				

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

No evidence of aspiration hazard.

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

No evidence of aspiration hazard.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

No classification.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

No classification.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

According to CLH-report there is no evidence of aspiration toxicity, and the hazard class was not within the scope of the Consultation of the CLH report

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

RAC proposes **no classification** of dicamba for aspiration toxicity based on lack of data.

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

Table 18: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Dicamba **Volume 1 – Level 2**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference

Acute neurotoxicity (oral). OECD 424 (1997). GLP Rat, Charles River Crl:CD®BR, 10/sex/group	Dicamba (technical material; purity: 86.9%) 0, 300, 600 or 1200 mg/kg bw. Single oral gavage dose. *The dose levels applied correspond to 261, 521 and 1043 mg/kg bw/day of pure dicamba.* Vehicle: corn oil Positive control: Acrylamide	**<u>1200 mg/kg bw</u>** 1/10 males found dead on day 1 *<u>Signs of neurotoxicity after 1.5 ± 1 hours:</u>* Rigidity in handling/body tone (8/10 males, 10/10 females), impairment of respiration (4/10 males, 5/10 females), flattened and/or raised posture (5/10 males, 6/10 females), impairment of gait (all animals), hypoalertness (7/10 males), ↓ rears/minute males, ↑ freezing in response to touch, abnormal righting reflex (9/9 males, 10/10 females), ↑ 86.5% tail flick latency time males, ↓ 29% fore limb grip strength males, ↓ activity both sexes during the first 10 to 15 minutes of session ↓ auditory startle *Body weight*:↓ 8.6% day 7 males *Body weight gain*: ↓ 25.9% day 0-7 males *Food consumption*: ↓ 12.8% day 0-7 males *<u>Signs of neurotoxicity after 7 days:</u>* Fore limb grip strength ↓ 15.0% males, Auditory startle: maximum and average input voltages to stimulus ↓ 59.10 and 53.5% respectively in males, 56% ↓ in females *<u>Signs of neurotoxicity after 14 days:</u>* No differences from control. **<u>600 mg/kg bw</u>** *Signs of neurotoxicity after 1.5 ± 1 hours:* Rigidity in handling/body tone (8/10 males, 8/10 females), impairment of respiration (2/10 males, 1/10 females), flattened and/or raised posture (5/10 males, 6/10 females), impairment of gait (all animals), hypoalertness (4/10 males, 2/10 females), ↓ rears/minute males, ↑ freezing in response to touch, abnormal righting reflex (10/10 males, 9/10 females), ↑ 54% tail flick latency time males, ↓ 19% fore limb grip strength males, ↓ activity both sexes during the first 10 to 15 minutes of the locomotor activity session *<u>Signs of neurotoxicity after 7 days:</u>* No effects. **<u>300 mg/kg bw</u>** *Signs of neurotoxicity after 1.5 ± 1 hours:* Rigidity in handling/body tone (5/10 females), raised posture (2/10 females), ↓ rears/minute males, ↑ freezing in response to touch (1/10 males, 2/10 females), abnormal righting reflex (7/10 males, 8/10 females), ↓ 15% fore limb grip strength males **No NOAEL (NOAEL < 300 mg/kg bw/day). All signs and measurements comparable to control by day 14.**	Minnema (1993)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		No treatment-related neuropathy.	
Acute delayed neurotoxicity. US-EPA FIFRA, Subdivision F, § 81-7 GLP Hen *Gallus gallus domesticus*, strain: Hisex Brown 10/group in control, low and mid dose group, positive control; 20/group high dose group.	Dicamba (technical material; purity: 86.82%). 0, 79 (¼ LD_{50}), 158 (½ LD_{50}), 316 mg/kg bw (LD_{50}) Single oral dose Vehicle: corn oil Positive control: TOCP *The dose levels applied correspond to 226, 327, 475, 688 and 998 mg/kg bw of pure dicamba for the LD_{50} determination, and to 69, 137, and 274 mg/kg bw of pure dicamba for the neurotoxicity assessment groups.*	**316 (274) mg/kg bw**: 9/20 animals died. *Body weight*: weight loss during the first two weeks of the experiment. Lesions of the sciatic nerve considered secondary to mild nerve entrapment resulting from recumbency not a direct toxic effect of dicamba. **158 (137) mg/kg bw**: 1/10 birds found dead day 5. *Body weight gain:*↓ 67% *Food consumption*: ↓ days 1 to 3 *Neuropathology*: comparable to control hens **79 (69) mg/kg bw**: No mortality. Body weight development similar to control. *Food consumption*: ↓ days 1 to 3 The LD_{50} expressed as technical dicamba is 316 mg/kg bw. NOAEL < 79 mg/kg bw. Effects at all doses: unsteadiness, inability to walk, collapsing when moved and lying on the pen floor with legs outstretched or lying on one side. **Effect was reversible.** **Does not induce delayed neurotoxicity in hens**	Roberts *et al* (1983)
Acute inhalation toxicity OECD 403 (1981)/GLP (study acceptable) CRL:(WI)BR Wistar rats 3 groups of 5 males and 1 group of 5 females	Dicamba (RC1176) Purity: 98.85% RTM/DCMB/03/20090612 Mean achieved doses: 5.01, 3.98, 4.50 mg/L, (nose-only) for 4 h.	LC_{50} females > 5.01 mg dicamba/L LC_{50} males 5.11 mg dicamba/L (technical material) In surviving animals (all groups), significant clinical signs commonly noted on the day of exposure and continuing during the observation period included laboured, noisy, gasping respiration and sneezing. In addition, ataxia, lethargy, hunched posture; tiptoe gait, eye partially closed, and emaciation were noted in some survivors during the first week of the observation period. No clinical signs were noted from Day 7 of the observation period.	Nagy K. 2010 KCA 5.2.3/03 (study acceptable)
Acute inhalation toxicity OECD 403 (2009)/GLP (study acceptable)	Dicamba Purity: 97.8% w/w 201410375 5.14 mg/L(nose-only) for 4 h.	LC_{50} > 5.14 mg dicamba/L for males and females (technical material) None of the rats died and all gained body weight during the study. Following exposure all rats exhibited irregular	Durnado J 2015 KCA 5.2.3/01 (study acceptable)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Sprague-Dawley derived, albino rats 1 group of 5 males and 5 females		respiration and hypoactivity. Additionally, two males had anogenital staining. All animals had recovered by day 3.	
Acute inhalation toxicity OECD 403 (1981)/GLP (study acceptable) Alpk:AP_fSD (Wistar-derived) rats 3 groups of 5 females and 5 males	Dicamba Tech. (SAN 837 Tech.) Purity 91.2% B2826511 Target concentrations: of 1, 2.5 and 5 mg/L air (males only at 1 and 2.5 mg/l). Analysed conc.: 1.011, 2.373 and 4.591 mg/L Achieved gravimetric concentration 1.182, 2.68 and 5.191 mg/L (nose-only) for 4 h	LC_{50} (males): 4.46 mg dicamba/L LC_{50} (females): >5.19 mg dicamba/L (technical material) At 2.68 mg/kg, wet fur (all animals) and stains around the nose (1/5) were observed. All animals displayed changes indicative of mild toxicity: decreased activity and salivation. Signs of moderate or mild toxicity (hunched posture, piloerection, salivation, decreased activity, coldness to touch, reduced foot withdrawal reflex, reduced response to sound) were present at 5.19 L. At 5.19 mg/L, one male was prostrate and all females had muscular rigidity. The respiratory effects, seen during and immediately after exposure in all animals exposed to 5.19 mg/L and 2.68 mg/L, were transient and most animals had recovered by day 3, although abnormal respiratory noise persisted in some animals exposed to 2.68 mg/L until day 4. All animals were symptom free from day 5.	Kilgour, 2001 KCA 5.2.3/02 (study acceptable)

Table 19: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Dicamba **Volume 1 – Level 2**

Type of study/data	**Test substance**	**Observations**	**Reference**
Developmental toxicity Test guideline not stated but complies largely to OECD 414 (2001) but with some notable deviations (see below) Oral (gavage) Rat, Charles River CD 25 mated females/group	Dicamba (Technical grade; batch: 52625110; purity 90.4%) 0, 64, 160 or 400 mg/kg bw/day on days 6-19 of gestation. *The dose levels applied correspond to 58, 145 and 362 mg/kg bw/day of pure dicamba.* Vehicle: corn oil	<u>Maternal toxicity</u> 400 (362) mg/kg bw/day: 4/25 deaths gestation day 7 & 8; ataxia, stiffening of the body when held, urine soaked fur, salivation and decreased motor activity; ↓ body weight gain (27% lower corrected maternal bw gain); ↓ food consumption (18.5% lower than controls, days 6-19). 4 deaths on GD7 and 8 (3 pregnant, 1 non- pregnant) 160 (145) mg/kg bw/day 10 % lower corrected maternal bw gain (not statistically significant) 64 (58) mg/kg bw/day No effects **Maternal NOAEL** 64 (58) mg/kg bw/day <u>Developmental toxicity</u> 400 (362) mg/kg bw/day: ↑ number of incompletely ossified frontal (s) and/or parietal(s) 64 (58) &160 (145) mg/kg bw/day: No effects **Developmental NOAEL** 160 (145) mg/kg bw/day	Smith (1981) (study acceptable)

Developmental toxicity US EPA 83-3 (complies largely to OECD 414, 2001) Oral (capsule) Rabbit, New Zealand White Hra:(NZW)SPF 20 inseminated females/group	Dicamba (Technical grade; batch: 52625110; purity 90.4%) 0, 30, 150 or 300 mg/kg bw/day on days 6-18 of gestation *The dose levels applied correspond to 27.1, 136 and 271 mg/kg bw/day of pure dicamba.*	**Maternal toxicity** 300 (271) mg/kg bw/day: 4/20 abortions; ataxia, rales, laboured breathing, perinasal substance, dried/no faeces, impaired righting reflex and decreased motor activity; ↓ body weight gain (42% lower than controls days 0 to 29); ↓ relative food consumption (13% lower than controls, days 0-29). Clinical observations first occurred on day 9 of presumed gestation, and one or more were generally observed in several does throughout the dosing and post dosing periods. 150 (136) mg/kg bw/day: 1/20 abortion; ataxia and decreased motor activity 30 (27.1) mg/kg bw/day No effects Maternal NOAEL:30 mg/kg bw/day Developmental toxicity 300 mg/kg bw/day: increased incidence of irregularly ossified internasals . High dosis (incidence) 300 mg/kg bw/day Pups: 3.9% Litter: 23.1% **HCD 1987-1989** Pups: 0-2.3% Litter: 0-14.3% **HCD 1990-1994** Pups: 0-5 (0-4.8%) Litter: 0-4 (0-26.7%) **HCD 1992-1994** Pups: 0-4.2% Litter: 0-26.7% 30, 150 mg/kg bw/day: No effects Developmental NOAEL 150 (136) mg/kg bw/day	Hoberman (1992) (study acceptable)

Two Generation Oral (continuous in diet) OECD 416 (1983) Rat, Crl:CD (SD) BR VAF/Plus 32/sex/group (F0) 28/sex/group (F1)	Dicamba (Technical material; batch 52103810; purity 86.9%) 0, 500, 1500 or 5000 ppm Vehicle: laboratory animal diet. The overall F0/F1 pre-mating doses correspond to 37.9, 113 and 389 mg/kg bw /day for males and 42.6, 130 and 424 mg/kg bw/day for females at 0, 500, 1500 or 5000 ppm, respectively. *The overall F0/F1 pre-mating means correspond to 32.9, 98.3 and 338 mg/kg bw/day of pure dicamba for males, and to 37.0, 113, 369 mg/kg bw/day of pure dicamba for females, at 500, 1500 and 5000 ppm, respectively*	***Parental toxicity*** 5000 ppm F0: mean achieved intake 347/390 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 9.6% (day 0-20: 3.2%) ↑ adjusted liver weight 13% females, 5% males F1: mean achieved intake, 432/458 mg/kg bw/day, males/ females respectively Clinical signs during lactation: tense/stiff body tone and slow righting reflex for a few days during the latter part of lactation ↓ body weight pregnancy day 0-14: 4.6% (F1A) and 23% (F1B) ↑ absolute liver weight 3% females, males 9.5% (relative) ↓ food consumption week 5-8 1500 ppm F0: mean achieved intake, 105/125 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 121/135 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14 (F1B): 15 % (day 0-20: 15%) 500 ppm F0: mean achieved intake, 35/41 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 40/44 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 9.6% (F1B) (day 0-20: 1.7%) but absolute body weight was not reduced Otherwise, no effects **NOAEL** 500 ppm (42.6 mg/kg bw/day) on the basis of decreased body weight during pregnancy (GD 0-14) at 1500 and 5000 ppm. Clinical signs during lactation, ↑ liver weights at 5000 ppm ***Reproductive toxicity*** No effects at any dose level NOAEL 5000 ppm (389 mg/kg bw/day) ***Offspring toxicity*** 5000 ppm F1: ↓mean pup body weight 24 % day 21, delayed sexual maturation of males by 2 days, ↑ relative liver weights 27%. F2A/B: ↓ body weight 26/30 % day 21, ↑ relative liver weights approx. 36%. 1500 ppm F1: ↓ mean pup body weight 4 % day 21 F2A/B: ↓ pup body weight 10/14 % day 21 500 ppm F2B: No effects **NOAEL**: 500 ppm (37.9 mg/kg bw/day) based on	Masters, 1993

		body weight effects at 1500 and 5000 ppm.	
Subchronic neurotoxicity study. OECD 424 (1997). GLP Rat, Charles River Crl:CD®BR, 10/sex/group (dietary)	Dicamba (technical material; purity: 86.9%) 0, 3000, 6000 and 12000 ppm Actual doses 0, 197.1, 401.5 and 767.9 mg/kg/day for the males and 253.4, 472.0 and 1028.9 mg/kg/day for females. Continuous in the diet for 13 weeks *The dose levels applied correspond to 171, 348 and 667 mg/kg bw/day of pure dicamba in males, and to 220, 410, 894 mg/kg bw/day of pure dicamba in females at 3000, 6000 and 12000 ppm, respectively.*	**12000 ppm (males 767.9 mg/kg bw/day, females 1028.9 mg/kg bw/day)**: *Body weight*: ↓ 5.5% males, 4.8% females, week 14 *Body weight gain*: ↓ 24.1% males, 37.9%, females week 1 *FOB*: ↑ frequency of rigid body tone when handled in weeks 4, 8 and 13 (greater in females than males). *Pathology*: No treatment-related changes in any of the tissues examined **6000 ppm (males 401.5 mg/kg bw/day, females 472 mg/kg bw/day)**: No treatment-related effects. **3000 ppm (males 197.1 mg/kg bw/day, females 253.4 mg/kg bw/day)**: No treatment-related effects. **NOAEL for neurotoxicity and systemic toxicity is 6000 ppm (401.5 mg/kg bw /day in males and 472 mg/kg bw/day in females), based on decreased body weight gain and neurobehavioral findings.**	Minnema (1994) (study acceptable)

13-week oral (capsule) toxicity OECD 409, 1998 GLP Dog: pure-bred Beagles 4/sex/group, plus an additional 4/sex/group for control and top dose 4-week recovery phase	Dicamba (technical material; batch B2826511; purity 90.4%) 0, 10, 50, 300 mg/kg bw/day Capsule administration. No vehicle 13-week duration plus 4-week recovery *Considering the purity of dicamba used for this study (90.4%), the applied doses referring to pure dicamba (100% purity) correspond to 9.0, 45, 274 mg/kg bw/day.*	**<u>300 (274) mg/kg bw/day:</u>** *Clinical observations*: Hind limb gait abnormalities noted from day 1: ataxia, stiff gait and sporadic transient collapse generally seen in the majority of the 300 mg/kg animals approximately 2 hours after dosing and persisting for up to 5 hours. The neurological screen at wk 6 and 13 showed abnormal locomotion and gait abnormalities in all animals. No effects detected following the recovery phase. *Mean bw gain:* ↓ 26 % in males and 44 % in females (not statistically significant) *Food consumption:* 90% of control for males and 84% of control for females *Haematology:* ↓ 9-17.7% RBC, Hct and Hb week 7 and 13 both sexes. ↑ 10.5% APPT activity in males and 7% in females at week 13, but signs of reversibility following recovery. *Clinical chemistry:* ↓ 24.6-32.4% cholesterol and phospholipids weeks 7 and 13. Partial improvement following recovery (no statistically significant differences from control). *Organ weights:* ↓ Not significantly decreased abs 17 % and 11 % rel testes weight Not toxicologically significant effects (↓ absolute and relative spleen weight for males due to high control value and ↑ kidney weight as percentage body weight in females, not evident after recovery). **<u>50 (45) mg/kg bw/day:</u>** No toxicologically significant findings. **<u>10 (9) mg/kg bw/day:</u>** No treatment-related effects. **NOAEL = 50 (45) mg/ kg bw/day based on effects on gait and behaviour, decreased food intake and body weight gain, minor alterations in the red blood cell parameters and disturbances in the serum lipid levels and decreased testes weight in the highest dose group.**	Jackson (2003) (study acceptable)

RC1176: 90-day oral capsule toxicity study in beagle dogs OECD 409 (1998) GLP Dog: Beagle 4/sex/group	0, 10, 50 and 300 mg/kg bw/day Dicamba (technical material; Lot: RTM/DCMB/03/20090612; purity >95%) *Doses corresponded to 0, 9.5, 47.5, 285 at 100, 500, and 2500 ppm, respectively when corrected for purity.*	**<u>At 300 (285) mg/kg bw/day :</u>** ***Clinical signs*** : Intermittent stiff gait and recumbency, slight and/or moderate uncoordination or ataxia and retching or emesis were consistently recorded. On occasion, the animals also displayed slightly to severely decreased activity, liquid faeces, increased salivation, minor tonic convulsions or tremors. All animals recovered by the following morning *Organ weight*: ↑ ovary absolute and relative weight (>40 %) ***Clinical chemistry parameters***: ↑ ALT in both sexes during week 13 (72%, p<0.01 in the males, and 143%, p<0.05 in the females).c ↓ cholesterol appeared to be lower than control; however, no statistically significant differences were noted when compared to control. ↓ triglyceride mean values in both males and females, attaining statistical significance in the males (-28%, p< 0.05).) ↓ ALKP mean values in females (-40%, p<0.05 at week 7), and -36%, p<0.01 at week 13. **<u>50 (47.5) mg/kg bw/day</u>** ↓ ALKP mean values (-30%, p<0.05 at week 7) ***<u>10 (9.5) mg/kg bw/day :</u>*** No toxicologically relevant effects **<u>NOAEL</u> was 50 (47.5) mg/kg bw/day based on clinical signs and parameters (stiff gait, uncoordination or ataxia and retching or emesis, decreased activity, liquid faeces, increased salivation, minor tonic convulsions or tremors, decreased values in the red blood count, haemoglobin and haematocrit at 300 mg/kg bw/day)**	Kubaszky R. (2010) (study acceptable)

4 week oral range-finding study Pre OECD guideline and GLP. No haematology, clinical chemistry or pathology conducted. Non-GLP Rat: Charles River CD® 5/sex/group (dietary)	Dicamba (Banvel technical; batch 52625110; purity 86.82%) Dietary study, 0, 5000, 7500, 10000, 12500, 15000 ppm. Doses correspond to 0, 568, 798,1053, 1353, 1649 for males and for females 0, 557, 840, 1085, 1364, 1654 mg/kg dicamba/day (technical material) at 0, 5000, 7500, 10000, 12500 and 15000 ppm 4-week duration *Corrected for purity corresponds to 493, 693, 914, 1175 and 1432 mg/kg bw/day for males at 5000, 7500, 10000, 12500 and 15000 ppm, respectively, and 484, 729, 942, 1184 and 1436 mg/kg bw/day for females at 5000, 7500, 10000, 12500 and 15000 ppm*	**15000 ppm (approx. 1649 mg/kg bw/day for males & 1654 mg/kg bw/day for females):** 3/5 males and 4/5 females impaired mobility in hind extremities. *Body weight gain*: ↓ 39.0% males, ↓ 23.0% females week 4 *Food consumption*: ↓ 35.6% males, ↓ 29.3% females week 1-4 **12500 ppm (approx. 1353 mg/kg bw/day for males & 1364 mg/kg bw/day for females):** 1/5 male impaired mobility in hind extremities. *Body weight*: ↓ 23.7% males, ↓ 12.8% females week 4 *Food consumption*: ↓ 24.9% males, ↓ 20.7% females week 1-4 **10000 ppm (approx. 1053 mg/kg bw/day for males & 1085 mg/kg bw/day for females):** *Body weight*: ↓ 11.2% males week 4 *Food consumption*: ↓ 16.9% males, 12 % females week 1-4 **7500 ppm (approx. 798 mg/kg bw/day for males & 840 mg/kg bw/day for females) and 5000 ppm (approx. 568 mg/kg bw/day for males & 557 mg/kg bw/day for females):** No adverse effects reported. **NOAEL: 840 mg dicamba/kg bw/day in females and 798 mg dicamba/kg bw/day in males (7500 ppm) based on reduced body weight gain and food consumption**	Goldenthal (1979) (range-finding study supportive of risk assessment)
Repeated dose 28-day inhalation. OECD 412 EC No. 440/2008 GLP Rat: Crl:WI(Han) Wistar 10/sex/group	Dicamba (BAS 183H Technical material; batch 0002B01BA-251; purity 93.9%) Nose only exposures to dust. 0, 0.001, 0.005, 0.05 mg/L, 6 hours/day, 5 days/week for 4 weeks. *Dose levels correspond to 0.00094, 0.0047 and 0.047 mg/L of pure dicamba*	**0.05 mg/L:** *Body weight gain :* ↓ 41 % in males, 13 % in females (not statistically significant in females) *Organ weights*: ↑ absolute (16 – 17%) and relative (17 – 20%) lung weights in males and females. *Histopathology:* minimal or slight hypertrophy or hyperplasia of the epithelium of single bronchi, bronchioles or terminal bronchioles in all males and females, minimal/slight bronchiolo-alveolar hyperplasia in 8/10 males and 9/10 females. **0.005 mg/L:** *Histopathology:* minimal multifocal bronchiolo-alveolar hyperplasia in 2/10 males. **0.001 mg/L:** No treatment-related adverse findings. **NOAEC for local toxicity at the respiratory tract was 0.001 (0.00094) mg/L. The NOAEC for general, systemic toxicity was 0.005 (0.0047) mg/L (decreased bw gain).**	Ma-Hock *et al* (2014) (study acceptable)

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

Neurotoxicity

In an acute neurotoxicity study in rats (Minnema, 1993) a single oral administration (gavage) of dicamba at dose levels of 0, 300, 600, or 1200 mg/kg bw resulted in one unscheduled death and in decreased mean body weight gain and food consumption in high dose males. Dose dependent neurobehavioral effects were apparent in all treated groups at 1.5 ± 1 hours after dosing. The overall effect of treatment could be described as a stimulus- or stress-induced rigidity based on the increased frequency in treated animals exhibiting rigidity in handling/body tone, impairment of respiration, flattened and/or raised posture, impairment of gait, hypoalertness, significantly decreased number of rears/minute, freezing in response to touch, abnormal righting reflex (uncoordinated, landing on side, landing on back), increased tail flick latency time, decreased forelimb and hind limb grip strength, and decreased activity during the first 10 to 15 minutes of the 40-minute locomotor activity session.
At the day 7 neurobehavioral evaluation, differences were restricted to a few parameters (forelimb grip strength, auditory startle) in high dose rats (statistically significant in males only). At the day 14 neurobehavioral examination there was no differences between dicamba-treated animals and vehicle control animals, demonstrating that the neurobehavioral changes were transient. There were no neurohistopathological findings that could be related to treatment. It was not possible to establish a NOAEL following a single high dose in rats.

Administration of single oral doses of dicamba to domestic hens at a dose level of 316 mg/kg bw (LD_{50}) was poorly tolerated (Roberts *et al*, 1983). However, there were none of the classical clinical signs of ataxia indicating delayed neurotoxicity at this or lower dose levels. The clinical signs of toxicity observed at all doses included unsteadiness, inability to walk, collapsing when moved and lying on the floor with legs outstretched or lying on one side. The first signs were noted within one hour of dosing and some birds were recumbent for up to 15 days before showing signs of recovery with animals in the lower dose groups recovering faster. In the high dose group, these clinical signs were accompanied by body weight loss and decreased food consumption during the first 10 to 14 days after treatment with recovery after this period of time. The microscopic examination revealed no neurohistopathological lesions in the brain and spinal cord of hens administered dicamba. Lesions of the sciatic nerve were restricted to the high dose level (316 mg/kg bw) and were considered secondary to nerve entrapment resulting from the recumbency rather than from a direct toxic effect of dicamba. The results of the study revealed no indication for delayed neurotoxicity.

It was not possible to establish a NOAEL following a single high dose, but in the subchronic neurotoxicity study a NOAEL of 401.5 mg/kg bw/day for neurotoxicity was determined (Minnema, 1994). The observed effects in the acute neurotoxicity study at 300 (261) mg/kg, which were generally observed 1.5 hours after administration only (Minnema, 1993), might be due to the higher systemic peak concentrations of dicamba after oral gavage compared to dietary administration of an even higher dose.

Three standard single dose inhalation studies performed with rats according to OECD 403 at doses ranging from 1 to 5.19 mg/L. In all three studies, evidence of specific target organ toxicity was seen in the form of clinical signs.

In the study by Nagy (2010), where Wistar rats were exposed to 3.98, 4.5 and 5.01 mg/L (nose only) signs of narcotic effects such as ataxia, lethargy and eyes partially closed were seen in animals in all groups during the first week after exposure. Ataxia and lethargy were not observed later than 1 day after dosing (Nagy 2010). In the study by Durnado (2015), all animals showed hypoactivity after dosing (5.14 mg/L) and in Kilgour (2001) decreased activity was noted in all animals (doses: 1.182, 2.676 and 5.191 mg/L), and in the highes dose group reduced foot withdrawal reflex and reduced response to noise were also observed.

Adverse clinical signs (ataxia, stiffening of the body when held, crusts around nose/muzzle) were recorded in the rat developmental toxicity study (Smith, 1981) on the first day of dosing at 400 (362) mg/kg bw. This dose level resulted in on 4 deaths on GD7 and 8. There were no adverse clinical signs at lower dose levels (64 and 160 mg/kg bw/day). In the rabbit developmental study ataxia was also observed at 300 (271) mg/kg bw/day and 150 (136) mg/kg bw/day up to the day efter last dosing (GD19) (Hobermann, 1992).

Clinical signs were not reported in the acute oral study and in the acute dermal study no clinical signs were oberved. Transiently abnormal gait including ataxia has also been observed in repeat studies in dogs at 300 (274) mg/kg bw/day (Jackson, 2003) and at at 300 (285) mg/kg bw/day (Kubaszky, 2010). In rats at a dose > 1000 mg/kg bw for 4 weeks impaired mobility of hind extremities was observed (Goldenthal 1979). In a 2-generation study in rats reported clinical signs during lactation included tense/stiff body tone and slow righting reflex for a few days during the latter part of lactation at 5000 ppm (424 mg/kg bw/day) (Masters, 1993).

Respiratory irritation
As described above, three standard single dose inhalation studies were performed in rats. In the study by Kilgour (2001), Alpk:AP_fSD (Wistar derived) rats were exposed nose only to aerosilised Dicamba at 1.182, 2.676 and 5.191 mg/L (measured concentration) for 4 h. The animals were devided in 3 groups of 5 males and 5 females. An LD_{50}

of 4.46 and >5.19 mg dicamba/L (technical material) was established. Changes indicative of irritation of the upper respiratory tract was seen at all doses and included increase in breathing depth and abnormal respiratory noice. At ≥ 2.68 mg/L, the breathing rate was reduced and at ≥ 5.19 mg/L laboured breathing was further observed. (Kilgour 2001).
Similar effects were seen in Nagy (2010), where CRL:(W1)BR Wistar rats were exposued to mean doses of 5.01, 3.98 and 4.5 mg/L nose only during 4 hours. In all groups, significant clinical signs commonly noted on the day of exposure and continuing during the observation period included laboured, noisy, gasping respiration and sneezing. Irregular respiration was also reported in the third study by Durnado (2015), where Sprague Dawley rats were exposed to 5.14 mg/L (nose only, 4 h).

A 28 day inhalational study is also available for Dicamba (Ma-hock et al., 2014). The study was performed according to OECD 412 on Crl:WI(Han) Wistar rats. The animals were exposed nose only to dust at doses of 0, 0.001, 0.005, 0.05 mg/L 6 hours/day, 5 days/week for 4 weeks. At the highest dose, the histopathology showed minimal or slight hypertrophy or hyperplasia of the epithelium of single bronchi, bronchioles or terminal bronchioles in all males and females and minimal/slight bronchiolo-alveolar hyperplasia in 8/10 males and 9/10 females. This indicates a local toxicity at the respiratory tract and the NOAEC for this endpoint was 0.001 (0.00094) mg/L. The NOAEC for general, systemic toxicity was 0.005 (0.0047) mg/L (decreased bw gain).

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

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. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed effects are considered.

STOT, SE - narcotic effects
In single dose oral studies in both the rat and hen there was no evidence of neuropathology at doses up to peri-lethal levels. In the hen adverse clinical signs were only observed at dose levels that induced some lethality. In the rat, adverse clinical signs at dose levels which did not induce lethality were seen within 1.5 hours of dosing at doses ≥ 300 mg/kg bw in the neurotoxicity study. These neurobehavioural effects include rigidity in handling/body tone, impairment of respiration, flattened and/or raised posture, impairment of gait (all animals), hypoalertness and abnormal righting reflex and reduced activity amongst others (see table 18). All effects were reversible and non-existing after 7 days. and in the absence of any evidence of neuropathology these transient effects in rats are considered not to be evidence of significant or severe toxicity or to be changes that clearly indicate functional disturbance of toxicological relevance.
In the acute inhalation studies, neurobehavioral effects after the single exposure were observed at all dose levels. These effects include ataxia and lethargy as well as reduced activity and reduced reflexes in forms of reduced foot withdrawal reflex and reduced response to noise.

Further, in several chronic studies signs of narcotic effects such as ataxia and reduced righting reflex were observed on the days after dosing.

The criteria for classifying substances as Category 3 for narcotic effects observed in animal studies are according to section 3.8.2.2.2 (b):

"Narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure."

Since effects as for example ataxia, lethargy, reduced reflexes and reduced activity were observed in rats after single exposure in both acute oral and acute inhalation studies and after dosing in chronic studies, a classification for narcotic effects is considered appropriateAs the effects are transient in nature, a STOT-SE category 3 H336 should apply.

STOT SE - Respiratory effects

In single dose inhalation studies, clear signs of respiratory tract irritation was found at all dose levels. Signs of irritation were increased in breathing depth and abnormal respiratory noice, reduced breathing rate and laboured breathing at the highest dose. Furthermore, historpathological changes in the lungs found in a 28-day inhalational study indicate local toxicity of dicamba in the respiratory tract that could explain the clinical signs of irritation.

According to the CLP regulation, classifying for respiratory tract irritation should be based on human data and animal data can be used part of a weight of evidence evaluation. According to section 3.8.2.2.1. (d), "*there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation".*

For dicamba, no human data is available for single exposure by inhalation. However, there are strong indications from the animal studies, that respiratory tract irritation occurs. This is based on the reversible effects on the respiration of the animals in the single dose inhalation studies, but also on histopathological changes found in the lungs in the 28-day inhalation study. This indicates that a classification as STOT, SE 3 H335 is warranted..

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

Proposed classification: STOT SE 3; H336: May cause drowsiness or dizziness and H335: may cause respiratory tract irritation.

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

Summary of the Dossier Submitter's proposal

The DS proposed the classification of dicamba as STOT SE3 H336 (may cause drowsiness or dizziness) and H335 (may cause respiratory tract irritation) based on the results of the acute inhalation toxicity studies.

Comments received during consultation

One MSCA supported the proposed classification of dicamba as STOT SE3 H336 (may cause drowsiness or dizziness) and STOT SE 3 H335 (may cause respiratory tract irritation). This MSCA also highlighted that there is an immediate action (sneezing and rhinitis) as the body tries to get rid of the substance and once it gets into the lower respiratory tract it causes morphological changes that presumably take somewhat longer to manifest. This MSCA also highlighted that this evidence also could potentially be relevant for classification for STOT RE.

One company downstream user and one company manufacturer commented that the STOT SE 3 classification is not required since narcotic effects or respiratory tract irritation were not noted in human data and that dicamba is highly water soluble, while narcotic effects are reported for fat soluble substances. The manufacturer company also commented that the narcotic effects noted in the animal studies are peripheral effects due to general toxicity, while the respiratory irritation observed is not sufficiently severe to trigger classification. The DS responded that the criteria for classification are met in the animal studies that report ataxia, lack of coordination and lethargy.

Assessment and comparison with the classification criteria

The table above summarises the animal studies on acute toxicity. The table below summarises other single exposure toxicity studies.

***Table**: Summary of single dose animal studies with dicamba other than acute toxicity studies.*

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td>Acute neurotoxicity (oral)

OECD 424

GLP

Charles River Crl:CD®BR rats

10/sex/group

Dicamba (technical material)

Purity: 86.9%

0, 300, 600 or 1200 mg/kg bw

Single oral gavage dose

The dose levels applied correspond to 261, 521 and 1043 mg/kg bw/day of pure dicamba

Vehicle: corn oil

Positive control: acrylamide</td><td>1/10 males found dead on day 1 at 1200 mg/kg bw

<u>Signs of neurotoxicity after 1.5 ± 1 hours</u>

<table>
<tr><th></th><th colspan="3">mg/kg bw</th></tr>
<tr><th></th><th>1200</th><th>600</th><th>300</th></tr>
<tr><td>Rigidity in handling/body tone</td><td>8 M
10 F</td><td>8 M
8 F</td><td>5 F</td></tr>
<tr><td>Impairment of respiration</td><td>4 M
5 F</td><td>2 M
1 F</td><td></td></tr>
<tr><td>Flattened and/or raised posture</td><td>5 M
6 F</td><td>5 M
6 F</td><td></td></tr>
<tr><td>Impairment of gait</td><td>10 M
10 F</td><td>10 M
10 F</td><td></td></tr>
<tr><td>Hypo alertness</td><td>7 M</td><td>4 M
2F</td><td></td></tr>
<tr><td>Rears/minute</td><td>0.7 vs 4.4 control</td><td>1.7 vs 4.4 control</td><td>2.1 vs 4.4 control</td></tr>
<tr><td>Abnormal righting reflex</td><td>9 M
10 F</td><td>10 M
9 F</td><td>7 M
8 F</td></tr>
<tr><td>Tail flick latency time</td><td>↑ 87% M</td><td>↑ 54% M</td><td></td></tr>
<tr><td>Fore limb grip strength</td><td>↓ 29% M</td><td>↓ 19% M</td><td>↓ 15% M</td></tr>
<tr><td>Auditory startle maximum input voltage</td><td>11.1 vs 33.4 (control) mV in M</td><td></td><td></td></tr>
<tr><td>Auditory startle average input voltage</td><td>2.1 vs 7.6 (control) mV in M
2.0 vs 5.4 (control) mV in F</td><td></td><td></td></tr>
</table>

<u>Signs of neurotoxicity after 7 days</u>

<table>
<tr><th></th><th>mg/kg bw</th></tr>
<tr><th></th><th>1200</th></tr>
<tr><td>Fore limb grip strength</td><td>↓ 15% M</td></tr>
<tr><td>Auditory startle maximum input voltage</td><td>623.6 vs 1524 (control) mV M</td></tr>
<tr><td>Auditory startle average input voltage</td><td>109.2 vs 2437.7 (control) mV M</td></tr>
</table>

No effects at 600 and 300 mg/kg bw/day

<u>Signs of neurotoxicity after 14 days</u>

No differences from control</td><td>KCA 5.7.1/01</td></tr>
<tr><td>Acute delayed neurotoxicity

US-EPA FIFRA</td><td><u>316 (274 pure) mg/kg bw</u>

9/20 animals died</td><td>KCA 5.7.2 / 01</td></tr>
</table>

GLP	Body weight: weight loss during the first two weeks of the experiment
Hen *Gallus gallus domesticus*,	<u>158 (137 pure) mg/kg bw</u>
	1/10 birds found dead day 5
10/group in control, low and mid dose group, positive control	Body weight gain:↓ 67%
	Food consumption: ↓ days 1 to 3
	<u>79 (69 pure) mg/kg bw</u>
20/group high dose group	No mortality
	Body weight development similar to control
Dicamba (technical material)	Food consumption: ↓ days 1 to 3
	<u>Effects at all doses</u>
Purity: 86.82%	Reversible unsteadiness, inability to walk, collapsing when moved and lying on the pen floor with legs outstretched or lying on one side
0, 79 (¼ LD_{50}), 158 (½ LD_{50}), 316 mg/kg bw (LD_{50})	Does not induce delayed neurotoxicity in hens
Single oral dose	
Vehicle: corn oil	
Positive control: TOCP	

Data shown in both the tables above suggest that dicamba, after a single dose, seems to be able to induce three different adverse effects; specifically: neurotoxicity, respiratory irritation, and narcotic effects.

Neurotoxicity

In an acute neurotoxicity study in rats a single dose of dicamba resulted in dose-dependent neurobehavioral alterations 1.5 h after dosing. These effects were stimulus- or stress-induced rigidity, impairment of respiration, flattened and/or raised posture, impairment of gait, hypo alertness, reduction in the number of rears/minute, freezing in response to touch, abnormal righting reflex, increased tail flick latency time, decreased forelimb and hind limb grip strength, and decreased activity during the first 10 to 15 minutes of the locomotor activity session (Table above). Most of these effects were notably reduced 7 days after exposure and all were absent 14 days after exposure demonstrating the transient nature of the neurotoxicity. No histopathological findings could be associated to treatment. These two points (lack of histopathological findings and transient nature of the neurological alterations) led RAC to not consider the results of this study sufficient to support classification.

The delayed neurotoxicity study in hens showed that dicamba induced several reversible effects, such as unsteadiness, inability to walk, collapsing when moved and lying on the pen floor with legs outstretched or lying on one side (Table above). However, again no histopathological lesions were noted and moreover these effects appeared at doses in the same order of magnitude of LD_{50}. Overall, RAC considers that the results of this study do not indicate that classification of dicamba as STOT SE is warranted.

No neurotoxicity or other systemic toxicity was reported in the acute oral or dermal toxicity studies. However, the acute inhalation toxicity studies reported neurotoxicity. KCA 5.2.3/02 study reported in all the animals decreased activity and salivation, hunched posture, piloerection, coldness to touch, reduced foot withdrawal reflex and reduced response to sound at 5.19 mg/L (Table "Summary of animal studies on acute toxicity with dicamba", above). However, these effects were reported at a dose level causing 4/10 mortalities, which in the opinion of RAC, precludes these effects for classification in order to avoid a double classification with acute toxicity. In study KCA 5.2.3/03 ataxia, lethargy, hunched posture, tiptoe gait, eye partially closed and emaciation in some survivors during the first week of the observation period but not later. Ataxia and lethargy were not observed later than 1 day after dosing. Two of the three doses considered in the KCA 5.2.3/03 study induced mortality in at least 1/5 animals. Finally, hypoactivity were reported at 5.14 mg/L in study KCA 5.2.3/01 but it is again to be noted that this dose is close to the proposed LD_{50} and therefore not suitable for classification for STOT SE.

Overall, RAC notes that the neurotoxic effects reported in the acute inhalation toxicity studies is a concern but is not strong enough to support classification of dicamba for STOT SE.

Respiratory irritation

Respiratory tract irritation (laboured breathing, changes in breathing depth and/or rate, abnormal respiratory noise) were noted at all three dose levels in the KCA 5.2.3/02 acute inhalation study in rats (Table "Summary of animal studies on acute toxicity with dicamba", above). These respiratory effects were transient and most animals had recovered by day 3. Similar effects (laboured, noisy, gasping respiration and sneezing) were reported in the KCA 5.2.3/03 acute inhalation toxicity. It is noteworthy that in contrast to the neurotoxicity reported in these studies, these respiratory effects were reported at doses causing low or no lethality.

Narcotic effects

Signs of narcotic effects such as ataxia, lethargy and eyes partially closed were seen in Wistar rats exposed (nose only) to 3.98, 4.5 and 5.01 mg/L of dicamba for 4 h in KCA 5.2.3/03 study (Table "Summary of animal studies on acute toxicity with dicamba", above). Lethargy and ataxia were not observed later than 1 day after dosing. In the KCA 5.2.3/01 acute inhalation toxicity study, all animals showed hypo activity after dosing (5.14 mg/L) and in KCA 5.2.3/02 study decreased activity was noted in all animals (doses: 1.182, 2.676 and 5.191 mg/L) and in the highest dose group reduced foot withdrawal reflex and reduced response to noise were also observed (Table "Summary of animal studies on acute toxicity with dicamba", above). Moreover, in the acute neurotoxicity study (Table above), hypo alertness was reported in 7/10 animals exposed to 1200 mg/kg bw dicamba and in 6/10 animals exposed to 600 mg/kg bw together with abnormal righting reflex in the majority of animals exposed to all tested dose levels. Overall, RAC notes

that there is sufficient evidence that dicamba induces narcotic effects after single exposure.

Comparison with the criteria

Narcotic effects

The criteria for classifying substances as Category 3 for narcotic effects observed in animal studies are, according to section 3.8.2.2.2 (b) of the Guidance on the Application of the CLP Criteria: "*Narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure*". Data from both tables above show that dicamba induced reversible ataxia, lethargy, eyes partially closed, hypo activity, decreased activity, reduced foot withdrawal reflex, reduced response to noise, hypo alertness and abnormal righting reflex. Considering that there are no guidance values for Category 3 and that evidence for narcotic effects at any dose level should be considered, RAC concludes that the conditions for classification in Category 3 are met.

Respiratory effects

There are no human data indicating respiratory effects of dicamba. However, there is strong evidence from acute inhalation toxicity studies with rats that reversible respiratory irritation occurs. This evidence includes increase in breathing depth and abnormal respiratory noise, reduced breathing rate and laboured breathing (Table "Summary of animal studies on acute toxicity with dicamba", above). Moreover, this evidence is supported by histopathological changes (slight hypertrophy or hyperplasia of the bronchi epithelium and minimal/slight bronchiolo-alveolar hyperplasia) in lungs found in a 28-day inhalational study, indicating local toxicity in the respiratory tract (Table "Summary for repeated dose toxicity studies in animals with dicamba", under STOT RE", below). Considering that there are no guidance values for Category 3 and evidence for respiratory tract irritation at any dose level should be considered, RAC concludes that classification for respiratory tract irritation is warranted.

Overall, RAC supports the DS's proposal for **classification of dicamba as STOT SE Cat 3, H335 (may cause respiratory tract irritation) and H336 (may cause drowsiness or dizziness).**

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) [equivalent to section 10.12 of the CLH report template]

Table 20: 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	Reference
Oral studies			

Dicamba **Volume 1 – Level 2**

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
4 week oral range-finding study Pre OECD guideline and GLP. No haematology, clinical chemistry or pathology conducted. Non-GLP Rat: Charles River CD® 5/sex/group (dietary)	Dicamba (Banvel technical; batch 52625110; purity 86.82%) Dietary study, 0, 5000, 7500, 10000, 12500, 15000 ppm. Doses correspond to 0, 551, 775, 1022, 1314, 1602 mg/kg bw/day for males and 541, 816, 1054, 1324, 1607 mg/kg bw/day for females at 5000, 7500, 10000, 12500, 15000 ppm, respectively 4-week duration *Corrected for purity corresponds to 493, 693, 914, 1175 and 1432 mg/kg bw/day for males at 5000, 7500, 10000, 12500 and 15000 ppm, respectively, and 484, 729, 942, 1184 and 1436 mg/kg bw/day for females at 5000, 7500, 10000, 12500 and 15000 ppm*	**15000 ppm (approx.1602 mg/kg bw/day for males & 1607 mg/kg bw/day for females):** 3/5 males and 4/5 females impaired mobility in hind extremities. *Body weight gain*: ↓ 39.0% males, ↓ 23.0% females week 4 *Food consumption*: ↓ 35.6% males, ↓ 29.3% females week 1-4 **12500 ppm (approx. 1304 mg/kg bw/day for males & 1324 mg/kg bw/day for females):** 1/5 male impaired mobility in hind extremities. *Body weight*: ↓ 23.7% males, ↓ 12.8% females week 4 *Food consumption*: ↓ 24.9% males, ↓ 20.7% females week 1-4 **10000 ppm (approx. 1022 mg/kg bw/day for males & 1054 mg/kg bw/day for females):** *Body weight*: ↓ 11.2% males week 4 *Food consumption*: ↓ 16.9% males, 12 % females week 1-4 **7500 ppm (approx.775 mg/kg bw/day for males & 816 mg/kg bw/day for females) and 5000 ppm (approx. 551 mg/kg bw/day for males & 541 mg/kg bw/day for females):** No adverse effects reported. **NOAEL: 775 mg dicamba/kg bw/day in females and 816 mg dicamba/kg bw/day in males (7500 ppm) based on reduced body weight gain and food consumption**	Goldenthal (1979) (range-finding study supportive of risk assessment)

Dicamba **Volume 1 – Level 2**

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
90-day oral toxicity. OECD 408, May 1981 GLP Rat; HanIbm: WIST (Wistar) 10/sex/group main groups; 10/sex control and top dose for recovery (dietary)	Dicamba (technical material; batch 52504710; purity 89.4%) 0, 500, 3000, 6000, 12,000 ppm. Equivalent to 0, 40.1, 239, 479, 1000 mg/kg bw/day (males); 0, 43.2, 266, 535 and 1065 mg/kg bw/day (females). Vehicle: diet 13-week duration plus 4-week recovery *Corrected for purity corresponds to 35.8, 213, 429, and 894 mg/kg bw/day in males, and 38.6, 238, 479, 952 mg/kg bw/day in females at 500, 3000, 6000 and 12000 ppm, respectively*	**12000 ppm (males 1000 mg/kg bw/day, females 1065 mg/kg bw/day)**: ↓ activity, transient hypothermia 20/20 males, 20/20 females *Body weight gain*: ↓ 28% males; 40.4% females (weeks 0-13). *Food consumption*: ↓ 13% both sexes weeks 0-13 *Haematology week 12*: ↑ 5.2% lymphocytes males; ↓ platelets 11.0% males, 12.4% females; ↓ partial thromboplastin times 7% males, 6% females; ↓ haemoglobin and RBC 3.8% females; ↑ 28.3% WBC females. *Clinical chemistry week 12*: 15% plasma proteins (males) and ↓ 16-23.3% globulins both sexes, ↑ 28.9-75.7% ALT, ALP and AST activities both sexes (note female ALP ↑ 75.7%); ↑ 136% GGT, ↑ 62.2% triglyceride, ↑ 31.6% cholesterol, ↑ 15.5% creatinine and ↑ 20.1% phosphorous values for females; ↓ 25.7% cholesterol, ↓ 47.6% triglycerides and ↓ 13.6 % glucose, ↑ 19.6% urea for males. *Week 17*: ↑ 38.9% ALP and ↑ 34.1% phosphorous in females. *Urinalysis:* ↑ 12/20 females uric acid crystals in urine week 12 (control 1/20) *Liver weights rel to bw week 13:* ↑ 23.0% males, 20.5% females (% bw). *Histopathology 13 weeks*: ↑ 4/10 females minimal to slight centrilobular hepatocyte hypertrophy and 5/10 females minimal to moderate hepatocellular pigmentation. ↓ adipose tissue after treatment in 1/10 male and 6/10 females, correlated with decreased terminal bodyweight. **6000ppm (males 479 mg/kg bw/day, females 535 mg/kg bw/day)**: ↑ 6/10 females uric acid crystals in urine week 12. **3000 ppm (males 239 mg/kg bw/day, females 266 mg/kg bw/day)**: No effects observed. **500 ppm (males 40.1 mg/kg bw/day, females 43.2 mg/kg bw/day)**: No effects observed. **NOAEL 6000 ppm (479 and 535 mg/kg bw/day in males and females, respectively).**	Doubovetzky (1997) (study acceptable)

Dicamba **Volume 1 – Level 2**

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Subchronic neurotoxicity study. OECD 424 (1997). GLP Rat, Charles River Crl:CD®BR, 10/sex/group (dietary)	Dicamba (technical material; purity: 86.9%) 0, 3000, 6000 and 12000 ppm Actual doses 0, 197.1, 401.5 and 767.9 mg/kg/day for the males and 253.4, 472.0 and 1028.9 mg/kg/day for females. Continuous in the diet for 13 weeks *The dose levels applied correspond to 171, 348 and 667 mg/kg bw/day of pure dicamba in males, and to 220, 410, 894 mg/kg bw/day of pure dicamba in females at 3000, 6000 and 12000 ppm, respectively.*	**12000 ppm (males** 767.9 **mg/kg bw/day, females** 1028.9 **mg/kg bw/day)**: *Body weight*: ↓ 5.5% males, 4.8% females week 14 *Body weight gain*: ↓ 24.1% males, 37.9% females week 1 *FOB*: ↑ frequency of rigid body tone when handled in weeks 4, 8 and 13 (greater in females than males). *Pathology*: No treatment-related changes in any of the tissues examined **6000 ppm (males 401.5 mg/kg bw/day, females 472 mg/kg bw/day)**: No treatment-related effects. **3000 ppm (males 197.1 mg/kg bw/day, females 253.4 mg/kg bw/day)**: No treatment-related effects. **NOAEL for neurotoxicity and systemic toxicity is 6000 ppm (401.5 mg/kg bw /day in males and 472 mg/kg bw/day in females), based on decreased body weight gain and neurobehavioral findings.**	Minnema (1994) (study acceptable)

Dicamba **Volume 1 – Level 2**

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Combined chronic toxicity/carcinogenicity. OECD 453, 87/302/EEC B.33 (1988) GLP Rat, Charles River CD (Sprague Dawley) 60/sex (50/sex/group main study, 10/sex/group interim kill after 12 months) (dietary)	Dicamba (technical material; purity 86.8%) Continuous in the diet 0, 50, 250, 2500 ppm for 115 weeks (males), 118 weeks (females). The doses correspond to 2.0, 10.0, and 99.1 mg/kg bw/day for males and 2.4, 12.1, and 120.1 mg/kg bw/day for females *Actual doses corrected for purity corresponds to 1.7, 8.7, and 83.0 mg/kg bw/day of pure dicamba for males, and to 2.1, 10.5, and 104 mg/kg bw/day of pure dicamba for females, at 50, 250, and 2500 ppm, respectively.*	**Non-neoplastic findings** **2500 ppm (males 99.1 mg/kg bw/day, females 120.1 mg/kg bw/day)**: *Food consumption:* ↑ 2.6% males during first year *Pathology*: ↑ incidence of liver necrosis in males (5/49 in control vs 11/50 at 2500 ppm), Slight ↑ hydronephrosis of kidney in males (1/49 in control vs 4/50 at 2500 ppm) and females (0/49 in control vs 3/49 at 2500 ppm) Slight ↑ cystic hyperplasia in the uterus (15/49 in control and 20/49 at 2500ppm) Carcinogenicity: ↑ incidence of thyroid parafollicular (C-cell) carcinoma in males ↑ increase in polyps in the uterus (4/60 in control, 8/60 at 2500 ppm) **250 ppm (males 10 mg/kg bw/day, females 12.1 mg/kg bw/day)**: Carcinogenicity: ↑ incidence of thyroid parafollicular (C-cell) carcinoma in males but within historical control range No other toxicologically significant treatment-related effects. ↑ increase in polyps in the uterus (4/60 in control, 8/60 at 2500 ppm) **50 ppm (males 2 mg/kg bw/day, females 2.4 mg/kg bw/day)**: No toxicologically significant treatment-related effects. **Neoplastic findings** NOAEL for carcinogenicity 250 ppm (equivalent to 10 mg/kg bw/day in males) based on increased incidence of thyroid parafollicular (C-cell) carcinoma in males outside historical control range. NOAEL supported by increase in polyps in the uterus at high dose. NOAEL systemic: 250 ppm (10 mg/kg bw/day) based on liver necrosis in and increase in cystic hyperplasia in the uterus at top dose The lowest survival at 104 weeks was 42 % in high dose males.	Goldenthal (1985) (study acceptable)

<table>
<tr>
<td>Carcinogenicity study. OECD 451 (1981), 87/302/EEC B.32 (1988)
GLP
Mouse, Charles River CD-1
52/sex/group (dietary)</td>
<td>Dicamba (technical material; purity 86.8%)
Continuous in the diet 0, 50, 150, 1000 and 3000 ppm for 89 weeks (males) or 104 weeks (females).

The average compound consumption was 5.5, 17.2, 108, and 358 mg/kg/day for the males and 5.8, 18.8, 121, and 364 mg/kg/day for females.

The average compound consumption then corresponds to 4.8, 14.9, 93.7 and 311 mg/kg bw/day of pure dicamba for males, and to 5.0, 16.3, 105, 316 mg/kg bw/day of pure dicamba for females, at 50, 150, 1000 and 3000 ppm, respectively.</td>
<td><u>Non-neoplastic findings</u>
<u>3000 ppm (males 358 mg/kg bw/day, females 364 mg/kg bw/day)</u>:
Body weight gain: ↓ females from week 25 (12% week 1-52, 17% week 1-104).
Pathology: slightly increased incidence of amyloidosis in males in heart, parathyroid, thyroid, spleen, kidney and adrenal
<table>
<tr><th rowspan="2">Dose (ppm)</th><th colspan="5">Males</th></tr>
<tr><th>0</th><th>50</th><th>150</th><th>1000</th><th>3000</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Thyroid, Amyloidosis</td><td>7/52</td><td>7/28</td><td>9/34</td><td>4/21</td><td>11/52</td></tr>
<tr><td>Parathyroid, Amyloidosis</td><td>5/52</td><td>5/28</td><td>5/34</td><td>4/21</td><td>11/52</td></tr>
<tr><td>spleen, Amyloidosis</td><td>4/52</td><td>6/31</td><td>10/38</td><td>5/23</td><td>11/52</td></tr>
<tr><td>adrenals, Amyloidosis</td><td>6/52</td><td>6/28</td><td>8/34</td><td>5/21</td><td>14/52</td></tr>
<tr><td>adrenals, medullary hyperplasia</td><td>16/52</td><td>5/28</td><td>7/34</td><td>5/21</td><td>7/52</td></tr>
<tr><td>heart , Amyloidosis</td><td>7/52</td><td>8/28</td><td>11/34</td><td>5/22</td><td>16/52</td></tr>
<tr><td>Kidney, glomeriolar amyloidosis</td><td>12/52</td><td>13/52</td><td>14/52</td><td>13/52</td><td>20/52</td></tr>
</table>
<u>1000 ppm (males 108 mg/kg bw/day, females 121 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.

<u>150 ppm (males 17.2 mg/kg bw/day, females 18.8 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.

<u>50 ppm (males 5.5 mg/kg bw/day, females 5.8 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.

<u>Neoplastic findings</u>
No treatment-related changes in neoplastic findings at any dose level.

NOAEL: 1000 ppm (equivalent to 108 mg/kg bw/day in males) based on slightly higher incidence of amyloidosis in amyloidosis in males in heart, parathyroid, thyroid, spleen, kidney and adrenal and 1000 ppm in females (121 mg/kg bw/day) based on decreased bw gain at 3000 ppm.</td>
<td>Crome (1988) (study acceptable)</td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		Termination week 89 survival 30% in males in the 150 and 3000 ppm groups; week 104 survival at least 35% all female groups. At 78 weeks the survival in all groups exceeded 50 %.	
13-week oral (capsule) toxicity OECD 409, 1998 GLP Dog: purebreed Beagles 4/sex/group, plus an additional 4/sex/group for control and top dose 4-week recovery phase	Dicamba (technical material; batch B2826511; purity 90.4%) 0, 10, 50, 300 mg/kg bw/day Capsule administration. No vehicle 13-week duration plus 4-week recovery *Considering the purity of dicamba used for this study (90.4%), the applied doses referring to pure dicamba (100% purity) correspond to 9.0, 45, 274 mg/kg bw/day.*	**<u>300 (274) mg/kg bw/day:</u>** *Clinical observations*: Hind limb gait abnormalities noted from day 1: ataxia, stiff gait and sporadic transient collapse generally seen in the majority of the 300 mg/kg animals approximately 2 hours after dosing and persisting for up to 5 hours. The neurological screen at wk 6 and 13 showed abnormal locomotion and gait abnormalities in all animals. No effects detected following the recovery phase. *Mean bw gain:* ↓ 26 % in males and 44 % in females (not statistically significant) *Food consumption:* 90% of control for males and 84% of control for females *Haematology:* ↓ 9-17.7% RBC, Hct and Hb week 7 and 13 both sexes. ↑ 10.5% APPT activity in males and 7% in females at week 13, but signs of reversibility following recovery. *Clinical chemistry:* ↓ 24.6-32.4% cholesterol and phospholipids weeks 7 and 13. Partial improvement following recovery (no statistically significant differences from control). *Organ weights:* ↓ Not significantly decreased abs 17 % and 11 % rel testes weight. Not toxicologically significant effects (↓ absolute and relative spleen weight for males due to high control value and ↑ kidney weight as percentage body weight in females, not evident after recovery). **<u>50 (45) mg/kg bw/day:</u>** No toxicologically significant findings. **<u>10 (9) mg/kg bw/day:</u>** No treatment-related effects. **NOAEL = 50 (45) mg/ kg bw/day based on effects on gait and behaviour, decreased food intake and body weight gain, minor alterations in the red blood cell parameters and disturbances in the serum lipid levels, decreased testes weigt in the highest dose group.**	Jackson (2003) (study acceptable)
1-year dietary toxicity EPA guideline 84-1 (1982). Similar to OECD 452, but no haematology examinations at 3 months.	Dicamba (technical material; Lot: 52625110; purity 86.8%) 0, 100, 500 and 2500ppm Dietary administration. 52-week duration coresponding to 2.03, 11.4 and 57 mg/kg bw/day for males, and 2.14, 11.4, and	**<u>2500 ppm (57 mg/kg bw/day males; 51 mg/kg bw/day females)</u>** *Clinical observations:* ↑ incidence and frequency of soft faeces during first 6 months (25-75% v 25% in controls). *Body weight:* ↓ during week 1, but recovered by week 5/6 (approx. 6.5% weight loss compared with pretreatment). No overall effect (weeks 0-50). *Food Consumption:* inappetance in 1 male and 1 female during first week: a further male did only eat	Blair (1986) (study acceptable)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
GLP Dog: Beagle 4/sex/group	51 mg/kg bw/day for females. *The applied doses referring to pure dicamba (100% purity) correspond to 1.8, 9.9, 50 mg/kg bw/day for males, and 1.9, 9.9, and 44 mg/kg bw/day for females at 100, 500, and 2500 ppm, respectively.*	small amount of food during first 3 weeks, but after being fed a slurry diet, stabilised by week 6. *Hematology :* ↓ statistically significant changes in the red blood cell values in high dose males at the 6 month investigation (↓ ~ 9% for haematocrit, erythrocytes and *haemoglobin).* *Clinical chemistry:* At 6 months females only: ↓ 10.9 % calcium, ↓ 6.9 % total protein, ↓ 24 % globulin, ↑ 31.3% Aspartate aminotransferase. *Organ weight:* ↓ ovary weight (30 % absolute/35 % reative), **500 ppm (11.4 mg/kg bw/day males and females):** *Body weight:*↓ week 1 (4.4 % weight loss compared with pretreatment), but recovered by week 2 and no overall effect (weeks 0-50). *Food consumption:* inappetance in 2 animals during first week of the study. **100 ppm (2.03 mg/kg bw/day males and 2.14 females mg/kg bw/day):** *Body weight:*↓ week 1 (3% weight loss compared with pretreatment), but recovered by week 2 and no overall effect (weeks 0-50). **The NOAEL was 500 ppm (11.4 for males and females)**	
RC1176: 90-day oral capsule toxicity study in beagle dogs OECD 409 (1998) GLP Dog: Beagle 4/sex/group	0, 10, 50 and 300 mg/kg bw/day Dicamba (technical material; Lot: RTM/DCMB/03/20090612; purity >95%) *Doses corresponded to 0, 9.5, 47.5, 285 at 100, 500, and 2500 ppm, respectively when corrected for purity*	**At 300 mg/kg bw/day :** ***Clinical signs*** : Intermittent stiff gait and recumbency, slight and/or moderate uncoordination or ataxia and retching or emesis were consistently recorded. On occasion, the animals also displayed slightly to severely decreased activity, liquid faeces, increased salivation, minor tonic convulsions or tremors. All animals recovered by the following morning ***Clinical chemistry parameters***: ↑ ALT in both sexes during week 13 (72%, p<0.01 in the males, and 143%, p<0.05 in the females).c ↓ cholesterol appeared to be lower than control; however, no statistically significant differences were noted when compared to control. ↓ triglyceride mean values in both males and females, attaining statistical significance in the males (-28%, p< 0.05).) ↓ ALKP mean values in females (-40%, p<0.05 at week 7), and -36%, p<0.01 at week 13.	Kubaszky R. (2010) (study acceptable)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		Haematology Significant effects in females: ↓ RBC (-17 to -20%) in weeks 7 and 13. ↓ Haemoglobin (-18%) in week 7. ↓ Haematocrit (-18%) in week 7 **50 mg/kg bw/day** ↓ ALKP mean values (-30%, p<0.05 at week 7) ***10 mg/kg bw/day :*** No toxicologically relevant effects **NOAEL was 50 (47.5) mg/kg bw/day based on clinical signs and parameters (stiff gait, uncoordination or ataxia and retching or emesis, decreased activity, liquid faeces, increased salivation, minor tonic convulsions or tremors, decreased values in the red blood count, haemoglobin and haematocrit at 300 mg/kg bw/day**	
Inhalation studies			
Repeat dose 28-day inhalation. OECD 412 EC No. 440/2008 GLP Rat: Crl:WI(Han) Wistar 10/sex/group	Dicamba (BAS 183H Technical material; batch 0002B01BA-251; purity 93.9%) Nose only exposures to dust. 0, 0.001, 0.005, 0.05 mg/L, 6 hours/day, 5 days/week for 4 weeks. *Dose levels correspond to 0.00094, 0.0047 and 0.047 mg/L of pure dicamba*	**0.05 mg/L:** *Body weight gain :* ↓ 41 % in males, 13 % in females (not statistically significant in females) *Organ weights*: ↑ absolute (16 – 17%) and relative (17 – 20%) lung weights in males and females. Not statistically significant changes in organ weight: ↓ thymus absolute and relative weight (15-19 %) in males and females. ↓ absolute and relative ovary weight (12-13%). ↑ absolute and relative adrenal weight (10 %) in females. *Histopathology:* minimal or slight hypertrophy or hyperplasia of the epithelium of single bronchi, bronchioles or terminal bronchioles in all males and females, minimal/slight bronchiolo-alveolar hyperplasia in 8/10 males and 9/10 females. **0.005 mg/L:** *Histopathology:* minimal multifocal bronchiolo-alveolar hyperplasia in 2/10 males. **0.001 mg/L:** No treatment-related adverse findings. **NOAEC for local toxicity at the respiratory tract was 0.001 (0.00094) mg/L. The NOAEC for general, systemic toxicity was 0.005 (0.0047) mg/L (decreased bw gain).**	Ma-Hock *et al* (2014) (study acceptable)

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Dermal studies			
28-day dermal OECD 410, 1981 GLP Rat: Alpk:AP_fSD (Wistar-derived) 10/sex/group	Dicamba (technical material; batch B2826511; purity 91.0%) 0, 30, 300, 1000 mg/kg bw/day for 21 days Vehicle: water used to make a paste 28-day duration, 21 applications. *Dose levels applied correspond to 27.3, 273 and 910 mg/kg bw/day of pure dicamba*	**1000 (910) mg/kg bw/day:** Histopathological signs of irritation in treated skin in 10/10 males and 10/10 females (Acanthosis/hyperkeratosis, inflammatory cell infiltration) **300 (273) mg/kg bw/day:** Histopathological signs of irritation in 10/10 males and 9/10 females, less severe than high dose. **30 (27.3) mg/kg bw/day:** Acanthosis/hyperkeratosis in 5/10 males and 1/10 females. **NOAEL for systemic toxicity > 1000 (910) mg/kg bw/day.**	Rattray (2002) (study acceptable)

Table 21: 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
None				

Table 22: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Observations	Reference
Developmental toxicity Test guideline not stated but complies largely to OECD 414 (2001) but with some notable deviations (see summary) Oral (gavage) Rat, Charles River CD 25 mated females/group	Dicamba (Technical grade; batch: 52625110; purity 90.4%) 0, 64, 160 or 400 mg/kg bw/day on days 6-19 of gestation Vehicle: corn oil *The dose levels applied correspond to 58, 145 and 362 mg/kg bw/day of pure dicamba*.	Maternal toxicity 400 (362) mg/kg bw/day: 4/25 deaths gestation day 7 & 8; ataxia, stiffening of the body when held, urine soaked fur, salivation and decreased motor activity; ↓ body weight gain (27% lower corrected maternal bw gain); ↓ food consumption (18.5% lower than controls, days 6-19). 160 (145) mg/kg bw/day 10 % lower corrected maternal bw gain (not statistically significant) 64 (58) mg/kg bw/day No effects	Smith (1981) (study acceptable)

Type of study/data	Test substance	Observations	Reference
		Maternal NOAEL 64 (58) mg/kg bw/day Developmental toxicity 400 (362) mg/kg bw/day: ↑ number of incompletely ossified frontal (s) and/or parietal(s) 64 (58) &160 (145) mg/kg bw/day: No effects Developmental NOAEL 160 (145) mg/kg bw/day	
Developmental toxicity US EPA 83-3 (complies largely to OECD 414, 2001) Oral (capsule) Rabbit, New Zealand White Hra:(NZW)SPF 20 inseminated females/group	Dicamba (Technical grade; batch: 52625110; purity 90.4%) 0, 30, 150 or 300 mg/kg bw/day on days 6-18 of gestation *The dose levels applied correspond to 27.1, 136 and 271 mg/kg bw/day of pure dicamba.*	Maternal toxicity 300 (271) mg/kg bw/day: 4/20 abortions; ataxia, rales, laboured breathing, perinasal substance, dried/no faeces, impaired righting reflex and decreased motor activity; ↓ body weight gain (42% lower than controls days 0 to 29); ↓ relative food consumption (13% lower than controls, days 0-29). 150 (136) mg/kg bw/day: 1/20 abortion; ataxia and decreased motor activity 30 (27.1) mg/kg bw/day No effects Maternal NOAEL: 30 mg/kg bw/day Developmental toxicity 300 mg/kg bw/day: increased incidence of irregularly ossified internasals. High dosis (incidence) Pups: 3.9% Litter: 23.1% **HCD 1987-1989** Pups: 0-2.3% Litter: 0-14.3% **HCD 1990-1994** Pups: 0-5 (0-4.8%) Litter: 0-4 (0-26.7%) **HCD 1992-1994** Pups: 0-4.2% Litter: 0-26.7% 30, 150 mg/kg bw/day: No effects Developmental NOAEL 150 (136) mg/kg bw/day	Hoberman (1992) (study acceptable)

Dicamba **Volume 1 – Level 2**

Type of study/data	Test substance	Observations	Reference
Two Generation Oral (continuous in diet) OECD 416 (1983) Rat, Crl:CD (SD) BR VAF/Plus 32/sex/group (F0) 28/sex/group (F1)	Dicamba (Technical material; batch 52103810; purity 86.9%) 0, 500, 1500 or 5000 ppm Vehicle: laboratory animal diet. The overall F0/F1 pre-mating doses correspond to 37.9, 113 and 389 mg/kg bw /day for males and 42.6, 130 and 424 mg/kg bw/day for females at 0, 500, 1500 or 5000 ppm, respectively. *The overall F0/F1 pre-mating means correspond to 32.9, 98.3 and 338 mg/kg bw/day of pure dicamba for males, and to 37.0, 113, 369 mg/kg bw/day of pure dicamba for females, at 500, 1500 and 5000 ppm, respectively*	***Parental toxicity*** 5000 ppm F0: mean achieved intake 347/390 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 9.6% (day 0-20: 3.2%) ↑ adjusted liver weight 13% females, 5% males F1: mean achieved intake, 432/458 mg/kg bw/day, males/ females respectively Clinical signs during lactation: tense/stiff body tone and slow righting reflex for a few days during the latter part of lactation ↓ body weight pregnancy day 0-14: 4.6% (F1A) and 23% (F1B) ↑ absolute liver weight 3% females, males 9.5% (relative) ↓ food consumption week 5-8 1500 ppm F0: mean achieved intake, 105/125 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 121/135 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14 (F1B): 15 % (day 0-20: 15%) 500 ppm F0: mean achieved intake, 35/41 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 40/44 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 9.6% (F1B) (day 0-20: 1.7%) but absolute body weight was not reduced. Otherwise, no effects **NOAEL** 500 ppm (42.6 mg/kg bw/day) on the basis of decreased body weight during pregnancy (GD 0-14) at 1500 and 5000 ppm. Clinical signs during lactation, ↑ liver weights at 5000 ppm ***Reproductive toxicity*** No effects at any dose level NOAEL 5000 ppm (389 mg/kg bw/day)	Masters, 1993

Type of study/data	Test substance	Observations	Refer-ence
		Offspring toxicity 5000 ppm F1: ↓mean pup body weight 24 % day 21, delayed sexual maturation of males by 2 days, ↑ relative liver weights 27%. F2A/B: ↓ body weight 26/30 % day 21, ↑ relative liver weights approx. 36%. 1500 ppm F1: ↓ mean pup body weight 4 % day 21 F2A/B: ↓ pup body weight 10/14 % day 21 500 ppm F2B: No effects **NOAEL**: 500 ppm (37.9 mg/kg bw/day) based on body weight effects at 1500 and 5000 ppm.	

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)

Dicamba is not a volatile compound (vapour pressure < 10^{-2} Pascal) and therefore no short term inhalation toxicity study is required.

Short-term toxicity was investigated in rats and dogs. Five oral studies are available of which three were dietary and two were with administration in capsules. Furthermore, one dermal toxicity study in rat and one rat inhalation study were available for evaluation of the short-term toxicity of dicamba. One not accepted repeated dose dermal toxicity study was also available for evaluation but not considered. Generally, the studies are old and therefore often missing to address potential neurotoxic and immunotoxic effects, genotoxicity by way of micronuclei formation and effects potentially related to changes in the hormonal system as is required in the data requirements. No immunotoxicity study was submitted. Dossier submitter evaluated that dicamba does not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic. A thorough review of the toxicology data for dicamba has shown no evidence of adverse effects on the immune system in rats, mice or dogs and functional assays in rats and goats confirmed lack of immunomodulation. Based on these findings it can be concluded that dicamba probably has no immunotoxic potential.

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Dietary administration of 0, 5000, 7500, 10000, 12500, and 15000 ppm dicamba to rats for 28 days resulted in reduced body weight gain and food consumption from 10000 ppm and above and clinical signs in form of impaired mobility of hindlimbs from 12500 ppm and higher. The NOAEL was 7500 ppm corresponding to 775 mg dicamba/kg bw/day in females and 816 mg dicamba/kg bw/day in males (Goldenthal, 1979).

Administration of dicamba to rats at dietary concentrations of 0, 500, 3000, 6000, and 12000 ppm for 90-days resulted in decrease of body weight gain and reduced food consumption at the highest dose level only. The liver was identified as target organ as indicated by an increased activity of hepatic enzymes, altered clinical chemistry parameters associated with the liver, increased relative liver weights as well as hepatocyte hypertrophy and pigmentation. In addition, a number of minor haematological changes were seen at 12000. Increased serum phosphate level changes were not reversible within 28 days following cessation of compound administration. Based on these results, the NOAEL for subchronic administration of dicamba to rats was determined to be 6000 ppm corresponding to 479 mg dicamba/kg bw/day in males and 535 mg dicamba/kg bw/day in females (Doubovetzky, 1997).

In dogs treated with dicamba in gelatine capsules at dose levels of 0, 10, 50 or 300 mg/kg bw/day (0, 9, 45 and 274 mg pure dicamba/kg/day) for 90-days treatment with 300 (274) mg/kg bw/day resulted in changes on the gait and

behaviour (stiff gait, uncoordination or ataxia and retching or emesis, decreased activity, liquid faeces, increased salivation, minor tonic convulsions or tremors), decreased food intake and body weight gain, and minor changes in the red blood cell parameters and in the serum lipid levels (decreased values in the red blood count, haemoglobin and haematocrit at 300 (274) mg/kg bw/day)). Not significantly decreased absolute (17 %) and (11 %) relative testes weight was observed without histopatological changes. Dosing with 50 (45) mg/kg bw/day resulted in slightly decreased serum lipid levels, which have not been considered to be an adverse effect. The NOAEL was considered to be 50 mg/kg bw/day in this (Jackson, 2003).

Dietary administration of dicamba to dogs for one year at dietary dose levels of 0, 100, 500, and 2500 ppm did result in statistically significant changes in the red blood cell values in high dose males at the 6 month investigation. These effects were not considered adverse at the PRAPeR 83 expert meeting. Further effects on body weight and food consumption changes were observed at 2500 ppm during the early phase of the study and were considered due to palatability problems. The dietary dose of 2500 ppm has been found to be the highest concentration of dicamba in the diet which dogs will consume. Effects on several clinical chemistry parameters were observed at 2500 ppm and a decreased ovary weight (around 30 %) Based on the result of this study, the NOAEL was 500 ppm, equivalent to a mean daily intake of approx. 11.4 mg/kg bw/day (Blair, 1986).

In a new study, dogs treated with dicamba in gelatine capsules at dose levels of 10, 50 and 300 mg/kg bw /day (0, 9.5, 47.5, 285 mg pure dicamba/kg bw/day) for 90-days. Clinical signs and parameters (stiff gait, uncoordination or ataxia and retching or emesis, decreased activity, liquid faeces, increased salivation, minor tonic convulsions or tremors, decreased values in the red blood count, haemoglobin and haematocrit were observed at 300 (285) mg/kg bw/day. NOAEL was 50 mg/kg bw/day (Kubaszky R. 2010).

The 21-day dermal study in rabbits treated with 0, 100, 500 or 2500 mg/kg bw/day was not accepted due to too few animals on study, too many accidental deaths and inadequate reporting of the study (Estes et al, 1979).

Dermal administration of 30, 300 or 1000 mg/kg bw/day (27.3, 273 and 910 mg/kg bw/day of pure dicamba) for 21 days in a 28 day period to male and female rats produced no systemic toxicity. At 300 and 1000 mg/kg bw/day there were histopathological changes in skin at the application site indicative of skin irritation with increased intensity in the high dose group. Males exposed to 30 mg/kg bw/day showed minimal histopathological signs of skin irritancy in a few animals while there were no effects in females at this dose level. The NOAEL for systemic toxicity in this study is considered to be 1000 mg/kg/day (Rattray, 2002).

In a 28-day study inhalation study, male and female rats were exposed to 0, 0.001, 0.005, 0.05 mg/L dicamba (0, 0.00094, 0.0047 and 0.047 mg/L of pure dicamba) for 20 days in total. In males, a significantly decreased body weight gain (41 %) was found at high dose. Absolute and relative thymus weight (15-17% in males and 19 % in females). In females, a non-significant decrease in absolute and relative ovary weight (12-13%) at the high dose. Based on these findings, systemic NOAEL was 0.005 (0.0047) mg/L. Local effects were also observed with increased mucous cell hyper-trophy (0.05 mg/L) found in the nasal cavity, increased epithelial alteration (≥0.001 mg/L) in larynx as well as squamous metaplasia (0.05 mg/L m). Effects observed in the lung were slightly increased lung weight (0.05 mg/L), increased incidence of alveolar histiocytosis + macrophage aggre-gation (0.05 mg/L) and multifocal bronchiolo-alveolar hyperplasia (≥0.005 mg/L) and bronchiolar hypertrophy or hyperplasia (0.05 mg/L). Based on these effects the local NOAEL was 0.001 (0.00094) mg/L (Ma-Hock et al., 2014).

Overall, the short term oral NOAEL was 50 mg/kg bw in dogs based on clinical symptoms, decreased body weight gain, haematology parameters and in rats oral NOAEL was 479 mg/kg bw/day based on decreased body weight gain, effects on liver including altered clinical chemistry parameters, relative weight and histopathology.

Dietary administration of technical dicamba to rats at dose levels of 0, 3000, 6000, and 12000 ppm corresponding to an average daily compound intake of 197.1, 401.5, and 767.9 mg/kg in males and 253.4, 472.0, and 1028.9 mg/kg in females (171, 348 and 667 mg/kg bw/day of pure dicamba in males, and 220, 410, 894 mg/kg bw/day of pure dicamba in female, respectively) for 3 months resulted in a slightly decreased body weight gain in high dose animals. The major neurobehavioral treatment-related effect in the high dose animals was an increased frequency of rigid body tone when handled throughout the study. More high-dose females than males were affected. The other findings in high dose rats may be related to rigidity. The effects included rigidity observed at weeks 4 and 13 during the landing splay test and during the righting reflex test at all post treatment FOB tests. An apparent, but non-significant,

increase in the mean latency to first step in male rats, an increased frequency of mildly impaired gait, and an increased frequency of abnormal righting reflex (i.e. uncoordinated, lands on side, or lands on back) was also observed in the high dose.
At week 13 fewer findings were observed and with lower incidence.
Administration of dicamba did not cause damage to the nervous tissues as indicated by the histopathology findings.
Based on the results of this study, the NOAEL for neurotoxicity and systemic toxicity was 6000 ppm, which is equivalent to a mean daily intake of 401 mg/kg bw and 472 mg/kg bw in males and females, respectively (Minnema, 1994).

For summary of longterm studies please see section 2.6.5 and for summary of developmental toxicity studies and 2-generation study please see 2.6.6.

Table 23: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	**Effective dose (mg/kg/day) (males/females)**	**Length of exposure**	**Extrapolated effective dose when extrapolated to 90-day exposure**	**Classification supported by the study**
Goldenthal (1979)	551/541	4 weeks	183,7/180	No
Doubouvetsky (1997)	1000/1065	90 days	1000/1065	No
Minnema (1994)	767.9 /1028.9	13 weeks	767.9 /1028.9	No
Jackson (2003)	300	13 weeks	300	No
Blair (1986)	57/51	52 weeks	228/204	No
Kubaszky (2010)	300	90 days	300	No
Ma-Hock (2014)	0.005 mg/L	28 days	0.0016 mg/L	No
Rattray (2002)	>1000	28 days	>333	No
Goldenthal (1985)	99.1/120.1 (systemic)	115 weeks (males, 118 weeks (females)	892/1081	No
Crome (1988)	>358/364	89 weeks (males), 104 weeks (females)	>2478/2944	No
Smith (1981)	160	14 days	26	No
Hoberman (1992)	150	14 days	24	No
Masters (1993)	113/130	2 generation study	113/130	No

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

According to the CLP regulation classification in STOT RE is required for substances that cause: "… consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health."
. Adverse clinical signs (ataxia, stiffening of the body when held, crusts around nose/muzzle) were recorded in the rat developmental toxicity study (Smith, 1981) on the first day of dosing at 400 (362) mg/kg bw. This dose level resulted in 4 deaths (3 pregnant, 1 non-pregnant) on GD 7 and 8. There were no adverse clinical signs at lower dose levels (64 and 160 mg/kg bw/day). In the rabbit developmental study ataxia was also observed at 300 (271) mg/kg bw/day and 150 (136) mg/kg bw/day up to the day efter last dosing (GD19) (Hobermann, 1992). Transiently abnormal gait including ataxia has also been observed in repeated dose studies in dogs at 300 (274) mg/kg bw/day (Jackson, 2003) and at at 300 (285) mg/kg bw/day (Kubaszky, 2010). In rats at a dose > 1000 mg/kg bw for 4 weeks impaired mobility of hind extremities was observed (Goldenthal 1979). In a 2-generation study in rats reported clinical signs during lactation included tense/stiff body tone and slow righting reflex for a few days during the latter part of lactation at 5000 ppm (424 mg/kg bw/day) (Masters, 1993).

Transient stiffness to handling and slow righting reflex was observed in rats in the 2-generation study at 5000 ppm corresponding to 424 mg/kg bw/day (Masters, 1993). Effects were only seen at **767.9** mg/kg bw/day when exposed via diet (Minnema, 1994). The neurobehavioural effects in this study were transient, unaccompanied by any evidence of morphological change and, consequently, are considered not to indicate significant or severe target organ

toxicity relevant for STOT-RE (please also see 2.6.2.10). For the inhalation study (Ma-Hock, 2014) was the extrapolated effective dose below the guidance values for STOT-RE, however, the effect at this dose was only seen on bw gain and not mean body weight.

Dicamba appears to be neurotoxic at doses below the guidance values for classification as STOT-RE. Nevertheless, STOT-RE classification in category 1 or 2 is not considered to be warranted since dicamba-induced neurotoxicity seems to be a transient effect.
Substances are classified as specific target organ toxicants following repeated exposure on the basis of "significant" or "severe" toxicity. In this context "significant " means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. "Severe" effects are generally more profound or serious than "significant" effects and are of a considerably adverse nature which significantly impact on health.

The effect on body weight gain was not considered significant or severe and classification for STOT-RE is not warranted for dicamba.

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

No classification.

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

Summary of the Dossier Submitter's proposal

Repeated dose toxicity of dicamba was assessed by the DS on the basis of data from short-term and long-term toxicity studies. This database included 4-week studies, 90-day studies, 1-year studies, carcinogenicity studies and combined chronic toxicity/carcinogenicity studies by the oral route in rats, mice, and dogs plus one 28-day study by the dermal route and one 28-day study by the inhalation route, both in rats. DS also reviewed the 2-generation reproductive toxicity study in rat and the developmental toxicity studies in rats and rabbits. Overall, the DS concluded that the neurotoxicity reported below the guideline values do not warrant classification of dicamba due to the transient nature of the findings and the lack of supporting histopathological evidence.

Comments received during consultation

One MSCA suggested that more attention be paid to the potential classification of dicamba for STOT RE based on: 1) The study on developmental toxicity in rats showing mortality at 400 mg/kg bw/day; 2) the abortions at 300 mg/kg bw/day in the developmental toxicity study in rabbits; 3) the multifocal bronchi-alveolar hyperplasia at 0.005 mg/L in the 28-day inhalation study in rats.

Assessment and comparison with the classification criteria

The table below summarises the short-term and long-term repeated dose toxicity studies.

***Table**: Summary for repeated dose toxicity studies in animals with dicamba.*

Method	Results			Reference
4 week oral range-finding study Non-GLP Charles River CD® rats 5/sex/group (dietary) Dicamba batch: 52625110 Purity: 86.82% Dietary study, 0, 5000, 7500, 10000, 12500, 15000 ppm Doses correspond, respectively, to 0, 551, 775, 1022, 1314, 1602 mg/kg bw/day for males and 541, 816, 1054, 1324, 1607 mg/kg bw/day for females	15000 ppm			KCA 5.3.1 / 01
		Males	Female	
	Impaired mobility in hind extremities	3/5	4/5	
	Body weight gain (week 4)	↓ 39.0%	↓ 23.0%	
	Food consumption (week 1-4)	↓ 35.6%	↓ 29.3%	
	12500 ppm			
		Males	Female	
	Impaired mobility in hind extremities	1/5	-	

Actual doses corrected for purity correspond, respectively, to 493, 693, 914, 1175 and 1432 mg/kg bw/day for males and 484, 729, 942, 1184 and 1436 mg/kg bw/day for females Vehicle: diet	Body weight gain (week 4) ↓ 23.7% ↓ 12.8% Food consumption (week 1-4) ↓ 24.9% ↓ 20.7% <u>10000 ppm</u> Males: Impaired mobility in hind extremities -; Body weight gain (week 4) ↓ 11.2%; Food consumption (week 1-4) ↓ 16.9% Females: - ; - ; ↓ 12% <u>7500 ppm and 5000 ppm</u> No adverse effects reported NOAEL = 7500 ppm (775 mg dicamba/kg bw/day in females and 816 mg dicamba/kg bw/day in males)	
90-day oral toxicity OECD 408 GLP HanIbm: WIST (Wistar) rats 10/sex/group main groups; Purity: 89.4% 0, 500, 3000, 6000, 12,000 ppm Equivalent, respectively, to 0, 40.1, 239, 479, 1000 mg/kg bw/day (males); 0, 43.2, 266, 535 and 1065 mg/kg bw/day (females). Actual doses corrected for purity correspond, respectively, to 35.8, 213, 429, and 894 mg/kg bw/day in males, and 38.6, 238, 479, 952 mg/kg bw/day in females Vehicle: diet 13-week duration plus 4-week recovery	<u>12000 ppm</u> ↓ activity, transient hypothermia 20/20 males, 20/20 females *Body weight gain:* ↓ 28% males and 40% females (weeks 0-13) *Food consumption:* ↓ 13% both sexes weeks 0-13 *Haematology week 12:* ↑ 5.2% lymphocytes males; ↓ platelets 11.0% males, 12.4% females; ↓ partial thromboplastin times 7% males, 6% females; ↓ haemoglobin and RBC 3.8% females; ↑ 28.3% WBC females *Clinical chemistry week 12:* 15% plasma proteins (males) and ↓ 16-23.3% globulins both sexes, ↑ 28.9-75.7% ALT, ALP and AST activities both sexes (note female ALP ↑ 75.7%); ↑ 136% GGT, ↑ 62.2% triglyceride, ↑ 31.6% cholesterol, ↑ 15.5% creatinine and ↑ 20.1% phosphorous values for females; ↓ 25.7% cholesterol, ↓ 47.6% triglycerides and ↓ 13.6% glucose, ↑ 19.6% urea for males. Week 17: ↑ 38.9% ALP and ↑ 34.1% phosphorous in females *Urinalysis:* ↑ 12/20 females uric acid crystals in urine week 12 (control 1/20) *Liver weights relative to bw week 13:* ↑ 23% males, 21% females (% bw) *Histopathology 13 weeks:* ↑ 4/10 females minimal to slight centrilobular hepatocyte hypertrophy and 5/10 females minimal to moderate hepatocellular pigmentation; ↓ adipose tissue after treatment in 1/10 male	KCA 5.3.2 / 01

	and 6/10 females, correlated with decreased terminal bodyweight <u>6000 ppm</u> ↑ 6/10 females uric acid crystals in urine week 12 <u>3000 ppm and 500 ppm</u> No effects observed NOAEL = 6000 ppm (479 and 535 mg/kg bw/day in males and females, respectively)	
Sub chronic neurotoxicity study OECD 424 GLP Charles River Crl:CD®BR rats 10/sex/group Dietary Dicamba (technical material) Purity: 86.9% 0, 3000, 6000 and 12000 ppm Equivalent to: 0, 197.1, 401.5 and 767.9 mg/kg/day for the males and 253.4, 472.0 and 1028.9 mg/kg/day for females Actual doses corrected for purity correspond to 171, 348 and 667 mg/kg bw/day in males and to 220, 410, 894 mg/kg bw/day in females Vehicle: diet	<u>12000 ppm</u> *Body weight:* ↓ 5.5% males, 4.8% females week 14 *Body weight gain:* ↓ 24.1% males, 37.9% females week 1 *FOB:* ↑ frequency of rigid body tone when handled in weeks 4, 8 and 13 (greater in females than males). *Pathology:* No treatment-related changes in any of the tissues examined <u>6000 ppm and 3000 ppm</u> No treatment-related effects NOAEL = 6000 ppm (401.5 mg/kg bw /day in males and 472 mg/kg bw/day in females)	KCA 5.7.1 / 02
Combined chronic toxicity/carcinogenicity OECD 453 87/302/EEC B.33 GLP Charles River CD (Sprague Dawley) rats	<u>NON-NEOPLASTIC FINDINGS</u> <u>2500 ppm</u> *Food consumption:* ↑ 2.6% males during first year Males: Liver necrosis (incidence borderline with top of 11/50 vs 5/49 control; Females:	KCA 5.5 / 02

60/sex (50/sex/group main study, 10/sex/group interim kill after 12 months) Dietary Dicamba (technical material) Purity 86.8% 0, 50, 250, 2500 ppm for 115 weeks (males), 118 weeks (females) Corresponds to 2.0, 10.0, and 99.1 mg/kg bw/day for males and 2.4, 12.1, and 120.1 mg/kg bw/day for females, respectively Actual doses corrected for purity correspond, respectively, to 1.7, 8.7, and 83.0 mg/kg bw/day for males, and to 2.1, 10.5, and 104 mg/kg bw/day for females Vehicle: diet	historical control data) Hydronephrosis of kidney (within historical control data): 4/50 vs 1/49 control; 3/49 vs 0/49 control Cystic hyperplasia in uterus: - ; 20/49 vs 15/49 control <u>250 ppm and 50 ppm</u> No toxicologically significant treatment-related non-neoplastic effects NOAEL for non-neoplastic findings = 250 ppm (10 mg/kg bw/day in males and 12.1 mg/kg bw/day in females)	
Carcinogenicity study OECD 451 (1981) 87/302/EEC B.32 GLP Charles River CD-1 mouse 52/sex/group Dietary Dicamba (technical material) Purity: 86.8% 0, 50, 150, 1000 and 3000 ppm for 89 weeks (males) or 104 weeks (females) Equivalent to 5.5, 17.2, 108, and 358 mg/kg/day for the males and 5.8, 18.8, 121, and 364 mg/kg/day for females, respectively	<u>NON-NEOPLASTIC FINDINGS</u> <u>3000 ppm</u> Body weight gain: ↓ females from week 25 (12% week 1-52, 17% week 1-104; p<0.07) Amyloidosis in: 0 ppm / 3000 ppm Thyroid 7/52 11/52 Parathyroid 5/52 11/52 Spleen 4/52 11/52 Adrenals 6/52 14/52 Heart 7/52 16/52 Kidney 12/52 20/52 <u>1000 ppm, 150 ppm and 50 ppm</u> No toxicologically significant treatment-related non-neoplastic effects NOAEL for non-neoplastic findings = 1000 ppm (equivalent to 108 mg/kg bw/day in males and 121 mg/kg bw/day)	KCA 5.5 / 01

Actual doses corrected for purity correspond, respectively, to 4.8, 14.9, 93.7 and 311 mg/kg bw/day of pure dicamba for males, and to 5.0, 16.3, 105, 316 mg/kg bw/day of pure dicamba for females Vehicle: diet		
13-week oral toxicity OECD 409 GLP Beagle dogs 4/sex/group, plus an additional 4/sex/group for control and top dose 4-week recovery phase Purity: 90.4% 0, 10, 50, 300 mg/kg bw/day No vehicle: the appropriate amount was weighed directly into the gelatine capsules No vehicle 13-week duration plus 4-week recovery Actual doses corrected for purity correspond, respectively, to 9.0, 45, 274 mg/kg bw/day	<u>274 mg/kg bw/day</u> *Clinical observations:* Hind limb gait abnormalities noted from day 1: ataxia, stiff gait and sporadic transient collapse generally seen in the majority of the 300 mg/kg animals approximately 2 hours after dosing and persisting for up to 5 hours. The neurological screen at week 6 and 13 showed abnormal locomotion and gait abnormalities in all animals. No effects detected following the recovery phase. *Mean bw gain:* ↓ 26% in males and 44% in females (not statistically significant) *Food consumption:* 90% of control for males and 84% of control for females *Haematology:* ↓ 9-18% RBC, Hct and Hb week 7 and 13 both sexes. ↑ 11% APPT activity in males and 7% in females at week 13, but signs of reversibility following recovery *Clinical chemistry:* ↓ 24.6-32.4% cholesterol and phospholipids weeks 7 and 13. Partial improvement following recovery (no statistically significant differences from control) <u>45 mg/kg bw/day and 9 mg/kg bw/day</u> No toxicologically significant findings NOAEL = 45 mg/ kg bw/day	KCA 5.3.2 / 02
1-year dietary toxicity EPA guideline 84-1 Similar to OECD 452 GLP Beagle dogs 4/sex/group Dicamba lot: 52625110 Purity: 86.8% 0, 100, 500 and 2500ppm dietary administration.	<u>2500 ppm</u> *Clinical observations:* ↑ incidence and frequency of soft faeces during first 6 months (25-75% v 25% in controls) *Body weight:* ↓ during week 1 but recovered by week 5/6 (approx. 7% weight loss compared with pre-treatment). No overall effect (weeks 0-50) *Food Consumption:* inappetance in 1 male and 1 female during first week: a further male did only eat small amount of food during first 3 weeks, but after being fed a slurry diet, stabilised by week 6 *Haematology:* ↓ statistically significant changes in the red blood cell values in high dose males at the 6-month investigation (↓ ~ 9% for haematocrit, erythrocytes, and haemoglobin)	KCA 5.3.2 / 03

Corresponding to 0, 2.03, 11.4 and 57 mg/kg bw/day for males, and 0, 2.14, 11.4, and 51 mg/kg bw/day for females, respectively. Actual doses corrected for purity correspond, respectively, to 1.8, 9.9, 50 mg/kg bw/day for males, and 1.9, 9.9, and 44 mg/kg bw/day for females Vehicle: diet	*Clinical chemistry:* At 6 months females only: ↓11% calcium, ↓7% total protein, ↓24% globulin, ↑31% Aspartate aminotransferase. *Organ weight:* ↓ ovary weight (30% absolute/35% relative) <u>500 ppm</u> *Body weight:* ↓ week 1 (4% weight loss compared with pre-treatment), but recovered by week 2 and no overall effect (weeks 0-50) *Food consumption:* inappetance in 2 animals during first week of the study <u>100 ppm</u> Body weight: ↓ week 1 (3% weight loss compared with pre-treatment), but recovered by week 2 and no overall effect (weeks 0-50) NOAEL = 500 ppm (11.4 mg/kg bw/day for both males and females)	
90-day oral toxicity study OECD 409 GLP Beagle dogs 4/sex/group 0, 10, 50 and 300 mg/kg bw/day Doses corresponded to 0, 9.5, 47.5, 285 at 100, 500, and 2500 ppm, respectively when corrected for purity Purity: >95% No vehicle: the test item is administered using Torpac Gelatin capsules	<u>300 mg/kg bw/day</u> *Clinical signs:* Intermittent stiff gait and recumbence, slight and/or moderate incoordination or ataxia and retching or emesis were consistently recorded. On occasion, the animals also displayed slightly to severely decreased activity, liquid faeces, increased salivation, minor tonic convulsions or tremors. All animals recovered by the following morning *Clinical chemistry parameters:* ↑ ALT (alanine aminotransferase) in both sexes during week 13 (72%, $p<0.01$ in the males, and 143%, $p<0.05$ in the females). ↓ triglyceride mean values in males (-28%, $p< 0.05$). ↓ ALKP (alkaline phosphatase) mean values in females -40% ($p<0.05$) at week 7 and -36% ($p<0.01$) at week 13 *Haematology:* Significant effects in females: ↓ RBC (17 to 20%) in weeks 7 and 13. ↓ Haemoglobin (18%) in week 7. ↓ Haematocrit (18%) in week 7 <u>50 mg/kg bw/day</u> ↓ ALKP mean values (30%, $p<0.05$ at week 7) <u>10 mg/kg bw/day</u> No toxicologically relevant effects NOAEL = 50 mg/kg bw/day	CA 5.3.2/01
Repeat dose 28-day OECD 412 EC No. 440/2008	<u>0.05 mg/L</u> *Body weight gain:* ↓ 41% in males *Organ weights:* ↑ absolute (16-17%) and relative (17-20%) lung weights in males and females	KCA 5.3.3 / 01

GLP Crl:WI(Han) Wistar rats 10/sex/group Purity: 93.9% Nose only exposures to dust 0, 0.001, 0.005, 0.05 mg/L Actual doses corrected for purity correspond to 0.00094, 0.0047 and 0.047 mg/L of pure dicamba 6 hours/day, 5 days/week for 4 weeks Desagglomerated and sieved test material was mixed with 1% Aerosil	*Histopathology:* minimal or slight hypertrophy or hyperplasia of the epithelium of single bronchi, bronchioles or terminal bronchioles in all males and females, minimal/slight bronchiolo-alveolar hyperplasia in 8/10 males and 9/10 females <u>0.005 mg/L</u> *Histopathology:* minimal multifocal bronchiolo-alveolar hyperplasia in 2/10 males <u>0.001 mg/L</u> No treatment-related adverse findings NOAEC for local toxicity at the respiratory tract = 0.001 mg/L NOAEC for general toxicity = 0.005 mg/L	
28-day dermal OECD 410 GLP Alpk:APfSD (Wistar-derived) rats 10/sex/group Purity: 91.0% 0, 30, 300, 1000 mg/kg bw/day Actual doses corrected for purity correspond, respectively, to 27.3, 273 and 910 mg/kg bw/day of pure dicamba Vehicle: water used to make a paste 28-day duration 21 applications	<u>910 mg/kg bw/day</u> Histopathological signs of irritation in treated skin in 10/10 males and 10/10 females (acanthosis/hyperkeratosis, inflammatory cell infiltration) <u>273 mg/kg bw/day</u> Histopathological signs of irritation in 10/10 males and 9/10 females, less severe than high dose <u>27.3 mg/kg bw/day</u> Acanthosis/hyperkeratosis in 5/10 males and 1/10 females NOAEL for systemic toxicity > 910 mg/kg bw/day	KCA 5.3.3 / 02
Developmental toxicity Complies largely to OECD 414 (2001) but with some notable deviations Oral (gavage) Charles River CD rats	<u>MATERNAL TOXICITY</u> <u>362 mg/kg bw/day</u> 4/25 deaths gestation day 7 & 8 Ataxia, stiffening of the body when held, urine soaked fur, salivation and decreased motor activity	KCA 5.6.2 / 02

25 mated females/group Purity: 90.4% 0, 64, 160 or 400 mg/kg bw/day on days 6-19 of gestation Actual doses corrected for purity correspond, respectively, to 0, 58, 145 and 362 mg/kg bw/day Vehicle: Mazola corn oil	↓ body weight gain (27% lower corrected maternal bw gain) ↓ food consumption (19% lower than controls, days 6-19) <u>145 mg/kg bw/day</u> 10% lower corrected maternal bw gain (not statistically significant) <u>58 mg/kg bw/day</u> No effects Maternal NOAEL = 58 mg/kg bw/day	
Developmental toxicity US EPA 83-3 (complies largely to OECD 414, 2001) Oral (capsule) New Zealand White rabbits 20 inseminated females/group Dicamba batch: 52625110 Purity: 90.4% 0, 30, 150 or 300 mg/kg bw/day on days 6-18 of gestation Actual doses corrected for purity correspond, respectively, to 27.1, 136 and 271 mg/kg bw/day No vehicle: gelatin capsules size 1	<u>MATERNAL TOXICITY</u> <u>271 mg/kg bw/day</u> 4/20 abortions (days 19, 21, 24 and 24) Ataxia, rales, laboured breathing, perinasal substance, dried/no faeces, impaired righting reflex, and decreased motor activity ↓ body weight gain (42% lower than controls days 0 to 29) ↓ relative food consumption (13% lower than controls, days 0-29) <u>136 mg/kg bw/day</u> 1/20 abortion (day 22) Ataxia and decreased motor activity <u>27.1 mg/kg bw/day</u> No effects Maternal NOAEL = 30 mg/kg bw/day	KCA 5.6.2 / 01
Two Generation Oral (continuous in diet) OECD 416 (1983) Crl:CD (SD) BR VAF/Plus rats 32/sex/group (F0) 28/sex/group (F1) Purity: 86.9% 0, 500, 1500 or 5000 ppm	<u>PARENTAL TOXICITY</u> <u>5000 ppm</u> *F0* Mean achieved intake 347/390 mg/kg bw/day, males/ females respectively ↓ Body weight pregnancy day 0-14: 10% (day 0-20: 3%) ↑ Adjusted liver weight 13% females, 5% males *F1* Mean achieved intake, 432/458 mg/kg bw/day, males/ females respectively	KCA 5.6.1 / 01

Vehicle: laboratory animal diet. The overall F0/F1 pre-mating doses correspond, respectively, to 37.9, 113 and 389 mg/kg bw /day for males and 42.6, 130 and 424 mg/kg bw/day for females The overall F0/F1 pre-mating means correspond, respectively, to 32.9, 98.3 and 338 mg/kg bw/day of pure dicamba for males, and to 37.0, 113, 369 mg/kg bw/day of pure dicamba for females Vehicle: diet	Clinical signs during lactation: tense/stiff body tone and slow righting reflex for a few days during the latter part of lactation ↓ Body weight pregnancy day 0-14: 5% (F1A) and 15% (F1B) ↓ Body weight pregnancy day 0-20: 0% (F1A) and 7% (F1B) ↑ Absolute liver weight 3% females, males 10% (relative) ↓ Food consumption week 5-8 <u>1500 ppm</u> *F0* Mean achieved intake, 105/125 mg/kg bw/day, males/ females respectively ↓ Body weight pregnancy day 0-14: 4% (day 0-20: 0%) *F1* Mean achieved intake, 121/135 mg/kg bw/day, males/ females respectively ↓ Body weight pregnancy day 0-14: 13% (F1A) and 15% (F1B) ↓ Body weight pregnancy day 0-20: 9% (F1A) and 15% (F1B) <u>500 ppm</u> *F0* Mean achieved intake, 35/41 mg/kg bw/day, males/ females respectively ↓ Body weight pregnancy day 0-14: 1% (day 0-20: 0%) *F1* Mean achieved intake, 40/44 mg/kg bw/day, males/ females respectively ↓ Body weight pregnancy day 0-14: 18% (F1A) and 6% (F1B) ↓ Body weight pregnancy day 0-20: 10% (F1A) and 2% (F1B) NOAEL = 500 ppm (42.6 mg/kg bw/day)

Comparison with the criteria

Dicamba was assessed in a number of repeated dose toxicity studies with rats, mouse and rabbits that did not identify clear target organs of toxicity. Alterations in body weight, hepatotoxicity with concomitant haematological and clinical chemistry alterations and neurotoxicity were found in several studies. The only study by inhalation identified mainly local toxicity in lungs, while the only dermal toxicity study did not induce systemic toxicity.

The table below summarises the LOAEL effects of all oral studies and LOEC of the inhalation study displayed in the table above. It is noted that among 12 available studies only 4 exhibited a LOAEL or LOEC below the guideline limit value for supporting classification.

***Table**: Summary of LOAEL or LOEC reported in the repeated dose toxicity studies with dicamba. All the information were taken from the Table above.*

Study	LOAEL (mg/kg bw/day)	Effect	Exposure	Guideline limit values Cat 1/Cat 2	Classification supported
KCA 5.3.1 / 01	1022	Reduced body weight gain and food consumption	4-weeks	30/300	No
KCA 5.3.2 / 01	1000	Hepatotoxicity	90-days	10/100	No
KCA 5.7.1 / 02	768	Neurotoxicity	13-weeks	10/100	No
KCA 5.5 / 02	99	Liver necrosis and increase in cystic hyperplasia in the uterus	115-weeks	1/10	No
KCA 5.5 / 01	358	Amyloidosis in several organs	89-weeks	1.4/14	No
KCA 5.3.2 / 02	274	Effects on gait and behaviour	13-weeks	10/100	No
KCA 5.3.2 / 03	57	Minor alterations in: haematology, clinical chemistry, ovary weight	1-year	2.3/23	No
CA 5.3.2/01	285	Clinical signs and parameters	90-days	10/100	No
KCA 5.3.3 / 01	0.05 mg/L	Decreased bw gain (41%)	28-days	0.06/0.6 mg/L	Yes
KCA 5.6.2 / 02	362	Corrected body weight (27%), ataxia, decreased motor activity	14-days	60/600	Yes
KCA 5.6.2 / 01	136/271	Ataxia and decreased motor activity	14-days	10/100	No
KCA 5.6.1 / 01	113	Decreased (15%) body weight during pregnancy	2-generation	F0 = 71 F1 = 66	No

The sub-acute toxicity study (KCA 5.3.3 / 01) by inhalation reported a LOEC borderline between Cat 1 and 2. However, according to the DAR, the animals had a 41% reduction

in body weight gain, mainly in the second half of the study, but the reduction was not reflected in absolute weight. RAC considers that this effect is not sufficiently marked to be described as adverse. Therefore, this study does not support classification.

The developmental toxicity study in rats (KCA 5.6.2 / 02) showed an LOAEL for maternal toxicity of 362 mg/kg bw/day based on a corrected body weight reduction of 27%, ataxia and reduced motor activity that would theoretically support a classification in Cat 2. However, this study presented deficiencies, such as a dosage volume of 1.0 mL/100 g instead of the maximum of 0.4 mL/100 g allowed in the Guideline and moreover it is noted that this dose of 362 mg/kg bw/day also caused 16% lethality and no histopathological support for the neurotoxicity was reported in this study or other repeated dose toxicity studies. Thus, RAC notes that the results of this study do not support a classification for STOT RE.

Overall, it is noted that dicamba causes neurotoxicity. However, since all neurotoxicity was reported above the guideline values RAC supports the DS's proposal for **no classification of dicamba for STOT RE.**

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

Table 24: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Test system **Test object** **TG/GLP**		**Concentration**	**Compound**[1] **Purity** **Batch. No.**	**Results**	**Reference**
In vitro					
Chromosome aberrations					
Mammalian Chromosomal Aberration Test OECD 473 (1997)/GLP	Human Lymphocytes	648, 1134, 1985 µg/mL (experiment I without S9, experiment II with S9), and 370.3, 648, 1134 µg/mL (experiment II without S9, experiment I with S9)	89.8 % P.MG2726410	Positive (-S9) Negative (+ S9)	Bohnenberger S, 2015 KCA 5.4.1/01 (acceptable)
Mammalian Chromosomal Aberration Test 2000/32/EC, B.10 ~OECD 473 (1997)/GLP	Chinese hamster ovary cells (CHO)	266, 524, 1039, 2069 µg/mL	Technical Dicamba 88.8% 52625110	Negative (+/- S9) Validity of the study is questioned.	Putman, DL, 1986 KCA 5.4.1/02 (supplemental)

[1] Test concentations are corrected for purity in all studies except for Verspeek-Rip 2010, Brown 2010a and b where the concentrations are given as technical dicamba.

Test system **Test object** **TG/GLP**		**Concentration**	**Compound[1]** **Purity** **Batch. No.**	**Results**	**Reference**
In vitro micronucleus test OECD 487 (2016)/GLP	Human Lymphocytes	50, 100, 250, 500, 1000, 1500 and 2000 µg/mL (±S9, 3 hours), 250, 500, 750, 1000, 1250, 1500, 1750, and 2000 µg/mL (-S9, 24h)	Technical Dicamba 89.8% P.MG2726410	Negative (+/- S9)	Whitwell, 2017a K-CA 5.4.1/02 (acceptable)
Gene mutations – Bacteria					
Bacterial Reverse Mutation Test EU 2000/32/EC, B.13/14 ~ OECD 471 (1997)/GLP	Salmonella typhimurium strains (TA98, TA100, TA1535, TA1537 and TA102	7.1, 35.4, 177, 885, 4425 µg/mL (experiment I), and 41.5 and 83.0 (TA102 only), 166, 332, 664 (all strains), 1328 and 2655 (all strains except TA102) µg/mL (experiment II)	Dicamba technical 88.5% 52504710	Negative (+/- S9)	Ballantyne, M, 1996 KCA 5.4.1/03 (acceptable)
Gene mutations – Mammalian cells					
Mammalian cell gene mutation test (forward mutation test) EU 2000/32/EC B.17~ OECD 476 (1997)/GLP	Mouse lymphoma L5178Y cells	226, 452, 904, 1356, 1808, and 1998 µg/mL	Dicamba (SAN 837) 90.4% B2826511	Negative (+/- S9)	Clay, P., 2001 KCA 5.4.1/04 (acceptable)
Mammalian cell gene mutation test (forward mutation test) OECD 476 (1997)/GLP	Mouse lymphoma L5178Y cells	10, 33, 100, 333, 1000, 1500, 1750, 2210 µg/mL (–S9) 10, 100, 333, 1000, 1250, 1500, 1750, 2000 µg/mL (+S9)	RC1176 (dicamba) 988.50 g/kg RTM/DCMB/03/20090612	Positive (+/- S9)	Verspeek-Rip CM, 2010 KCA 5.4.1/05 (acceptable)
Mammalian cell gene mutation test (forward mutation test) OECD 476 (1997)/GLP	Mouse lymphoma L5178Y cells	Exp. 1: 65.6 – 2100 µg/mL (-/+S9) Exp. 2: 21.9 – 1400 µg/mL (-S9), 175 – 2100 µg/mL (+S9) Exp. 3: 175 – 2100 µg/mL (-S9)	RC1176 (dicamba) 988.50 g/kg RTM/DCMB/03/20090612	Positive (+/- S9)	Brown R, 2010a KCA 5.4.1/06 (acceptable)
Mammalian cell gene mutation test (forward mutation test)	Mouse lymphoma L5178Y cells	Exp. 1: 65.6 – 2100 µg/mL (-/+S9)	dicamba PAS 3 99% TM/DCMB/PURE/20090612	Positive (- S9) Negative (+/S9)	Brown R, 2010b KCA 5.4.1/07 (acceptable)

Test system **Test object** **TG/GLP**		**Concentration**	**Compound[1]** **Purity** **Batch. No.**	**Results**	**Reference**
OECD 476 (1997)GLP		Exp. 2: 43.8 – 2100 µg/mL (-S9), 175 – 2100 µg/mL (+S9)			
Other genotoxic effects					
No tests					
QSAR					
	DEREK Nexus (multiple end-points not limited to genotoxicity), Vega suite (mutagenicity models) and ToxTree (structural alerts for in vivo micronuceus formation). Additionally the OECD QSAR Toolbox was used to assess DNA and protein binding and for functional group profiling.			Alert for *in vivo* micronuclei formation in rodents (as potential H-acceptor-path3- H-acceptor) from ToxTree and the OECD QSAR Toolbox	Lorez C, Booth E (2016).

Table 25: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Test system **Test object** **TG/GLP**		**Concentration**	**Compound** **Purity** **Batch. No.**	**Results**	**Reference**
In vivo – somatic cells (non-heritable)					
Gene mutations					
No tests					
Chromosome aberrations					
Bone Marrow cytogenetic assay No TG ~2000/32/EEC B.11~ OECD 475/No GLP	Male and female Sprague-Dawley rats	Dicamba Technical 208, 416 or 832 mg/kg bw	Dicamba ≥ 99% Not specified	Negative	Hrelia, P. et al. (1994) KCA 5.4.2/01 supplemental

Mammalian Erythrocyte Micronucleus Test 2000/32/EC, B.12 ~ OECD 474/GLP	Male and female CD-1 mice	Dicamba Technical 1300 mg/kg bw	Dicamba technical 88.5% 52504710	Negative	Marshall, R. (1996) KCA 5.4.2/02 (acceptable)
Other genotoxic effects					
Rat Alkaline Comet Assay OECD 489, 2016/GLP.	Male Crl:CD(SD) rats.	Dicamba 37.5, 75 and 150 mg/kg/day	Dicamba, Batch nr P.MG2726410 89.8% w/w	Negative in liver Positive in duodenum, with concurrent increase in hedgehog cells	Barfield, W (2019), Envigo XB29VC (acceptable)
Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays OECD 488, 2013/GLP	Male Muta™Mouse	0, 1000, 3000 or 10000 ppm (calculated as 171, 454 and 1443 mg/kg/day, respectively), diet	Dicamba, Batch nr P.MG2726410 89.8% w/w	Negative	Ueda, M. (2020) BioSafety Research Center Inc. (acceptable)
In vivo – germ cells (heritable)					
No tests					

Other studies relevant for genotoxicity / germ cell mutagenicity

Other studies					
Rat Histopathological Follow-up Study OECD 489, 2016/GLP.	Male Crl:CD (SD) rats.	Dicamba 37.5, 75 and 150 mg/kg/day	Dicamba, Batch nr P.MG2726410 89.8% w/w	There was no detectable increase in apoptotic/necrotic cells in the stomach or duodenum related to treatment with dicamba	Herring, T. (2019) Envigo NS52VW
Dicamba techn. (BAS 183 H; SAN837 techn.): Follow up study to determine potential ex-vivo effects during comet tissue processing Not GLP	Crl:CD(SD) rats.	Dicamba 75 mg/kg bw/day (gavage)	Dicamba, Batch nr P.MG2726410 89.8% w/w	Positive in duodenum. Inconclusive regarding direct or indirect damage.	Barfield,W. (2020) Covance MM44NB
[14C]Dicamba: Duodenum Kinetics in Rats GLP	Male Crl:CD (SD) rats	Dicamba (oral) 75 mg/kg bw/day	Dicamba, Batch nr P.MG2726410 89.8% w/w	Results supports exposure of duodenum after oral exposure to dicamba in rats	Hilton, A. (2020) Covance MT42NJ

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Assay type	Conditions	Result	References	*Reliability*
In vitro assays				
bacterial mutation	*S Typhimurium* TA98 TA100, ± S9	-	Shirasu *et al.* (1982);	*Overview publication contains no details citing earlier publication by same author[2]: both publications combined considered* ***not reliable****: lack of details on test compounds (unclear whether dicamba acid or salt was tested, no source/purity), methods (no information on source of cells, on concentrations used, on vehicle, on negative/positive controls), result documentation (no numerical data at all for dicamba)*
			Moriya *et al.* (1983)	***Not reliable****: lack of details on test compounds (no source, purity), methods (no onformation on source of cells, on concentrations used; uncertainty about positive controls), result documentation (information limited to +/- response, no numerical data)*
	S Typhimurium TA97, TA98, TA100, TA102, ± S9	-	Hrelia *et al.* (1990);	***Not reliable****: lack of details on test compounds (no source, purity), methods (no onfromation on source/cultivation of cells, essentially no information on study design except strains and +/-S9), result documentation (only negative response, no numerical data)*
			Mersch-Sundermann *et al.* (1994)	*Overview publication not containing any details citing earlier publication by same author for data on dicamba[3]: both publications combined considered* ***reliable with restrictions****: reasonable documentation of test compounds and methods but only limited documentation of results (+/- response with very little numerical data)*
	S Typhimurium TA98 TA100,TA1535, TA1537, TA1538 ± S9; maize ±1S†	-	Eisenbeis *et al.* (1981);	***Not reliable****: lack of details on test compounds (no source/purity; test material likely to be commercial products – not active ingredients as such), methods (no information on concentrations used, number of replicates or experiments), result documentation (only negative response, essentially no numerical data)*
			†Plewa *et al.* (1984);	*Cites Gentile et al 1982[4] for part of method description; both publications together still considered* ***not reliable****: lack of details on test compounds (no purity; unclear description of sources; active ingredient and commercial product used but product not identified), methods (apparently same positive control compounds used +/- S9 for all but one strain; uncertainty whether negative controls were included into each experiment; no information on number/range of concentrations used); results (mostly +/- response with only very sporadic numerical*

[2] *Shirasu Y, Moriya M, Kato K, Furuhashi A, Kada T; Mutagenicity screening of pesticides in the microbial system; Mutation Research (1976) 40: 19-30*

[3] *Mersch-Sundermann V, Dickgiesser N, Hablizel U, Gruber B; Examination of mutagenicity or organic microconatimations on the environment – I Communication: The mutagenicity of selected herbicides and insecticides with the Salmonella-microsome test (Ames test) in consideration of the pathogenic potence of contaminated ground- and drinking water; Zbl Bakt Hyg B (1988), 186:247-260*

[4] *Gentile JM, Gentile GJ, Bultman J, Sechriest R, Wagner ED, Plewa MJ; An evaluation of the genotoxic properties of insecticides following plant and animal activation; Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis; 1982, 101(1):19-29*

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Assay type	Conditions	Result	References	*Reliability*
				data); partly exotic study design (additional experiments with bacteria treated with extracts from plants grown on water/pesticide mixtures = 1S experiments)
			Kier *et al.* (1986) and references therein	*Review paper citing data from other publications/reports; for dicamba data, Simmon 1978 is cited – however citation cannot be clearly identified, as 28 references by Simmon et al 1978 are provided (all apparently US-EPA reports without referenced title providing an indication which of these reports contains data on dicamba); nevertheless a summary report by Simmon 1979[5] is available that is expected to include the data cited for dicamba within Kier et al 1986; the 1979 report by Simmon is considered* ***reliable (Ames part):*** *reasonably good documentation on test compounds (no purity and slight uncertainty whether active ingredient or product was tested but active ingredient considered likely), methods (only number of replicates per experiment missing and positive controls in absence of S9 only included in one experiment in 4 strains and none in the 5th strain; positive control +S9 always included) and results (numerical data available on negative/positive controls, treatment concentrations for all 3 experiments with dicamba)*
	S Typhimurium TA1535, TA1536, TA1537, TA1538, *E coli, WP2* use of S9 not stated	-	Shirasu (1975)	*Apparently refers to same data as Shirasu 1982 above (again no information on methods/numerical results) – therefore also considered* ***not reliable*** *(details see Shirasu 1982)*
	S Typhimurium TA98, TA100, TA1535, TA1537, TA1538, *E coli WP2 ±S9*	-	Poole *et al.* (1977)	*Abstract only (co-author is VF Simmon) – no data provided within abstract but considered to refer to the same experiments as reported within Simmon 1979[5] (same EPA Contract number 68-01-2458 as abstract by Poole et al 1977) – see above under Kier et al 1986*
	S Typhimurium TA100, TA1535, TA1537, TA1538; *E coli WP2uvrA* ±S9	-	Waters *et al.* (1980); Waters *et al.* (1981)	*Review papers not containing any detailed results on dicamba - for Ames results of dicamba, expected to refer to data as reported by Simmon 1979[5] – for evaluation of Simmon 1979 see Kier et al 1986 above*
	S Typhimurium TA98, TA100,TA1537, TA1538, *E coli WP2uvrA ±S9*	-	Simmon (1980)	*identical to Simmon 1979[5] (same EPA-600/1-79-041) – for evaluation of Simmon 1979 see Kier et al 1986 above*
	S Typhimurium TA98, TA100, TA1535, TA 1537, TA1538, *E coli WP2uvrA,* ± S9	-	Waters *et al.* (1982); Sandhu *et al.* (1985)	*Review papers not containing any detailed results on dicamba - for Ames results of dicamba, expected to refer to data as reported by Simmon 1979[5] – for evaluation of Simmon 1979 see Kier et al 1986 above*

[5] *Simmon VF, In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides, EPA-600/1-79-041, October 1979*

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Assay type	Conditions	Result	References	*Reliability*
mutation, DNA repair, mitoic recombination, or relative toxicity assays	SOS chromotest *E coli* PQ37±S9	-	Xu & Schurr (1990);	*For method description Xu et al 1989[6] cited – both publications together still considered **not reliable**: lack of details on test compounds (no purity, no direct info on source), and results (mostly +/- response with essentially no detailed numerical data for dicamba); reasonably good description of methods (in Xu 1989) but no positive controls used and no information on concentrations tested*
			Mersch-Sundermann *et al.* (1994)	*Review paper comparing results of Ames and SOS chromotest results reported elsewhere; for dicamba data Mersch-Sundermann et al 1988[3] (Ames results – evaluation of reliability see Mersch-Sundermann 1994 above) and Mersch-Sundermann et al 1989[7] (SOS Chromotest results) are cited; SOS chromotest part together with the 1989 publication combined considered **reliable with restrictions**: reasonable documentation of test compounds and methods but only limited documentation of results (only SOSIP value but no info on responses at individual dicamba concentrations)*
	pol A E. coli p3478, W3110	+	Leifer *et al.* (1981) [and references therein]; Simmon (1980); Waters *et al.* (1980); Waters *et al.* (1981); Waters *et al.* (1982)	*Review paper citing data from other publications; for dicamba a report from Simmon (1978) is referenced which is considered to contain the data that is also contained in Simmon 1980 (= Simmon 1979); the three papers by Waters et al are also considered to refer to the data contained in Simmon 1980 (=1979); for growth inhibition part within Simmon 1979: considered (borderline) **not reliable**: only slight uncertainties about test compound (probably active ingredients, no purity), good description of methods (however no information on number of replicates or experiments; likely single experiment) and results (numerical data provided for test compound concentrations and positive/ negative controls); however shortcomings make evaluation of relatively weak dicamba response difficult (increase in ratio of growth inhibition zone for DNA-repair incapable/capable strain at top concentration vs. negative control but less strong than positive control) and no information on variability or reproducibility; no statistical analysis; also no clear criteria for definition of positive response was provided (no historical controls; absolute values of growth inhibition zones for dicamba smaller than negative and positive controls)*
	*S Typhimurium,uvr*B rec;	-	Sandhu *et al.* (1985)	*Publication contains no actual data on dicamba and refers to an EPA testing program – the latter is considered to be represented by Simmon 1979 (= Simmon 1980); evaluation of Ames part within Simmon 1979, see under Kier et al 1986*

[6] *Xu H, Microtitration SOS Chromotest: A new approach in genotoxicity testing, Toxicity Assessment: An International Journal (1989), 4:105-114*

[7] *Mersch-Sundermann V, Hofmeister A, Müller G, Hof H, Examination of mutagenicity of organic microcontaminations of the environment – III Communication: The mutagenicity of selected herbicides and insecticides with the SOS-Chromotest, Zbl Hyg (1989), 189:135-146*

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Assay type	Conditions	Result	References	*Reliability*
	rec assay *B subtilus H17, M45*	+	Leifer *et al.* (1981) and references therein; Simmon (1980); Waters *et al.* (1980), (1981) and (1982)	*See discussion on growth inhibition part of Simmon 1979 under Leifer et al 1981 above (same type of growth inhibition experiment both for E.coli W3110/p3478 and B. subtilis H17/M45)*
	rec assay *B subtilis* M45	-	Shirasu (1975)	*Contains no actual data on dicamba (just listed as one of several compounds being tested and apparently found negative); several papers cited within Shirasu 1975 (Kada 1972/1974[8]) or by the same author published later (Shirasu 1982, Shirasu 1976) also contain no actual data on dicamba: all papers together still considered* ***not reliable****: very little information on test compound (not entirely clear whether its dicamba acid or dimethylamine salt; no source/purity), methods (no information on vehicle/applied concentrations or on positive/negative controls, no statistical analysis) and results (no numerical data at all)*
	S Typhimurium various strains, T_4 AP72 bacteriophage *E coli K, B,*	-	Andersen *et al.* (1972)	***Not reliable****: lack of details on test compounds (only range of purities for several compounds, no detailed information on supplier), methods (source of cells given but S.typhimurium strains for Ames part not identified; no information on test concentrations or vehicles used, on time between exposure to response evaluation, on replicates/number of experiments; unclear whether positive/negative controls included in each experiment), result documentation (only +/- response for Ames part – no numerical data; only numerical data for one dose for bacteriophage experiments)*
	S. cerevisiae D3 ±S9	-	Sandhu *et al.* (1985); Simmon (1980); Waters *et al.* (1980); Waters *et al.* (1981); Waters *et al.* (1982); Poole *et al.* (1977); Zimmerman *et al.* (1984) and references therein	*All publication expected to rely on data reported by Simmon 1979[5] (=1980); for experiments with S. cervisiae: considered borderline* ***reliable with restrictions****: only slight uncertainties about test compound (probably active ingredients, no purity), reasonable description of methods (however no information on number of replicates - likely single culture; some short-comings vs. OECD test guideline: 4 instead of 5 concentrations used; no positive control in dicamba experiments; only single direct acting positive control in other experiments not needing S9 activation) and results (numerical data provided for test compound concentrations and positive/negative controls; reasonable variation for available positive/negative controls with clear distinction between +/- responses)*

[8] *Kada T, Tutikawa K, Sadaie Y; In vitro and host-mediated 'rec-assay' procedures for screening chemical mutagens; and phloxine, a mutagenic red dye detected; Mutation Research, 1972, 16:165-174*
Kada T, Moriya M, Shirasu Y, Screening of pesticides for DNA interactions by ‚rec-assay' and mutagenesis testing, and frameshift mutagens detected, Mutation Research (1974) 26:243-248

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Assay type	Conditions	Result	References	*Reliability*
	S. cerevisiae D4± S9, maize ±1S	±	Plewa *et al.* (1984)	*Cites Gentile et al 1982[4] for part of method description; both publications together still considered* ***not reliable****: lack of details on test compounds (purity; unclear description of sources; active ingredient and commercial product used but product not identified), methods (no information on concentration levels used, number of replicates, solvent concentration in negative controls; assumption single culture and single experiment; as compared to OECD 481 short treatment period); results (mostly +/- response with only very sporadic numerical data; no data at all for positive controls); partly exotic study design (additional experiments with S. cerevisiae treated with extracts from plants grown on water/pesticide mixtures = 1S experiments); variation in negative controls overlaps with criteria for positive response; some concurrent negative control responses outside reported 'normal' negative control ranges*
	S. cervisiae D7± S9	-	Hrelia et al. (1990);	***Not reliable****: lack of details on test compounds (no source, purity), methods (no source/cultivation of cells, essentially no information on study design except strains and +/-S9), result documentation (only negative response, no numerical data)*
chromosome aberration	Swiss albino mouse spleen cells	+	Amer & Aly, (1997);	***Not reliable****: lack of details on test compounds (no source/purity for in vitro part), methodological short comings (experiments only in absence of S9, no positive controls, time between start of exposure and harvest too short for chromosome aberrations to be visible in 1st experiment, no cytotoxicity info for 1st experiment, only single concentration in 2nd experiment; only 50 vs. recommended 300 metaphases evaluated per experiment/concentration), result documentation (only limited numerical data; doubts about correct presentation of cytotoxicity data) and plausibility (stronger 'response' in 1st experiment as compared to 2nd experiment at same concentration)*
	CHO cells	+	*Gonzalez et al (2011)*	***Not reliable:*** *details see reliability discussion for Gonzalez et al publications under point 5.4.1. lack of details on test compounds (no purity).*
SCE	human peripheral blood lymphocytes ±S9	+	Hrelia *et al.* (1990)	***Not reliable****: lack of details on test compounds (no source, purity), methods (no source/cultivation of cells, essentially no information on study design, cell type used and +/-S9), result documentation (only positive response, no numerical data)*
	human peripheral blood lymphocytes ±S9	-	Perocco *et al.* (1990)	***Not reliable*** *(borderline): reasonable description of test compound, methods and results but some short-comings (uncertainty whether blood from single or several donors used per experiment; no positive controls; 30 metaphases from apparently single culture scored per experiment/concentration less than recommended 50)*

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Assay type	Conditions	Result	References	*Reliability*
	Swiss albino mouse spleen cells	+	Amer & Aly (1997);	***Not reliable**: lack of details on test compounds (no source/purity for in vitro part), methodological short comings (experiments only in absence of S9, no positive controls, time between start of exposure and harvest too short for SCE to be visible; only 25 vs. recommended 50 metaphases evaluated per experiment/concentration), result documentation (only limited numerical data; no information on cytotoxicity) and implausibility (positive effects reported for experimental design with too short period between start of exposure and harvest for SCEs being visible)*
	human lymphocytes (in whole blood cultures)	+	Gonzalez *et al.* (2006)	***Not reliable:** details see reliability discussion for Gonzalez et al publications under point 5.4.1. lack of details on test compounds (no purity).*
	CHO cells	+	Gonzalez *et al.* (2007)	***Not reliable:** details see reliability discussion for Gonzalez et al publications under point 5.4.1. lack of details on test compounds (no purity).*
	CHO cells	+	*Gonzalez et al (2009)*	***Not reliable:** details see reliability discussion for Gonzalez et al publications under point 5.4.1. lack of details on test compounds (no purity).*
Unscheduled DNA synthesis	human lung fibroblasts (WI-38) ±S9	-	Simmon (1980); Waters *et al.* (1981); Waters *et al.* (1982); Sandhu *et al.* (1985)	*All publication expected to rely on data reported by Simmon 1979[5] (=1980); UDS part of Simmon 1979 considered **reliable**: reasonably good documentation on test compounds (no purity and slight uncertainty whether active ingredient or product was tested but active ingredient considered likely), methods (source of cells absent, number of cells used not reported) and results (statistics only included in text; no clear criteria provided what is considered a positive response); otherwise no relevant deviations from OECD482*
	human peripheral blood lymphocytes +S9	+	Hrelia *et al.* (1990)	***Not reliable**: lack of details on test compounds (no source, purity), methods (no information on source/cultivation of cells, essentially no information on study design, cell type used; only that it was done +/-S9), result documentation (only positive response +S9, no numerical data) – Hrelia et al (1994) indicates that the UDS results mentioned within Hrelia et al (1990) are the same as those reported within Perocco et al 1990 (below)*
	human peripheral blood lymphocytes ±S9	+	Perocco *et al.* (1990)	***Not reliable**: reasonably good description of test compounds and methods but methodological (no positive controls, no statistics, no criteria for positive response, no cytotoxicity) and reporting shortcomings (no numerical data, only dpm shown graphically but no information on dpm/µg DNA, no information on cytotoxicity, no dose-relationship, variability between donors partly larger than between negative control and dicamba treated cultures)*

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Assay type	Conditions	Result	References	*Reliability*
COMET	CHO cells	-	Sorensen *et al.* (2005)	*Two different experimental designs: assumption (not clearly described) that one part was direct treatment of cells with pesticides and second part was treatment of cells with pesticides (and/or degradation products) after pre-incubation with vehicle or clays – both parts are considered* ***not reliable****; Both parts: only minor short-comings for test compound (no purity) and test system (source of cells not provided)* *Direct treatment of cells with dicamba: some methodological (no information on vehicle, on exact dose levels used – only range given, on number of cultures; apparently no negative controls, experiments done in absence of S9 only) and reporting short-comings (no information on cytotoxicity, results only presented graphically, no negative/positive control data, no individual experiment results)* *Clay pre-treatment: methodological and reporting shortcomings: unclear description how claimed concentrations in genotox part (up to 7.3 mM) are achieved from pre-treatment samples (about 4.5 mM); results only presented graphically, no positive control data, no individual experiment results, apparently no true negative control (dicamba in vehicle without clay in pre-treatment was considered negative control)*
	CHO cells	+	Gonzalez *et al.* (2007)	***Not reliable:*** *details see reliability discussion for Gonzalez et al publications under point 5.4.1*
GreenScreen HC assay (Gentronix Ltd.)	*GADD45a-GFP GFP* induction, -S9	-	Knight *et al.* (2009)	*Publication of screening tests with large number of compounds including dicamba by using three high throughput in vitro test systems (limited relevance):* ***considered reliable with restrictions within limitations normal for screening tests****: lack of details on test compounds (no clear source, purity), methods (no source of cells, apparently all experiments done without metabolic activation, information on replicates only for HepG2 part, no information on number of experiments) and results (essentially only +/- response; no detailed results on genotoxicity parameters nor on cytotoxicity)*
CellSensor p53RE-bla HCT-116 assay (Invitrogen Corp.)	HCT-116 cells p53 response, -S9	-	Knight *et al.* (2009)	
CellCiphr Cytotox Profiling Panel-p53 endpoint. (Cellumen Inc.)	HepG2 cells p53 activation, -S9	-	Knight *et al.* (2009)	

Assay type	Conditions: route, dose	Result	Reference	Reliability
In vivo assays				
chromosome aberration	non inbred white mice, ♂, oral gavage, 50 or 500 mg/kg, bone marrow	±	Kurinnyi *et al.* (1982)	***Not reliable****: insufficient description of test compound (no source/purity; product tested - not active ingredient), of methods (no information on animal strain/sex/ group size, on mode/number of applications, on experimental timings, no positive controls, two dose levels only for dicamba product, apparently only one negative control vs. totally 57 treated groups) and result documentation (only % aberrant metaphases; no details whether these in/exclude gaps or other aberrations, no individual animal data)*

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Assay type	Conditions: route, dose	Result	Reference	Reliability
	SD rats ♂&♀, oral gavage, 832 mg/kg (80% of LD50), 416, 208 mg/kg	-	Hrelia et al. (1994)	***Reliable***[9]*: good description of test compound, methods and documentation of results (but no individual animal data); however some deviations to current OECD 475 (mainly: 4 vs. recommended 5 animals/sex/group – however no relevant sex difference – therefore totally 8 animals/group, 100 vs. recommended 200 metaphases scored/animal – totally 800/group vs. recommended 1000/group in absence of relevant sex difference)*
	Swiss mice, ♂ i.p., 20mg/kg, (1/10 LD50), spleen, testes	+	Aly (1995)	***Not reliable*** *(ip and oral part of publication – apparently not peer-reviewed): reasonable description of test compound (only purity lacking) but lack of details/shortcomings on methods (no information on animal strain or on health status prior to*
	Swiss mice, ♂ oral gavage, 1, 3 or 5 days 119 mg/kg/day (1/10 LD50), bone marrow, spleen, testes	+	Aly (1995)	*treatment, on dye used for bone marrow and spermatocytes, on colchicine dose, on how authors ensured that spermatocytes were in metaphase and no illustration of cell preparation quality; no positive controls included nor criteria for positive response; insufficient description of statistics; slides apparently not coded for spleen and bone marrow) and on results (inadequate description of structural aberrations [spleen, bone marrow], inadequate description of aberrations for spermatocytes [except tetraploid]; only 50 metaphases scored/animal and tissue vs. recommended 200, only single dose level used; no information on target organ toxicity e.g. by MI);* *ip treatment part – further short-comings: implausible results: strongest response seen too early at 6 h after application (<<1.5 cell cycles - corresponding to 15-24 h); effect by solvent (DMSO) alone (vs. untreated group) – side effects by vehicle not excluded;* *oral application part – further short-comings: no information on housing condition of animals (repeated treatment), on treatment of vehicle controls, on stability of dicamba in vehicle, timing of sacrifice after application (appropriate or not?)*
	Swiss albino mice; i.p.; 11 or 20 mg/kg, bone marrow	+	Amer & Aly (1997)	***Not reliable****: reasonable description of test compound but lack of details for methods (no information sex, exact age or bw of animals) and for results (only means but no individual animal data; no information on cytotoxicity e.g. mitotic index), methodological short-comings (no positive controls; only 4/5 mice of unknown sex/group for negative controls/treated group, respectively, vs. recommended 5/sex/group; only 50 metaphases evaluated/animal vs. recommended 200; slides not coded; no info on target organ exposure or toxicity), and positive response at too early time point (6h <<1.5x cell cycle) considered implausible (also causes doubts on less strong positive response at later/more appropriate time points)*

[9] *Considered ‚Acceptable in the view of other supporting studies' in the last EU review*

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Assay type	Conditions: route, dose	Result	Reference	Reliability
Sex-linked recessive lethal assay	*D. melanogaster*	-	Valencia (1981); Waters *et al.* (1980); Waters *et al.* (1981); Waters *et al.* (1982); Sandhu *et al.* (1985)	***Not reliable*** *(publications by Waters and Sandhu refer to data by Valencia 1981): lack of details for test compounds (no purity/source), for methods (no information on number of flies treated or mated; unclear description of experimental design or measurements) and result reporting (no positive control data); no information at all about methods/measurements/results (except +/- response) of dominant lethal part of report (only two compounds tested as test procedure considered too time-consuming to be considered further)*
Dominant lethal assay	*D. melanogaster*	-	Valencia (1981)	
DNA unwinding assay	SD rats ♂ i.p. 13.3, 17.8 or 26.6 mg/k (1/6, 1/4.5 or 1/3 LD_{50}) liver DNA	+	Perocco *et al.* (1990)	***Not reliable:*** *reasonably good description of test compound but methodological short-comings (no information on number of rats used; uncertainties about vehicle/application volumes in treated groups; different exposure period in negative/treated groups vs. positive control; likely introduction of DNA damage post-treatment by harsh DNA isolation procedures; no criteria for positive response) and reporting shortcomings (no information at all on variability; values partly presented graphically only); post-treatment DNA damage not excluded (considerable damage in negative controls already), which is likely to be sensitive to slight variations in experimental procedure – uncertainty whether variations between treated (or positive control) and negative control by maximum factor of 2 are truly treatment-related; no difference in rate of DNA-unwinding between treated or positive control vs. negative control*

Some of the additional not reliable publications report negative and some positive findings. However the lack of details in the description of methods and results often prevent a meaningful evaluation of the published results and/or the study design/methods used are considered flawed (e.g. leading to implausible results).

Table 26: 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 studies available	

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

In vitro

The submitted *in vitro* genotoxicity tests cover the three endpoints gene mutation and structural as well as numerical chromosome abberations.

Dicamba technical was tested in one reverse gene mutagenicity tests in five S. typhimurium strains (Ballantyne 1996) and in four forward gene mutation tests in mouse lymphoma L5178Y cells (Clay 2001, Verspeek-Rip 2010, Brown 2010a, Brown 2010b) and in three assays for chromosome damage using Human Lymphocytes and Chinese hamster ovary cells (Bohnenberger 2015, Putman 1986, Whitwell, 2017a).

The original three *in vitro* tests presented in the DAR 2010 (Ballantyne 1996, Clay 2001, Putman 1986) were all considered negative under the conditions of the performed studies. The five new tests (one mammalian chromosomal aberration test, one *in vitro* MN test and three mammalian forward gene mutation test) submitted for the purpose of renewal show a positive result in 4 tests and negative result in one test (*in vitro* micronucleus).

In two of the new mammalian forward gene mutation test (Verspeek-Rip 2010, Brown 2010a), dose-related genotoxic responses were recorded both in the absence and presence of S9-mix at cytotoxic concentrations (RTGs of 5-24% with S9 and 17-42% without S9). A third mammalian gene mutation test (Brown 2010b) performed with highly purified dicamba (99%) was positive in the absence of S9-mix at cytotoxic concentrations (RTGs 15-27% without S9). A genotoxic response was also observed with S9-mix but only at RTGs of 6-9% which according to the TG 490 should not be considered positive as the increase in MF occurred only below 10% RTG. Overall no genotoxic response was observed at concentrations below 1750 µg/mL (-S9) and 2000 µg/mL (+S9) after 3-4 hours exposure; and 700 µg/mL (-S9) after 24 hours exposure. Increases in MF exceeding the GEF value were observed at cytotoxic concentrations only, beginning at RTGs of 42% (-S9) and 24% (+S9) followed by a steep dose -related increase in MFs with increasing cytotoxicity.

In the old study testing for forward mutations (Clay 2001), isolated statistically significant increases in MFs were observed in the presence and absence of S9-mix. The result was not reproduced in the subsequent experiment and was relatively small (less than a 2-fold increases over solvent control levels) and Dicamba was therefore considered non-mutagenic in this test.

A position paper (Holmes 2010) to address the significance of the results in the mammalian forward gene mutation tests was submitted. It thoroughly discuss all mammalian forward gene mutation tests for dicamba and its metabolite OH- dicamba and states that the positive results are artefactual to cytotoxicity caused by oxidative stress with a threshold concentration below which they cannot occur. RMS is not convinced by this argumentation as Salmonella strains TA100 and TA102 are considered susceptible to oxidative stress and tests in these organisms were negative.

In the old study testing for *in vitro* mammalian chromosomal aberration (CA) (Putman 1986), dicamba did not induce an increase in CA when Chinese hamster ovary cells were treated in the absence or presence of S9-mix in doses up to the limit of solubility (maximum dose was 2330 µg/ml). A new study; however (Bohnenberger 2015) produced a statistically significant increase in CA in the absence of S9 mix, clearly exceeding the range of the laboratory historical solvent control after 22 hours treatment with 1262.9 µg/mL. One higher concentration in this

experiment was not evaluated due to clear cytotoxicity. After a 4 hour treatment, at 2210.0 µg/mL also in the absence of S9-mix, a statistically significant increase in CA was observed, but was clearly within the range of the laboratory historical solvent control data. In the presence of S9-mix no statistically significant increases in CA was observed.

Dicamba did not induce micronuclei in cultured human peripheral blood lymphocytes following treatment in the absence and presence of an aroclor induced rat liver metabolic activation system (S-9) after 3 hours of treatment in an *In Vitro* Human Lymphocyte Micronucleus Assay. In the 24 hour treatment a statistically significant increase in MNBN at 1250 µg/mL (0.60%) was within range of the historical control 95th percentile (0.1-0.85 %). At 250 µg/mL one culture was statistically significantly increased outside the 95th percentile histrorical control range (0.9 %) but within the historical control range (the other culture was 0.4%). Vehicle historical control,: mean +/- SD: 0.37 +/- 0.18, range 95th%ile: 0.10-0.84, / observed range: 0.1-1.0. Furthermore, there was no dose response in the study and the concurrent control was in the low end of the range (0.15%). Therefore, these small statistical increases were not considered to be biologically relevant. Concentrations were analysed up to 2000 µg/mL, a recommended regulatory maximum concentration for in vitro micronucleus assays. Dicamba was concluded to be negative in this assay (Whitwell, 2017a).

In vivo, there was no evidence of CA at the maximum dose of 832 mg/kg bw in rats, corresponding to 80 % of the $LD_{50,}$ however the test was only supplemental due to limitations of study design (Hrelia, et al.1994). Dicamba did not induce micronuclei in the polychromatic erythrocytes of the bone marrow in mice treated with two doses of 1300 mg/kg bw/day (techn), which produced limited mortality (Marshall, 1996). ADME data in mice indicates target tissue was reached as dicamba was measured in blood 16 (approx. 1% of applied dose) and 96 hours (approx. 0.1 % of applied dose) after exposure to 89 mg/kg bw (Atallah and Yu (1980)). As elimination of dicamba is fast, the levels were low after 96 hours. The tested dose in the MN study (1300 mg/kg bw) was somewhat higher than the dose used in the mouse ADME study. Based on these studies, dicamba is not denoted clastogenic or aneugenic.

An *in vivo* comet assay study was performed to address the the above discussed conflicting results found in *in vitro* gene mutation studies with dicamba. In the study, male Crl:CD(SD) rats were treated orally (gavage) with daily doses of 0 (vehicle), 37.5, 75 and 150 mg/kg bw/day of dicamba at 0 and 24 h (Barfiels, 2019). A positive control group was included (Ethyl Methanesulphonate). Animals were sacrificed at 2 h after the 2nd application and cell suspensions were prepared from the duodenum (site of contact tissue) and the liver. DNA strand breaks were assessed by comparing the % tail intensity and evidence for any overt toxicity to concurrent and historical control data. Further, the number of hedgehog cells per 150 cells were noted (cells with > 80% DNA in tail). The systemic availability of dicamba was confirmed in blood samples taken at 1 and 2 h after the 2nd application at the high dose level. Duodenum and liver tissue samples were evaluated microscopically and histopathologically. No increase in tail intensity nor hedgehogs was seen at all three dose levels in the liver confirming a complete absence of genotoxicity. An increase in tail intensity and in the number of hedgehog cells was seen at the low and mid dose level in the duodenum (see table below). Excessive toxicity (gross DNA debris) prevented the evaluation of the top dose level in the duodenum. Histopathology evaluations within the Comet assay did not indicate relevant fixed markers of treatment in the duodenum nor the liver.

Table 27: Median tail intensity and number of hedgehog cells scored in the liver and duodenum in rats after exposure to Dicamba in Barfield et al., 2019.

Dicamba	Number of cells scored	Median tail intensity (%)	Number of hedgehog cells°
Liver			
0 (vehicle)	900	0.53	0
37.5	900	0.49	0
75	900	0.39	0
150	900	0.50	0
Positive control	450	50.28***	0
Duodenum			
0 (vehicle)	900	0.51	0
37.5	900	21.75***	63
75	900	38.73***	72
150	900	-	-
Positive control	450	50.46***	0

*** p <0.001

° mean number of hedgehogs encountered while scoring 150 cells

A follow-up study to the Comet assay was performed to investigate potential causes or modes of action for the inconclusive findings observed in the duodenum within the Comet assay and to clarify whether the absence of histopathological findings indicating cellular damage in the duodenum within the Comet assay may be due to the relatively short time period between the last treatment and sacrifice (2 h). This short time period may be insufficient for cellular damage leading to sufficient morphological changes in the cells/tissue to become visible by standard histopathological methods. In this follow-up study groups of male rats were treated similarly as in the Comet assay (2 daily gavage applications at 0, 37.5 and 75 mg/kg bw/day) but were sacrificed at 2, 6, 24 or 48 h after the 2nd application. This would allow more time for any cellular/ tissue damage in the duodenum and stomach (as a point of contact tissues) to become manifest as cell death or other morphological changes via standard histopathological evaluation and by staining for specific apoptosis markers (TUNEL, caspase 3). In this follow-up study, no indications of any adverse effects of treatment were seen in the duodenum nor in the stomach up to 48 h after 2nd (72 h after 1st) application – neither in cell/tissue morphology (e.g. necrotic or apoptotic changes) nor with specific staining for apoptosis markers (Herring, 2019). These results indicate that, whatever causes the effects seen in the duodenum within the Comet assay, does not cause cellular or tissue damage within the duodenum within this follow-up study.

A second follow-up study was conducted to investigate if any ex-vivo effects may have caused the increases in %tale intensity observed in the initial Comet test using different tissue processing methods in a test similar to Comet Assay using male Crl:CD(SD) rats. Groups of 3 animals were treated with Dicamba at 75 mg/kg/day, orally by gavage on two occasions, the second dose being administered approximately 24 hours after the first dose. Tissues were sampled at 0.5, 1, 2, 4 and 6 hours post treatment. In addition to this, one group received three 25 mg/kg doses (separated by 30-minute intervals) on two consecutive days; the animals were terminated 2 hours after their final dose. The vehicle control group received 0.5% methylcellulose (group 1), the positive control group received 200 mg/kg ethyl methanesulphonate (EMS) on a single occasion approximately 3 hours before sampling. Furthermore, the pH (intracellular and in the mincing buffer), osmolarity (in the mincing buffer) and histopathological examination were also performed to assess any ex-vivo effects which may have caused the increases in %TI observed in the initial Comet test. The result of this study confirmed the increse in TI after dicamba exposure but was inconclusive regarding if the observed effect on DNA damage was direct or indirect (Barfield, 2020).

To finally conclude on the potential of Dicamba to induce gene mutations (reporter gene: lacZ) in the duodenum, notifiers conducted a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (OECD TG 488, 2013) with transgenic male mice (Muta™Mouse). Dicamba was administered to groups of 7 male transgenic mice orally for 28 consecutive days via the diet and, after 3 days of manifestation period, the mutant frequencies in the duodenum were determined. Dose levels of 0, 1200, 3000 and 7000 ppm corresponding to 176, 431 and 924 mg/kg bw/day, respectively, were selected for the transgenic rodent assay. A positive control group received 100 mg/kg bw/day N-ethyl-N-nitrosourea for two consecutive days via gavage followed by a 10 day manifestation period. At 7000 ppm food consumption and body weight development were slightly reduced achieving statistical significance on days 1-3 and 15, respectively. No effects of treatment were seen at 1200 and 3000 ppm and duodenum weights

were unaffected at all dose levels. There were no treatment-related macroscopic changes and no histopathological findings in the duodenum. There were no significant differences in the mutant frequencies in the duodenum in any of the groups treated with dicamba as compared to the negative control group. The mutant frequencies in the duodenum in the positive control group were statistically significantly increased (Ueda, 2020).

In a kinetic study, the rate and route of excretion of radioactivity and the absorption kinetics in duodenal sections were investigated, at intervals, following two daily oral doses of [14C]Dicamba (75 mg/kg) to 20 male rats.

Table 28: Experimental design

Group	Treatment	Sacrifice time after final dose (h)	Males	Sampling
1	Dicamba: 7.5 mg/mL, 75 mg/kg bw (by oral gavage on 2 days)	0.5	4	At sacrifice: blood, gastrointestinal tract, duodenum, liver
2		1	4	
3		2	4	
4		4	4	
5		6	4	Urine: 1, 2, 4, 6, 24 h (post 1st dose); 1, 2, 4, 6 (post 2nd dose); Feces: 24 h (post 1st dose), 6 h (post 2nd dose) At sacrifice: cage wash, blood, gastrointestinal tract, duodenum, liver

Following two daily oral doses of [14C]Dicamba (75 mg/kg) in 0.5% (w/v) methyl cellulose solution to 20 male rats radioactivity was rapidly absorbed with maximum mean whole blood (35.6 µg equiv/g, 161 nmol equiv/g), plasma (53.4 µg equiv /g, 242 nmol equiv/g), duodenum sections (20.9 µg equiv/g, 94.6 nmol equiv/g) and liver (17.4 µg equiv/g, 78.7 nmol equiv/g) concentrations occurring at 0.5 hours after the second dose (first samping interval). Following two oral doses of [14C]Dicamba (75 mg/kg) mean concentrations in duodenum sections were greatest in section A (immediately after the stomach). Mean concentrations generally declined between sections A to B and sections B to C between 0.5 – 2 hours post dose. At 0.5 hours post dose mean concentrations in section A were 20.9 µg equiv/g (94.6 nmol equiv/g) declining to 6.62 µg equiv/g (30 nmol equiv/g) at 2 hours post dose. Mean concentrations in section B at 0.5 hours were 13.3 µg equiv/g (60.2 nmol equiv/g) declining to 5.33 µg equiv/g (24.1 nmol equiv/g) at 2 hours post dose. Mean concentrations in section C at 0.5 hours were 11.6 µg equiv/g (52.5 nmol equiv/g) declining to 5.92 µg equiv/g (26.8 nmol equiv/g) at 2 hours post dose. At 4 and 6 hours post dose mean concentrations between each of the sections were generally similar. Following a single oral dose of [14C]Dicamba to male rats mean concentrations of radioactivity in urine was maximal at 4 hours post dose (4680 µg equiv/g) declining to the lowest observed concentrations at 24 hours (111 µg equiv/g). Following the second oral dose of [14C]Dicamba greatest concentrations were observed at 2 hours (6040 µg equiv/g) post dose. Mean concentrations of [14C]Dicamba in liver were maximal (17.4 µg equiv/g, 78.7 nmol equiv/g) at 0.5 hours (first sampling time) and declined over time but were still measurable (0.983 µg equiv/g, 4.45 nmol equiv/g) at 6 hours post dose (final sampling time) which indicates exposure in liver is comparable with the duodenum.

In conclusion dispite the initial variations seen between the three duodenum sections, the study demonstrates that all sections of the duodenum were exposed to dicamba in rats and a difference in tissue exposure does not seem to be the cause for the difference in Comet assay response (Hilton, 2020).

A comprehensive literature search and discussion on *in vitro*/*in vivo* genotoxicity was performed by Syngenta in September 2009[10] and included in the DAR. The published results are contradictory but there is evidence for a slight DNA daminging capacity by dicamba. For SCE four out of five studies were positive and for unscheduled DNA synthesis two out of three studies were positive. One *in vitro* chromosome aberration study was positive and among the *in vivo* chromosom aberration studies published, three out of five studies were positive and 1/5 inconclusive. The quality of the published studies is not without deficiencies (e.g. information of purity missing) and the reporting on methods is usually sparse/lacking and it cannot be entirely ruled out that some of the positive genotoxicity results are false positive results.

[10] Dicamba Statement. Comprehensive literature search and discussion on *in vitro*/*in vivo* gentoxicity. September 2009. Syngenta

In the latest literature search, 4 relevant studies were identified, which were conducted by Gonzalez et al (2006; 2007; 2009; 2011). These studies showed positive results for increased SCE frequency, MN formation and increased comet width and comet length in vitro. These results were seen for both dicamba and the product Banvel. The conclusions of all four papers indicated a genotoxic effect of Dicamba and Banvel. However, all four papers show major limitations and therefore a genotoxic effect in vitro cannot be concluded based on these papers.

Three (Q)SAR programs were selected working on different basis of expert knowledge rules and statistical methods for the assessment of genotoxicity. These were; DEREK Nexus (multiple endpoints not limited to genotoxicity), Vega suite (mutagenicity models) and ToxTree (structural alerts for in vivo micronuceus formation). Additionally the OECD QSAR Toolbox was used to assess DNA and protein binding and for functional group profiling. Hence, overall, using these tools genotoxicity endpoints of in vitro and in vivo mutagenicity, chromosome damage and DNA/protein binding were considered. An alert for *in vivo* micronuclei formation in rodents (as potential H-acceptor-path3- H-acceptor) from ToxTree and the OECD QSAR Toolbox was observed for dicamba (Lorez C, Booth E (2016)).

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

Classification in category 1A or B is not considered relevant because there are no positive evidence from human epidemiological studies or evidence of dicamba inducing heritable mutations in the germ cells of humans or mammals.

Classification in category 2 may be based on positive results of a least one valid *in vivo* mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of an *in vivo* valid mammalian somatic cell genotoxicity test, supported by positive *in vitro* mutagenicity results.
In vitro results can lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Gene mutation tests *in vitro* in bacteria (Ames) were negative, while in mammalian cells conflicting results are seen *in vitro* (one negative and three positive gene mutation studies – the positive effects being in presence of clear cytotoxicity); available *in vitro* tests for cytogenetic endpoints also show variable results for dicamba – one positive and one negative *in vitro* chromosome aberration study and one negative *in vitro* micronucleus study. *In vivo* studies covering structural and numerical chromosome aberrations (chromosome aberration study in rats, micronucleus study in mice) do not indicate any genotoxic potential of dicamba *in vivo*. In order to address the conflicting *in vitro* results, an *in vivo* Comet assay was performed. The study clearly demonstrates a lack of genotoxicity in the liver, while increases in tail intensity was seen in the duodenum, as a site-of-contact tissue. This increase was accompanied by marked increase in hedgehog cells at low doses of dicamba and tissue toxicity in histopathological analysis at high doses. The positive result in duodenum was confirmed in a follow-up study based on elements of the guideline for the Comet Asssay.

However, a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, conducted to address the positive findings in the Comet assay, was clearly negative in duodenum up to a dose (924 mg/kg bw/day) a dose near the limit dose of 1000 mg/kg bw/day. Taking into account that a Comet assay detects DNA damage and the TGR Assay detects mutations and the latter was negative, it is not considered likely dicamba causes gene mutations in vivo. On that basis, the criteria of a classification for mutagenicity in category 2 is not considered met.

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

No classification.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS assessed the germ cell mutagenicity using an extended data base containing *in vitro* (mammalian chromosomal aberration test with human lymphocytes and Chinese hamster ovary cells, micronucleus test with human lymphocytes, bacterial reverse mutation test with five different strains of *Salmonella typhimurium* and mammalian cell gene mutation test with mouse lymphoma L5178Y cells) and *in vivo* (bone marrow cytogenetic assay with rats, mammalian erythrocyte micronucleus test with mice, Comet Assay in rats and transgenic rodent somatic and germ cell gene mutation assays) assays.

The *in vitro* results were contradictory, with a number of negative and positive results. *In vivo* tests were positive, showing DNA damage in the Comet assay, but no *in vivo* tests were positive for showing gene mutation, which led the DS to propose no classification of dicamba for germ cell mutagenicity.

Comments received during consultation

One company downstream user and one company manufacturer supported the conclusion for no classification of dicamba for germ cell mutagenicity despite the positive result in the Comet assay in the duodenum. The DS replied to both comments that the negative results from the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays overrule the results from the Comet assay.

Assessment and comparison with the classification criteria

The next 2 tables below summarise, respectively, *in vitro,* and *in vivo* mutagenicity and genotoxicity studies with dicamba.

***Table**: Summary of mutagenicity/genotoxicity in vitro studies with dicamba*

Method	Tested concentrations	Results	Reference
Mammalian Chromosomal Aberration Test OECD 473 GLP Human lymphocytes	648, 1134, 1985 µg/mL (experiment I without S9, experiment II with S9) 370.3, 648, 1134 µg/mL (experiment II without S9, experiment I with S9) Purity: 89.8% Solvent: 0.5% DMSO	Positive controls: ethylmethane sulfonate and cyclophosphamide Cytotoxicity at top dose Experiment 1 -S9: **Negative** +S9: **Negative** Experiment 2 -S9: **Positive** +S9: **Negative**	K-CA 5.4.1/01

Mammalian Chromosomal Aberration Test EC B.10 OECD 473 GLP Chinese hamster ovary cells (CHO)	266, 524, 1039, 2069 µg/mL Purity: 88.8% Solvent: DMSO	No cytotoxicity Top dose = Limit of solubility **Negative (+ S9)** **Negative (- S9)**	KCA 5.4.1 / 02
In vitro micronucleus test OECD 487 GLP Human lymphocytes	50, 100, 250, 500, 1000, 1500 and 2000 µg/mL (±S9, 3 hours) 250, 500, 750, 1000, 1250, 1500, 1750, and 2000 µg/mL (-S9, 24h) Purity: 89.8% Solvent: 1% DMSO	Positive control: cyclophosphamide **Negative (+ S9)** **Negative (- S9)**	KCA 5.8.1 / 10
Bacterial Reverse Mutation Test B.13/14 OECD 471 GLP *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537 and TA102)	7.1, 35.4, 177, 885, 4425 µg/mL (experiment I) 41.5 and 83.0 (TA102 only), 166, 332, 664 (all strains), 1328 and 2655 (all strains except TA102) µg/mL (experiment II) Purity: 88.5% Solvent: DMSO	Positive controls: 2-aminoanthracene, 2-nitrofluorene, sodium azide, 9-aminoacridine and glutaraldehyde Cytotoxicity at the two highest doses **Negative (+ S9)** **Negative (- S9)**	KCA 5.4.1 / 03
Mammalian cell gene mutation test (forward mutation test) B.17 OECD 476 GLP Mouse lymphoma L5178Y cells	226, 452, 904, 1356, 1808, and 1998 µg/mL Purity: 90.4% Solvent: DMSO	Positive controls: ethylmethane sulfonate Moderate cytotoxicity **Negative (+ S9)** **Negative (- S9)**	KCA 5.4.1 / 04
Mammalian cell gene mutation test (forward mutation test) OECD 476	10, 33, 100, 333, 1000, 1500, 1750, 2210 µg/mL (-S9) 10, 100, 333, 1000, 1250, 1500, 1750, 2000 µg/mL (+S9)	Positive controls: methyl methane sulfonate and cyclophosphamide **Positive (+ S9)** **Positive (- S9)**	KCA 5.4.1/05

GLP Mouse lymphoma L5178Y cells	Purity: 988.50 g/kg Solvent: DMSO		
Mammalian cell gene mutation test (forward mutation test) OECD 476 GLP Mouse lymphoma L5178Y cells	Experiment 1: 65.6- 2100 µg/mL (-/+S9) Experiment 2: 21.9- 1400 µg/mL (-S9), 175-2100 µg/mL (+S9) Experiment 3: 175-2100 µg/mL (-S9) Purity: 988.50 g/kg Solvent: DMSO	Positive controls: ethyl methane sulfonate and cyclophosphamide **Positive (+ S9)** **Positive (- S9)**	KCA 5.4.1/06
Mammalian cell gene mutation test (forward mutation test) OECD 476 GLP Mouse lymphoma L5178Y cells	Experiment 1: 65.6-2100 µg/mL (-/+S9) Experiment 2: 43.8-2100 µg/mL (-S9), 175-2100 µg/mL (+S9) Purity: 99% Solvent: DMSO	Positive controls: ethyl methane sulfonate and cyclophosphamide **Negative (+ S9)** **Positive (- S9)**	KCA 5.4.1/07

The database of *in vitro* genotoxicity tests cover three endpoints, chromosome aberrations and gene mutations in bacteria and mammalian cells (Table above). Positive results were found in the presence of S9 in the mammalian chromosomal aberration test with human lymphocytes, in two independent studies of mammalian cell gene mutations with mouse lymphoma L5178Y (both with and without S9) and in a third one only in absence of S9. In contrast, negative results were found in the absence of S9 in the mammalian chromosomal aberration test with human lymphocytes, in a mammalian chromosomal aberration test with Chinese hamster ovary cells (both with and without S9), mammalian cell gene mutations with mouse lymphoma L5178Y in the presence of S9 and both presence and absence of S9 in *in vitro* micronucleus test with human lymphocytes, mammalian cell gene mutations with mouse lymphoma L5178Y and a bacterial reverse mutation test with Salmonella.

Additionally, a QSAR study with ToxTree and OECD QSAR Toolbox alerted for *in vivo* micronuclei formation in rodents, while DEREK Nexus and Vega suite did not raise any concerns.

Table: Summary of mutagenicity/genotoxicity *in vivo* studies with dicamba.

Method	Tested concentrations	Results	Reference
Bone Marrow cytogenetic assay EEC B.11 OECD 475	208, 416 or 832 mg/kg bw Purity ≥ 99%	**Negative**	KCA 5.4.2 / 01

No GLP Male and female Sprague-Dawley rats	Vehicle: Water with 20% gum arabic		
Mammalian Erythrocyte Micronucleus Test EC, B.12 OECD 474/GLP Male and female CD-1 mice	1300 mg/kg bw Purity = 88.5% Gavage Vehicle: Corn oil	**Negative**	KCA 5.4.2 / 02
Rat Alkaline Comet Assay OECD 489 GLP Male Crl:CD(SD) rats	37.5, 75 and 150 mg/kg/day Purity = 89.8% w/w Vehicle: 0.5% methylcellulose	**Negative** in liver **Positive** in duodenum, with concurrent increase in hedgehog cells	K-CA 5.4.2/01
Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays OECD 488 GLP Male Muta™Mouse 7 males/group	0, 1200, 3000 or 7000 ppm (calculated as 176, 431 and 924 mg/kg/day, respectively Diet Purity = 89.8% w/w Vehicle: diet	Positive control: N-ethyl-N-nitrosourea 7000 ppm: food consumption and body weight development slightly reduced (days 1-3 and 15) No effects of treatment were seen at 1200 and 3000 ppm Duodenum weights were unaffected at all dose levels. No treatment-related macroscopic changes and no histopathological findings in the duodenum **Negative**	K-CA 5.4.2/04

The database of *in vivo* tests included 2 chromosome aberration tests, a Comet assay, and a somatic and germ cell mutation assay with transgenic rodents (Table above). The results of both bone marrow cytogenetic assays in rodents were negative at doses of 832 mg/kg bw (approximately 80% of LD_{50}) and 1300 mg/kg bw. Toxicokinetic information indicates that the target tissue was reached at these doses.

The Comet assays in rats were negative in the liver, but in the duodenum, the lowest and middle dose induced an increase in tail intensity and number of hedgehog cells (the top dose could not be assessed due to excessive toxicity) (Table above). A rat histopathological follow-up supportive study in which animals were sacrificed up to 48 hours after a second dicamba dose showed no detectable increase in apoptotic/necrotic cells in the stomach or duodenum related to treatment with dicamba. These results indicate that whatever causes the effects seen in the duodenum with the Comet assay, does not cause cellular or tissue damage within the duodenum within this follow-up study. A second follow-up supportive study confirmed the increase in tail intensity after dicamba exposure but was inconclusive regarding whether the observed effect on DNA damage was direct or indirect.

To finally conclude on the potential of dicamba to induce gene mutations in the duodenum, a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (OECD TG 488) with transgenic male mice (Muta™Mouse) was conducted (Table above). There were no significant differences in the mutant frequencies in the duodenum in any of the groups treated with dicamba as compared to the negative control group. A supportive study with 14C-dicamba demonstrated that the duodenum was exposed to dicamba in rats.

The applicant also performed a comprehensive literature search and discussion on *in vitro/in vivo* genotoxicity (see the CLH-report for details). The published results are contradictory but there is evidence for a slight DNA damaging capacity by dicamba. For sister chromatid exchange 4/8 studies were positive and for unscheduled DNA synthesis 2/3 studies were positive too. One *in vitro* chromosome aberration study was positive and among the *in vivo* chromosome aberration studies published, 3/5 studies were positive and 1/5 inconclusive. The quality of the published studies is not without deficiencies (e.g., information on purity is missing) and the reporting on methods is usually sparse/lacking and it cannot be entirely ruled out that some of the positive genotoxicity results are false positive results.

Comparison with the criteria

There was no positive evidence from human epidemiological studies or evidence of dicamba inducing heritable mutations in the germ cells of humans or mammals. Therefore, classification in Categories 1A or 1B is not warranted. Classification within category 2 may be based on positive results of *in vivo* mammalian somatic cell genotoxicity test, supported by positive *in vitro* mutagenicity results.

Gene mutation tests *in vitro* in bacteria (Ames) were negative, while in mammalian cells conflicting results are seen *in vitro*. There were one negative and three positive gene mutation studies (the positive effects being in the presence of clear cytotoxicity), while available *in vitro* tests for cytogenetic endpoints also show variable results for dicamba (one positive and one negative *in vitro* chromosome aberration study and one negative *in vitro* micronucleus study).

In vivo studies addressing structural and numerical chromosome aberrations (chromosome aberration study in rats, micronucleus study in mice) do not indicate any genotoxic potential of dicamba *in vivo*. An *in vivo* Comet assay demonstrated a lack of genotoxicity in the liver but not in the duodenum; this was confirmed in follow-up studies. However, a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay was clearly negative

in the duodenum up to a dose (924 mg/kg bw/day) near the limit dose of 1000 mg/kg bw/day.

Overall, taking into account that a Comet assay detects DNA damage and the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay detects mutations and the latter was negative, RAC notes that it is very unlikely that dicamba causes gene mutations *in vivo* and consequently the criteria of a classification for mutagenicity in category 2 are not considered to have been met.

In conclusion, RAC supports the DS's proposal for **no classification of dicamba for germ cell mutagenicity.**

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

Table 29: 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**	**Reference**
Combined chronic toxicity/carcinogenicity. OECD 453, 87/302/EEC B.33 (1988) GLP Rat, Charles River CD (Sprague Dawley) 60/sex (50/sex/group main study, 10/sex/group interim kill after 12 months)	Dicamba (technical material; purity 86.8%) Continuous in the diet 0, 50, 250, 2500 ppm for 115 weeks (males), 118 weeks (females) The doses correspond to 2.0, 10.0, and 99.1 mg/kg bw/day for males and 2.4, 12.1, and 120.1 mg/kg bw/day for females *Corrected for purity the doses correspond to 1.7, 8.7, and 83.0 mg/kg bw/day of pure dicamba for males, and to 2.1, 10.5, and 104 mg/kg bw/day of pure dicamba for females, at 50, 250, and 2500 ppm, respectively.*	**Non-neoplastic findings** **2500 ppm (males 99.1 mg/kg bw/day, females 120.1 mg/kg bw/day)**: *Food consumption:* ↑ 2.6% males during first year *Pathology*: ↑ incidence of liver necrosis in males (5/49 in control vs 11/50 at 2500 ppm), Slight ↑ hydronephrosis of kidney in males (1/49 in control vs 4/50 at 2500 ppm) and females (0/49 in control vs 3/49 at 2500 ppm) Slight ↑ cystic hyperplasia in the uterus (15/49 in control and 20/49 at 2500ppm) Carcinogenicity: ↑ incidence of thyroid parafollicular (C-cell) carcinoma in males ↑ increase in polyps in the uterus (4/60 in control, 8/60 at 2500 ppm) **250 ppm (males 10.0 mg/kg bw/day, females 12.1 mg/kg bw/day)**: Carcinogenicity: ↑ incidence of thyroid parafollicular (C-cell) carcinoma in males but within historical control range No other toxicologically significant treatment-related effects. **50 ppm (males 2.0 mg/kg bw/day, females 2.4 mg/kg bw/day)**: No toxicologically significant treatment-related effects. **Neoplastic findings** NOAEL for carcinogenicity 250 ppm (equivalent to 10 mg/kg bw/day in males) based on increased incidence of thyroid parafollicular (C-cell) carcinoma in males from 250 ppm, which showed a positive trend. The observations were also outside historical control range. NOAEL supported by increase in polyps in the uterus at high dose. NOAEL systemic: 250 ppm (10 mg/kg bw/day) based on ↑ incidence of liver necrosis in males, increase in cystic hyperplasia in the uterus at 2500 ppm. The lowest survival at 104 weeks was 42 % in high dose males.	Goldenthal (1985)

<table>
<tr>
<td>Carcinogenicity study. OECD 451 (1981), 87/302/EEC B.32 (1988)
GLP
Mouse, Charles River CD-1
52/sex/group</td>
<td>Dicamba (technical material; purity 86.8%)
Continuous in the diet 0, 50, 150, 1000 and 3000 ppm for 89 weeks (males) or 104 weeks (females) corresponding to 5.5, 17.2, 108, and 358 mg/kg/day for the males and 5.8, 18.8, 121, and 364 mg/kg/day for females.

The average compound consumption then corresponds to 4.8, 14.9, 93.7 and 311 mg/kg bw/day of pure dicamba for males, and to 5.0, 16.3, 105, 316 mg/kg bw/day of pure dicamba for females, at 50, 150, 1000 and 3000 ppm, respectively.</td>
<td><u>Non-neoplastic findings</u>
<u>3000 ppm (males 358 mg/kg bw/day, females 364 mg/kg bw/day)</u>:
Body weight gain: ↓ females from week 25 (12% week 1-52, 17% week 1-104).
Pathology: slightly increased incidence of amyloidosis in males in heart, parathyroid, thyroid, spleen, kidney and adrenal
<table>
<tr><th>Dose (ppm)</th><th colspan="5">Males</th></tr>
<tr><th></th><th>0</th><th>50</th><th>150</th><th>1000</th><th>3000</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Thyroid, Amyloidosis</td><td>7/52</td><td>7/28</td><td>9/34</td><td>4/21</td><td>11/52</td></tr>
<tr><td>Parathyroid, Amyloidosis</td><td>5/52</td><td>5/28</td><td>5/34</td><td>4/21</td><td>11/52</td></tr>
<tr><td>spleen, Amyloidosis</td><td>4/52</td><td>6/31</td><td>10/38</td><td>5/23</td><td>11/52</td></tr>
<tr><td>adrenals, Amyloidosis</td><td>6/52</td><td>6/28</td><td>8/34</td><td>5/21</td><td>14/52</td></tr>
<tr><td>adrenals, medullary hyperplasia</td><td>16/52</td><td>5/28</td><td>7/34</td><td>5/21</td><td>7/52</td></tr>
<tr><td>heart , Amyloidosis</td><td>7/52</td><td>8/28</td><td>11/34</td><td>5/22</td><td>16/52</td></tr>
<tr><td>Kidney, glomeriolar amyloidosis</td><td>12/52</td><td>13/52</td><td>14/52</td><td>13/52</td><td>20/52</td></tr>
</table>
<u>1000 ppm (males 108 mg/kg bw/day, females 121 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.

<u>150 ppm (males 17.2 mg/kg bw/day, females 18.8 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.

<u>50 ppm (males 5.5 mg/kg bw/day, females 5.8 mg/kg bw/day)</u>:
No toxicologically significant treatment-related effects.

<u>Neoplastic findings</u>
No treatment-related changes in neoplastic findings at any dose level.
NOAEL: 1000 ppm (equivalent to 108 mg/kg bw/day in males) based on slightly higher incidence of amyloidosis in males in heart, parathyroid, thyroid, spleen, kidney and adrenal and 1000 ppm in females (121 mg/kg bw/day) based on decreased bw gain at 3000 ppm.</td>
<td>Crome (1988)</td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Termination week 89 survival 30% in males in the 150 and 3000 ppm groups; week 104 survival at least 35% all female groups. At 78 weeks the survival in all groups exceeded 50 %.	

Table 30: Summary table of human data on long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Prospective cohort study	Dicamba as a pesticide but not further specified	The study investigates potential association between lung cancer incidence and exposure to agricultural pesticides among the Agricultural Health Study cohort of licensed pesticide applicators while controlling for known risk factors for lung cancer. 57284 pesticide applicators and 32333 spouses.	There was no difference in the incidence of lung cancer in any of the dicamba exposure groups when compared to the never exposed group, while the low dicamba exposure group had a lower incidence than the never exposed group. Therefore the difference between the low (<24.5 days lifetime exposure) and high dicamba exposure group is considered due to an incidentally low incidence in the low dicamba exposure group and not to indicate a relevant increase in the high exposure group.	Alavanja MC, Dosemeci M, Samanic C, Lubin J, Lynch CF, Knott C, Barker J, Hoppin JA, Sandler DP, Coble J, Thomas K, Blair A; Pesticides and lung cancer risk in the agricultural health study cohort; published; Am J Epidemiol (2004) 160:876-85.
Case-control study	Dicamba as a pesticide but not further specified	The study investigates the risk of developing prostate cancer in relation to exposure to specific pesticides.	No statistically significant risk of prostate cancer was observed for ever exposure to dicamba, while a significant excess risk was observed for high exposure to dicamba (OR=2.70; 95% CI: 1.01–7.20) based on eight exposed cases. Considering that the 'ever' vs. 'never' use of dicamba did not reveal an increased risk for prostate cancer, the only small number of cases in the dicamba 'high' exposure group and the general limitations of the	Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP, Le ND; Prostate cancer risk and exposure to pesticides in British Columbia farmers; published; Prostate (2011) 71:168-83

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			study as such, the statistically significant association between high dicamba exposure and prostate cancer risk is considered not to indicate a relevant carcinogenic potential of dicamba.	
Case-control	Dicamba	Canadian incident case (non-Hodgkin's lymphoma; n=517 or 513) - control (n=1506) study among men in a diversity of occupations. An initial postal questionnaire was followed by a telephone interview for those reporting pesticide exposures of 10 hours/year or more and a 15% random sample of the remainder	A significantly increased risk for non-Hodgkin's lymphoma by exposure to Dicamba (odds ratio 1.88; 95% CI 1.32-2.68) and exposure to mixtures containing Dicamba (odds ratio 1.96; 95% CI 1.40-2.75). When they distinguished between those exposed to Dicamba, but not to DEET (N,N-diethyl-m-toluamide), and those exposed to both compounds, they calculated odds ratios of 1.39 (95% CI 0.77-2.50) and 1.84 (95% CI 1.23-2.75), respectively. Limitations of the study include differential response rates between cases (61.7%) and controls (48.0%) and the potential for recall bias.	McDuffie H.H., Pahwa P., McLaughlin J.R., Spinelli J.J., Fincham S., Dosman J.A., Robson D., Skinnider L.F. and Choi N.W. (2001) Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in men: Cross-Canada Study of Pesticides and Health. Cancer Epidemiology, Biomarkers and Prevention 10, 1155-1163. McDuffie H.H., Pahwa P., Robson D., Dosman J.A., Fincham S., Spinelli J.J. and McLaughlin J.R. (2005) Insect Repellents, Phenoxyherbicide Exposure, and Non-Hodgkin's Lymphoma. J Occup Environ Med. 47: 806-816.
Case-control	Dicamba	US incident case (non-Hodgkin's lymphoma; n=1321) - control (n=1057) study among men and women identified by random digit dialing and Medicare eligibility files.	In a subset of 679 cases and 510 controls carpet dust samples were analysed for Dicamba, which was found in homes of 15% of cases and 20% of controls. No elevation in risk was detected among the respondents who had the highest dust levels and highest self-reported exposures.	Hartge P., Colt J.S., Severson R.K., Cerhan J.R., Cozen W., Camann D., Zahm S.H., and Davis S. (2005) Residential herbicide Use and Risk of Non- Hodgkin Lymphoma. Cancer Epidemiol Biomarkers Prev 14(4) 934-937
Prospective cohort study	Dicamba	Investigation of cancer incidence among pesticide applicators exposed to dicamba in the Agricultural Health Study, a prospective cohort	A total of 41969 applicators were included in the analysis and 22036 (52.5%) reported ever having used dicamba. When	Samanic C., Rusiecki J., Dosemeci M., Hou L., Hoppin J.A., Sandler D.P., Lubin

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		of licensed pesticide applicators in North Carolina and Iowa	the reference group comprised low exposure applicators a positive trend in the risk between lifetime exposure days and lung cancer was noted but none of the individual point estimates was elevated. An elevated risk for colon cancer was also noted at the high exposure level. No increases for any cancer risk including lung and colon cancer were seen when comparing the high with the no exposure group. Although associations between exposure and lung and colon cancer were observed, the authors did not find clear evidence for an association between dicamba exposure and cancer risk. There was no apparent risk for non-Hodgkin lymphoma.	J., Blair A. And Alavanja C.R. (2006) Cancer Incidence among Pesticide Applicators Exposed to Dicamba in the Agricultural Health Study. Environmental Health perspectives 114 (10) 1521-1526.

Table 31: 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 studies available	

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

Dicamba was tested for long-term effects in a mouse and in a rat study.

Dietary administration of dicamba to CD-1 mice at dietary dose levels of 0, 50, 150, 1000, and 3000 ppm (corresponding to 5.5, 17.2, 108, and 358 mg/kg/day for males and 5.8, 18.8, 121, and 364 for females, respectively) for at least 89 weeks resulted in a slight reduction in body weight gain in high dose females (> 10%). Differential white blood cell count of blood smears at termination revealed a marked decrease of neutrophils and an increase of lymphocytes counts in treated female mice at ≥150 ppm but witout clear dose-response. A tendency to increased incidence of amyloidosis was observed in several tissues in high dose males only. Body weight gain was decreased in females at 3000ppm. In females, a significantly higher incidence of combined lymphoid tumours was observed at 150 and 1000 ppm. The incidence at 3000 ppm was not significantly increased.

		Males					Females				
	Dose [ppm]	**0**	**50**	**150**	**1000**	**3000**	**0**	**50**	**150**	**1000**	**3000**
	No. exam.	52	52	52	52	52	52	51	52	52	52
Lymphoid leukaemia		1	0	0	0	0	0	0	1	0	0
Lymphosarcoma		0	4	2	0	1	2	4	8	7	5
Pleomorphic lympho-sarcoma		-	-	-	-	-	1	1	2	2	2
Combined lymphosarcoma							3	5	10	9	7
Combined lymphoid tumors							3(6%)	5 (10%)	**11* (21%)**	**9*(17%)**	7 (13%)
Histiocytic sarcoma		-	-	-	-	-	2	2	0	1	2
Myeloid leukemia		-	-	-	-	-	0	1	1	1	0

*:p<0.05, pairwise comparison. HCD: 7.7-34.6%

Incidence of combined lymphoid tumors in this study was found to be up to 21 %. This is within the background incidence observed in acceptable historical control data (7.7-34.6 %). HCD studies were performed within a 5-year period and with same strain/supplier of animals and the same laboratory as the current dicamba study. Because of the lack of dose response and the incidence were within historical controls, dicamba was not considered to have a tumourigenic potential at dosage levels up to 3000 ppm in mice. The NOAEL in this study was 1000 ppm (mean value 121/108 for females/males mg/kg bw/day) (Crome, 1988).

Dietary administration of dicamba to rats at dietary concentrations of 0, 50, 250, 2500 ppm (corresponds to 2.0, 10.0, and 99.1 mg/kg bw/day for males and 2.4, 12.1, and 120.1 for females, at 50, 250, and 2500 ppm, respectively) for up to 27 months resulted in slightly increased food consumption observed in high dose males mainly during the first year of treatment. Also, in males there was a slight increase in liver necrosis and increase in cystic hyperplasia in the uterus at high dose. There was also a marginally decreased survival rate (42%) (Goldenthal,1985).

The dicamba rat study was performed 1981 to 1983. 6 separate HCDs were provided by Syngenta:

HCD source/description	**Years performed (in life)**	**Lab./strain**	**Duration (months) (dicamba study: 26.5)**	**Number of studies**	**Acceptability**
1. HCD: Historical control from studies done by the laboratory, in which the dicamba study was performed in, over the period of 1975-1979 in Charles River CD rats are available. Information is lacking on tumor incidence of individual studies for the HCD from 1975 to 1979 (only a mean and a range is given). Since the Goldenthal study is from 1981-1983, the HCD are not collected within a 5 year but rather 10 year period. It is not known if incidences are based on terminal kill animals only or includes also interrrim kill animals for all studies. Data for lymphoma, polyps in uterus and c-cell carcinoma available.	1975-1979,	Performing lab/CD rats (Sprague Dawley)	Exact duration unknown (In the introduction text to this HCD collection, the studies are described as 24 months studies)	Unknown but 1010 animals	**Acceptable but with uncertainties.**
2. HCD: Historical control data collected in 1983 and 1985 from the performing laboratory. These data seem to be lacking in confirmed available information of breeder and other details and for data from 1983 the strain is not available. The strain in the HCDs from 1985 is CD rats. It was not possible to confirm when exactly the studies	Data collected 1983 and 1985, exact years not known	Performing lab/CD rats (Sprague Dawley)	Exact duration unknown (The CROs updated HCD have shown that only studies of 24	1983:10 1985:9	**Acceptable but with uncertainties.**

were performed either. Data were collected in 1983 and in 1985, but no more information is available on the time these studies were actually conducted. Syngenta has some indirect information which may support that for the 1985 HCD, the studies were performed in close proximity to the inlife period of the dicamba study. This was mostly deduced and not actually confirmed. RMS finds the HCD collected in 1983 of less credibility than the HCD from 1975-1979. The HCD collected in 1985 also lack information but are considered more useful than the HCDs collected in 1983. It is not known if incidences are based on terminal kill animals only or includes also interrrim kill animals for all studies. Please refer to Vol 3, study B6.5/03 for more details. Data for lymphoma and c-cell carcinoma available. Data available for pheochromocytoma and uterus polyps (1985 only)			month duration are included in HCDs).		
3. HCD: Studies x and y are considered acceptable for use as HCD (X started 2 years prior and Y started 4 years after the study with dicamba according to applicant). The data was also from the performing laboratory and on the same strain of rat. Study x: results for given group size (60 for males, 55 for females) includes only animals from terminal sacrifice and animals dying during the study; interim sacrifice animals not included (interim sacrifice had only been done for control and high dose groups) Study y: results for given group size (70) does also not include animals from the interim sacrifice; thyroid tumors and malignant lymphomas were not seen in the interim sacrifice groups. Thyroid c-cell hyperplasia was also not seen at interim sacrifice. Data for lymphoma and c-cell carcinoma available.	1979 and 1987	(study x and y) Performing lab/CD rats (Sprague Dawley)	24	2	**Acceptable**
4. HCD: Notifier supplied HCD for polyps and Thyroid effects from RITA (Registry of Industrial Toxicology Anamial data) on SD rats. For effects on thyroid: for males ranges of incidences of thyroid gland C-cell adenomas were 3.3-38.3% and 0-8.3% for C-cell carcinomas. For polyps (glandular) the range was 0-5.8% and for polyps (endometrial) the range was 0-36.5%. These data are only considered supplementary by RMS since they were from different unknown laboratories and HCD are colleted in a period of time exceeding way above the 5 or even 10 years around the time when the dicamba study was conducated (1981-1983) since data are collected from 1985 to 2010. Please also refer to position paper Vol 3, B.6.5/04 and B.6.5/05. Data for c-cell carcinoma and polyps available.	1985-2010	RITA (Registry of Industrial Toxicology Anamial data): Sprague Dawley	24-26	39 studies for uterus polyp and 40 studies for thyroid tumors	**Supplementary.**
5. HCD: National Toxicology Program (NTP). Data collected from NTP labs/female Sprague Dawley. Please see position paper 6.5/05 for more information in Vol 3. Data for c-cell carcinoma available.	1998-2004	NTP/ Sprague Dawley, females	24	9	**Supplementary**
6. HCD: **6.** Historical control data from studies done by the laboratory, in which the	1976-1986	Performing lab/CD rats	24	29 (this is the number of control	**Acceptable**

dicamba study was performed in, over the period of 1977-1994 an in Sprague Dawley rats are available. Since the dicamba study is from 1981-1983, the HCD are not collected within 5 years of the study but rather over around 17 years. Notifier further submitted data within ± 5 years (initiated 1976-1986), which were used for comparison to dicamba data and are considered acceptable and the most reliable of the HCDs submitted. However, ±5 years may be considered too long a time period. Please also refer to position paper B.6.5/05 in Vol 3 for further clarification by notifier. Data represents both administration by diet and gavage. Information of body weigt and other details of study conduct are missing for the single studies. Data for c-cell carcinoma available, liver necrosis and kidney nephrosis and lymphoma.		(Sprague Dawley)		groups from totally 20 studies initiated 1976-1986, as a number of studies had more than one control group).	

Mixed malignant lymphoma tumors were observed in high dose (6.7%) with significant trend analysis: during the first evaluation it was found appropriate to combine the different types of malignant lymphomas and they were then considered within historical control range. The incidence of the 1975-1979 historical control data set from the laboratory the dicamba study was performed in (using Charles River CD rats, 24 month duration, based on data from 1010 males) for malignant lymphoma (no differentation into histio- or lymphocytic or mixed) had a mean incidence of 3.8% with a range of 0-8.6% in individual studies. However, if the other historical control data are used (1985), the ranges for malignant lymphoreticular lymphoma at the laboratory was 0-7.2% for studies reported/data collected in 1985. In the X,Y studies Range was between 0-1.7%. The incidence observed in this study is in this respect within the available historical control range.

Since no mixed malignant lymphomas were observed at interim kill, the incidence could also be calculated out of the animals at terminal kill. In this case the incidences of lymphomas would be 0% (0/50), 0% (0/50), 8% (4/50) and 8% (4/50). The incdence would then be within the 1975-1979 HCDs and outside the 1985 HCDs. HCDs from performing study restricted to starting 1976-1986 is 0-9.1 %.

However, a discussion of the usefulness of the HCDs as well as how to calculate the incidences would be considered necessary by RMS

Thyroid parafollicular (C-cell) carcinoma:

in high dose males, an increased incidence of thyroid parafollicular (C-cell) carcinoma was observed. No significant difference was found according to pairwise comparison, whereas a significant trend was observed. In the last evaluation it was discussed that often parafollicular tumours develop upon functional changes of the thyroid. Changes in the incidence of parafollicular adenoma and parafollicular hyperplasia would therefore be expected. However, neither the incidence of parafollicular adenoma (2, 5, 5 and 3 at 0, 50, 250, and 2500 ppm, respectively) nor of parafollicular hyperplasia (28, 27, 37 and 26) was affected by treatment. Likewise, the weighted grade of parafollicular hyperplasia was comparable between all groups (1.9, 2.0, 1.9 and 2.0). Therefore, at the last evaluation, it was considered unlikely that the increased incidence of parafollicular carcinoma is related to treatment.

Thyroid	No. exam.	60	60	60	60	60	60	60	60
0-12 months									
Parafollicular cell carcinoma		0/11	0/11	1/12	0/10	0/11	0/11	0/10	0/11
Parafollicular hyperplasia, mild		1	1			1		1	
12 months to termination									
Parafollicular cell hyperplasia		28/49	27/49	37/48	26/50	35/49	36/49	39/50	35/49
-trace		4	3	2	3	3	6	4	0
-mild		24	24	35	21	30	29	34	34
-moderate		0	0	0	2	2	1	1	1

Follicular adenoma		0/49	1/49	1/48	1/50	-	-	-	-
Parafollicular cell adenoma		2/49	5/49	5/48	3/50	5/49	1/49	3/50	6/49
Follicular carcinoma		0/49	1/49	0/48	0/50	-	-	-	-
Parafollicular cell carcinoma		1/49	0/49	1/48	5/50	0/49	1/49	0/50	0/49
Parafollicular cell carcinoma (/total)		1/60	0/60	2/60	**5/60a**				
Parafollicular cell carcinoma (%)		1.7	0	3.3	8.3				

a: positive trend analysis

It should be noted that the incident of thyroid parafollicular (C-cell) carcinoma in this study is 8.3 % (5/60) in the high dose group and the incidence in the 250 ppm group is 3.3 % (2/60). In the historical control data from 1975-1979 the range is 0.0-2.0 % incidence (mean 0.2%). In the other historical control data from collected 1983, the range is 0-2.9 (but these HCDs are considered less reliable) and 0-1.7% for data collected in 1985. In the studies x and y the incidence is 0%. Thus the incidence in the study is above the incidence found in all these HCD for mid and high dose group males. In the HCD from RITA (unknown laboratories and collected over a periode of 25 years for males, ranges of incidences of thyroid gland C-cell adenomas were 3.3-38.3% and 0-8.3% for C-cell carcinomas. The RITA HCDs are supplementary. The latest historical controls supplied by the notifier are spanning ±5 years around the dicamba study, but not 2.5 years centered around the study. The range of incidence of parafollicular cell carcinoma in these studies are 0-5 % in males, with a mean and standard deviation of 0.3 ± 1. These historical control data are acceptable and expected to be the most relevant. Only the high dose is outside the range of these HCDs. HCDs from the NTP in females are considered supplementary.
Notifier argues that the longer in-life periode in the dicamba study (26.5 months for males) versus 24 months in HCD may have led to higher incidence of carcinomas in the dicamba study and RITA data may be more relevant. RMS acknowledge that in-life periode may affect the HCD range.
Notifier argues that perhaps collecting data over a larger time periode from different laboratories is less important than using a longer treatment periode. It is difficult to know what may affect the incidence of c-cell tumors more. The dicamba study had a duration of 26.5 months which is longer than the studies where HCD have been colleted from. However, using HCD from different laboratories may introduce many possible confounding factors (e.g. animal vendor, type of bedding, possible chemical contaminations, or differences in the feed composition, many different pathologists, tissue trimming) which may also affect the range. Furthermore, only 1 study among the 40 studies taken from the RITA database had a c-cell carcinoma incidence of 8.3% (25 months duration). The highest incidence after that was 6.0 % (which was actually from a 24 months study). There were 9 studies in total with a duration of 25-26 weeks. Of these studies 7 of them had an incidence of 2 % or less and 1 had an incidence of 5%. So 8.3% represents the most extreme control group even among the studies of longer than 24 monts duration (25-26 months) and from unknown laboratories and also collected over a time period of 25 years.

The increase in parafollicular cell carcinoma was not accompanied by increases in hyperplasia or adenomas.
Furthermore, there were no indication of early onset of tumors and no indication of thyroid effects from the short term studies. Taken together, this decrease the level of concern regarding the carcinogenicity concern for humans

However, considering both that the incidence in the high dose was above the most appropriate HCD as well as a significant trend was observed, the observed increase in thyroid parafollicur carcinoma cannot be excluded to be treatment related.

Thyroid parafollicular c-cell carcinoma	**Years (in life)**	**Lab./strain**	**Males; Range (%), mean ± SD**	**Duration (months) (dicamba study: 26.5)**
1. HCD	1975-1979,	Performing lab/CD rats (Sprague Dawley)	0-2, 0.2	Exact duration unknown (mentioned as 2 year studies)
2. HCD	Data collected 1983 and 1985, exact years not known	Performing lab/CD rats (Sprague Dawley)	0-1.7 (1985) 0-2.9 (1983)	Exact duration unknown (mentioned as 2 year studies)

3. HCD	1979 and 1987	(study x and y) Performing lab/CD rats (Sprague Dawley)	0	24
4. HCD	1985-2010	RITA (Registry of Industrial Toxicology Anamial data): Collected from different labs/Sprague Dawley	0-8.3, 2.5 ±2.5	24-26
5. HCD	1998-2004	NTP/ Sprague Dawley, females	0-8	24
6. HCD	1976-1986	Performing lab/CD rats (Sprague Dawley)	0-5, 0.3 ±1	24

In females, pheochromocytoma of the adrenal medulla was observed in the incidence: 1/47, 4/48, 3/46 and 5/46. No adrenal medulla pheochromocytoma were observed before 12 months of age and therefore RMS considers it appropriate to calculate the incidence out of the number of animals who died after 12 months or were killed at termination. Historical control data were supplied by Syngenta and collected in 1985 (acceptability of HCD are discussed above). Incidence in females was outside HCD range (0-8.3%) in the high dose (11%) but without clear dose-response (not statistically significant trend or by pairwise comparison). Because of the lack of dose-response and lack of increased finding of adrenal medullary hyperplasia, in females, the increased incidence of pheochromocytoma of the adrenal medulla may be considered incidental. Also, if it is considered acceptable to calculate the incidence out of 60 animals, the incidence in high dose group is 8.3% (5/60) which is just inside HCD range. In males, the incidence was also above HCD in some groups, but the highest incidence was found in controls and therefore not considered treatment related.

In females, 4/60 (6.7%), 5/60 (8.3%), 5/60 (8.3%) and 8/60 (13.3%) polyps in the uterus was observed untill terminal sacrifice so the overall incidence of uterine polyps in the high dose group was slightly higher than concurrent and historical control data from the same laboratory (0-8.3 % in the HCDs collected 1975-1979) but did not reach statistical significance. The increase in high dose group may be treatment related. Uterine polyps are a benign age related tumor in rats which may not have an etiology relevant for women (Davis, 2012)[11] but according to ECHA CLP guidance (2017) only if a mode of action of tumour development is conclusively determined not to be operative in humans may the carcinogenic evidence for that tumour be discounted. To the knowledge of RMS, this is not the case for uterine polyps at this time. No early onset was observed at 12 months (only 2 rats with polyps seen at 50 ppm). However, the finding may be considered supportive for a classification.

Effects observed in humans:

The only source of human information on carcinogenicity of dicamba is epidemiology. A general difficulty for epidemiology papers, and for the evaluation of any dicamba associated adverse health effects, is that exposure to dicamba alone normally cannot be evaluated. This is because dicamba is often used in mixtures with other herbicide active ingredients with often lower dicamba-content in these mixture products as compared to the other active ingredients. It is therefore almost impossible to consider the effect of exposure to dicamba alone without the influence of other active substances/co-formulants. Furthermore, it is difficult to attribute health effects including cancer to dicamba-containing products since humans are exposed to a great number of environmental chemicals.

Lung cancer: Statistical significance was only seen when comparing high dicamba exposure (as lifetime exposure days) with low dicamba exposure but not with no dicamba exposure (Alavanjaet al., 2004). The statistical significance is therefore considered more of an artefact - due to the fact that the low dicamba exposure groups had a lower risk for lung cancer than the no exposure group – than indicating an actual effect of dicamba.

No lung effects were seen in any repeated dose toxicity study in animals.

[11] Davis, B (2012). Endometrial Stromal Polyps in Rodents: Biology, Etiology, and Relevance to Disease in Women. Toxicologic Pathology.

Therefore, the published findings on lung cancer are considered of insufficient relevance to be considered for human risk assessment of dicamba.

Prostate cancer: Statistical significance was seen in only one publication when a low number of cases with high dicamba exposure were compared to never exposure but not for 'ever' use of dicamba (data based on British Columbia Cancer Registry) (Band et al, 2011).
Additionally, there are a number of other publications that investigate the association between prostate cancer risk and pesticide including dicamba exposure[12] (data based on the Agricultural Health Study – applicators and spouses): none of these found an association between prostate cancer and dicamba exposure. Additionally two reviews evaluate the overall evidence of an association of pesticide exposure and prostate cancer and conclude that there is no relevant association[13].
Therefore, the reported association of high dicamba exposure and prostate cancer is considered not relevant for human risk assessment considering that this was not confirmed by a considerable number of other epidemiology publications or any similar finding in animal studies.

An apparent association between exposure to dicamba, either alone or in combination with other pesticides, and Non-Hodgkin's lymphoma in agricultural workers was identified by McDuffie et al (2001;2005) but was not confirmed by Samanic et al (2005). Similarly, Hartge et al (2006) found no correlation between the use of dicamba and Non-Hodgkin's lymphoma in a residential environment.
Two reasonably well-designed and reported publications did not find an association between exposure to dicamba and NHL covering exposure to dicamba from residential use (home and garden, case-control study) and from use on the field (pesticide applicators on farms, prospective cohort study) – Samanic et al (2006) and Hartge et al (2005).
The other two publications from the same primary author (McDuffie et al 2001/2005) were based on the same data set and reported a weak association of dicamba exposure (various professions) with NHL (case-control design).
However the design and reporting limitations of the McDuffie publications are considered somewhat more marked as compared to the other two publications: e.g. no information on period of data collection or whether pesticide exposure preceded NHL diagnosis or not, potential recall bias (inherent for case-control studies), risk of statistically significant associations occurring by chance considering the large numbers of associations evaluated (but details missing exactly how many compounds/associations were evaluated). Due to the fact that subjects had variable occupations potentially made a correct assessment of pesticide exposure particularly difficult in the McDuffie et al papers.
Therefore considering a weight of evidence in the evaluation whether dicamba was associated with an increased risk for NHL, more weight is placed on the results by Hartge et al and Samanic et al vs McDuffie et al – also as the association seen by McDuffie et al were relatively weak as well. Therefore, dicamba is considered not to be associated with a relevantly increased risk for NHL based on the epidemiology papers discussed above.
The last publication by Samanic et al (2006) additionally reports a slightly increased risk for lung and colon cancer when the highest dicamba-exposed group is compared with the low-exposed group but not when any dicamba-exposed group (including the highest exposed group) is compared to the no-exposure group. The same data set concerning lung cancer is also discussed in the Alavanja et al 2004 publication (summarised as above). As the low-exposure group for dicamba contained more non-smokers than the high- or no–exposure groups and consequently had a markedly lower risk for lung (and colon) cancer as compared to the no-exposure group, the reported increase of risk for the highest exposure group is considered more an artefact to the low risk within the low-exposure group and not to indicate a relevant increase of risk due to high dicamba expsoure.
Therefore, the additional 4 epidemiology papers discussed above are considered not to indicate a relevant risk for cancer associated with dicamba.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Table 32: Compilation of factors to be taken into consideration in the hazard assessment

[12] Barry et al 2011 and 2012, Koutros et al 2011
[13] Mink et al 2008, Weichental et al 2010

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
Mouse, Charles River CD-1	No treatment-related changes in neoplastic findings							
Rat, Charles River CD (Sprague Dawley)	uterine polyps (0-8.3%)	No	No		single	No	oral	Not known
Rat, Charles River CD (Sprague Dawley)	Thyroid parafollicular (C-cell) carcinoma (0-2.9%)	No	NA		single	No	oral	Not known

Regarding the increased incidence of c-cell carcinoma there was a lack of concurrent histopathological findings/increase in c-cell adenoma in the thyroid from the database, an unknown mode of action and the increase in thyroid parafollicular (C-cell) carcinoma was observed only in one species and in one gender without indication of early onset. The factors mentioned above weaken the available evidence and decrease the level of concern regarding the carcinogenicity concern for humans. However, based on the dose-related increased incidence of thyroid parafollicular (C-cell) carcinoma in male rats, which were above the incidence found in the HCD for high dose group males and in addition with a significant trend analysis, RMS considers the increase in these tumors cannot be exluded to be treatment related and a classification for Carc Cat 2 is suggested. The finding of increased number of polyps in female rats may be considered supportive.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Based on the dose-related increased incidence of thyroid parafollicular (C-cell) carcinoma in male rats (although not accompanied by increases in hyperplasia or adenomas), observed above the incidence found in the HCD for high dose group males and a significant trend analysis, RMS considers the increase in these tumors may be treatment related. Since the increase in thyroid parafollicular (C-cell) carcinoma was observed in one species and in one gender, a classification for Carc Cat 2 is suggested by RMS.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS considered that the dose-related incidence of thyroid parafollicular (C-cell) carcinoma observed in males in the carcinogenicity study in rats, together with the statistically significant positive trend, suggests that these tumours are treatment related. Moreover, the incidence of these parafollicular (C-cell) carcinoma at the top dose were also above the incidence found in several of the Historical Control Data (HCD) provided. Since the increase in the incidence of this thyroid parafollicular (C-cell) carcinoma was only

observed in one species and in one sex, the DS proposed classification for Carcinogenicity in Category 2.

Comments received during consultation

One MSCA supported the proposed classification of dicamba as Carc. 2 H351 (suspected of causing cancer).

One company-downstream user expressed that dicamba should not be classified as Carc. Cat 2 since thyroid parafollicular (C-cell) carcinoma found at 250 ppm and above should be considered an age- and not substance-related effect. According to this comment, the incidence of thyroid C-cell carcinomas in male rats only slightly exceeded the range of the HCD. In general, in older animals, spontaneously formed tumours increase with every additional week of exposure. In the case of the reported study, the HCD did not cover the most critical time period (2.5 and 3.5 months longer than HCD for males and females, respectively). In addition, since no pre-neoplastic lesions were recorded, this commenter considered that a relationship to treatment is unlikely. Finally, the commenter highlighted that amyloidosis found in the study should not be used to support the classification for Carc. Cat 2. The DS agreed that the HCD were not ideal, but the dose-response relationship along with a statistically significant positive trend test suggest that there is a treatment-related effect. The DS also clarified that amyloidosis was not used to support the proposal for classification.

A company manufacturer considered a Carc. Cat 2 classification not to be required for dicamba based on: 1) the longer duration of the study compared to the studies contained in the relevant HCD database, which impacts on the assessment of the thyroid-c-cell carcinomas and uterus polyps because this may underestimate the incidence of these two tumours with an age-related component, and 2) the non-relevance of amyloidosis for supporting carcinogenicity. This commenter also considered that since none of the available HCD fully match the dicamba study, a weight of evidence approach is needed. In relation to this, the manufacturer noted that: 1) there is no relationship between treatment and incidence of thyroid-c-cell hyperplasia and adenomas; 2) the combined incidence of thyroid c-cell adenoma and carcinoma in dicamba treated groups were well within the range of the HCD from the laboratory performing the study, despite the longer in-life phase of the dicamba study; 3) there is no indication of early onset of thyroid C-cell carcinomas; 4) there were no pre-neoplastic findings in sub-chronic studies; 5) there were no tumours in female rats. Finally, the commenter noted that the absence of a relevant dose-response relationship for the number of animals with tumours and the number of tumours (benign, malignant, or total tumours) observed do not support a carcinogenic potential of dicamba.

The DS replied that the 8.3% incidence of thyroid-c-cell carcinomas represented the most extreme control group from even among the studies of longer duration and from unknown laboratories, which were collected over a time period of 25 years and also despite the lack of pre-neoplastic lesions there is a dose-response relationship with a positive trend test, suggesting that there is a treatment-related effect. The DS noted that the case of uterus polyps is a borderline effect that is difficult to interpret with the available HCD. Finally, the DS also clarified that amyloidosis was not being used to support the proposal for classification.

An MSCA provided an additional epidemiological study (Lerro *et al*., 2020) available from the open scientific literature showing an association with dicamba use and liver and intrahepatic bile duct cancer. The DS responded that the study was not included in the CLH-report because peroxisome proliferation (a mechanism of tumour formation not considered relevant for humans) is mentioned as a possible mode of action. Nevertheless, RAC summarised this study in the table "Summary table of human data on dicamba carcinogenicity", below and considered the main findings in the weight of evidence analysis.

Assessment and comparison with the classification criteria

The database contained two animal studies (a carcinogenicity study in mice plus a combined chronic toxicity/carcinogenicity in rats) and human data (two prospective cohort studies plus three case-control studies).

Carcinogenicity study in mice (KCA 5.5 / 01)

The study was conducted according to OECD TG 451 and was GLP compliant. Groups of 7-week-old CD-1 mice (52/sex/group) were administered 0, 50, 150, 1000 and 3000 ppm dicamba (purity 86.8%) per day. These doses were equivalent to 0, 4.8, 14.9, 93.7 and 311 mg pure dicamba/kg bw/day, respectively, for males and to 0, 5.0, 16.3, 105, 316 mg pure dicamba/kg bw/day, respectively, for females. Male mice were killed following 89 completed weeks of treatment when the male survival approached 30% in animals administered with 150 and 3000 ppm. Females were killed after 104 weeks of treatment when the survival was at least 35% in all groups. Non neoplastic findings are summarised in the table "Summary for repeated dose toxicity studies in animals with dicamba" in the section "STOT RE", above and included a slight body weight gain reduction in high dose in females. All other parameters of systemic toxicity were unaffected. Increased incidences of amyloidosis in thyroid, parathyroid, spleen, adrenals, heart and kidney were also reported (Table "Summary for repeated dose toxicity studies in animals with dicamba").

Neoplastic findings in this study are summarised in the table below. In females, a significantly higher incidence of combined lymphoid tumours was observed at 150 ppm (pairwise comparison: p=0.006, 21.2%) and 1000 ppm (pairwise comparison: p=0.036, 17.3%). The incidence at 3000 ppm was not significantly increased (pairwise comparison: p=0.125, 13.4%). The effect was not dose-related and the incidences were within the background control data for this strain of mice in the laboratory. The DS assessed the quality of the HCD and determined that they were valid for evaluating the findings in the dicamba study.

***Table**: Multicentric tumours and combined lymphoid tumours in the carcinogenicity study in mouse with dicamba*

	0 ppm	50 ppm	150 ppm	1000 ppm	3000 ppm
MALES					
Number	52	52	52	52	52
Lymphoid leukaemia	1	0	0	0	0
Lymphosarcoma	0	4	2	0	1

FEMALES					
Number	52	51	52	52	52
Lymphoid leukaemia	0	0	1	0	0
Lymphosarcoma	2	4	8	7	5
Pleomorphic lympho-sarcoma	1	1	2	2	2
Combined lymphosarcoma	3	5	10	9	7
Combined lymphoid tumours	3	5	**11***	**9***	7
Histiocytic sarcoma	2	2	0	1	2
Myeloid leukemia	0	1	1	1	0

*:p<0.05
HCD: 7.7-34.6% (within 5 years from the start of the study, range: 13.5-34.6%)

Combined chronic toxicity/carcinogenicity in rats (KCA 5.5 / 02)

The study was conducted according to OECD TG 453 and was GLP compliant. Groups of Sprague Dawley rats (60/sex/group) were administered 0, 50, 250 and 2500 ppm dicamba (purity 86.8%) daily. These doses were equivalent to 0, 1.7, 8.7, and 83 mg pure dicamba/kg bw/day, respectively, for males and to 0, 2.1, 10.5, and 104 mg pure dicamba/kg bw/day, respectively, for females. Ten rats/sex/group were sacrificed after 12 months. The remaining animals were sacrificed after 115 weeks for the males and 118 weeks for the females. Survival was (marginally) less than 50% in all dosed male groups and in mid dose females at 104 weeks. Non-neoplastic findings are summarised in the table "Summary for repeated dose toxicity studies in animals with dicamba" and included, at the top dose, increases in incidences of liver necrosis in males and hydronephrosis of kidney in males and females (in both cases the incidences within the HCD of the performing facility) and increases in the incidence of cystic hyperplasia in the uterus.

The CLH-report contains an array of up to 6 different HCD, four of which were considered acceptable (two of them with uncertainties) by the DS and other two only supplementary. However, RAC assessed the reliability of the provided HCD and considered only 2 of them to be valid; specifically, those obtained by the same performing laboratory and within a temporal frame of ±5 years. The table below summarises the main features of the HCD accepted by RAC.

***Table**: HCD considered in the assessment. All the HCD were obtained in the performing lab with CD rats (Sprague Dawley)*

Number	Description	Years	Duration (months)	Number studies
1	Study x: started 2 years prior the study with dicamba. Results for given group size (60 for males, 55 for females) includes only animals from terminal sacrifice and animals dying during the study Study y: started 4 years prior the study with dicamba. Results for the given group size (70) does also not include animals from the interim sacrifice.	1979 and 1987	24	2

2	HCD within ± 5 years (dicamba study is 1981-1983). This HCD does not include HCD 1 (see above)	1976-1986	24	29[#]

[#]this is the number of control groups from a total of 20 studies initiated (a number of studies had more than one control group)

Mixed malignant lymphoma tumours were observed at the high dose (6.7%, 4/60) in males which were statistically significant by trend analysis (Table below). This incidence was within the range reported for HCD 2 (in the Table below). It is noted that the notifier considers likely that the HCDs are compiled of all animals and therefore do not find it appropriate to exclude interim-sacrifice animals from the calculation.

***Table**: Incidences of neoplastic findings in the lymphoreticular system in the combined chronic toxicity/carcinogenicity in rats with dicamba*

	MALES				**FEMALES**			
ppm:	**0**	**50**	**250**	**2500**	**0**	**50**	**250**	**2500**
Number of examined animals	60	60	60	60	60	60	60	60
Leukaemia, granulocytic	-	-	-	-	2	0	0	1
Lymphoma, histiocytic, malignant	0	0	2	0	3	0	0	0
Lymphoma, lymphocytic, malignant	0	0	2	0	3	0	2	0
Lymphoma, mixed, malignant	0	0	0	**4a**	-	-	-	-

[a] = positive trend analysis
HCD 1 (see Table 11): Incidence of mixed malignant lymphoma: range 0-1.7%
HCD 2 (see Table 11): Incidence of mixed malignant lymphoma: range 0-9.1%

In females, pheochromocytoma of the adrenal medulla was observed with the following incidences: 1/47, 4/48, 3/46 and 5/46 (Table 13). No adrenal medulla pheochromocytoma were observed before 12 months of age and therefore the DS considered it appropriate to calculate the incidence out of the number of animals who died after 12 months or were killed at termination. The incidences showed no clear dose-response relationship (and were not statistically significant either by trend test or by pairwise comparison). Because of the lack of dose-response relationship and lack of any increases in the incidences of adrenal medullary hyperplasia in females, the increased incidence of pheochromocytoma of the adrenal medulla may be considered incidental.

The overall incidence of uterine polyps in the high dose group was slightly higher than in concurrent controls and in historical control data (0-8.3%) (Table below). In females, incidences of polyps in the uterus of 6.7, 8.3, 8.3 and 13.3% (assuming 60 animals/group at each dose), respectively, were observed up to terminal sacrifice but did not reach statistical significance.

***Table**: Incidences of neoplastic and non-neoplastic findings in adrenal medulla and uterus in the combined chronic toxicity/carcinogenicity in rats with dicamba*

	MALES				**FEMALES**			
ppm:	**0**	**50**	**250**	**2500**	**0**	**50**	**250**	**2500**
Adrenal medulla pheochromocytoma (%) 12 months to termination	14/48 (29)	9/48 (19)	12/47 (26)	14/49 (29)	1/47 (2)	4/48 (8)	3/46 (6)	5/46 (11)
Adrenal medulla hyperplasia, trace or mild (0-12 months)	2/10	0/11	0/11	0/8	0/9	0/10	1/10	1/10
Adrenal medulla hyperplasia, trace or mild (12 months -termination)	8/48	7/48	12/47	9/49	2/47	2/48	1/46	1/46
Polyp in uterus (%)					4/60 (6.7)	5/60 (8.3)	5/60 (8.3)	8/49 (16)

Died/sacrificed: 0-12 month	0/11	2/11	0/10	0/11
Died/sacrificed: 12 months to termination	4/49 (8)	3/49 (6)	5/50 (10)	8/49 (16)
Uterus Adenocarcinoma	0/49	1/49	1/49	0/49
Uterus squamous cell carcinoma	1/49	0/49	0/49	1/49

In high dose males, an increase in the incidence of thyroid parafollicular (C-cell) carcinoma was observed (Table below). No significant difference was found according to a pairwise comparison, whereas a statistically significant trend was observed. Changes in the incidence of parafollicular adenoma and parafollicular hyperplasia would be expected. However, neither the incidence of parafollicular adenoma nor of parafollicular hyperplasia was affected by treatment (Table below). Furthermore, there was no indication of early onset of tumours and no indication of thyroid effects from the short-term studies. It should be noted that the incidence of thyroid parafollicular (C-cell) carcinoma in this study at the top dose exceeds both HCD (see the Table below).

***Table**: Incidences of neoplastic and non-neoplastic findings in thyroid in the combined chronic toxicity/carcinogenicity in rats with dicamba*

	MALES				**FEMALES**			
ppm:	**0**	**50**	**250**	**2500**	**0**	**50**	**250**	**2500**
<u>0-12 months</u>								
Parafollicular cell carcinoma	0/11	0/11	1/12	0/10	0/11	0/11	0/10	0/11
Parafollicular hyperplasia, mild	1	1			1		1	
<u>12 months to termination</u>								
Parafollicular cell hyperplasia:	28/49	27/49	37/48	26/50	35/49	36/49	39/50	35/49
trace	4	3	2	3	3	6	4	0
mild	24	24	35	21	30	29	34	34
moderate	0	0	0	2	2	1	1	1
Follicular adenoma	0/49	1/49	1/48	1/50	-	-	-	-
Parafollicular cell adenoma	2/49	5/49	5/48	3/50	5/49	1/49	3/50	6/49
Follicular carcinoma	0/49	1/49	0/48	0/50	-	-	-	-
Parafollicular cell carcinoma	1/49	0/49	1/48	5/50	0/49	1/49	0/50	0/49
Parafollicular cell carcinoma (/total)	1/60	0/60	2/60	**5/60[a]**				
Parafollicular cell carcinoma (%)	1.7	0	3.3	**8.3**				

[a] = positive trend analysis
HCD 1 (see Table 11): Incidence of thyroid parafollicular (C-cell) carcinoma: mean 0%
HCD 2 (see Table 11): Incidence of thyroid parafollicular (C-cell) carcinoma: mean 0.3±1%, range 0-5%

Finally, under the conditions of the study no treatment-related effects were observed on overall tumour incidence (Table below).

***Table**: Overall incidence of neoplastic findings in the combined chronic toxicity/carcinogenicity in rats with dicamba*

	MALES	**FEMALES**

ppm:	0	50	250	2500	0	50	250	2500
Number of animals	60	60	60	60	60	60	60	60
Animals with benign tumours	49	17	41	45	48	49	53	52
Animals with malignant tumours	13	14	17	17	25	20	18	17
Animals with tumours	59	50	46	52	51	51	55	52
Number of neoplastic events	89	71	78	73	98	92	96	108
Number of malignant neoplastic events	17	19	19	20	32	26	20	20
Number of neoplastic events	106	90	97	92	130	118	116	128

Human data

Human data was extracted from the open scientific literature and is summarised in the table below. A general challenge of these studies for the evaluation of any adverse effect of dicamba on human health is that the exposure of dicamba alone normally cannot be evaluated. This is because dicamba is used very often with other herbicide components with higher concentrations than those for dicamba and control for potential confounding by these exposures was limited in the studies identified.

The human data summarised in the table below have found potential associations between dicamba exposure and incidence of several types of tumours such as lung, prostate, liver and intrahepatic bile duct, lymphocytic leukaemia, and non-Hodgkin's lymphoma. However, all studies had several methodological weaknesses in addition to the co-exposure challenge stated above. These additional weaknesses include a low number of exposed individuals and/or few observed cases in the exposed, no dose-response relationship among different exposure groups or lack of reproducibility among different studies. Overall, the studies summarised in the table below did not provide robust evidence for an association between cancer and exposure to dicamba.

***Table**: Summary table of human data on dicamba carcinogenicity*

Type of study/data	Observations	Reference
Prospective cohort study	There was no difference in the incidence of lung cancer in any of the dicamba exposure groups when compared to the never exposed group.	Alavanja *et al.,* 2004
Case-control study	No statistically significant risk of prostate cancer was observed for ever exposure to dicamba, while a significant excess risk was observed for high exposure to dicamba (odds ratio = 2.70; 95% CI: 1.01-7.20) based on eight exposed cases. Considering that the 'ever' vs. 'never' use of dicamba did not reveal an increased risk for prostate cancer, the small number of cases in the dicamba 'high' exposure group and the general limitations of the study as such, the statistically significant association between high dicamba exposure and prostate cancer risk is considered not to indicate a relevant carcinogenic potential for dicamba.	Band et al., 2011
Case-control	A significantly increased risk for non-Hodgkin's lymphoma by exposure to dicamba (odds ratio 1.88; 95% CI: 1.32-2.68) and exposure to mixtures containing dicamba (odds ratio 1.96; 95% CI: 1.40-2.75). When those exposed to dicamba	McDuffie *et al.*, (2001) McDuffie *et al.*, 2005

	but not to DEET were distinguished from those exposed to both these substances, the odds ratios were 1.39 (95% CI: 0.77-2.50) and 1.84 (95% CI: 1.23-2.75), respectively. Limitations of the study include differential response rates between cases (61.7%) and controls (48.0%) and the potential for recall bias.	
Case-control	In a subset of 679 cases and 510 controls carpet dust samples were analysed for dicamba, which was found in homes of 15% of cases and 20% of controls. No elevation in risk was detected among the respondents who had the highest dust levels and highest self-reported exposures.	Hartge *et al.*, 2005
Prospective cohort study	49 922 applicators (52.9% exposed to dicamba) of which 6702 in the highest quartile of exposure to dicamba. Regarding cancer sites with an indication of an exposure-related trend the relative risk in the highest exposure quartile category and the p for trend by exposure quartile were: 1) elevated risk of liver and intrahepatic bile duct cancer (28 exposed cases, relative risk 1.80, CI: 1.26–2.56, Ptrend< 0.001); 2) elevated risk of chronic lymphocytic leukaemia (93 exposed cases, relative risk 1.20, CI: 0.96–1.50, Ptrend = 0.01); and, 3) reduced risk of risk of myeloid leukaemia (55 exposed cases, relative risk 0.73, CI: 0.51–1.03, P trend = 0.01). The associations for liver cancer and myeloid leukaemia remained after lagging exposure of up to 20 years. Associations with lung and colon cancer were not apparent. Due to a low number of exposed cases, only less detailed analyses (two exposure categories only) were performed for the following: 1) acute/other lymphocytic leukaemia (13 exposed cases, relative risk = 4.59, CI: 2.11-19.98, P trend < 0.001); 2) mantle cell lymphoma (18 exposed cases, relative risk = 3.47, CI: 2.06-5.85, P trend = 0.12)	Lerro *et al.,* 2020
Prospective cohort study	A total of 41969 applicators were included in the analysis and 22036 (52.5%) reported ever having used dicamba. When the reference group comprised low exposure applicators a positive trend (p = 0.02) in the risk between lifetime exposure days and lung cancer (relative risk of 2.16 with 95% CI: 0.97–4.82). An elevated risk for colon cancer was also noted at the high exposure level (relative risk = 3.29; 95% CI: 1.40–7.73; p-trend = 0.02). There was no apparent risk for non-Hodgkin lymphoma. Although associations between exposure and lung and colon cancer were observed, the authors did not find clear evidence for an association between dicamba exposure and cancer risk.	Samanic *et al.*, 2006

Comparison with the criteria

RAC notes that the study by Lerro *et al*. (2020), summarised in the table above, is especially relevant due to the large cohort considered. In this study, indications of an association with dicamba exposure with an elevated risk of liver and intrahepatic bile duct cancers, acute lymphocytic leukaemia, chronic lymphocytic leukaemia and mantle cell lymphoma were observed. However, adjustment for potential confounding by other concomitantly used chemicals was limited to one pesticide.

The observed elevated risk of liver and bile duct cancer was based on relatively few exposed cases (n=28). Moreover, looking separately at the liver and intrahepatic bile ducts, only intrahepatic bile duct cancer demonstrated an elevated risk with dicamba and this was based on 10 exposed cases only. There was a positive trend for liver and bile duct cancer overall after a 20-year exposure lag as well as for bile duct cancer. However, the latter was based on only 3 cases in the highest exposure category.

Dicamba was also associated with an elevated risk of lymphocytic leukaemias. However, there were few exposed cases (n=13) and these findings did not remain after lagging exposure more than 5 years; which suggest either a short latency or a spurious finding due to few cases. The positive association between dicamba and mantle cell lymphoma was based on 18 cases and there was limited evidence of a monotonic exposure-response trend.

Classification for carcinogenicity within group 1A is largely based on human evidence. In the opinion of RAC, the study by Lerro *et al*. (2020) and the remaining information summarised in the table above do not provide evidence of an established causal relationship between exposure to the agent and human cancer and therefore classification of dicamba as Carc. Cat 1A is not warranted.

Dietary administration of dicamba to mice resulted in a significantly higher incidence of combined lymphoid tumours at 150 and 1000 ppm but not at 3000 ppm (Table 10). Even though the findings at 150 ppm and 1000 ppm were statistically significant compared to controls, the effect was not dose-related and the incidences were within the background control data for this strain of mice in the laboratory. Therefore, RAC does not consider these tumours compound-related and they cannot support a classification for carcinogenicity.

Dietary administration of dicamba to rats resulted in an increase in the incidence of pheochromocytoma in the adrenal medulla of female rats (Table "Incidences of neoplastic and non-neoplastic findings in adrenal medulla and uterus in the combined chronic toxicity/carcinogenicity in rats with dicamba", above). However, these tumours were noted only at month 12 and not at the end of the study and no dose-response relationship was observed which led RAC to not consider such tumours as treatment-related and consequently the findings are not sufficient for supporting a classification of dicamba for carcinogenicity.

Dietary administration of dicamba to rats cause a dose-related increase in incidence of polyps in the uterus (Table "Incidences of neoplastic and non-neoplastic findings in adrenal medulla and uterus in the combined chronic toxicity/carcinogenicity in rats with dicamba", above). However, since no statistically significant differences in the incidence of controls and the exposed groups were noted and since these uterine polyps are benign

tumours and may not be relevant for women, RAC does not consider these findings sufficiently convincing for supporting a classification for carcinogenicity.

The dose-response relationship for incidence of malignant lymphoma in male rats showed a positive trend at 2500 ppm (Table "Incidences of neoplastic findings in the lymphoreticular system in the combined chronic toxicity/carcinogenicity in rats with dicamba", above). However, the incidence at this top dose only exceeded the top range of one of the two provided sets of HCD and there were no statistically significant differences between the incidences at any dose and concurrent controls. These reasons led RAC to consider this trend as an artefact and do not consider these malignant lymphomas sufficient for supporting classification.

Parafollicular C-cell carcinoma in thyroids was reported after dietary administration of dicamba. The incidence of this tumour observed a dose-response relationship and exceeded the two different HCD at the top (2500 ppm) and only one of them at the mid dose (250 ppm) and also at the top dose a statistically significant trend was noted (Table "Incidences of neoplastic and non-neoplastic findings in thyroid in the combined chronic toxicity/carcinogenicity in rats with dicamba", above). However, as also indicated in this table, the increased incidence of parafollicular C-cell carcinoma in high dose males was not accompanied by an increase in parafollicular adenoma and parafollicular hyperplasia, which is usually associated with an increase of parafollicular carcinoma. Furthermore, none of these increases in tumour incidences was statistically significant in pairwise comparisons. RAC also notes that the provided HCD considered studies with durations of 24 months, while in this study the duration of the study for males and females were 29 and 30 months, respectively. It suggests that the incidence of these parafollicular tumours could have been higher in the HCD, including studies of comparable duration to the duration used in the dicamba study. All these reasons suggests that the parafollicular C-cell carcinomas were not likely to be associated with treatment and consequently do not support classification.

In addition to all the above stated factors RAC also notes that no treatment-related effects were observed on overall tumour incidence in the carcinogenicity study in rats with dicamba (Table "Overall incidence of neoplastic findings in the combined chronic toxicity/carcinogenicity in rats with dicamba" above).

RAC also notes that the highest daily dietary dose used in the rat carcinogenicity study was 104 mg/kg/day, without any effect reported on e.g., body weights, whereas the NOAEL in the 90 days dietary rat study is reported to be about 500 mg/kg/day, and the rats tolerated the top dose of 952 mg/kg/day. Therefore, the carcinogenic potential of dicamba has not been fully investigated in rats due to inadequate dosing.

Overall, RAC notes that there is some indication of concern for carcinogenic potential due to the thyroid tumours reported in rats but proposes **no classification of dicamba for carcinogenicity due to inconclusive data.**

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 [equivalent to section 10.10.1 of the CLH report template]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two Generation Oral (continuous in diet) OECD 416 (1983) Rat, Crl:CD (SD) BR VAF/Plus 32/sex/group (F0) 28/sex/group (F1)	Dicamba (Technical material; batch 52103810; purity 86.9%) 0, 500, 1500 or 5000 ppm Vehicle: laboratory animal diet. The overall F0/F1 premating doses correspond to 37.9, 113 and 389 mg/kg bw /day for males and 42.6, 130 and 424 mg/kg bw/day for females at 0, 500, 1500 or 5000 ppm, respectively. *Corrected for purity, the overall F0/F1 premating means correspond to 32.9, 98.3 and 338 mg/kg bw/day of pure dicamba for males, and to 37.0, 113, 369 mg/kg bw/day of pure dicamba for females, at 500, 1500 and 5000 ppm, respectively*	***Parental toxicity*** 5000 ppm F0: mean achieved intake 347/390 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 9.6% (day 0-20: 3.2%) ↑ adjusted liver weight 13% females, 5% males F1: mean achieved intake, 432/458 mg/kg bw/day, males/ females respectively Clinical signs during lactation: tense/stiff body tone and slow righting reflex for a few days during the latter part of lactation. ↓ body weight pregnancy day 0-14: 4.6% (F1A) and 23% (F1B) ↑ absolute liver weight 3% females, males 9.5% (relative) ↓ food consumption week 5-8 1500 ppm F0: mean achieved intake, 105/125 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 121/135 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14 (F1B): 15 % (day 0-20: 15%) 500 ppm F0: mean achieved intake, 35/41 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 40.6/44 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 9.6% (F1B) (day 0-20: 1.7%) but absolute body weight was not decreased. Otherwise, no effects **NOAEL** 500 ppm (42.6 mg/kg bw/day) on the basis of decreased body weight during pregnancy (GD 0-14) at 500, 1500 and 5000 ppm. Clinical signs during lactation, ↑ liver weights at 5000 ppm ***Reproductive toxicity*** No effects at any dose level NOAEL 5000 ppm (389 mg/kg bw/day) ***Offspring toxicity*** 5000 ppm F1: ↓mean pup body weight 24 % day 21, delayed sexual maturation of males by 2 days, ↑ relative liver weights 27%.	Masters (1993)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		F2A/B: ↓ body weight 26/30 % day 21, ↑ relative liver weights approx. 36%. 1500 ppm F1: ↓ mean pup body weight 4 % day 21 F2A/B: ↓ pup body weight 10/14 % day 21 500 ppm F2B: No effects **NOAEL**: 500 ppm (37.9 mg/kg bw/day) based on body weight effects at 1500 and 5000 ppm.	

Table 33: 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
The Ontario Farm Family Health Study (OFFHS), a retrospective investigation of the effect of pesticide exposures on reproductive health. No OECD guideline used	Dicamba in an unspecified form	The study investigated the relationship between farm couple exposures to pesticides during pregnancy and the development of subsequent health problems in their offspring including: persistent cough or bronchitis, asthma, and allergies or hay fever. A total of 3405 children were included in the study, of whom 341 were reported to have allergy, 104 persistent cough or bronchitis and 173 reported to have asthma. For 1196 children (35%) there was no pesticide use on the farm during pregnancy.	Although not statistically significant, the reported use of dicamba led to odds ratios above 1.6 for persistent cough or bronchitis. The study offers weak support for the hypothesis that indirect exposure to dicamba during pregnancy is associated with the development of persistent cough or bronchitis and no support for an association for asthma, and allergies or hay fever during childhood.	Weselak M, Arbuckle TE, Wigle DT, Krewski D; In utero pesticide exposure and childhood morbidity; published; Environmental Research (2007) 103:79-86;
The Ontario Farm Family Health Study (OFFHS), a retrospective investigation of the effect of pesticide exposures on reproductive health.	Dicamba in an unspecified form	Couples living year-round on family-run farms with sales above a threshold figure were eligible for inclusion in the OFFHS if they were married or living as married, and the wife was at most 44 years of age. Of the 2946 eligible couples that met the eligibility criteria, 1893 (64%) returned all three	Gender specific results showed significantly elevated adjusted odds ratios (OR) for birth defects for male offspring in relation to reported farm use of dicamba during the pre-conception period (OR = 2.42, 95% CI: 1.06–5.53), although the dicamba association did not reach statistical significance in the GEE analysis that allowed for familial	Weselak M, Arbuckle TE, Wigle DT, Walker MC, Krewski D; Pre- and post-conception pesticide exposure and the risk of birth defects in an Ontario farm population; published; Reproductive toxicology (2008) 25:472-80;

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No OECD guideline used		questionnaires and identified a total of 5853 pregnancies. A total of 53% of the husbands and 6% of the wives were the farm operator.	correlation (OR = 2.34, 95% CI: 0.97–5.67).	

Table 34: 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	

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 two-generation rat reproduction study was conducted in rats. Deviations from OECD TG 416 (2001) were the following: Sperm analysis was performed for 8 (F0) and 7 (F1) males from each group instead of the recommended 10 animals/group; Sperm parameters were only examined in proven males. Uterus, spleen, ovary and thyroids in parental animals and spleens in pups were not weighted. Due to relatively low fertility in all groups of the F1 generation, number of litters were <20 in most groups (except high dose group in the 1st mating). As the latter was considered unrelated to treatment and correlated with a high body weight of females, this is considered not to impair the scientific validity of the study. In addition numbers of litter were 20/19 in the 1st/2nd mating of the F1 generation at the top dose level – therefore sufficiently high to reveal a potential effect of treatment. Since there were effects on sexual development, AGD should have been determined in F2 pups. Qualitative depletion of primordial follicles should have been investigated as well as enumeration of the number of promordial follicles and small growing follicles for comparison between treated and control ovaries.

One randomly selected pup/litter should be selected for examination of thymus, brain and spleen according to OECD TG 416 (2001). In this study, selection was made on the basis of body weight at Day 21 post partum; within each litter, the pup with the median weight for the respective sex was chosen. Estrus cycle data were not collected for the recommended 2 weeks but for most animals only 7 days, data was not summarised and it was very difficult/imposible to assess any patterns. Clinical signs were not summarised but only shown on individual level but sorted by group.

The two-generation rat reproduction study conducted with administration of dicamba at dose levels of 0, 500, 1500, and 5000 ppm (correspond to 37.9, 113 and 389 mg/kg bw /day for males and 42.6, 130 and 424 mg/kg bw/day for females at 0, 500, 1500 or 5000 ppm, respectively.) resulted in slight parental toxicity at 1500 ppm and above indicated by decreased body weight gain of F_1 females during gestation (F_0 only seen at 5000 ppm) and by clinical signs in F_1 females during lactation at 5000ppm (increased body tone and slowed righting reflex) and by increased liver weights in F_0 and F_1 adults at 5000 ppm. The increased liver weights were not accompanied by histopathological findings.

Developmental toxicity was observed by reduced pup weights in the top dose group of 5000 ppm at birth and reduced body weight gain at 1500 and 5000 ppm. Increased liver weights were observed in high dose weanlings. A slight delay of sexual maturation was observed in F_1 males as indicated by delayed cleavage of the balanopreputial skinfold. A covariance analysis was done: The aim of the analysis was to compare the developmental landmark (balano-preputial skinfold cleavage) between the treated groups and the control via analysis of covariance (ANCOVA), using bodyweight at 4 weeks as the covariate. There was a strongly significant relationship between bodyweight at 4 weeks and time to balanopreputial separation when parallel linear models were fitted to all four treatment groups (P = 0.001). The ANCOVA comparison of time to balanopreputial separation between

the treatment groups, with adjustment for bodyweight at 4 weeks, was not statistically significant: P = 0.117. This suggests that the previously observed difference in the time to balano-preputial skinfold cleavage between the 5000 ppm group and the control group was related to the reduced bodyweight at 4 weeks in the 5000 ppm group.

Reproductive performance was not affected by treatment. A reduced fertility was observed in all F_1 groups including controls. Therefore, a second mateing was performed where previously unsuccessful males were mated with successful females and *vice versa*. Fertility was reduced again without any dose-relationship. Analysis of the combined mateing revealed a comparable number of successfully mating males and females in all groups. Oestrus cycle determinations prior to mating as well as sperm analysis revealed no effects that could be related to dosing.

NOAEL F0 and F1 parental generation was 500 ppm (equivalent to a daily dose of approx. 42.6 mg/kg bw/day) based on decreased body weight gain at 1500 (F1) and 5000 ppm.

Developmental NOAEL was 500 ppm (equivalent to a daily dose of approx. 37.9 mg/kg bw/day) based on dose-related reduced weight in pups at 5000 and 1500 ppm.

The ability to reproduce and to deliver and rear offspring was not affected up to the highest dose tested (5000 ppm, approx. 389 mg/kg bw in males and 424 mg/kg bw/day in females) (Masters, 1993).

Effects in humans:
Although not statistically significant, the reported use of dicamba led to odds ratios above 1.6 for persistent cough or bronchitis. The study offers weak support for the hypothesis that indirect exposure to dicamba during pregnancy is associated with the development of persistent cough or bronchitis and no support for an association for asthma, and allergies or hay fever during childhood. The authors reecoomend using this study for hypothesis generation as it has limitations (Weselak et al, 2007). Gender specific results showed significantly elevated adjusted odds ratios (OR) for birth defects for male offspring in relation to reported farm use of dicamba during the pre-conception period (OR = 2.42, 95% CI: 1.06–5.53), however, the dicamba association did not reach statistical significance in the GEE analysis that allowed for familial correlation (OR = 2.34, 95% CI: 0.97–5.67). The evidence of an association between dicamba exposure and birth defects was weak in males and considering the limitations of the study, the authors also recommended to treat the results with caution as the findings should serve primarily to generate hypotheses (Weselac et al, 2008).

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

No treatment related effects were observed on sexual function or fertility hence a classification is not proposed.

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

Table 35: 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	Reference
Developmental toxicity Test guideline not stated but complies largely to OECD 414 (2001) but with some notable deviations (see below) Oral (gavage) Rat, Charles River CD 25 mated females/group	Dicamba (Technical grade; batch: 52625110; purity (90.4%) 0, 64, 160 or 400 mg/kg bw/day on days 6-19 of gestation Vehicle: corn oil *The dose levels applied correspond to 58, 145 and 362 mg/kg bw/day of pure dicamba*.	<u>Maternal toxicity</u> 400 (362) mg/kg bw/day: 4/25 deaths gestation day 7 & 8; ataxia, stiffening of the body when held, urine soaked fur, salivation and decreased motor activity; ↓ body weight gain (27% lower corrected maternal bw gain); ↓ food consumption (18.5% lower than controls, days 6-19). 4 deaths on GD7 and 8 (3 pregnant, 1 non- pregnant) 160 (145) mg/kg bw/day 10 % lower corrected maternal bw gain (not statistically significant) 64 (58) mg/kg bw/day No effects **Maternal NOAEL:** 64 (58) mg/kg bw/day <u>Developmental toxicity</u> 400 (362) mg/kg bw/day: ↑ number of incompletely ossified frontal (s) and/or parietal(s) 64 (58) &160 (145) mg/kg bw/day: No effects **Developmental NOAEL:** 160 (145) mg/kg bw/day	Smith (1981) (study acceptable)

Developmental toxicity US EPA 83-3 (complies largely to OECD 414, 2001) Oral (capsule) Rabbit, New Zealand White Hra:(NZW)SPF 20 inseminated females/group	Dicamba (Technical grade; batch: 52625110; purity 90.4%) 0, 30, 150 or 300 mg/kg bw/day on days 6-18 of gestation *The dose levels applied correspond to 27.1, 136 and 271 mg/kg bw/day of pure dicamba.*	<u>Maternal toxicity</u> 300 (271) mg/kg bw/day: 4/20 abortions; ataxia, rales, laboured breathing, perinasal substance, dried/no faeces, impaired righting reflex and decreased motor activity; ↓ body weight gain (42% lower than controls days 0 to 29); ↓ relative food consumption (13% lower than controls, days 0-29). 150 (136) mg/kg bw/day: 1/20 abortion; ataxia and decreased motor activity 30 (27.1) mg/kg bw/day No effects **Maternal NOAEL**: 30 (27.1) mg/kg bw/day <u>Developmental toxicity</u> 300 (271) mg/kg bw/day: increased incidence of irregularly ossified internasals . High dosis (incidence) Pups: 3.9% Litter: 23.1% **HCD 1987-1989** Pups: 0-2.3% Litter: 0-14.3% **HCD 1992-1994** Pups: 0-4.2% Litter: 0-26.7% **HCD 1990-1994** Pups: 0-5 (0-4.8%) Litter: 0-4 (0-26.7%) 30, 150 mg/kg bw/day: No effects **Developmental NOAEL:** 150 (136) mg/kg bw/day	Hoberman (1992) (study acceptable)

Table 36: 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**
The Ontario Farm Family Health Study (OFFHS), a	Dicamba in an unspecified form	The study investigated the relationship between farm couple exposures to pesticides during pregnancy and the development of	Although not statistically significant, the reported use of dicamba led to odds ratios above 1.6 for persistent cough or bronchitis. The study offers	Weselak M, Arbuckle TE, Wigle DT, Krewski D; In utero pesticide exposure and childhood morbidity;

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
retrospective investigation of the effect of pesticide exposures on reproductive health. No OECD guideline used		subsequent health problems in their offspring including: persistent cough or bronchitis, asthma, and allergies or hay fever. A total of 3405 children were included in the study, of whom 341 were reported to have allergy, 104 persistent cough or bronchitis and 173 reported to have asthma. For 1196 children (35%) there was no pesticide use on the farm during pregnancy.	weak support for the hypothesis that indirect exposure to dicamba during pregnancy is associated with the development of persistent cough or bronchitis and no support for an association for asthma, and allergies or hay fever during childhood.	published; Environmental Research (2007) 103:79-86;
The Ontario Farm Family Health Study (OFFHS), a retrospective investigation of the effect of pesticide exposures on reproductive health. No OECD guideline used	Dicamba in an unspecified form	Couples living year-round on family-run farms with sales above a threshold figure were eligible for inclusion in the OFFHS if they were married or living as married, and the wife was at most 44 years of age. Of the 2946 eligible couples that met the eligibility criteria, 1893 (64%) returned all three questionnaires and identified a total of 5853 pregnancies. A total of 53% of the husbands and 6% of the wives were the farm operator.	Gender specific results showed significantly elevated adjusted odds ratios (OR) for male offspring in relation to reported farm use of dicamba during the pre-conception period (OR = 2.42, 95% CI: 1.06–5.53), although the dicamba association did not reach statistical significance in the GEE analysis that allowed for familial correlation (OR = 2.34, 95% CI: 0.97–5.67).	Weselak M, Arbuckle TE, Wigle DT, Walker MC, Krewski D; Pre- and post-conception pesticide exposure and the risk of birth defects in an Ontario farm population; published; Reproductive toxicology (2008) 25:472-80;

Table 37: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
None				

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

The developmental toxicity of dicamba was investigated in two prenatal developmental toxicity studies, one in rats and one in rabbits. Both studies predate the current OECD Test Guideline Number 414 (2001) and in rabbits do not include the recommended extended dosing period (i.e. from implantation to one day prior to the day of scheduled kill). In rabbit dosing was performed GD 6-18 and in rats dosing was administered GD 6-19. The rat study (Smith, 1981) has other notable deviations from the guideline including the use of corn oil as a vehicle administered at 1 mL/100g body weight (guideline recommendation ≤ 0.4 mL/100g), the lack of maternal body

weight monitoring (body weight was recorded for gestation days 0, 6 and 20 only and the guideline requirement is for at least every 3 days) and, an insufficient number of foetuses examined for soft tissue alterations (only one third of each litter was examined and the guideline requirement is for one half). The number of corpora lutea was not reported.

Administration of dicamba to pregnant rats at dose levels of 0, 64, 160, and 400 mg/kg bw/day (Correspond to 64 (58), 160 (145) and 400 (362) mg/kg bw/day of technical dicamba) from day 6 through day 19 of gestation resulted in maternal toxicity at 400 (362) mg/kg bw as indicated by mortality, clinical signs (e.g. ataxia, decreased motor activity, stiff body when held), and food consumption. Decreased corrected body weight gain at mid and high dose was also observed in the dams. Based on these findings, the maternal NOAEL was 64 (58) mg/kg in this study. An increase in the number of incompletely ossified frontal (s) and/or parietal(s) was observed in the high dose fetuses but was not statistically significant. The increase in incomplete ossification may be related to maternal toxicity, as a slight general delay in development of the fetuses. This was corroborated by a slightly reduced fetus weight (ca 6 %) also observed in the high dose. Therefore, the developmental NOAEL was changed to 160 (145) mg/kg bw/day (Smith, 1981).

Administration of Dicamba at dose levels of 0, 30, 150, 300 mg/kg (*Correspond to 27.1, 136 and 271 mg/kg bw/day of pure dicamba*) to inseminated rabbits during days 6 to 18 of gestation resulted in maternal toxicity at dose levels ≥ 150 (136) mg/kg bw/day indicated by mortality, body weight loss, reduced food consumption, and a significant increased incidence of abortions at 300 (271) mg/kg and ataxia and decreased motor activity. Reproductive parameters were not affected by treatment. The incidence of irregularly ossified internasals in the high dose group (3.9 fetal/ 23.1% litter) were increased compared with control (0%). Even though the incidence of irregularly ossified internasals are inside the historical control range of the 1990-1994 studies (but not the 1987-1989 studies), the increase in this variation was statistically significant, only found in high dose animals and moreover, in three different litters. The incidence found in the study (23 % for litters) is also well above the mean of the historical controls (3.5 and 7% for litter in the historical controls). Therefore, it cannot be ruled out that the increased incidence of irregularly ossified internasals is treatment related and the NOAEL for development is therefore 150 (136) mg/kg bw/day. Based on the findings of the study, the maternal NOAEL was 30 (27.1) mg/kg bw/day (Hobermann, 1992).

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

In the classification system, adverse effects on development of the offspring include 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.
In rat and rabbit prenatal developmental toxicity studies, maternal toxicity was demonstrated but there was no effect on foetal viability or body weight and no evidence of any treatment-related malformations or increased incidences of external or visceral variations. A slight increase in number of incompletely ossified frontal (s) and/or parietal(s) were observed in rat fetuses but at a dose where maternal toxicity was observed (4 deaths, ataxia, stiffening of the body when held, urine soaked fur, salivation and decreased motor activity, decreased body weight gain and food consumption). It is therefore not considered justified to classify dicamba as a developmental toxicant. Bearing in mind the limitations of the available epidemiology studies, the findings were not considered enough for classification.

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

Table 38: 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**	**Reference**
Two Generation (Oral) OECD 416 (1983) Rat, Crl:CD (SD) BR VAF/Plus 32/sex/group (F0) 28/sex/group (F1)	Dicamba (Technical grade; batch: 52103810; purity 86.9%) 0, 500, 1500 or 5000 ppm Continuous in diet. Vehicle: laboratory animal diet The overall F0/F1 premating doses correspond to 37.9, 113 and 389 mg/kg bw /day for males and 42.6, 130 and 424 mg/kg bw/day for females at 0, 500, 1500 or 5000 ppm, respectively. *The overall F0/F1 premating means correspond to 32.9, 98.3 and 338 mg/kg bw/day of pure dicamba for males, and to 37.0, 113, 369 mg/kg bw/day of pure dicamba for females, at 500, 1500 and 5000 ppm, respectively*	***Parental toxicity*** 5000 ppm F0: mean achieved intake 347/390 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 9.6% ↑ adjusted liver weight 13% females, 5% males F1: mean achieved intake, 432/458 mg/kg bw/day, males/ females respectively Clinical signs during lactation: tense/stiff body tone and slow righting reflex ↓ body weight pregnancy day 0-14: 4.6% (F1A) and 22.8% (F1B) ↑ absolute liver weight 3% females, males 9.5% (relative) ↓ food consumption week 5-8 1500 ppm F0: mean achieved intake, 105/125 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 121/135 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14 (F1B): 15 % 500 ppm F0: mean achieved intake, 35/41 mg/kg bw/day, males/ females respectively F1: mean achieved intake, 40/44 mg/kg bw/day, males/ females respectively ↓ body weight gain pregnancy day 0-14: 10% (F1B), but absolute body weight was not decreased Otherwise, no effects NOAEL < 500 ppm (42.6 mg/kg bw/day) on the basis of decreased body weight during pregnancy (GD 0-14) at 500, 1500 and 5000 ppm. Clinical signs during lactation, ↑ liver weights at 5000 ppm ***Reproductive toxicity*** No effects at any dose level NOAEL 5000 ppm (389 mg/kg bw/day) ***Offspring toxicity*** 5000 ppm F1: ↓mean pup body weight 24 % day 21, delayed sexual maturation of males by 2 days, ↑ relative liver weights 27%. F2A/B: ↓ body weight 26/30 % day 21, ↑ relative liver weights approx. 36%. 1500 ppm	Masters (1993)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		F1: ↓ mean pup body weight 4 % day 21 F2A/B: ↓ pup body weight 10/14 % day 21 500 ppm F2B: ↓ pup body weight 10 % day 21 No other effects NOAEL 500 ppm (37.9 mg/kg bw/day) based on body weight effects at 1500 and 5000 ppm.	

Table 39: Summary table of human data on effects on or via lactation

Type o data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
None				

Table 40: 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
None				

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

The two generation reproduction study (Masters, 1993) has been described previously. The results showed that administration of 5000 ppm affected the lactating female with clinical signs in F1 females during late lactation (tense/stiff body tone and slow righting reflex). The body weight gain of the females (F0 & F1) was reduced during gestation. Other systemic effects included increased liver weights in F0 and F1 adults and weanling pups. Probably, as a consequence of the reduced maternal body weight during gestation, pup body weights were reduced at birth. Subsequent growth of the pups during lactation was reduced resulting in a slight delay in the time of cleavage of the balano-preputial skin fold in males. There was no indication of impaired nursing behaviour or decreased pup viability during lactation even in the presence of maternal clinical signs. The results of the study do not indicate any direct, adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk.

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

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. There were no effects to warrant classification of dicamba for effects on or via lactation.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

In the classification system, reproductive toxicity is subdivided under two main headings:
(a) Adverse effects on sexual function and fertility
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.
(b) Adverse effects on development of the offspring.
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.

In the rat, dietary exposure of two generations of rats to 5000 ppm dicamba (equivalent to approximately 389 mg/kg bw/day) had no adverse effect on sexual function or fertility or on development of the offspring although it did elicit systemic toxicity in adults and offspring.

A slight increase in number of incompletely ossified frontal (s) and/or parietal(s) were observed in rat fetuses but at a dose where maternal toxicity was observed (4 deaths, ataxia, stiffening of the body when held, urine soaked fur, salivation and decreased motor activity, decreased body weight gain and food consumption). It is therefore not considered justified to classify dicamba as a developmental toxicant.

Classification of dicamba as a reproductive toxicant is not warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of dicamba for fertility and sexual function based on lack of effects noted in a 2-generation reproductive toxicity study in rats conducted according to OECD TG 416.

The DS proposed no classification of dicamba for development based on the lack of effects on foetal viability or treatment-related malformations reported in the developmental toxicity studies in rats and rabbits.

The DS proposed no classification of dicamba for effects on or via lactation based on the lack of indication of impaired nursing behaviour.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

2-generation reproductive toxicity study (KCA 5.6.1 / 01)

The two-generation rat reproduction study was conducted in rats observing OECD TG 416 but with some deviations (see details in CLH-report) that did not compromise the scientific validity of the study.

The study was conducted with administration of dicamba at dose levels of 0, 500, 1500, and 5000 ppm. These dose levels corresponded to an overall F0/F1 pre-mating means of 0, 32.9, 98.3 and 338 mg/kg bw/day of pure dicamba for males, respectively, and to 0, 37.0, 113, 369 mg/kg bw/day of pure dicamba for females, respectively. The parental

toxicity is summarised in the table "Summary for repeated dose toxicity studies in animals with dicamba" in the section "STOT RE", above and resulted in slight parental toxicity at 1500 ppm and above indicated by: 1) decreased body weight gain of F1 females during gestation (F0 only seen at 5000 ppm); 2) clinical signs in F1 females during lactation at 5000 ppm (increased body tone and slowed righting reflex); and, 3) increased liver weights in F0 and F1 adults at 5000 ppm. The increased liver weights were not accompanied by histopathological findings.

Developmental toxicity was observed as a reduction in pup body weights in the top dose group of 5000 ppm (24% in F1 and 26-30% in F2 by day 21) and at 1500 ppm (4% in F1 and 10-14% in F2 by day 21). However, RAC notes that the weight at the moment of birth was unaltered and changed through weaning concurrently with a reduction in food and water consumption, which suggests that this effect was indeed not a developmental effect. Increases in liver weights were observed at the high dose (27% in F1 and 36% in F2), but not at other doses.

Reproductive performance was not affected by treatment. Reduced fertility was observed in all F1 groups, including the controls. Therefore, a second mating was performed where previously unsuccessful males were mated with successful females and vice versa. Fertility was again reduced without any dose-relationship. Analysis of the combined mating revealed a comparable number of successfully mating males and females in all groups. The oestrus cycle determinations prior to mating as well as sperm analysis revealed no effects that could be related to dosing.

Developmental toxicity study in rats (KCA 5.6.2 / 02)

This study has notable deviations from the guideline including the use of corn oil as a vehicle administered at 1 mL/100 g body weight (guideline recommendation ≤ 0.4 mL/100 g), the lack of maternal body weight monitoring (body weight was recorded for gestation days 0, 6 and 20 only and the guideline requirement is for at least every 3 days) and an insufficient number of foetuses examined for soft tissue alterations (only one third of each litter was examined and the guideline requirement is for one half to be examined). The number of *corpora lutea* was not reported.

Administration of dicamba to pregnant rats at dose levels of 0, 58, 145 and 362 mg pure dicamba/kg bw/day from day 6 through day 19 of gestation resulted in maternal toxicity at 362 mg/kg bw/day as indicated by mortality, clinical signs (e.g. ataxia, decreased motor activity, stiff body when held), and food consumption (the table "Summary for repeated dose toxicity studies in animals with dicamba" in the section "STOT RE", above). As indicated in this table, decreased corrected body weight gain at mid and high dose was also observed in the dams. An increase in the number of incompletely ossified frontal(s) and/or parietal(s) was observed in the high dose foetuses, but this was not statistically significant. No developmental effects were noted at 58 and 145 mg/kg bw/day.

Developmental toxicity study in rabbits (KCA 5.6.2 / 01)

The developmental toxicity study of dicamba in rabbits do not include the recommended extended dosing period (i.e., from implantation to one day prior to the day of scheduled kill). Administration of 0, 27.1, 136 and 271 mg pure dicamba/kg bw/day to inseminated rabbits during days 6 to 18 of gestation resulted in maternal toxicity at 271 mg/kg

bw/day indicated by body weight loss (42% days 0-29), reduced food consumption (13% days 0-29), and a significant increased incidence of abortions (4/20) and ataxia and decreased motor activity. At 136 mg/kg bw/day ataxia and decreased motor activity and 1 abortion was recorded. Reproductive parameters were not affected by treatment. The incidence of irregularly ossified internasals in the high dose group (3.9% foetal/23.1% litter) were increased compared with control (0%). The incidence of irregularly ossified internasals were inside the HCD range of the 1990-1994 studies and inside the HCD range of the 1992-1994 studies (this latter corresponding to the same laboratory using, the same rabbit strain and supplier and within a temporal frame closer to the dicamba study). However, the incidences of irregularly ossified internasals exceeded the range of a third HCD formed with studies performed between 1987-1989 (between 3-5 years earlier than the dicamba study).

Human data

The DS also included 2 epidemiological studies that are summarised in the table below. Although not statistically significant, the reported use of dicamba led to odds ratios above 1.6 for persistent cough or bronchitis. The study offers weak support for the hypothesis that indirect exposure to dicamba during pregnancy is associated with the development of persistent cough or bronchitis and no support for an association for asthma, and allergies during childhood. Gender specific results showed significantly elevated adjusted odds ratios for birth defects for male offspring in relation to reported farm use of dicamba during the pre-conception period (odds ratio = 2.42; 95% CI = 1.06-5.53). The evidence of an association between dicamba exposure and birth defects was weak in males and considering the limitations of the study, the authors also recommended that the results be treated with caution.

***Table**: Summary table of human data on dicamba reproductive toxicity*

Type of study/data	Observations	Reference
Retrospective investigation of the effect of pesticide exposures on reproductive health	A total of 3405 children were included in the study, of whom 341 were reported to have allergy, 104 persistent cough or bronchitis and 173 reported to have asthma. For 1196 children (35%) there was no pesticide use on the farm during pregnancy. Although not statistically significant, the reported use of dicamba led to odds ratios above 1.6 for persistent cough or bronchitis.	Weselak *et al*., 2007
Retrospective investigation of the effect of pesticide exposures on reproductive health	Gender specific results showed significantly elevated adjusted odds ratios for birth defects for male offspring in relation to reported farm use of dicamba during the preconception period (odds ratio = 2.42), although the dicamba association did not reach statistical significance in the GEE analysis that allowed for familial correlation.	Weselak *et al*., 2008

Comparison with the criteria

<u>Fertility and sexual function</u>

The two-generation reproductive toxicity study showed no treatment related effects. Therefore, RAC supports the DS's proposal for **no classification of dicamba for sexual function and fertility.**

Development

Classification of a substance as a human reproductive toxicant in Category 1A must be largely based on human data. The epidemiological data provided in the CLH-report and summarised in the table above have limitations that do not enable a causal relationship between dicamba exposure and developmental toxicity to be established. Thus, classification as Repr. 1A is not warranted.

The developmental toxicity study in rats was shown, after administration of 362 mg/kg bw/day, to induce an increase of incompletely ossified frontal(s) and parietal(s) bones. However, these alterations were reported at a dose causing 16% mortality (4/25 does) and clinical signs (ataxia, stiffening of the body when held, urine soaked fur, salivation and decreased motor activity, decreased body weight gain and food consumption). Due to this maternal toxicity and the lack of statistical significance in the differences between incidences in control and exposed animals, these alterations in ossification were not considered to constitute evidence for developmental toxicity.

The developmental toxicity study in rabbits showed increased incidences of irregularly ossified internasals at a dose of 271 mg/kg bw/day. This dose causes maternal toxicity consisting in clinical observations (ataxia, rales, laboured breathing, and decreased motor activity among others) and reduced body weight gains. The incidence of irregularly ossified internasals marginally exceeded the range of one set of HCD generated with studies performed 3-5 years earlier than the dicamba study. However, the incidence of these variations was within the two different sets of HCD provided by the notifier and that data, especially the HCD from 1992-1994, should be given greater weight, since they are from the same laboratory, the same rabbit strain and supplier and from within a time frame closer to the dicamba study. There were no treatment-related malformations or increases in incidences of external or visceral malformations. The abortions at this top dose (4/25) could also be attributed to maternal toxicity.

Overall, RAC supports the DS's proposal for **no classification of dicamba for developmental toxicity.**

Effects on or via lactation

There were no indications of impaired nursing behaviour or decreased pup viability during lactation even in the presence of maternal toxicity signs in the 2-generation reproductive toxicity study. This study does not provide indications that dicamba could alter the quality of the breast milk. There were no toxicokinetic indications that lead to the assumption that dicamba is being transferred to breast milk at significant levels. Overall, RAC supports the DS's proposal for **no classification of dicamba for effects on or via lactation.**

2.6.7 Summary of neurotoxicity

Table 41: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference

Acute neurotoxicity (oral, gavage). OECD 424 (1997). GLP Rat, Charles River Crl:CD®BR, 10/sex/group	Dicamba (technical material; purity: 86.9%) 0, 300, 600 or 1200 mg/kg bw. Single oral gavage dose. *The dose levels applied correspond to 261, 521 and 1043 mg/kg bw/day of pure dicamba.* Vehicle: corn oil Positive control: Acrylamide	**<u>1200 mg/kg bw</u>** 1/10 males found dead on day 1 *<u>Signs of neurotoxicity after 1.5 ± 1 hours:</u>* Rigidity in handling/body tone (8/10 males, 10/10 females), impairment of respiration (4/10 males, 5/10 females), flattened and/or raised posture (5/10 males, 6/10 females), impairment of gait (all animals), hypoalertness (7/10 males), ↓ rears/minute males, ↑ freezing in response to touch, abnormal righting reflex (9/9 males, 10/10 females), ↑ 86.5% tail flick latency time males, ↓ 29% fore limb grip strength males, ↓ activity both sexes during the first 10 to 15 minutes of session ↓ auditory startle *Body weight*:↓ 8.6% day 7 males *Body weight gain*: ↓ 25.9% day 0-7 males *Food consumption*: ↓ 12.8% day 0-7 males *<u>Signs of neurotoxicity after 7 days:</u>* Fore limb grip strength ↓ 15.0% males, Auditory startle: maximum and average input voltages to stimulus ↓ 59.10 and 53.5% respectively in males, 56% ↓ in females *<u>Signs of neurotoxicity after 14 days:</u>* No differences from control. **<u>600 mg/kg bw</u>** *Signs of neurotoxicity after 1.5 ± 1 hours:* Rigidity in handling/body tone (8/10 males, 8/10 females), impairment of respiration (2/10 males, 1/10 females), flattened and/or raised posture (5/10 males, 6/10 females), impairment of gait (all animals), hypoalertness (4/10 males, 2/10 females), ↓ rears/minute males, ↑ freezing in response to touch, abnormal righting reflex (10/10 males, 9/10 females), ↑ 54% tail flick latency time males, ↓ 19% fore limb grip strength males, ↓ activity both sexes during the first 10 to 15 minutes of the locomotor activity session *<u>Signs of neurotoxicity after 7 days:</u>* No effects. **<u>300 mg/kg bw</u>** *Signs of neurotoxicity after 1.5 ± 1 hours:* Rigidity in handling/body tone (5/10 females), raised posture (2/10 females), ↓ rears/minute males, ↑ freezing in response to touch (1/10 males, 2/10 females), abnormal righting reflex (7/10 males, 8/10 females), ↓ 15% fore limb grip strength males	Minnema (1993)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		No NOAEL. (NOAEL < 300 mg/kg bw/day). All signs and measurements comparable to control by day 14. **No treatment-related neuropathy.**	
Acute delayed neurotoxicity (gavage). US-EPA FIFRA, Subdivision F, § 81-7 GLP Hen *Gallus gallus domesticus*, strain: Hisex Brown 10/group in control, low and mid dose group, positive control; 20/group high dose group.	Dicamba (technical material; purity: 86.82%). 0, 79 (¼ LD_{50}), 158 (½ LD_{50}), 316 mg/kg bw (LD_{50}) Single oral dose Vehicle: corn oil Positive control: TOCP *The dose levels applied correspond to 226, 327, 475, 688 and 998 mg/kg bw of pure dicamba for the LD_{50} determination, and to 69, 137, and 274 mg/kg bw of pure dicamba for the neurotoxicity assessment groups.*	**316 (274) mg/kg bw**: 9/20 animals died. *Body weight*: weight loss during the first two weeks of the experiment. Lesions of the sciatic nerve considered secondary to mild nerve entrapment resulting from recumbency not a direct toxic effect of dicamba. **158 (137) mg/kg bw**: 1/10 birds found dead day 5. *Body weight gain:*↓ 67% *Food consumption*: ↓ days 1 to 3 *Neuropathology*: comparable to control hens **79 (69) mg/kg bw**: No mortality. Body weight development similar to control. *Food consumption*: ↓ days 1 to 3 The LD_{50} expressed as pure dicamba is 274 mg/kg bw of pure dicamba (100%) and 316 mg/kg be for technical dicamba. NOAEL < 79 mg/kg bw. Effects at all doses: unsteadiness, inability to walk, collapsing when moved and lying on the pen floor with legs outstretched or lying on one side. **Effect was reversible.** **Does not induce delayed neurotoxicity in hens**	Roberts *et al* (1983)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Subchronic neurotoxicity study (dietary). OECD 424 (1997). GLP Rat, Charles River Crl:CD®BR, 10/sex/group	Dicamba (technical material; purity: 86.9%) 0, 3000, 6000 and 12000 ppm Actual doses 0, 197.1, 401.5 and 767.9 mg/kg/day for the males and 253.4, 472.0 and 1028.9 mg/kg/day for females. Continuous in the diet for 13 weeks *The dose levels applied correspond to 171, 348 and 667 mg/kg bw/day of pure dicamba in males, and to 220, 410, 894 mg/kg bw/day of pure dicamba in females at 3000, 6000 and 12000 ppm, respectively.*	**12000 ppm (males 767.9 mg/kg bw/day, females 1028.9 mg/kg bw/day)**: *Body weight*: ↓ 5.5% males, 4.8% females week 14 *Body weight gain*: ↓ 24.1% males, 37.9% females week 1 *FOB*: ↑ frequency of rigid body tone when handled in weeks 4, 8 and 13 (greater in females than males). *Pathology*: No treatment-related changes in any of the tissues examined **6000 ppm (males 401.5 mg/kg bw/day, females 472 mg/kg bw/day)**: No treatment-related effects. **3000 ppm (males 197.1 mg/kg bw/day, females 253.4 mg/kg bw/day)**: No treatment-related effects. **NOAEL for neurotoxicity and systemic toxicity 6000 ppm (**401.5 mg/kg bw /day in males and 472 mg/kg bw/day in females), based on decreased body weight gain and neurobehavioral findings.	Minnema (1994)

2.6.8 Summary of other toxicological studies

2.6.8.1

Single oral administration (gavage) of dicamba at dose levels of 0, 300, 600, and 1200 mg/kg bw to rats (*corresponding to 261, 521 and 1043 mg/kg bw/day of pure dicamba*) resulted in one unscheduled death and in decreased mean body weight gain and food consumption in high dose males. Dose dependent neurobehavioral effects were recorded in all treated groups at 1.5 ± 1 hours after dosing. The overall effect of treatment was a stimulus- or stress-induced rigidity, a consideration based on the increased frequency in treated animals exhibiting rigidity in handling/body tone, impairment of respiration, flattened and/or raised posture, impairment of gait, hypoalertness, significantly decreased number of rears/minute, freezing in response to touch, abnormal righting reflex (uncoordinated, landing on side, landing on back), increased tail flick latency time, decreased forelimb and hind limb grip strength, and decreased activity during the first 10 to 15 minutes of the 40-minute locomotor activity session.

At the day 7 neurobehavioral evaluation, differences were restricted to a few parameters (forelimb grip strength, auditory startle) in high dose rats. At the day 14 neurobehavioral examination there were no apparent differences between dicamba-treated animals and vehicle control animals, indicating that the neurobehavioral changes were transient. There were no neurohistopathological findings that could be related to treatment. Based on neurobehavioral effects were observed at all tested doses, no NOAEL could be established (Minnema, 1993).

Administration of single oral doses of dicamba to domestic hens at a dose level of 316 mg/kg bw (LD_{50}) was poorly tolerated (Roberts *et al*, 1983). However, there was none of the classical clinical signs of ataxia indicating delayed neurotoxicity at this or lower dose levels. The clinical signs of toxicity observed at all doses included unsteadiness, inability to walk, collapsing when moved and lying on the floor with legs outstretched or lying on one side. The first signs were noted within one hour of dosing and some birds were recumbent for up to 15 days before showing signs of recovery with animals in the lower dose groups recovering faster. In the high dose group, these clinical signs were accompanied by body weight loss and decreased food consumption during the first 10 to 14 days after treatment with recovery after this period of time. The microscopic examination revealed no neurohistopathological lesions in the brain and spinal cord of hens administered dicamba. Lesions of the sciatic nerve were restricted to the high dose level (316 mg/kg bw) and were considered secondary to nerve entrapment resulting from the recumbency rather than from a direct toxic effect of dicamba. Clinical signs were observed at all doses and no NOAEL was found in this study. The results of the study revealed no indication for delayed neurotoxicity.

Dietary administration of technical dicamba to rats at dose levels of 0, 3000, 6000, and 12000 ppm (0, 197.1, 401.5 and 767.9 mg/kg/day for the males and 253.4, 472.0 and 1028.9 mg/kg/day for females) for 3 months resulted in a slightly decreased body weight gain in high dose animals. The major neurobehavioral treatment-related effect in the high dose animals was an increased frequency of rigid body tone when handled throughout the study. More high-dose females than males were affected. The other findings in high dose rats may be related to rigidity. The effects included rigidity observed at weeks 4 and 13 during the landing splay test and during the righting reflex test at all post treatment FOB tests. An apparent, but non-significant, increase in the mean latency to first step in male rats, an increased frequency of mildly impaired gait, and an increased frequency of abnormal righting reflex (i.e. uncoordinated, lands on side, or lands on back) was also observed in the high dose.
At week 13 fewer findings were observed and with lower incidence.
Administration of dicamba did not cause damage to the nervous tissues as indicated by the histopathology findings. Based on the results of this study, the NOAEL for neurotoxicity and systemic toxicity was 6000 ppm, which is equivalent to a mean daily intake of 401.5 mg/kg bw and 472 mg/kg bw in males and females, respectively (Minnema, 1994).

It was not possible to establish a NOAEL following a single high dose, but in the subchronic neurotoxicity study a NOAEL of 401.5 mg/kg bw/day for neurotoxicity was determined (Minnema, 1994). The observed effects in the acute neurotoxicity study at 300 (261) mg/kg, which were generally observed 1.5 hours after administration only (Minnema, 1993), might be due to the higher systemic peak concentrations of dicamba after oral gavage compared to dietary administration of an even higher dose.

Clinical signs in the form of neurobehavioral effects were recorded in other studies as well (please see 2.6.2.10 for further discussion).

2.6.8.2 Toxicity studies of metabolites and impurities

Toxicity studies of metabolites

Study type (reference)	**EU agreed end-point14**	**Proposed end-point**	**Classification according to Regulation (EC) No 1272/2008 as amended**	**Reference**
NOA405873 (5-OH dicamba)				

[14] Dicamba: EFSA Journal 2011; 9(1):1965)
Final addendum to the Draft Assessment Report (DAR), November 2010

Acute short-term toxicity				
Acute oral toxicity rat, gavage (NOA405873) TG 423 (1996)/GLP	LD50 >2000 mg/kg bw (males, females)	-	None	Sommer, 2001 KCA 5.8.1/01
Acute oral toxicity study in rats (5-hydroxydicamba) TG 423/GLP	LD50 >2000 mg/kg bw (females)		None	Tavaszi J. 2010, KCA 5.8.1/11
90 Day subacute feeding studies in the male and female albino rat and the male and female purebred Beagle dog. TG 408 (1998)/ before GLP	This study is considered to be acceptable with reservations only.			Wazeter FX 1966, KCA 5.8.1/02 (Supplemental)
Genotoxicity *in vitro*				
Ames test (S. typhimurium and E. coli) 2000/32/EC, B.13/B.14 (2000)~TG 471 (1997)/GLP	Negative (+/- S9)	-	None	Deparade 2001, KCA 5.8.1/03
Ames test (S. typhimurium and E. coli) TG 471 (1997)/GLP	Negative (+/- S9)		None	Verskeep-Rip C.M. 2010. KCA 5.8.1/12
Gene mutation in mammalian cells (mouse lymphoma L5178Y cells) B.17 (2000)~TG 476/GLP	Equivocal (+S9), Positive (-S9)	-	None	Clay 2002; KCA 5.8.1/04
Mouse Lymphoma Mutagenicity Assay B.17 (2000))~TG 476/GLP	Positive (+ S9) Positive (-S9)	-	None (in absence of an effect in the *in vivo* study)	Ogorek 2002a; KCA 5.8.1/05
Gene mutation in mammalian cell (L5178Y mouse lymphoma cells) TG 476 (1997)/GLP	Positive (+S9) Negative (-S9)			Verspeek-Rip C.M. 2010, KCA 5.8.1/14
Cytogenetic test on Chinese hamster cells B.10 (2000)/TG OECD 473 (1997)/GLP	Positive (+ S9) Positive (-S9)			Ogorek B 2002b, KCA 5.8.1/06
Chromosome aberrations in vitro human peripheral lymphocytes TG 473 (1997)/GLP	Negative (+/- S9)		None	Buskens C.A.F. 2010, KCA 5.8.1/13
In vitro micronucleus test TG 487 (2016)/GLP	Negative (+/-S9)		None	Whitwell 2017b; KCA 5.4.1/03
Genotoxicity *in vivo*				
Mouse bone marrow micronucleus test	Negative	-	None	Fox 2003; KCA 5.8.1/07

B.12 (2000)/TG OECD 474/GLP				
Unscheduled DNA synthesis in rat liver B.39 (2000)/TG 486 (1997)/GLP	Negative	-	None	Clay 2004; KCA 5.8.1/08
In vivo Comet assay genotoxicity study TG 489 (2016)/ TG474 (1997)/GLP	Negative	-	None	Watters, 2019
NOA414746 (DCSA)				
Multi-(Q)SAR genotoxicity assessment of dicamba and ites metabolite DCSA (NOA414746)	-	no indication that DCSA is more toxic than parent	Not applicable	Lorez C, Booth E (2016)., K-CA 5.8.1/01

The metabolite 5-OH dicamba (NOA 405873) was not acutely toxic to mammals and no toxic response was identified in rats and dogs up to the highest dose tested (250 ppm) in dietary subchronic (90-days) studies (Wazeter, 1966).

The acute toxicity of 5-OH Dicamba was investigated with respect to the oral route (Sommer, 2001; Tavaszi, 2010). Two studis on 5-OH Dicamba in rats was performed. One in accordance with GLP and OECD 423 (1996) and a new study in accordance with GLP and OECD 423 (2001) both with minor deviations not considered to compromise the validity of the studies. The resulting LD_{50} was found to be greater than 2000 mg/kg bw for males and females. Based on the result, no classification for acute oral toxicity is required for 5-OH Dicamba (NOA 405873 tech.) in accordance with Regulation (EC) No.1272/2008.
Two reverse mutagenicity tests have been conducted (Deparade, 2001; Verskeep-Rip, 2010a), one of which was not in the DAR. 5-OH Dicamba was found negative in both tests with and without metabolic activation which indicates that 5-OH Dicamba does not induce point mutations by base substitutions or frame shift in the genome of *Salmonella typhimurium* and *Escherichia Coli*.

Gene mutations was tested in mammalian cells in two studies already included in the DAR and a new study submitted for the RAR: 5-OH dicamba was tested in *in vitro* mammalian cell mutation assay in L5178Y in two experiments. 5-OH dicamba induced statistically significant increases in the mutant frequency at the tk locus of mouse lymphoma compared to solvent control at the highest doses tested (2000, 2370 µg/ml without S9 and 1000 2000, 2370 µg/ml with S9). A dose related increase was seen in the absence of metabolic activation in both experiments. In the presence of S9-mix, the dose relationship was less clear. NOA 405873 is mutagenic in L5178Y TK± cells treated in vitro in the abscence of S9-mix and equivocal in the presence of S9 (Clay, 2002).

In the second study, 5-OH dicamba was tested in *in vitro* mammalian cell mutation assay in L5178Y in two experiments. In the presence of metabolic activation significant increases were only observed at concentrations ≥ 10 mM (corresponding to 2370 µg/ml) or the increase was not reproducible, however, a positive trend was observed in both experiments. In the absence of S9-mix reproducible increases in the mutant frequency were observed at doses ≥ 600 µg/ml. Significant positive trends were observed both in presence and absence of S9 (Ogorek 2002b). In the new study 5-OH dicamba the test item was tested up to concentrations of 2370 and 1800 µg/mL without and with S9. 5-OH dicamba was mutagenic in mouse lymphoma L5178Y test under the experimental conditions in the presence of metabolic activation but not in absence of metabolic activation (Verspeek –Rip, 2010b).

Chromosome aberrations in vitro was investigated in two studies. In a new study submitted for the RAR, the ability of 5-OH dicamba to induce chromosome aberrations in human peripheral lymphocytes was investigated in

two independent experiments. 5-OH dicamba did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations with and without S9, in either of the two independent repeated experiments. No effects of 5-hydroxydicamba on the number of polyploid cells and cells with endoreduplicated chromosomes were observed both with and without S9. Under the experimental conditions reported, it was concluded that the test substance 5-OH dicamba did not induced structural chromosomal aberrations in human lymphocytes in vitro (Buskens, 2010).

Chromosome aberrations (CA) were investigated in vitro in Chinese hamster ovary cells. The cytotoxicity test was performed as an integral part of the mutagenicity test. The highest concentration of 1250 µg/ml without S9-mix with enough cells for scoring caused 29% suppression of mitotic activity (MI).
In the absence of metabolic activation, a significant increase of cells with specific chromosomal aberrations compared to the negative control was found at 1250 µg/ml. Higher concentrations could not be measured due to toxicity. In the presence of metabolic activation, a significant increase of cells with specific chromosomal aberrations compared to the negative control was found at the highest concentration of 5000 µg/mL (which exceeds 10 mM). The increase in cells with specific chromosomal aberrations was outside the historical control range both with and without S9. Under the condition of this in vitro chromosome aberration assay, NOA 405873 induced chromosome aberrations in CHO cells (Ogorek, 2002b).

NOA405873 did not induce micronuclei in cultured human peripheral blood lymphocytes following treatment in the absence and presence of an aroclor induced rat liver metabolic activation system (S-9) in an *In Vitro* Human Lymphocyte Micronucleus Assay. Concentrations were analysed up to 2000 µg/mL, a recommended regulatory maximum concentration for in vitro micronucleus assays. NOA405873 concluded to be negative in this assay (Whitwell, 2017).

In vivo genotoxicity was tested in three assays. In an *in vivo* study, 5-OH Dicamba was investigated for its genotoxic ability in the unscheduled DNA synthesis test. 5-OH Dicamba did not induce DNA repair (measured by unscheduled DNA synthesis) in the rat liver and is therefore denoted as not genotoxic under the conditions of this assay (Clay, 2004).
The *in vivo* mutagenicity was also investigated in the Mouse micronucleus test. Any positive induction of micronuclei in the polychromatic erythrocytes of the bone marrow in mice treated orally with a single dose of 5-OH Dicamba was within the range of negative control values and not indicative of a positive response. The assay is classified as negative under the conditions of the study (Fox, 2003).
Further, a *in vivo* comet assay study was performed. Six animals/group of young adult out-bred Han Wistar Crl:WI(Han) male rats were exposed to 0 (vehicle control), 500, 1000 or 2000 mg 5-OH dicamba/kg/day by oral gavage at 0 and 23 hours after (Watters, 2019) in the main experiment. A positive control was included. In the positive control group (Ethyl methanesulfonate 150 mg/kg, single oral administration at 21 hours (Day 2), 3 males were allocated. Bioanalysis showed exposure at all doses. Liver and duodenum were sampled on Day 2, equivalent to approximately 24 hours after first dosing. The samples were examined for % increase in tail intensity, number of hedgehog cells and for histopathology as indication of cellular toxicity. 5-OH dicamba did not induce DNA damage in the liver of male rats treated up to 2000 mg/kg/day (the maximum recommended dose for *in vivo* comet studies). In the duodenum, the group mean tail intensity values for all groups treated with 5-OH dicamba exceeded the group mean concurrent vehicle control data with a statistically significant dose-response relationship ($P \leq 0.05$). However, of these group mean increases, only the group mean tail intensity value of the highest dose group (2000 mg/kg/day) was found to be statistically significant ($P \leq 0.05$) compared to the concurrent vehicle control group, and within this group only 3 animals showed tail intensity values above those observed in the concurrent vehicle control group. In addition, all animals in all test article treated dose groups fell within the historical vehicle control 95% reference range of 0.24-5.60 for this tissue with individual animal responses for the concurrent vehicle control towards the lower end of that range. The findings of increased tail intensity were associated with clear histopathological changes in the duodenum including villi degeneration/atrophy and eosinophilic material in the lumen, the severity of both increased in a general dose-response relationship. Moreover, the increase in the highest dose group compared to the control dose group was about 2% tail DNA. Such a small absolute increase is not of biological relevance. The conclusion is that the metabolite is not genotoxic in this study.

In conclusion, 5-OH dicamba (NOA405873) has a low acute oral toxicity (LD_{50} >2000 mg/kg bw) and is unlikely to be genotoxic *in vivo*.

Based on SAR modelling, DCSA (NOA414746) is expected to show a similar genotoxicity as the parent dicamba. An alert for for *in vivo* micronuclei formation in rodents (as potential H-acceptor-path3- H-acceptor) from ToxTree and the OECD QSAR Toolbox was observed for bot dicamba and DCSA (NOA414746). Additionally – as it was found in rat metabolism studies – DCSA (NOA414746) already contributed to the toxicological properties detected in the toxicity studies with dicamba. However, it is only a minor urine metabolite <10 % and no studies were submitted investigating the general toxicity *in vitro* or *in vivo* of this metabolite.

2.6.8.3 Supplementary studies on the active substance

Commonly, indicators of immunotoxicity include changes in haematological parameters, serum globulin levels, alterations in immune system organ weights such as spleen and thymus, and histopathological changes in immune organs such as spleen, thymus, lymph nodes and bone marrow.
Dicamba does not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic and a detailed review of the repeat exposure toxicity database for dicamba revealed no evidence of an adverse effect on the immune system. A thorough review of the toxicology database for dicamba has shown no evidence of adverse effects on the immune system in rats, mice or dogs and functional assays in rats and goats confirmed lack of immunomodulation. Based on these findings within the dicamba toxicology database and published literature, it can be concluded that dicamba probably has no immunotoxic potential.

2.6.8.4 Endocrine disrupting properties

Please see point 2.10

2.6.9 Summary of medical data and information

The expected effect of poisoning with dimethylamine salt of dicamba is described in a publication (Moon et al, 2014). The main effects observed after voluntary ingestion of dicamba (for committing suicide) was mental status change followed by nausea, vomiting, and anorexia. Gastric lavage and administered charcoal may contribute to the development of gastrointestinal symptoms such as nausea, vomiting, or sore throat.
Repetitive EKG has been performed in only four among 10 patients with QTc prolongation because of relatively short hospitalization period. In the four patients with repeated EKG evaluations, QTc prolongation disappeared by discharge. Despite the absence of apparent tissue hypoperfusion during hospitalization, 76.9% of patient had an elevated lactate, which may be explained by the uncoupling effect in mitochondria demonstrated *in vitro* studies. The blocking oxidative phosphorylation results in accumulation of pyruvate that is converted into lactate.
Most presenting symptoms had subsided within 1 day of ingestion. This rapid wane of symptoms may be explained by low tissue accumulation and rapid elimination of dimethylamine salt of dicamba despite its lipid solubility. If a patient has renal insufficiency, however, the excretion of dicamba herbicide may be delayed and the clinical symptoms may be prolonged.

Blood and urine samples were obtained during the acute phase of intoxication from a 30.22 kg woman who ingested 100 ml of a formulation containing 2,4-D (20.1%) and dicamba (1.9%). Assumed ingestion was 12.29 g of 2,4-D and 1.16 g of dicamba. The best fitting model was a two-compartment model for dicamba. The half-life of dicamba was calculated to be approximately 15 hours and the volume of distribution was 23.4 liters. Dicamba was the preferred chemical for elimination until the relative concentration of the 2 chemicals favoured 2,4-D (Shared extra urinary excretion route) (Young and Haley, T.J., 1977).

A farmer sprayed a wheat field with a 1% Banvel M spray broth using a knapsack sprayer for half an hour (Banvel M contains 340g MCPA and 30g Dicamba per litre concentrate). When he was spraying against the wind his face and arms were contaminated. The following day he suffered from nausea, bloating, loss of appetite and palpitation of the heart. Six days later the symptoms were vomiting and abdominal pain. The family doctor prescribed Metoclopramid (Paspertin□). Eight days after the exposure a gastrocopy revealed hemorrhagic gastro-duodenitis which had resolved at follow up five weeks later. No laboratory confirmation of exposure to the two herbicides was performed (Huepp and Hesselmann , 1979).

In a prospective study from patients notified to the Poisons Unit, Guy's Hospital, St Thomas' Street, London from 1984 to 1987.Blood and urine analysis were done in all cases (HPLC with limit of sensitivity 10 mg/l for dicamba). 12 Patients had ingested dicamba. The formulations ingested contained more than one herbicide in most cases.

Plasma dicamba concentration was 0.02 g/l or less in 4 patients. The article reports the relation between blood herbicide concentration and the effect of alkaline diuresis on outcome of patients following acute poisoning. There was no indication that dicamba had contributed to toxicity in any patient (Flanagan et al 1990).

Information from manufacturing plant personnel, data collected on humans (public literature) and direct observations (information on adverse health incidences in public databases), information from epidemiology studies (public literature) indicate a low toxic potential of dicamba. Clinical signs after intentional ingestion were transient, non-specific and reversible (with symptomatic or even no treatment). Except of the irritating properties to eyes (and skin) no marked systemic toxicity is expected. Standard first aid measures and symptomatic medical treatment are recommended after accidental or intentional exposure.
There is no specific antidote for dicamba poisoning. Most patients were discharged without complication after hydration and administration of sodium bicarbonate for elevation of creatinine kinase and metabolic acidosis.
The acute toxicities of dicamba herbicide ingestion in patients were managed with supportive treatment such as hydration and sodium bicarbonate, and most symptoms had subsided within 2 days after ingestion. However, physicians should take into account potential complications such as gastrotintestinal tract corrosion, rhabdomyolysis, and acute pancreatitis.

The only source of human information on carcinogenicity of dicamba is epidemiology. An apparent association between exposure to dicamba, either alone or in combination with other pesticides, and Non-Hodgkin's lymphoma in agricultural workers was identified by McDuffie et al (2001;2005) but was not confirmed by Samanic et al (2005). Similarly, Hartge et al (2006) found no correlation between the use of dicamba and Non-Hodgkin's lymphoma in a residential environment.

Lung cancer: Statistical significance was only seen when comparing high dicamba exposure (as lifetime exposure days) with low dicamba exposure but not with no dicamba exposure. The statistical significance is therefore considered more of an artefact - due to the fact that the low dicamba exposure groups had a lower risk for lung cancer than the no exposure group – than indicating an actual effect of dicamba.

No lung effects were seen in any repeated dose toxicity study in animals.

Therefore the published findings on lung cancer are considered of insufficient relevance to be considered for human risk assessment of dicamba.

Prostate cancer: Statistical significance was seen in only one publication when a low number of cases with high dicamba exposure were compared to never exposure but not for 'ever' use of dicamba (data based on British Columbia Cancer Registry).
Additionally there are a number of other publications that investigate the association between prostate cancer risk and pesticide including dicamba exposure[15] (data based on the Agricultural Health Study – applicators and spouses): none of these found an association between prostate cancer and dicamba exposure. Additionally two reviews evaluate the overall evidence of an association of pesticide exposure and prostate cancer and conclude that there is no relevant association[16].
Therefore the reported association of high dicamba exposure and prostate cancer is considered probably not relevant for human risk assessment considering that this was not confirmed by a considerable number of other epidemiology publications or any similar finding in animal studies.

Hypothyroidism: Significance was only seen for 'ever' exposure of dicamba in pesticide applicators but not for the intensity weighted dicamba exposure (Goldner et al, 2013). It was also not supported by a similar finding in spouses (also from AHS data set). Therefore the published association for 'ever' use of dicamba in pesticide applicators to hypothyroidism is considered insufficiently relevant for human risk assessment.

Birth defects in male offspring: A statistically significant association between pre-conception dicamba exposure and (any) birth defect was only seen for male offspring when not adjusting for familial correlation (no association was seen when adjusting for familial correlation for male offspring, or for female or all offspring with exposure during pre-conception or offspring with post-conception exposure). Already the authors conclude that this might be due to chance (Weselak et al, 2008). The evidence of an association between dicamba exposure and birth defects was weak in males and considering the limitations of the study, the authors also recommended to treat the results with caution as the findings should serve primarily to generate hypotheses (Weselac et al, 2007).

[15] Barry et al 2011 and 2012, Karami et al 2013, Koutros et al 2011 and 2013
[16] Mink et al 2008, Weichental et al 2010

2.6.10 Toxicological end points for risk assessment (reference values)

Table 42: 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
Goldenthal, 1985	Combined chronic toxicity/carcinogenicity. OECD 453, 87/302/EEC B.33 (1988) GLP Rat, Charles River CD (Sprague Dawley) 60/sex (50/sex/group main study, 10/sex/group interim kill after 12 months)	Dicamba (technical material; purity 86.8%) Continuous in the diet 0, 50, 250, 2500 ppm for 115 weeks (males), 118 weeks (females) The doses correspond to 2.0, 10.0, and 99.1 mg/kg for males and 2.4, 12.1, and 120.1 mg/kg for females *Actual doses correspond to 1.7, 8.7, and 83.0 mg/kg bw/day of pure dicamba for males, and to 2.1, 10.5, and 104 mg/kg bw/day of pure dicamba for females, at 50, 250, and 2500 ppm, respectively.*	↑ incidence of thyroid parafollicular (C-cell) carcinoma in males	NOAEL for carcinogenicity 250 ppm (equivalent to 10.0 in males)	2500 ppm (99.1 mg/kg bw/day)	*2.6.5*
Hoberman, 1992	Developmental toxicity US EPA 83-3 (complies largely to OECD 414, 2001) Oral (capsule) Rabbit, New Zealand White Hra:(NZW)SPF 20 inseminated females/group	Dicamba (Technical grade; batch: 52625110; purity 90.4%) 0, 30, 150 or 300 mg/kg bw/day on days 6-18 of gestation The dose levels applied correspond to 27.1, 136 and	1/20 abortion; ataxia and decreased motor activity	30 (27.1) mg/kg bw/day	150 (136) mg/kg bw/day	*2.6.6*

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
		271 mg/kg bw/day of pure dicamba.				

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

ADI was previously based on the multigeneration study in rats by Master et al (1993) as it was the most sensitive study, i.e. the study with the lowest and most relevant NOAEL. Since, at the re-evaluation, a new NOAEL of 10.0 mg/kg bw/day (carcinogenicity) has been proposed at a lower dose in the chronic study in rats (Goldenthal, 1985), it is suggested to use this value for the derivation of the ADI. An UF of 150 is proposed to ensure a margin of safety to the carcinogenic effect of at least 1000 based on the carcinogenic effect (increase in thyroid parafollicular (C-cell) carcinoma) observed in this study.

Based on the NOAEL of 10.0 mg/kg bw/day and a safety factor of 150, to achieve a margin of safety above 1000, an ADI can be calculated:

ADI = NOAEL/UF = 10 mg/kg bw/day/150 = 0.07 mg/kg bw/day (rounded)

Rounding from 0.06666666667 to 0.07 is < 10 %.

Margin of safety relative to LOAEL will in this case be:

LOAEL/reference value: 99.1 mg/kg bw/day/0.07= 1415.7

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

RMS proposes keeping the acute reference dose from the previous evaluation only corrected for the purity of dicamba tested in the study:

The acute oral LD_{50} in the rat was below 2000 mg/kg and the compound is classified as harmful. The acute neurotoxicity study showed neurobehavioral findings upon single treatment of rats. In the rabbit developmental toxicity study clinical signs were observed in dams at ≥ 150 mg/kg/day with a NOAEL of 30 mg/kg/day (Hoberman, 1992). Therefore, the criteria may be fulfilled to allocate an ARfD.
The proposed ARfD is derived from the NOAEL of 30 (27.1) mg/kg bw/day established in the teratology study in rabbits and a safety factor of 100.

ARfD = NOAEL/safety factor = 30 mg/kg bw/day/100 = 0.30 mg/kg bw/day

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

AOEL was previously based on the Teratology study in rabbits: NOAEL = 30 mg/kg bw/day (Hobermann, 1992). However since during the re-evaluation a NOAEL for Carcinogenicity has been proposed, setting a new AOEL is considered required. At the re-evaluation, a new NOAEL of 10.0 mg/kg bw/day (carcinogenicity) has been proposed at a lower dose in the chronic study in rats (Goldenthal, 1985), it is suggested to use this value for the derivation of the AOEL. An UF of 150 should be used because of the carcinogenic effect (increase in thyroid parafollicular (C-cell) carcinoma) observed in this study.

Based on the NOAEL of 10.0 mg/kg bw/day and a safety factor of 150, to achieve a margin of safety above 1000, an AOEL can be calculated:

AOEL = NOAEL/UF = 10 mg/kg bw/day/150 = 0.07 mg/kg bw/day (rounded)

Rounding from 0.06666666667 to 0.07 is < 10 %.

Margin of safety relative to LOAEL will in this case be:

LOAEL/reference value: 99.1 mg/kg bw/day/0.07 = 1415.7

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

ARfD is suggested as a value for AAOEL: NOAEL/safety factor = 30 mg/kg bw/day/100 = 0.3 mg/kg bw/day.

2.6.11 Summary of product exposure and risk assessment

Syngenta representative product (A7254B containing 480 g/L dicamba):
According to the GAP the highest dose used is 0.288 kg as/ha with a minimum volume for this use of 200 l/ha.
Dermal absorption:

Undiluted: 0.39 %

Diluted: 3.7 %

Operator Exposure:
Safe use can be demonstrated for operators wearing work wear during mixing and loading and application.

Work wear during mixing and loading and application:

Exposure % of AOEL: 5.86 %

Exposure % of AAOEL: 6.83 %

Bystander/resident exposure :
Safe use can be demonstrated for residents and bystanders for both children and adults.

Exposure with default input values:

Resident exposure for children % of AOEL: 5.14 %

Resident exposure for adult % of AOEL: 1.78 %

Bystander exposure for children % of AAOEL: < 1.13 %

Bystander exposure for adult % of AAOEL: < 0.33 %

Worker exposure:
Safe use can be demonstrated for workers wearing work clothing:

Worker exposure % of AOEL: 2.13 %

Rotam representative product dicamba 700SG (OCEAL/FH-048):

According to GAP the highest dose used is 0.280 kg as/ha and a minimum volume for this use of 200 l/ha.

Dermal absorption:

Concentrate: 0.1%

Dilution: 6%

Operator exposure:

Safe use can be demonstrated with use of work wear during mixing, loading and application.

PPE: Workwear during mixing, loading and application:

Exposure % of AOEL: 5.38%

Exposure % of AAOEL: 7.25 %

Resident/bystander exposure:

Safe use can be shown for residents/bystanders with default input parameters.

Resident exposure for children % of AOEL: 7.06 %

Resident exposure for adult % of AOEL: 2.61 %

Bystander exposure for children % of AAOEL: < 2 %

Bystander exposure for adult % of AAOEL: < 1 %

Worker exposure:

Safe use can be shown for workers with default input parameters and wearing work clothing.

Potential exposure:

Worker exposure % of AOEL: 30 %

For a worker wearing clothes:

Worker exposure % of AOEL: 3.36 %

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Syngenta/Rotam
Storage stability of dicamba was demonstrated for a period of 36 months at -18°C in crop commodities with high water and high starch content.
Storage stability of 5-OH-dicamba (NOA405873) was demonstrated for a period of 36 months at -18°C in crop commodities with high water and high starch content.
Storage stability of dicamba and DCSA (NOA414746) was demonstrated in milk, muscle (meat), fat, liver and kidney at -12°C or below for up to 18 months.

Only the results in high water, high starch and the animal commodities for dicamba and 5-OH-dicamba are relevant to the representative uses in this submission.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Syngenta/Rotam

Plants
In the DAR (2007, 2010) metabolism in plants were studied in several commodities. In the EFSA opinion from 2011 it was concluded:

The metabolism in plants was investigated in cereals (wheat, sugar cane) and in the pulse/oilseed plant group (soya, cotton), using ^{14}C-dicamba labelled on the phenyl moiety applied by foliar spraying (wheat), or by droplet applications by means of a micro-syringe to a limited number of leaves (sugar cane, soya, cotton). In sugar cane, soya and cotton, where the characterization of the residues was investigated shortly after the application (6 to 28 days), dicamba remains the major component of the residues, accounting for 22-29% of the TRR in sugar cane leaves, 44 - 94 % of the TRR in soya beans, and 72 % of the TRR in cotton seed. Other identified metabolites were observed in low proportions (< 2 % TRR), except 5-OH-dicamba, which represented 47 % and 20 % of the TRR in sugar cane leaves, 12 and 28 days after application, respectively. In wheat, dicamba seems to be more extensively metabolised, accounting for 10 % of the TRR in immature plant (forage), and 2 % and 16 % of the TRR respectively in straw and grain at harvest. 5-OH-dicamba is detected as the major metabolite in wheat forage (65 % TRR), but it represents less than 4 % TRR in grain and straw at harvest. Both the parent compound and 5-OH-dicamba were observed in free and conjugated form. Considering the different structures identified, the following metabolic pathway in plants was proposed. The metabolism of dicamba proceeds first by hydroxylation to form 5-OH-dicamba, or by demethylation to the DCSA metabolite, both compounds being further degraded to DCGA. The proposed metabolism is shown in Figure 1:

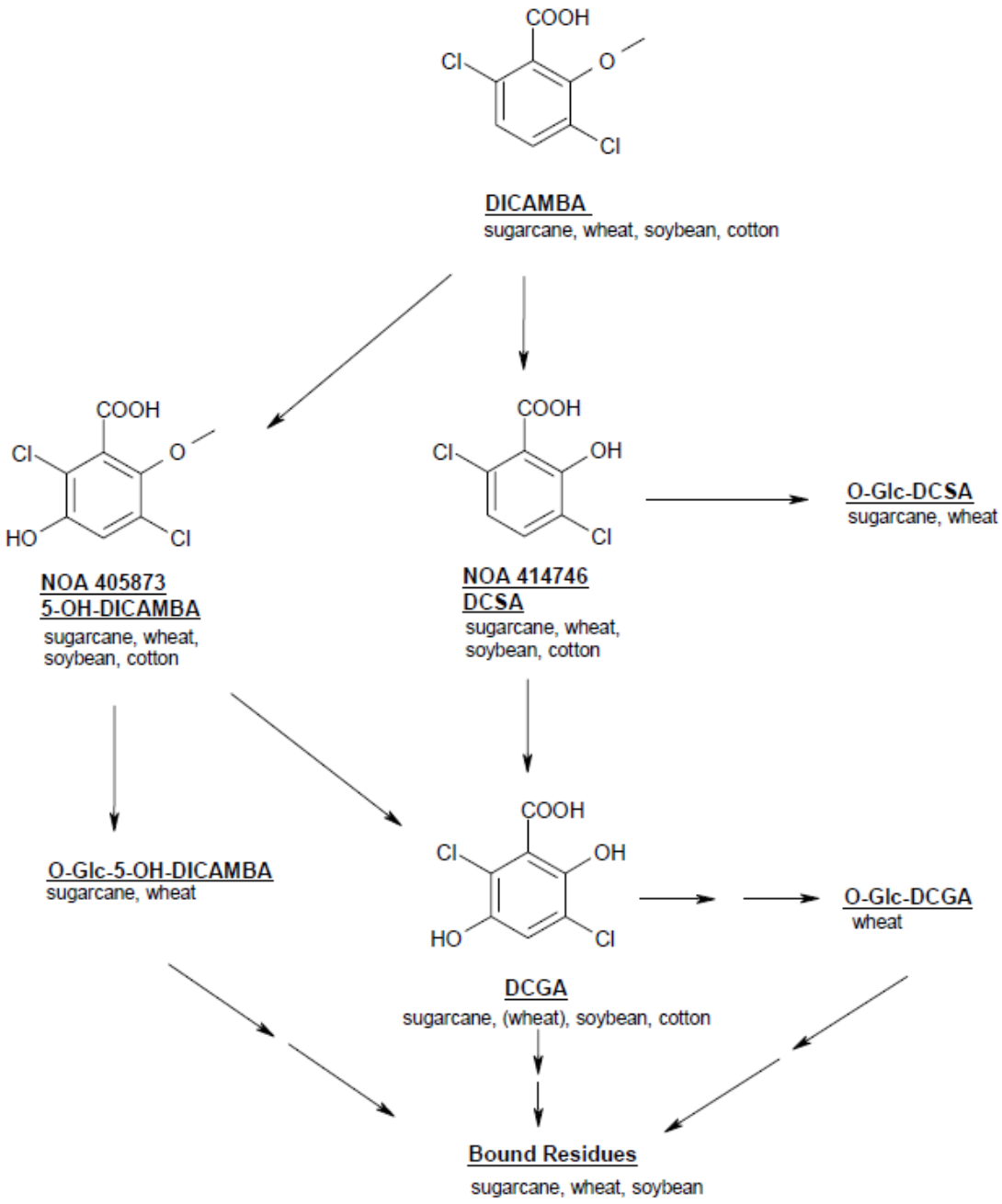


Figure 1: Proposed metabolic pathways of dicamba in plants

Animal

In the DAR (2007, 2010) metabolism in animals was studied in poultry, cow and goat. In the EFSA opinion from 2011 it was concluded:

Metabolism in animals was considered in cow, goat and poultry, using 14C-dicamba. The transfer in fat, milk and eggs was limited, the highest TRRs being observed in kidney and liver. Dicamba (free and conjugated) was by far the major compound identified in all animal matrices, accounting for more than 50 % of the TRR. In addition, DCSA was also observed in ruminants, but only in kidney and liver, up to 21 % of the TRR. 5-OH-dicamba was not detected in animal matrices, except in urine and excreta, but at insignificant levels and proportions (< 0.01 %

TRR). Having regard to the high levels of 5-OH-dicamba in grass, and consequently its significant intake by ruminants (*c.a.* 1.5 mg/kg bw/day), the PRAPeR TC 50 meeting of experts discussed whether a specific metabolism study using this metabolite needs to be required. The experts were of the opinion that a similar pathway to the parent is expected for 5-OH-dicamba, this metabolite being probably more extensively excreted than the parent compound since it is more polar. This assertion is supported by the results of the cow feeding study conducted with 5-OH-dicamba, where this metabolite was almost not detected in any matrices, except in kidney, at the 5N dose rate. It was therefore concluded that a specific ruminant metabolism study should not be required for 5-OH-dicamba.

The metabolism of ^{14}C-dicamba follows the same pathway in both poultry and ruminants:

- O-demethylation of dicamba to DCSA.
- Conjugation of DCSA with glucuronic acid.
- Decarboxylation of DCSA to 2,5-dichlorophenol (DCP).
- Decarboxylation of DCSA followed by substitution by an amino group to from 2-amino-3,6-dichlorophenol (2A36DCP).
- Hydroxylation of dicamba to 5-OH-dicamba.

A metabolism study in pigs is not required as the metabolism in the ruminant and rat is similar.

The proposed metabolic pathway of dicamba in animals is shown in Figure 2.

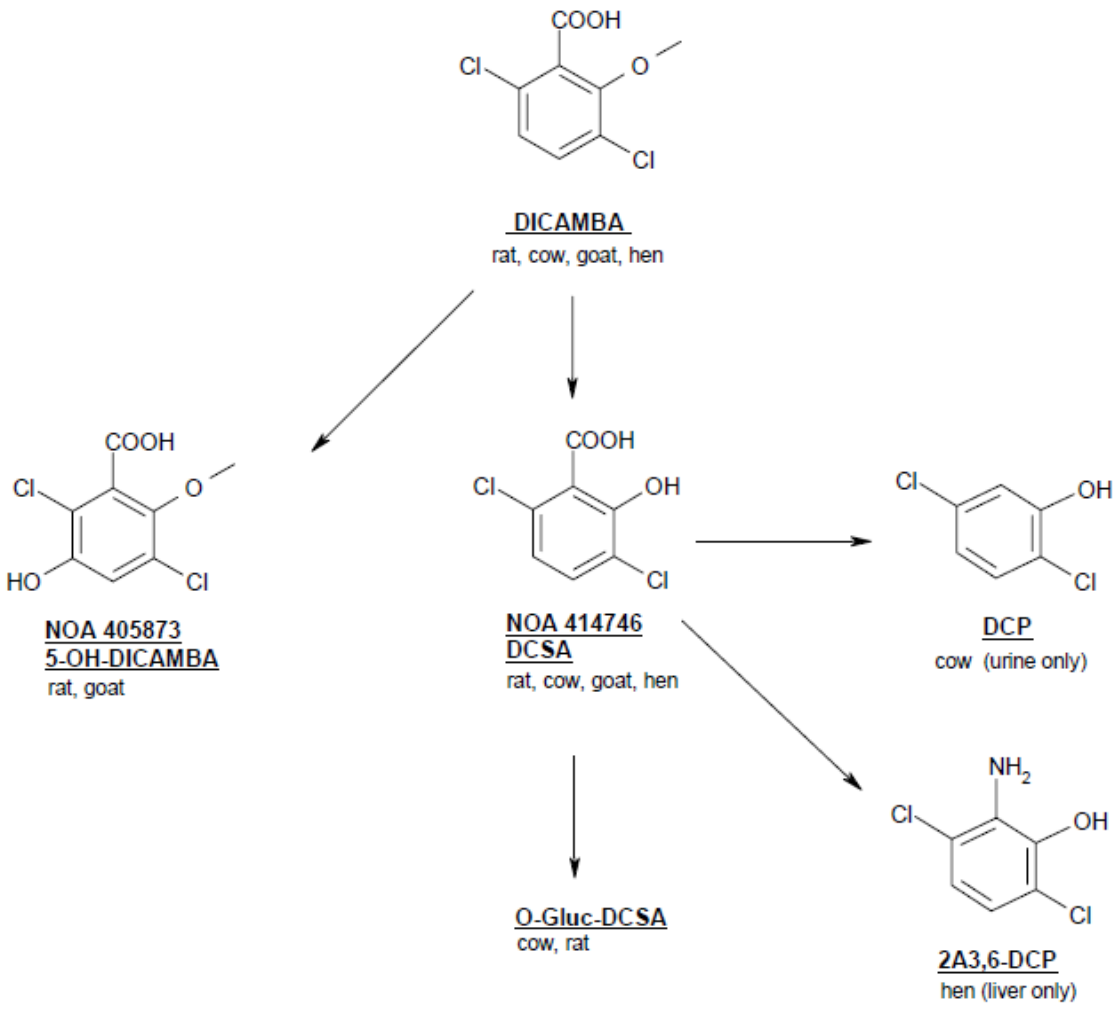


Figure 2: Proposed metabolic pathways of dicamba in animals

Fish
It can be expected that there is no potential for residues in commercial fish diet since dicamba is hydrophilic (Log P_{OW} -0.15 at PH 7) and therefore no data are required.

2.7.3 Definition of the residue

Syngenta and Rotam

Definition of the residue in plants
The metabolism in plants was investigated in cereals (wheat, sugarcane), soybean and cotton as representatives of pulses, oilseeds and cereals and was peer reviewed under Directive 91/414/EEC.
It is proposed to set the residue definition for enforcement to:

The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba.

Similarly, it is proposed that the residue definition for risk assessment is set to:

The sum of dicamba, 5-OH-dicamba and their conjugates, expressed as dicamba.

Definition of the residue in animal products
The metabolism in ruminants and poultry was peer reviewed under Directive 91/414/EEC.
It is proposed that the residue definition for enforcement is set to:

The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba.

Similarly it is proposed that the residue definition for risk assessment is set to:

The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba.

Definition of the residue in processed commodities

Syngeta and Rotam
The effect of hydrolysis on the nature of the residue of parent dicamba was investigated and peer reviewed under Directive 91/414/EEC. No breakdown or reaction products were formed during hydrolysis under representative processing conditions.

Syngenta
A new study investigating the effect of hydrolysis on the nature of the residue of 5-OH-dicamba was submitted – this has not previously been reviewed in the EU. No breakdown or reaction products were formed during hydrolysis under representative processing conditions.
No change to the definition of residue is proposed.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Syngenta

In Table 43 the applied GAPs for Syngenta is shown.

Table 43: Applied GAPs from Syngenta

Crop	Outdoor/ Protected	Growth Stage	Max. No. of Applica-tions	Minimum Application Interval (days)	Max. Application		Minimum PHI (days)
					Rate (g a.s./ha)	Water (L/ha)	
Barley	Outdoor (NEU)	BBCH 21-29	1	na	96	200-400	na[(a)]
Maize	Outdoor (NEU)	BBCH 12-19	1	na	288	200-500	na[(a)]
	Outdoor (SEU)	BBCH 12-19	1	na	288	200-500	na[(a)]
Oat	Outdoor (NEU)	BBCH 21-29	1	na	96	200-400	na[(a)]
Rye	Outdoor (NEU)	BBCH 21-29	1	na	96	200-400	na[(a)]
Sorghum	Outdoor (NEU)	BBCH 12-18	1	na	210	200-400	na[(a)]
	Outdoor (SEU)	BBCH 12-18	1	na	210	200-400	na[(a)]
Triticale	Outdoor (NEU)	BBCH 21-29	1	na	96	200-400	na[(a)]
Wheat	Outdoor (NEU)	BBCH 21-29	1	na	96	200-400	na[(a)]
	Outdoor (SEU)	BBCH 10-32	1	na	120	200-400	na[(a)]

na = not applicable

[(a)] It is more appropriate for cereal crops to indicate the application timing using growth stage rather than a pre-harvest interval.

The representative crops included in the original EU review of dicamba were maize and pasture; the use pattern for maize was at a more critical GAP (360 g as/ha) than the one being proposed by Syngenta. New data have therefore been provided by Syngenta to support the new representative GAP for maize. The representative crops included in the original EU review of dicamba did not include wheat, rye, triticale, barley, oat and sorghum. Trials have therefore been provided to support the GAPs for these crops.

Rotam

The representative use on maize is shown in Table 44.

Table 44: Applied GAP from Rotam in maize

Crop	Outdoor/ Protected	Growth Stage	Max. No. of Applica-tions	Minimum Application Interval (days)	Max. Application		Minimum PHI (days)
					Rate (g a.s./ha)	Water (L/ha)	
Maize	outdoor	16	1	-	350	200-400	60**

* latest possible growth stage at application

** critical parameter is the growth stage compared to minimum PHI, which is an indicative data

For maize the formulation used in the residue trials submitted by the original notifier was a 48% Soluble Liquid (SL) formulation. The proposed formulation is a 70% Soluble Granule (SG) formulation. Both formulations were applied in trials at a rate producing 360 g dicamba/ha (worst case compared to the current intended use at 350 g dicamba/ha). Both are water based formulation applied at practically identical gaps and are hence likely to produce similar residues. Trials with Dicamba 700 SG were conducted to confirm comparability of residues data to that in the DAR from the 48% SL formulation.

Syngenta/Rotam

The applied GAPs from Syngenta in cereals except maize corresponds to the critical GAP, while the applied GAP in maize from Rotam corresponds to the critical GAP for maize. However, the applied GAP from Syngenta is within the ± 25 % of the residue trials conducted in the initial DAR, so the same residue trials can be used in this evaluation, see table 45.

Maize (Syngenta)
Maize is a major crop both in the northern and in southern EU. Therefore, eight trials are necessary from each region. Ten trials are available from north and twelve trials are available from south in accordance with the applied GAP ± 25%. Eight of the trials from north were evaluated in the initial DAR, while two were new. For three of the trials evaluated in the initial DAR the LOQ for the method was 0.05 mg/kg for dicamba and 5-OH-dicamba, respectively. In the submission for renewal the LOQ for dicamba and 5-OH-dicamba in the same three trials were presented as 0.01 mg/kg. The residues for dicamba and 5-OH-dicamba were < 0.01 mg/kg in two trials, while it was 0.02 mg/kg in the third trial in the submission for renewal while the residues in the initial DAR were presented as < 0.05 mg/kg for both dicamba and 5-OH-dicamba, respectively. RMS has asked Syngenta for clarification on that point. Syngenta agree on that. So in this evaluaton the residues for dicamba and 5-OH dicamba were all < 0.05 mg/kg in those three trials instead of 2x <0.01 and 0.02 mg/kg as presented by Syngenta in the submission for renewal.

Maize (Rotam)
Rotam rely on the data submittet by Syngenta in the initial DAR. The proposed formulation from Rotam is a 70% Soluble Granule (SG) formulation while the formulation used in the residue trials submittet by Syngenta is a 48% Soluble Liquid (SL) formulation. To show that the residues are comparable when using a SG formulation compared to a SL formulation, Rotam has conducted 4 residue trials. Both formulations are water based and applied at a rate of 350 g as/ha or 360 g as/ha.

Table 45: Summary of residue trials in maize used in the calculation of the MRL

Crop	**Region/ Indoor** (a)	**Residue levels** (mg/kg) **observed in the supervised residue trials relevant to the supported GAPs** (b)	**Recommendations/comments** (OECD calculations)	**MRL** proposals (mg/kg)	**HR** (mg/kg) (c)	**STMR** (mg/kg) (d)
Representative uses						
Rotam						
Maize grain	NEU	Mo: 2 x < 0.01 RA: 2x 0.1* *Rotam rely on Syngentas trials from initial DAR* Mo: 5 x < 0.01; 3 x <0.05* RA: 5x 0.02 , 3 x 0.1	Mo: dicamba. Since the residues are below the LOQ of 0.01 or 0.05, the MRL is set at the highest LOQ of 0.05* mg/kg RA: The sum of dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba	0.05*	Mo: 0.02 RA: 0.1	Mo: 0.01 RA: 0.02
Maize grain	SEU	Mo: 2 x < 0.01 RA: 2x 0.1** *Rotam rely on Syngentas trials from initial DAR* Mo: 4 x <0.01 RA: 4 x 0.02		0.01*	Mo: 0.01 RA: 0.02	Mo: 0.01 RA: 0.02
Syngenta						
Maize grain	NEU	Mo: 7x <0.01; 3x <0.05* RA: 7x <0.02, 3x 0.1	Mo: dicamba. Since the residues are below the LOQ of 0.01 or 0.05, the MRL is set at 0.05* mg/kg RA: The sum of dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba RA	0.05*	Mo: 0.05 RA: 0.1	Mo: 0.01 RA: 0.02
Maize grain	SEU	Mo: 12x <0.01 RA: 12x 0.02		0.01*	Mo: 0.01 RA: 0.02	Mo: 0.01 RA: 0.02
Maize stover	NEU	MO: NA RA: <0.01,< 0.01, <0.01, 0.02, 0.027, 0.065, 0.076, 0.1, 0.1, 0.1, 0.525			RA: 0.53	RA: 0.02
Maize stover	SEU	MO: NA RA: <0.02, <0.02, 0.02, 0.02, 0.029, 0.029, 0.03, 0.03, 0.05, 0.08, 0.095			RA: 0.095	RA: 0.029

*For these three trials the LOQ is 0.05 mg/kg for the method used in the determination, while Syngenta has written that the residues of dicamba and 5-OH-dicamba were <0.01 in two trials and 0.02 in one trial. RMS has used the residues as reported in the evaluation in the initial DAR, i.e. <0.05 mg/kg.
**These residue are not used in the calculation for the value for risk assessment, since the LOQ is 0.1 mg/kg

Wheat, barley, oats, rye, triticale (Syngenta)
Wheat, barley, oat, rye, triticale are major crops in northern and southern EU so normally 8 trials are required in each region (SANCO 7525/VI/95 – rev.10.2). Barley, oats, rye and triticale are applied for in the northern EU while wheat is also applied for in the southern EU. Data from barley, oats, rye, triticale and wheat trials can be extrapolated to support the other crops with the same GAP, since the final application is made before the edible part of the crop is formed. Eight trials are availble for barley, five trials are available for oats and nine trials are available for wheat (one from north and eight from south).

Sorghum (Syngenta)
Sorghum is a minor crop in Northern Europe and a major crop in Southern Europe. Consequently 4 trials are necessary from north and 8 trials are necessary from south (SANCO 7525/VI/95 – rev.10.2).

The proposed representative uses of dicamba lead to calculated MRLs of 0.05* mg/kg in maize grain and 0.3 mg/kg in small grain cereals. These do not exceed the established MRLs of 0.5 mg/kg (maize), 7.0 mg/kg (barley), 0.5 mg/kg (oats and rye) or 2.0 mg/kg wheat (including triticale). For sorghum the MRL is calculated to 0.2 mg/kg, which is less than the current MRL of 4 mg/kg. The MRL of 4 mg/kg is an Codex MRL implemented in the EU legislation with Regulation (EU) No. 441/2012.

The summary residue trials are presented in Table46.

Table 46: Summary of residue trials Wheat, barley, oats, rye, triticale used in the calculation of the MRL

Crop	**Region/ Indoor** (a)	**Residue levels** (mg/kg) **observed in the supervised residue trials relevant to the supported GAPs** (b)	**Recommendations/comments** (OECD calculations)	**MRL** proposals (mg/kg)	**HR** (mg/kg) (c)	**STMR** (mg/kg) (d)
Representative uses						
Wheat grain Barley grain Oats grain (combined)	NEU	Mo: 3 x <0.01, 3 x 0.02, 0.03, 0.052, 0.06, 0.06, 0.076, 0.117, 0.142, 0.146 RA: 3 x <0.02, 3 x <0.03, 0.04, 0.062, 0.07, 0.086, 0.10, 0.127, 0.153, 0.167	OECD calculations Mo: Dicamba (MRL: 0.103) RA: The sum of dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba	0.3	Mo: 0.146 RA: 0.167	Mo: 0.041 RA: 0.051
Wheat grain	SEU	Mo: 5 x <0.01, 2 x 0.02, 0.07 RA: 5 x <0.02, 0.03, 0.06, 0.15	OECD calculations Mo: Dicamba (MRL: 0.25) RA: The sum of dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba	0.1	Mo: 0.07 RA: 0.15	Mo: 0.01 RA: 0.02
Wheat straw Barley straw Oats straw (combined)	NEU		No MRL calculated for feed items			
Wheat straw	SEU					

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

In the framework of the original EU review of dicamba, the dietary burden calculations were performed according to EU guideline 7031/VI/95 rev.4. Based on the representative uses, which were maize and grass the intake for poultry and ruminants was found to be above the trigger value of 0.1 mg/kg diet on dry weight basis. Therefore, feeding studies were submitted and evaluated in the original DAR.
The worst case for dairy- and beef cattle consisting of 100 % pasture is 9.05 mg/kg in fresh diet corresponding to 45 mg kg feed (dry matter) or 1.65 mg/kg bw/day for dairy cattle and 45 mg kg feed (dry matter) or 1.94 mg/kg bw/day beef cattle. Worst-case residue in chicken feed containing 70 % grain was 0.01 mg/kg in fresh diet corresponding to 0.012 mg/kg dry matter or 0.0007 mg/kg bw/day.

Syngenta

Dietary burden calculations
The dietary burden has been calculated for poultry, dairy cattle, beef cattle for the supported representative crops of barley, maize, oats, rye, triticale, wheat and sorghum or their processed products by using the EFSA animal burden calculator from 2016.
According to the OECD feeding table barley, oat, triticale, wheat and rye forage, hay and silage are not considered relevant crops as the representative use for dicamba is on barley, oat, rye, triticale and wheat for grain production only (OECD).
The dietary inputs for the calculation are summarised in Table 47. The highest residues in the residue trials (HR) are used to calculate the maximum potential dietary intake except for feed commodities that are bulked, where the STMR is used, or processed, where the STMR-P is used, as detailed in Table 47. The STMR values in residue trials have been used to calculate the median potential dietary intake.

Table 47: Input values used in the dietary burden calculation

Commodity	**Maximum dietary burden**		**Median dietary burden**	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Barley, Straw	1.78	HR	0.25	STMR
Corn, Field, Forage/Silage	0.62	HR	0.31	STMR
Corn, Field, Stover	0.1	HR	0.301	STMR
Oat, Straw	1.78	HR	0.25	STMR
Rye, Straw	1.78	HR	0.25	STMR
Sorghum, Forage	0.60	HR	0.32	STMR
Sorghum, Stover	0.80	HR	0.355	STMR
Sorghum, Silage	0.60	HR Forage data used as surrogate	0.32	STMR Forage data used as surrogate
Triticale, Straw	1.78	HR	0.25	STMR
Wheat, Straw	1.78	HR	0.25	STMR
Barley, Grain	0.167	HR	0.04	STMR
Corn, Field, Grain	0.05	HR	0.01	STMR
Oat, Grain	0.167	HR	0.04	STMR
Rye, Grain	0.167	HR	0.04	STMR
Sorghum, Grain	0.34	HR	0.05	STMR
Triticale, Grain	0.167	HR	0.05	STMR
Wheat, Grain	0.167	HR	0.051	STMR

Commodity	**Maximum dietary burden**		**Median dietary burden**	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Brewer's grain (wheat or barley), Dried (By-products group)	0.17	EFSA default processing factor of 3.3 for barley malt is used in calculation [Median value x PF malt 0.051 x 3.3 =0.17]	0.17	EFSA default processing factor of 3.3 for barley malt is used in the calculation [Median value x PF malt 0.051 x 3.3 = 0.17]
Corn, Field, Milled Byprods.	0.02	EFSA default processing of 1 used [Median value x PF 0.02 x 1 = 0.02]	0.02	EFSA default processing of 1 used [Median value x PF 0.02 x 1 = 0.02]
Corn, Field, Hominy Meal	0.12	EFSA default processing of 6 used [Median value x PF 0.02 x 6 = 0.12]	0.12	EFSA default processing of 6 used [Median value x PF 0.02 x 6 = 0.12]
Corn, Field, Gluten Feed	0.05	EFSA default processing of 2.5 used [Median value x PF 0.02 x 2.5 = 0.05]	0.05	EFSA default processing of 2.5 used [Median value x PF 0.02 x 2.5 = 0.05]
Corn, Field, Gluten Meal	0.02	EFSA default processing of 1 used [Median value x PF 0.02 x 1 = 0.02]	0.02	EFSA default processing of 1 used [Median value x PF 0.02 x 1 = 0.02]
Wheat, Gluten Meal	0.092	EFSA default processing factor of 1,8 is used in calculation [Median value x PF malt 0.051 x 1,8 = 0.092]	0.092	EFSA default processing factor of 1,8 is used in calculation [Median value x PF malt 0.051 x 1,8 = 0.092
Wheat, Milled By-prods.	0.36	EFSA default processing factor of 7 is used in calculation [Median value x PF malt 0.051 x 7 = 0.357]	0.36	EFSA default processing factor of 7 is used in calculation [Median value x PF malt 0.051 x 7 = 0.357]

The results of the dietary burden calculation are reported in Table 48.

Table 48: Results of the dietary burden calculation

Animals	**Median burden** (mg/kg bw)	**Maximum burden** (mg/kg bw)	**Above 0.004 mg /kg bw**	**Maximum burden** (mg/kg DM)	**Highest contributing commodities**	Previous assessment Maximum burdens (mg/kg bw DM)
Beef cattle	0,017	0,032	Yes	1,32	Corn, field forage/silage	1.94 mg/kg bw/day
Dairy cattle	0,023	0,042	Yes	1,09	Corn, field forage/silage	1.65 mg/kgbw/day
Ram/Ewe	0,012	0,045	Yes	1,36	Barley straw	Not calculated
Lamb	0,017	0,058	Yes	1,36	Barley straw	
Pig (breeding)	0,009	0,014	Yes	0,63	Corn, field forage/silage	
Pig (finishing)	0,007	0,012	Yes	0,40	Wheat milled bypdts	Not calculated
Poultry broiler	0,008	0,025	Yes	0,36	Wheat milled bypdts	0.00073 mg/kg bw/day
Poultry layer	0,014	0,038	Yes	0,56	Wheat straw	
Turkey	0,008	0,020	Yes	0,28	Wheat milled bypdts	

It is seen that the trigger value 0.004 mg/kg bw/day is exceeded in all animals. For ruminants the intake is far below the values calculated in the framework of the initial DAR, while for poultry the exposure is higher than calculated in the initial DAR.

Rotam

The applicant has only used the new trials conducted in maize in 2010 in the dietary burden calculation. However, the LOQ for 5-OH dicamba was 0.1 mg/kg. Thus the value for risk assessment that should be used in the dietary burden calculation is too high. Therefore, RMS has used the same values as was used for Syngenta in the dietary burden calculation.

Dietary burden calculation

Table 49: Input values used in the dietary burden calculation

Commodity	**Maximum dietary burden**		**Median dietary burden**	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Corn, Field, For-age/Silage	0.62	HR	0.31	STMR
Corn, Field, Stover	0.1	HR	0.301	STMR
Corn, Field, Grain	0.05	HR	0.01	STMR
Corn, Field, Milled Byprods.	0.05	EFSA default processing of 1 used [Median value x PF 0.02 x 1 = 0.02]	0.02	EFSA default processing of 1 used. [Median value x PF 0.02 x 1 = 0.02]
Corn, Field, Hominy Meal	0.12	EFSA default processing of 6 used [Median value x PF 0.02 x 6 = 0.12]	0.12	EFSA default processing of 6 used [Median value x PF 0.02 x 6 = 0.12]
Corn, Field, Gluten Feed	0.05	EFSA default processing of 2.5 is used [Median value x PF 0.02 x 2.5 = 0.05]	0.05	EFSA default processing of 2.5 is used [Median value x PF 0.02 x 2.5 = 0.05]
Corn, Field, Gluten Meal	0.02	EFSA default processing of 1 used [Median value x PF 0.02 x 1 = 0.02]	0.02	EFSA default processing of 1 used [Median value x PF 0.02 x 1 = 0.02]

Table 50: Results of the dietary burden calculation

Animals	**Median burden** (mg/kg bw)	**Maximum burden** (mg/kg bw)	**Above 0.004 mg /kg bw**	**Maximum burden** (mg/kg DM)	**Highest contributing commodities**	Previous assessment Maximum burdens (mg/kg bw DM)
Beef cattle	0.015	0.030	Yes	1.27	Corn. field forage/silage	1.94 mg/kg bw/day
Dairy cattle	0.019	0.037	Yes	0.97	Corn. field forage/silage	1.65 mg/kgbw/day
Ram/Ewe	0.001	0.002	No	0.05	Corn. field gluten feed	Not calculated
Lamb	0.002	0.002	No	0.05	Corn. field gluten feed	
Pig (breeding)	0.004	0.009	Yes	0.37	Corn. field forage/silage	
Pig (finishing)	0.001	0.002	No	0.06	Corn. field gluten feed	Not calculated
Poultry broiler	0.001	0.004	No	0.05	Corn. field milled bypdts	0.00073 mg/kg bw/day
Poultry layer	0.008	0.015	Yes	0.22	Corn. field forage/silage	
Turkey	0.002	0.004	No	0.06	Corn. field hominy meal	

It is seen that the trigger value 0.004 mg/kg bw/day is exceeded in all animals besides ram/ewe/lamb. For ruminants the intake is far below the values calculated in the framework of the initial DAR while for poultry the exposure is higher than calculated in the initial DAR.

Feeding studies

Rotam

Two feeding studies in livestock on the active substance dicamba were performed and included in the DAR of dicamba (2007). There was one feeding study in ruminants (dairy cattle) and one feeding study in poultry (laying hens).

Both studies were considered as reliable but it is not required to present them in the current submission for renewal of dicamba approval.

RMS: the dietary burden calculation show that the exposure is higher than 0.004 mg/kg lgv/day for both ruminants and poultry. Consequently, feeding studies are nescessary.

Poultry

Syngenta
A feeding study with dicamba in poultry was evaluated under Council Directive 91/414/EEC and is presented in the dicamba draft Assessment Report (Vol.3, Annex B, Section B.7.1, February 2007) and the results are summarised in Table 51.

Table 51: Poultry feeding study evaluated in the initial DAR

Commodity	**Results from Livestock Feeding Study**					**Median Residue (mg/kg)[c]**	**Highest Residue (mg/kg)[d]**	**Calculated MRL (mg/kg)**	**CF for RA[e]**
	Dose level (mg/kg bw/day)[a] [mg/kg diet]	No of animals	DoR (E or RA)[b]	Mean Residue (mg/kg)	Max Residue (mg/kg)				
EU Reviewed Data *(Report No. 107-203 and 74; DAR, 2007)*									
Poultry Fat	0.15 [2]	10	E & RA	<0.01	<0.01	<0.01	<0.01	<0.01	--
	0.46 [6]	10	E & RA	<0.01	<0.01				
	1.5 [20]	10	E & RA	0.01	0.025				
Poultry Skin	0.15 [2]	10	E & RA	n.a.	n.a.	<0.01	<0.01	<0.01	--
	0.46 [6]	10	E & RA	<0.01	<0.01				
	1.5 [20]	10	E & RA	0.034	0.068				
Poultry Liver	0.15 [2]	10	E & RA	<0.01	<0.01	<0.01	<0.01	<0.01	--
	0.46 [6]	10	E & RA	0.015	0.023				
	1.5 [20]	10	E & RA	0.031	0.053				
Poultry Meat	0.15 [2]	10	E & RA	n.a.	n.a.	<0.01	<0.01	<0.01	--
	0.46 [6]	10	E & RA	<0.01	<0.01				
	1.5 [20]	10	E & RA	0.01	0.013				
Eggs	0.15 [2]	10	E & RA	n.a.	n.a.	<0.01	<0.01	<0.01	--
	0.46 [6]	10	E & RA	<0.01	<0.01				
	1.5 [20]	10	E & RA	<0.01	<0.01				

n.a.: Not analysed
(a): Based on average weight of 1.645 kg animal consuming 0.125 kg feed DM/day.
(b): Residue definition used for presented results; E = enforcement, RA = risk assessment. Method AM-0685 was used which determined dicamba, salts & conjugates
(c): Median residue value according to the enforcement residue definition, derived by interpolation/extrapolation from the feeding study for the median dietary burden (FAO, 2009).
(d): Highest residue value (tissues, eggs) or mean residue value (milk) according to the enforcement residue definition, derived by interpolation/extrapolation of the maximum dietary burden between the relevant feeding groups of the study (FAO, 2009).
(e): The median conversion factor for enforcement to risk assessment.
(*): Indicates that the MRL is set at the limit of analytical quantification.

The study was reviewed within the framework of Directive 91/414/EEC and was considered to be acceptable; There are no new requirements or guidance applicable to this submission under Regulation (EC) 1107/2009, therefore the original endpoints and assessment are still valid. No further feeding studies in poultry are required to support the renewal of dicamba.

Ruminants

Syngenta
Feeding studies with dicamba and 5-OH-dicamba separately in lactating ruminants were evaluated under Council Directive 91/414/EEC and are presented in the dicamba draft Assessment Report (Vol.3, Annex B, Section B.7.1, February 2007) and the results are summarised in Table 52.

At the time of this review, a data gap was identified; the method of analysis for animal products had not been fully validated (EFSA Journal 2011;9(1):1965) so MRLs were proposed only. With this submission method GRM022.03A has been sufficiently validated as well as independent validated for analysis in animal matrices. MRLs for animal commodities have subsequently been set (Reg. (EU) No. 441/2012).

According to the results of the dietary burden calculation, lambs demonstrated the highest dietary exposure to residues of dicamba and 5-OH-dicamba of a maximum dietary burden of 0.058 mg/kg/bw/d. The calculated exposure of lambs is sixteen times lower than the lowest dose level in the feeding studies where residues in ruminant tissues and milk were calculated to be <LOQ (<0.01 mg/kg).
No residues above the LOQ are expected in ruminant tissues or milk following the representative uses of dicamba supported by Syngenta. Residues all fall below the established MRLs for ruminant tissues and milk.

Table 52: Overview of ruminant dicamba feeding study evaluated for inclusion of dicamba in Annex I Directive 91/414/EEC

Commodity	**Results from livestock Feeding Study**					**Median Residue (mg/kg)[c]**	**Highest Residue (mg/kg)[d]**	**Calculated MRL (mg/kg)**	**CF for RA[e]**
	Dose level (mg/kg bw/day)[a] [mg/kg diet]	No of animals	DoR (E or RA)[b]	Mean Residue (mg/kg)	Max Residue (mg/kg)				
EU Reviewed Data (*Report No. 379; DAR, 2007*)									
Ruminant meat	0.93 [40]	3	--[f]	<0.01	<0.01	<0.01	<0.01	<0.01	--
	2.78 [120]	3	--[f]	0.012	0.014				
	9.3 [400]	3	--[f]	0.030	0.037				
Ruminant fat	0.93 [40]	3	--[f]	0.023	0.046	<0.01	<0.01	<0.01	--
	2.78 [120]	3	--[f]	0.025	0.034				
	9.3 [400]	3	--[f]	0.047	0.059				
Ruminant liver	0.93 [40]	3	--[f]	0.026	0.029	<0.01	<0.01	<0.01	--
	2.78 [120]	3	--[f]	0.066	0.070				
	9.3 [400]	3	--[f]	0.207	0.207				
Ruminant kidney	0.93 [40]	3	--[f]	0.154	0.174	<0.01	<0.01[g]	<0.01 [g]	--
	2.78 [120]	3	--[f]	0.282	0.288				
	9.3 [400]	3	--[f]	0.646	0.885				
Milk	0.93 [40]	3	--[f]	0.02	0.029	<0.01	<0.01	<0.01	--
	2.78 [120]	3	--[f]	0.035	0.055				
	9.3 [400]	3	--[f]	0.177	0.294				

n.r.: Not required - only the mean values are considered for calculating MRLs in milk
(a): Based on a 570 kg animal consuming 13.2 kg feed DM/day.
(b): Residue definition used for presented results; E = enforcement, RA = risk assessment
(c): Median residue value according to the enforcement residue definition, derived by interpolation/extrapolation from the feeding study for the median dietary burden (FAO, 2009).
(d): Highest residue value (tissues, eggs) or mean residue value (milk) according to the enforcement residue definition, derived by interpolation/extrapolation of the maximum dietary burden between the relevant feeding groups of the study (FAO, 2009).
(e): The median conversion factor for enforcement to risk assessment.
(f): Residues were determined as dicamba and DCSA together.

(g): Highest residue by interpolation = 0.011 mg/kg, however this is for residues of dicamba and DCSA together therefore residues according to E & RA definition are expected to be <0.01 mg/kg.
(*): Indicates that the MRL is set at the limit of analytical quantification.

Samples in the first study (report number 379) were analysed using method AM-0659. This method determined residues of dicamba and the metabolite DSCA together as a common moiety (methyl ester of dicamba). The residue definition for both monitoring and risk assessment is proposed as the sum of dicamba, and the salts and conjugates of dicamba expressed as dicamba, therefore the results from this study will give a worst case for residues.

Samples in the second study where 5-OH-dicamba was administered, were analysed for residues of 5-OH-dicamba only. The residue definition for risk assessment in plants includes 5-OH dicamba therefore a feeding study dosed with 5-OH dicamba is relevant. However, this metabolite is not included in the residue definition for animal products for either monitoring or risk assessment as the ruminant metabolism studies indicated that significant residues of 5-OH dicamba were unlikely to be found in the edible animal commodities (5-OH dicamba was only found in excreta at significant levels). The feeding study data supports the conclusions of the metabolism studies. The results of this study will not impact on any proposals for residue values in animal products and have not been discussed further.

Pigs

Syngenta
The calculated dietary exposure of dicamba for pigs is 0.014 mg/kg/bw/d, which is lower than that calculated for ruminants (lambs). The metabolism of dicamba in ruminants was similar to that seen in the rat. Metabolism and feeding studies in pigs are therefore not required, as data for ruminants can be used to address the potential for residues in pigs.
Significant residues in tissues of pigs are therefore not expected and it is anticipated that they would fall below the established MRLs for swine.

Fish

Syngenta, Rotam
As the accumulation of compounds of relatively low lipophilicity (log Pow < 3 (dicamba= -0.15, PH 7)) *via* the diet is known to be negligible, neither fish metabolism nor fish feeding data are needed.

2.7.6 Summary of effects of processing

Rotam
Not required, since no significant residues (all residue < 0.01 mg/kg) occur in the plant or plant product for further processing and TMDI < 10% of the ADI (EU-Guidelines (Lundehn, Appendix E, 7035/VI/95 rev. 5; 22/07/1997)).

Syngenta
As quantifiable residues of dicamba and 5-OH-dicamba are expected in treated crops, studies investigating the nature of residues in processed commodities are required.

Conditions	Identified Compounds (%)	Report Reference	Source
EU Reviewed Data			
Pasteurisation (20 min, 90°C, pH 4)	Dicamba (100.7)	RJ3333B	Denmark, 2007
Baking, boiling, brewing (60 min, 100°C, pH 5)	Dicamba (105.1)		
Sterilisation (20 min, 120°C, pH 6)	Dicamba (107.6)		

The effect of processing on the nature of dicamba and 5-OH-dicamba were investigated in two separate studies. Studies simulated representative hydrolytic conditions for pasteurisation (20 minutes at 90ºC, pH4), boiling/brewing/baking (60 minutes at 100ºC, pH5) and sterilisation (20 minutes at 120ºC, pH6).
The studies showed that no breakdown or reaction products were formed during hydrolysis of dicamba or 5-OH-dicamba under representative processing conditions.
It can be concluded that the nature of residues in processed commodities and hence the relevant residues for enforcement and risk assessment in processed commodities are expected to be the same as for primary crops.

The distribution of residues in peel/pulp was not deemed relevant to this submission since no representative crop uses have inedible peel.

Magnitude of residue studies have previously been evaluated for barley and oats under Directive 91/414/EC. Processing factors have been derived for barley and oats.

Processing studies have been conducted in barley, oats and wheat and these have not been previously submitted for evaluation under Directive 91/414/EEC. The studies presented have investigated the transfer of dicamba and total dicamba (dicamba + 5-OH-dicamba) residues in processes representative of major industrial procedures for barley (preparation of alcoholic beverages), and for minor industrial procedures and domestic or home procedures; pearling for barley, rolled oats for oats and flour and wholemeal bread production, gluten and starch separation and wheat germ extraction.
In barley, residues of dicamba and dicamba + 5-OH-dicamba did not concentrate in malt, beer or pearl barley. In oats, residues of dicamba and dicamba + 5-OH-dicamba concentrated slightly in rolled oats. In wheat, no detectable residues were observed in the pre-processing RAC grain samples; slight concentration was observed in coarse bran (in one study) and waste by-products. As a result, no processing factors were calculated for wheat commodities.
An overall summary of processing factors for dicamba and dicamba + 5-OH-dicamba in processed barley and oat commodities is presented in Table 53.

Table 53: Summary of processing factors for dicamba and dicamba + 5-OH-dicamba from studies presented

Crop	Processed Commodity	Number of Studies	Median Processing Factor	
			dicamba	Dicamba + 5-OH-dicamba
Barley	Malt (all types)	8	1.00	1.00
	Beer	4	0.34	0.48
	Pearl barley	5	0.50	0.67
Oats	Rolled oats	4	1.33	1.33
Wheat	Flour production Wholemeal Bread Gluten & starch separation Wheat Germ Extraction	2	Not calculated	Not calculated

2.7.7 Summary of residues in rotational crops

Rotam
Dicamba is degraded rapidly in soil with a DT_{90} of 24.9 days and a DT_{50} of 6.66 days. The predominant metabolite was DCSA, which also is degraded rapidly with a DT_{50} of 4.9 days and a DT_{90} of 16.1 days. Therefore, no studies in rotational or succeeding crops are required according to EU-Guidelines (Lundehn, Appendix C, 7524/VI/95 rev. 2; 22/07/1997) where it is stated that "*From existing results on the residue behaviour of the active substance in soil, a test is carried out to determine whether after 100 days less than 10 % of active substance and bioavailable metabolites can be detected*". If it is not case, as for dicamba, it is not required to presented residues in rotational crops (including metabolism and magnitude).

Syngenta
The metabolism of dicamba in rotational crops was considered during the EU evaluation using C^{14} phenyl-U labelled-dicamba. The studies were evaluated under Council Directive 91/414/EEC and are presented in the dicamba draft Assessment Report (Vol.3, Annex B, Section B.7.9, February 2007).

Comments made by EFSA at the last review indicated residues from confined rotational crops were adequately characterised. Syngenta has conducted a new rotational crop study and this confirms the high total radioactive residues (TRRs) at 30 DAT (0.027-0.886 mg/kg) and progressive and marked decline in subsequent 111 DAT (0.017–0.097 mg/kg) and 285 DAT (0.001–0.016 mg/kg) rotational intervals that were observed in the earlier studies.

Parent was the principal residue identified in all 30 DAT commodities (≤67% TRR; ≤0.204 mg/kg) except that of wheat straw and grain (≤1.9% TRR; 0.002 mg/kg). NOA405873 (5-OH-dicamba) was the principal identified metabolite in wheat forage, hay and straw (≤56.3% TRR; ≤0.342 mg/kg) but was present at much lower levels in all other commodities (≤5.5% TRR; ≤0.005 mg/kg).

By the time of 111 DAT rotational crop harvests, residues of parent and all identified metabolites had declined to ≤0.007 mg/kg (except for NOA405873 in wheat hay, 0.017 mg/kg).
By the time of 285 DAT rotational crop harvests, residues of parent and all the above identified metabolites were not detectable (except for NOA414746 in wheat hay, 0.001 mg/kg).
The decline in parent and identified metabolite residues in successive rotational intervals was accompanied by an increase in the proportion of the total radioactive residue associated with naturally incorporated radioactivity (≤ 41.7% TRR identified as ^{14}C-glucose).
These studies demonstrate that the metabolic pathway in rotational crops is identical to that previously elucidated in the primary metabolism studies.

Four limited rotational field trials were conducted to investigate the magnitude of residues in rotational crops. Residues of parent dicamba and 5-OH-dicamba were observed in barley commodities (whole plant, straw, grain) and carrot (tops and leaves). The magnitude and distribution of residues is consistent with those seen in the confined rotational studies. Residues of parent dicamba and 5-OH-dicamba were seen at or below the limit of quantification (LOQ) in three of the four trials conducted. In one trial only, residues of parent and 5-OH-dicamba were observed in some feed items, predominantly at the 30 DAT plant-back interval; these declined over time across the later plant-back intervals. The impact of these results on the animal dietary burden has been considered within this submission. The proposed definition of the residue in succeeding crops is therefore consistent with the definition of the residue for risk assessment in primary crops.

2.7.8 Summary of other studies

As there is currently no guidance available to conduct such studies an assessment on the effect on residue levels in pollen and other bee products has not been conducted. Besides, treatment is taking place before flowering.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

The ADI and ARfD for dicamba are summarised in the table below.

Table 54: ADI and ARfD values for dicamba

End-Point	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.07 mg/kg bw/d	chronic study in rats (NOAEL:10 mg/kg bw/day)	150	Goldenthal, 1985
Acute Reference Dose (ARfD)	0.3 mg/kg bw	Rabbit developmental toxicity study (NOAEL)	100	Hoberman,1992

TMDI
TMDI has been calculated using EFSA PRIMo vers. 3. The residues for cereals and maize are lower than the existing MRLs in Regulation 396/2005. Therefore the current MRL for all commodities are used in the calculation. The results are shown in table 2.7.9-2. As can be seen from the table the highest exposure is for GEMS/Food G1 accounting for 84% of the proposed ADI of 0.07 mg/kg bw/d.

IESTI
The estimates of acute intake were conducted with the EFSA model PRIMO (EFSA model for chronic and acute risk assessment - rev. 3_0)

An IESTI risk assessment was performed, using 97.5th percentile dietary intake values. MRL values were used as an input for the crops and commodities included in this dossier. The summary of the calculation is presented in Table 53. The highest IEST amounted to 21% of the ARfD for milk and milk products.

Table 55: Calculation of the chronic and acute exposure for dicamba using the MRLs set in Regulation 2015/845

European Food Safety Authority
EFSA PRIMo revision 3.0; 2017/12/11

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day): **0,07** ARfD (mg/kg bw): **0,3**

Source of ADI: Source of ARfD:

Year of evaluation: Year of evaluation:

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment | Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI: ---

TMDI/NEDI/IEDI calculation (based on average food consumption)

Calculated exposure (% of ADI)	MS Diet	Exposure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from MRLs set at the LOQ (in % of ADI)	Exposure resulting from commodities not under assessment (in % of ADI)
84%	GEMS/Food G11	58,62	53%	Soyabeans	10%	Wheat	8%	Barley		84%
75%	NL toddler	52,40	43%	Milk: Cattle	11%	Wheat	5%	Maize/corn		75%
74%	GEMS/Food G10	51,49	47%	Soyabeans	11%	Wheat	6%	Barley		74%
59%	GEMS/Food G08	41,14	28%	Soyabeans	12%	Wheat	9%	Barley		59%
56%	GEMS/Food G15	39,35	25%	Soyabeans	13%	Wheat	8%	Barley		56%
55%	GEMS/Food G07	38,46	25%	Soyabeans	12%	Wheat	6%	Barley		55%
48%	GEMS/Food G06	33,63	21%	Wheat	17%	Soyabeans	2%	Sugar canes		48%
41%	UK infant	28,99	28%	Milk: Cattle	7%	Wheat	3%	Peas (without pods)		41%
38%	NL child	26,77	17%	Milk: Cattle	12%	Wheat	3%	Soyabeans		38%
38%	FR toddler 2 3 yr	26,64	21%	Milk: Cattle	9%	Wheat	5%	Beans (with pods)		38%
37%	FR child 3 15 yr	26,07	16%	Milk: Cattle	13%	Wheat	3%	Beans (with pods)		37%
35%	DE child	24,26	14%	Milk: Cattle	12%	Wheat	2%	Apples		35%
30%	UK toddler	20,82	15%	Milk: Cattle	11%	Wheat	2%	Peas (without pods)		30%
28%	DK child	19,28	13%	Wheat	9%	Milk: Cattle	4%	Rye		28%
26%	RO general	18,12	14%	Wheat	8%	Milk: Cattle	0,7%	Maize/corn		26%
25%	ES child	17,78	13%	Wheat	9%	Milk: Cattle	1%	Beans (with pods)		25%
24%	DE general	16,54	9%	Milk: Cattle	5%	Wheat	5%	Barley		24%
22%	SE general	15,59	9%	Wheat	9%	Milk: Cattle	0,5%	Beans (with pods)		22%
21%	DE women 14-50 yr	14,98	9%	Milk: Cattle	6%	Wheat	2%	Barley		21%
21%	IT toddler	14,90	19%	Wheat	0,6%	Peas (without pods)	0,5%	Beans (with pods)		21%
20%	PT general	14,25	11%	Wheat	4%	Soyabeans	1%	Beans (without pods)		20%
20%	NL general	13,94	6%	Milk: Cattle	6%	Wheat	3%	Barley		20%
19%	FR infant	12,97	12%	Milk: Cattle	3%	Beans (with pods)	2%	Wheat		19%
18%	ES adult	12,83	7%	Wheat	5%	Barley	4%	Milk: Cattle		18%
17%	IE adult	12,17	7%	Wheat	3%	Milk: Cattle	1%	Peas (without pods)		17%
14%	IT adult	9,88	12%	Wheat	0,9%	Beans (with pods)	0,4%	Peas (without pods)		14%
13%	FR adult	9,29	6%	Wheat	3%	Milk: Cattle	1%	Beans (with pods)		13%
10%	UK vegetarian	7,21	6%	Wheat	2%	Milk: Cattle	0,8%	Peas (without pods)		10%
9%	DK adult	6,35	4%	Milk: Cattle	3%	Wheat	0,7%	Peas (without pods)		9%
9%	UK adult	6,23	5%	Wheat	2%	Milk: Cattle	0,7%	Peas (without pods)		9%
9%	LT adult	6,02	3%	Wheat	3%	Milk: Cattle	0,8%	Rye		9%
7%	IE child	4,84	3%	Wheat	3%	Milk: Cattle	0,5%	Beans (without pods)		7%
7%	FI 3 yr	4,76	3%	Wheat	0,7%	Barley	0,5%	Rye		7%
5%	FI 6 yr	3,81	3%	Wheat	0,6%	Barley	0,4%	Rye		5%
3%	FI adult	2,15	0,9%	Wheat	0,5%	Rye	0,4%	Coffee beans		3%
1%	PL general	0,88	0,3%	Beans (without pods)	0,3%	Apples	0,2%	Potatoes		1%

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of is unlikely to present a public health concern.

Acute risk assessment /children | **Acute risk assessment / adults / general population**

Details - acute risk assessment /children | Details - acute risk assessment/adults

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

Acute risk assessment /children | **Acute risk assessment / adults / general population**

Hide IESTI new calculations | Show IESTI new calculations

IESTI new calculations:
The calculation is performed with the MRL and the peeling/processing factor (PF), taking into account the residue in the edible portion and/or the conversion factor for the residue definition (CF). For case 2a, 2b and 3 calculations a variability factor of 3 is used. Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.
Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.

Show results for all crops

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
21%	Milk: Cattle	0,5 / 0,5	62
19%	Asparagus	5 / 5	56
13%	Barley	7 / 7	39
12%	Beans (with pods)	4 / 4	35
11%	Peas (without pods)	4 / 4	33
11%	Beans (without pods)	4 / 4	32
10%	Wheat	2 / 2	29
8%	Soyabeans	10 / 10	23
8%	Lentils (fresh)	4 / 4	23
5%	Peas (with pods)	4 / 4	14
4%	Sorghum	4 / 4	13
4%	Apples	0,1 / 0,1	11
3%	Chamomille	40 / 40	8,0
3%	Chamomille	40 / 40	8,0
3%	Chamomille	40 / 40	8,0

Expand/collapse list

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
18%	Soyabeans	10 / 10	55
13%	Asparagus	5 / 5	38
11%	Barley	7 / 7	34
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
6%	Milk: Cattle	0,5 / 0,5	19
6%	Wheat	2 / 2	17
5%	Rooibos	40 / 40	16
5%	Rooibos	40 / 40	16
5%	Beans (without pods)	4 / 4	16
4%	Lentils (fresh)	4 / 4	13
4%	Peas (without pods)	4 / 4	13

IESTI new
Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
23%	Asparagus	5 / 5	68
0,7%	Cucumbers	0,05 / 0,05	2,0
0,6%	Cauliflowers	0,05 / 0,05	1,7
0,6%	Mandarins	0,05 / 0,05	1,7
0,6%	Celeriacs/turnip rooted	0,05 / 0,05	1,7
0,6%	Witloofs/Belgian endives	0,05 / 0,05	1,7
0,5%	Kohlrabies	0,05 / 0,05	1,6
0,5%	Swedes/rutabagas	0,05 / 0,05	1,6
0,5%	Tomatoes	0,05 / 0,05	1,5
0,5%	Buckwheat and other	0,3 / 0,3	1,5
0,5%	Carrots	0,05 / 0,05	1,4
0,4%	Head cabbages	0,05 / 0,05	1,3
0,4%	Kales	0,05 / 0,05	1,3
0,4%	Sweet corn	0,07 / 0,07	1,3
0,4%	Plums	0,05 / 0,05	1,3

Total number of commodities found exceeding the ARfD/ADI in children and adult diets (IESTI new calculation)

IESTI new
Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
30%	Asparagus	5 / 5	89
18%	Soyabeans	10 / 10	55
11%	Barley	7 / 7	34
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
8%	Chamomille	40 / 40	24
6%	Milk: Cattle	0,5 / 0,5	19
6%	Wheat	2 / 2	17
5%	Rooibos	40 / 40	16
5%	Rooibos	40 / 40	16
5%	Beans (without pods)	4 / 4	16
4%	Lentils (fresh)	4 / 4	13
4%	Peas (without pods)	4 / 4	13

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
17%	Beans (with pods) / boiled	4 / 4	50
14%	Soyabeans / soy milk	10 / 10	42
11%	Peas (without pods) / cann	4 / 4	32
8%	Barley / cooked	7 / 7	25
8%	Wheat / milling (flour)	2 / 2	24
5%	Soyabeans / boiled	10 / 4	15
4%	Barley / milling (flour)	7 / 7	13
4%	Maize / oil	0,5 / 12,5	12
4%	Wheat / milling (wholemea	2 / 2	11
3%	Sugar canes / sugar	1 / 1	9,2
2%	Sugar beets (root) / sugar	0,05 / 0,6	5,5
2%	Apples / juice	0,1 / 0,1	5,4
2%	Potatoes / fried	0,05 / 0,05	4,7
1%	Pumpkins / boiled	0,05 / 0,05	4,4
1%	Broccoli / boiled	0,05 / 0,05	3,9

Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
17%	Barley / beer	7 / 1,4	50
7%	Beans (without pods) /	4 / 4	21
5%	Peas (with pods) / boiled	4 / 4	14
4%	Peas (without pods) /	4 / 4	13
3%	Wheat / bread/pizza	2 / 2	8,8
3%	Wheat / pasta	2 / 2	7,6
2%	Maize / oil	0,5 / 12,5	6,3
1%	Wheat / bread	2 / 2	3,4
1%	Apples / juice	0,1 / 0,1	3,3
0,9%	Pumpkins / boiled	0,05 / 0,05	2,8
0,7%	Sugar beets (root) / sugar	0,05 / 0,6	2,2
0,7%	Cauliflowers / boiled	0,05 / 0,05	2,1
0,6%	Celeries / boiled	0,05 / 0,05	1,7
0,4%	Broccoli / boiled	0,05 / 0,05	1,2
0,4%	Rooibos leaves / infusion	40 / 0,4	1,2

IESTI new
Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)

IESTI new
Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity. As short term intake of residues of is unlikely to preser
For processed commodities, no exceedance of the ARfD/ADI was identified.

2.7.10 Proposed MRLs and compliance with existing MRLs

EU MRLs for dicamba are currently detailed in Regulation (EU) 2015/845. EU MRLs have not been reviewed under Article 12 of Regulation (EC) 396/2005. EU MRLs for commodities relevant to this submission are detailed in the following table, with established and proposed values. No new EU MRLs are currently proposed.

Maize
MRLs for maize grain have been proposed. Both results from the residue trials performed by Rotam and Syngenta have been included. The highest LOQ have been used to set the MRL, see Table 56.

Table 56: MRL calculations for dicamba on maize grain – representative GAPs

Region	Outdoor / Pro-tected	Residue Data (mg/kg)	MRL OECD Method (mg/kg)	MRL OECD Rounded (mg/kg)
Northern EU	Outdoor	<0.01, <0.01 <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.05, <0.05, <0.05	0.05*	0.05*
Southern EU	Outdoor	<0.01 < 0.01, <0.01, <0.01, <0.01, <0.01, <0.01, <0.01,<0.01, <0.01, <0.01, <0.01, <0.01, <0.01	0.01*	0.01*

* The highest LOQ is used to set the MRL.

There is an existing EU MRL of 0.5 mg/kg (Commission Regulation (EU) 845/2015) for dicamba on maize. The data presented in Table 56 from trials supporting the representative GAP indicate that all residues will be within the existing EU MRL of 0.5 mg/kg.

Dicamba residue calculations for risk assessment
STMR and HR values for maize grain have been proposed for northern and southern Europe for the combined residues of dicamba and 5-OH-dicamba for the trials performed by Syngenta only, since the LOQ for 5-OH dicamba was 0.1 for the residue trials performed by Rotam. The STMR is the median residue and the HR is the highest residue value found. The values are presented in Table 57.
STMR and HR values for maize forage and stover as potential livestock feed items have also been proposed for northern and southern Europe and are presented in Table 57.

Table 57: STMR and HR calculations for dicamba + 5-OH-dicamba on maize grain, forage and stover – representative GAP

Region	Outdoor / Pro-tected	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)
Grain				
Northern EU	Outdoor	<0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.1, <0.1, 0.1	0.02	0.1
Southern EU	Outdoor	<0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02	0.02	0.02
Northern + Southern EU	Outdoor	<0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.02, <0.1, <0.1, <0.1	0.02	0.1
Forage				
Northern EU	Outdoor	0.02, 0.048, 0.243, 0.376, 0.417, 0.617	0.31	0.617
Southern EU	Outdoor	0.023, 0.026, 0.028, 0.039, 0.05, 0.05, 0.137	0.05	0.137
Stover				
Northern EU	Outdoor	0.02, 0.027, 0.065, 0.076, 3x 0.1, 0.525	0.301	0.525
Southern EU	Outdoor	<0.02, <0.02, 0.02, 0.02, 0.029, 0.029, 0.03, 0.03 0.05, 0.08, 0.095	0.029	0.095

Dicamba residue calculations to derive conversion factors
Residue values of dicamba and 5-OH-dicamba derived from the supervised residue trials have been used to calculate MRLs and derive STMR and HR values for risk assessment calculations. No conversion factors have been used for these calculations. Conversion factors have been determined using these residue data and are summarised in Table 58.

Table 58: Dicamba residue conversion factor calculations

Crop	**Zone**	**Individual residue values (mg/kg)**		
		Dicamba	**5-OH-dicamba**	**Conversion factor**
Maize grain	NEU/SEU	20 x <0.02	20 x <0.02	20 x 1.00
		0.02	0.02	1.00
		<0.01	0.01	1.00
Median conversion factor (maize grain): 1.00				

Barley, oat, rye and wheat

Dicamba residue calculations for MRL setting
An MRL for all small grain cereals (barley, oat, rye and wheat) has been calculated for northern and southern Europe according to the OECD calculator (OECD Series on pesticides No. 56, ENV/JM/MONO (2011)2, 1 March 2011) for parent dicamba only. In accordance with SANCO 7525/VI/95 – rev.10.1, data from barley, oat, rye and wheat trials can be extrapolated to support the other small grain cereal crops. Since the residue trials presented in this dossier on barley, oats and wheat in Northern EU were conducted following the same GAP, the data has been combined to calculate a single MRL value for all small grain cereal crops in Northern EU. For the Southern EU, the MRL for small grain cereals is based on data in wheat.

In these calculations a single data point from each trial supporting the representative GAP has been considered. Where two or more values are available from duplicate analysis for the same trial following applications according to the GAP, the mean has been used. Where two or more values are available from duplicate sampling for the same trial following applications according to the GAP, the highest has been used. The calculated outputs are presented in Table 59.

Table 59: MRL calculations for dicamba on cereal grain – representative GAP

Region	**Commodity**	**Outdoor / Protected**	**Residue Data (mg/kg)**	**MRL OECD Method (mg/kg)**	**MRL OECD Rounded (mg/kg)**
Northern EU	Barley, oats, wheat	Outdoor	<0.01, <0.01, <0.01, 0.02, 0.02, 0.02, 0.03, 0.052, 0.06, 0.07, 0.076, 0.117, 0.142, 0.146	0.25	0.3
Southern EU	Wheat	Outdoor	<0.01, <0.01, <0.01, <0.01, <0.01, 0.02, 0.02, 0.07	0.11	0.15

There are existing EU MRLs of 7.0 mg/kg for dicamba in barley, 0.5 mg/kg in oats and rye, and 2.0 mg/kg in wheat (Commission Regulation (EU) 845/2015). The data presented in Table 59 from trials supporting the representative GAP indicate that residues in small grain cereals will be within the existing EU MRLs.

Dicamba residue calculations for risk assessment

STMR and HR values for barley, oats, rye and wheat (including triticale) grain and straw have been calculated for northern and southern Europe for the combined residues of dicamba and 5-OH-dicamba. The STMR is the median residue and the HR is the highest residue value found. In accordance with SANCO 7525/VI/95 – rev.10.1 data from barley, oat, rye and wheat trials can be extrapolated to support the other small grain cereal crops when the final application is made before the edible part of the crop is formed. Since the residue trials presented in this dossier on barley, oats and wheat were conducted following the same GAP in Northern EU, the data has been combined to calculate single STMR and HR values for small grain cereal crops in Northern EU. For the Southern EU, the STMR and HR values for small grain cereals are based on data in wheat.

In these calculations a single data point from each trial supporting the representative GAP has been considered. Where two or more values are available from duplicate analysis for the same trial following applications according to the GAP, the mean has been used. Where two or more values are available from duplicate sampling for the same trial following applications according to the GAP, the highest has been used. The calculated outputs for grain and straw are presented in Table 60.

Table 60: STMR and HR calculations for dicamba and 5-OH-dicamba on cereal grain and straw – representative GAPs

Region	Commodity	Outdoor / Protected	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)
Grain					
Northern EU	Barley, oats, wheat	Outdoor	<0.02, <0.02, <0.02, 0.03, 0.03, 0.03, 0.04, 0.062, 0.07, 0.086, 0.10, 0.127, 0.153, 0.167	0.051	0.167
Southern EU	Wheat	Outdoor	<0.02, <0.02, <0.02, <0.02, <0.02, 0.03, 0.06, 0.15	0.02	0.15
Straw					
Northern EU	Barley, oats, wheat	Outdoor	<0,02, <0.02, <0.02, 0.02, 0.025, 0.026, 0.03, 0.05, 0.078, 0.098, 0.112, 0.12, 0.13, 0.34	0.04	0.34
Southern EU	Wheat	Outdoor	0.05, 0.06, 0.07, 0.20, 0.29, 0.34, 1.32, 1.78	0.245	1.78

Dicamba residue calculations to derive conversion factors

Residue values of dicamba and 5-OH-dicamba derived from the supervised residue trials have been used to calculate MRLs and derive STMR and HR values for risk assessment calculations. No conversion factors have been used for these calculations.

Conversion factors have been determined using these residue data and are summarised in Table 61.

Table 61: Dicamba residue conversion factor calculations

Crop	Zone	Individual residue values (mg/kg)		
		Dicamba	**5-OH-dicamba**	**Conversion factor**
Barley grain	NEU	0.07	0.03	0.43
		0.02	<0.01	0.50
		0.02	<0.01	0.50
		<0.01	<0.01	1.00
		0.02	<0.01	0.5
		0.06	<0.01	0.17
		0.03	<0.01	0.33
		<0.01	<0.01	1.00
Median conversion factor (barley grain): 0.50				
Oats grain	NEU	0.146	0.021	0.14
		0.052	<0.01	0.19
		0.142	0.011	0.08
		0.076	<0.01	0.13
		0.117	<0.01	0.09
Median conversion factor (oats grain): 0.13				
		<0.01	<0.01	1
		<0.01	<0.01	1
		<0.01	<0.01	1

Crop	Zone	Individual residue values (mg/kg)		
		Dicamba	**5-OH-dicamba**	**Conversion factor**
		<0.01	<0.01	1
		<0.01	<0.01	1
		0.02	0.01	0.50
		<0.01	<0.01	1
		0.07	0.08	1.14
		0.02	0.04	2.00
Median conversion factor (wheat grain): 1.00				
Barley straw	NEU	0.15	0.19	1.27
		0.01	<0.01	1.00
		0.02	0.03	1.50
		<0.01	<0.01	1.00
		0.02	0.01	0.50
		0.06	0.07	1.17
		0.05	0.07	1.40
		<0.01	0.01	1.00
Median conversion factor (barley straw): 1.085				
Oats straw	NEU	0.088	0.01	0.11
		0.016	<0.01	0.63
		0.1	0.012	0.12
		0.067	0.011	0.16
		0.015	<0.01	0.67
Median conversion factor (oats straw) 0.16				
		<0.01	0.013	1.30
		0.01	0.04	4.00
		<0.01	0.05	5.00
		0.02	0.18	9.00
		0.02	0.27	13.50
		0.03	0.31	10.33
		0.01	0.06	6.00
		0.18	1.6	8.89
		0.12	1.2	10.00
Median conversion factor (wheat straw): 6				

Sorghum

For MRL setting, the definition of the residue for dicamba is parent dicamba and its salts (free and conjugated). For risk assessment purposes it is the combined residues of dicamba and 5-OH-dicamba (free and conjugated). MRL calculations are presented below.

Dicamba residue calculations for MRL setting

MRLs for sorghum grain have been calculated for northern and southern Europe according to the OECD calculator (OECD Series on pesticides No. 56, ENV/JM/MONO (2011)2, 1 March 2011) for parent dicamba only. In these calculations a single data point from each trial (from either formulation if two formulations were used in side by side plots within a single trial) supporting the representative GAP has been considered. Where two or more values are available from duplicate analysis for the same trial following applications according to the GAP, the mean has been used. Where two or more values are available from duplicate sampling for the same trial following applications according to the GAP, the highest has been used. The calculated outputs are presented in Table 62.

Table 62: MRL calculations for dicamba on sorghum grain – representative GAPs

Region	Outdoor / Protected	Residue Data (mg/kg)	MRL OECD Method (mg/kg)	MRL OECD Rounded (mg/kg)
Northern EU	Outdoor	0.02, 0.02, 0.04, 0.04	0.09	0.09
Southern EU	Outdoor	<0.01, <0.01, <0.01, 0.02, 0.02, 0.028, 0.03, 0.04, 0.043, 0.15	0.203	0.2

There is an existing EU MRL of 4.0 mg/kg (Commission Regulation (EU) 845/2015) for dicamba on sorghum. The data presented in Table 62 from trials supporting the representative GAP indicate that all residues will be within the existing EU MRL of 4.0 mg/kg.

Dicamba residue calculations for risk assessment

STMR and HR values for sorghum grain have been calculated for northern and southern Europe for the combined residues of dicamba and 5-OH-dicamba. The STMR is the median residue and the HR is the highest residue value found. In these calculations a single data point from each trial (from either formulation if two formulations were used in side by side plots within a single trial) supporting the representative GAP has been considered. Where two or more values are available from duplicate analysis for the same trial following applications according to the GAP, the mean has been used. Where two or more values are available from duplicate sampling for the same trial following applications according to the GAP, the highest has been used. The calculated outputs are presented in Table 63. STMR and HR values for sorghum forage and stover as potential livestock feed items have also been calculated for northern and southern Europe. The calculated outputs are presented in Table 63. Dicamba + 5-OH-dicamba residues in grain, forage and stover in samples from northern and southern EU were similar and combined calculations of the STMR and HR from the two regions are presented.

Table 63: STMR and HR calculations for dicamba + 5-OH-dicamba on sorghum grain, forage and stover – representative GAP

Region	Outdoor / Protected	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)
Grain				
Northern EU	Outdoor	0.03, 0.04, 0.06, 0.07	0.05	0.07
Southern EU	Outdoor	0.02, 0.02, 0.02, 0.03, 0.04, 0.042, 0.06, 0.068, 0.08, 0.34	0.041	0.34
Northern + Southern EU	Outdoor	0.02, 0.02, 0.02, 0.03, 0.03, 0.04, 0.04, 0.042, 0.06, 0.06, 0.07, 0.068, 0.08, 0.34	0.041	0.34
Forage				
Northern EU	Outdoor	0.02, 0.28, 0.36, 0.56	0.32	0.56
Southern EU	Outdoor	0.02, 0.06, 0.07, 0.60	0.065	0.60
Northern + Southern EU	Outdoor	0.02, 0.02, 0.06, 0.07, 0.28, 0.36, 0.56, 0.60	0.175	0.60
Stover				
Northern EU	Outdoor	0.10, 0.23, 0.48, 0.66	0.355	0.66
Southern EU	Outdoor	0.02, 0.02, 0.03, 0.06, 0.10, 0.27, 0.295, 0.80	0.08	0.80
Northern + Southern EU	Outdoor	0.02, 0.02, 0.03, 0.06, 0.10, 0.10, 0.23, 0.27, 0.295, 0.48, 0.66, 0.80	0.165	0.80

Dicamba residue calculations to derive conversion factors
Residue values of dicamba and 5-OH-dicamba derived from the supervised residue trials have been used to calculate MRLs and derive STMR and HR values for risk assessment calculations. No conversion factors have been used for these calculations.

Conversion factors have been determined using these residue data and are summarised in Table 64.

Table 64: Dicamba residue conversion factor calculations

Crop	**Zone**	**Individual residue values (mg/kg)**		
		Dicamba	**5-OH-dicamba**	**Conversion factor**
Sorghum grain	NEU/SEU	0.04	0.02	0.50
		0.02	<0.01	0.50
		0.04	0.03	0.75
		0.02	0.02	1.00
		0.028	0.014	0.50
		0.043	0.025	0.58
		<0.01	<0.01	1.00
		<0.01	<0.01	1.00
		0.15	0.19	1.27
		0.02	0.01	0.50
		0.03	0.03	1.00
		0.04	0.04	1.00
		0.02	0.02	1.00
		<0.01	<0.01	1.00
Median conversion factor (sorghum grain): 1				
Sorghum forage	NEU/SEU	0.21	0.15	0.71
		0.10	0.18	1.8
		0.03	0.02	0.67
		0.19	0.37	1.95
		<0.01	<0.01	1
		0.03	0.04	1.33
		0.34	0.26	0.76
		0.04	0.02	0.5
Median conversion factor (sorghum forage): 0.88				
Sorghum stover	NEU/SEU	0.37	0.11	0.30
		0.07	0.16	2.29
		0.04	0.06	1.5
		0.24	0.42	1.75
		<0.01	<0.01	1.00
		0.02	0.01	0.5
		0.51	0.29	0.57
		0.05	<0.01	0.2
		0.08	0.19	2.38
		0.125	0.17	1.36
		0.09	<0.01	0.11
		0.01	<0.01	1.00
Median conversion factor (sorghum stover): 1				

Animal products

In Table 65 the exsisting MRLs in animal products are shown. Since the dietary burden calculation showed that all MRLs should be set to < 0.01 mg/kg. The excisting MRLs can be kept when dicamba is used in accordance with the applied uses. Therefore, no modification is necessary.

Table 65: MRLs for dicamba set in Regulation 2015/845 for animal products

1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	
1010000	. Tissues from	
1011000	. (a) swine	
1011010	. Muscle	0.05*
1011020	. Fat tissue	0.07
1011030	. Liver	0.7
1011040	. Kidney	0.7
1011050	. Edible offals (other than liver and kidney)	0.7
1011990	. Others	0.05*
1012000	. (b) bovine	
1012010	. Muscle	0.5
1012020	. Fat tissue	0.07
1012030	. Liver	0.7
1012040	. Kidney	0.7
1012050	. Edible offals (other than liver and kidney)	0.7
1012990	. Others	0.5
1013000	. (c) sheep	
1013010	. Muscle	0.05*
1013020	. Fat tissue	0.07
1013030	. Liver	0.7
1013040	. Kidney	0.7
1013050	. Edible offals (other than liver and kidney)	0.7
1013990	. Others	0.05*
1014000	. d) goat	
1014010	. Muscle	0.05*
1014020	. Fat tissue	0.07
1014030	. Liver	0.7
1014040	. Kidney	0.7
1014050	. Edible offals (other than liver and kidney)	0.7
1014990	. Others	0.05*
1015000	. (e) equine	
1015010	. Muscle	0.05*
1015020	. Fat tissue	0.07
1015030	. Liver	0.7
1015040	. Kidney	0.7
1015050	. Edible offals (other than liver and kidney)	0.7
1015990	. Others	0.05*
1016000	. (f) poultry	
1016010	. Muscle	0.02
1016020	. Fat tissue	0.04
1016030	. Liver	0.07
1016040	. Kidney	0.07
1016050	. Edible offals (other than liver and kidney)	0.07
1016990	. Others	0.05*
1017000	. (g) other farmed terrestrial animals	
1017010	. Muscle	0.05*
1017020	. Fat tissue	0.07
1017030	. Liver	0.7
1017040	. Kidney	0.7
1017050	. Edible offals (other than liver and kidney)	0.7
1017990	. Others	0.05*
1020000	. Milk	
1020010	. Cattle	0.5
1020020	. Sheep	0.2
1020030	. Goat	0.2
1020040	. Horse	0.2
1020990	. Others	0.2
1030000	. Birds eggs	0.05*
1030010	. Chicken	0.05*
1030020	. Duck	0.05*
1030030	. Geese	0.05*
1030040	. Quail	0.05*
1030990	. Others	0.05*
1040000	. Honey and other apiculture products	0.05*
1050000	. Amphibians and Reptiles	0.05*
1060000	. Terrestrial invertebrate animals	0.05*
1070000	. Wild terrestrial vertebrate animals	0.05*

Pesticide residue	Legislation	Entry in force
Dicamba	Reg. (EU) 2015/845	04-06-2015

1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	
1010000	. Tissues from	
1011000	. (a) swine	
1011010	. Muscle	0.05*
1011020	. Fat tissue	0.07
1011030	. Liver	0.7
1011040	. Kidney	0.7
1011050	. Edible offals (other than liver and kidney)	0.7
1011990	. Others	0.05*
1012000	. (b) bovine	
1012010	. Muscle	0.5
1012020	. Fat tissue	0.07
1012030	. Liver	0.7
1012040	. Kidney	0.7
1012050	. Edible offals (other than liver and kidney)	0.7
1012990	. Others	0.5
1013000	. (c) sheep	
1013010	. Muscle	0.05*
1013020	. Fat tissue	0.07
1013030	. Liver	0.7
1013040	. Kidney	0.7
1013050	. Edible offals (other than liver and kidney)	0.7
1013990	. Others	0.05*
1014000	. d) goat	
1014010	. Muscle	0.05*
1014020	. Fat tissue	0.07
1014030	. Liver	0.7
1014040	. Kidney	0.7
1014050	. Edible offals (other than liver and kidney)	0.7
1014990	. Others	0.05*
1015000	. (e) equine	
1015010	. Muscle	0.05*
1015020	. Fat tissue	0.07
1015030	. Liver	0.7
1015040	. Kidney	0.7
1015050	. Edible offals (other than liver and kidney)	0.7
1015990	. Others	0.05*
1016000	. (f) poultry	
1016010	. Muscle	0.02
1016020	. Fat tissue	0.04
1016030	. Liver	0.07
1016040	. Kidney	0.07
1016050	. Edible offals (other than liver and kidney)	0.07
1016990	. Others	0.05*
1017000	. (g) other farmed terrestrial animals	
1017010	. Muscle	0.05*
1017020	. Fat tissue	0.07
1017030	. Liver	0.7
1017040	. Kidney	0.7
1017050	. Edible offals (other than liver and kidney)	0.7
1017990	. Others	0.05*
1020000	. Milk	
1020010	. Cattle	0.5
1020020	. Sheep	0.2
1020030	. Goat	0.2
1020040	. Horse	0.2
1020990	. Others	0.2
1030000	. Birds eggs	0.05*
1030010	. Chicken	0.05*
1030020	. Duck	0.05*
1030030	. Geese	0.05*
1030040	. Quail	0.05*
1030990	. Others	0.05*
1040000	. Honey and other apiculture products	0.05*
1050000	. Amphibians and Reptiles	0.05*
1060000	. Terrestrial invertebrate animals	0.05*
1070000	. Wild terrestrial vertebrate animals	0.05*

Pesticide residue	Legislation	Entry in force
Dicamba	Reg. (EU) 2015/845	04-06-2015

2.7.11 Proposed import tolerances and compliance with existing import tolerances

No MRLs exist as a consequence of import tolerances to the EU. Only Codex MRLs have been adopted.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

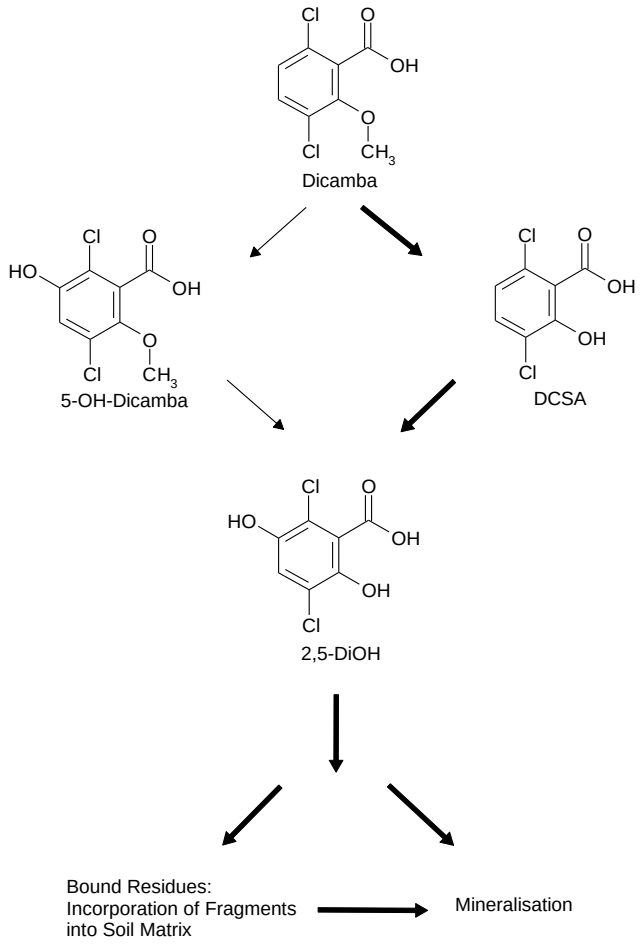
2.8.1.1 Route of degradation in soil

Data on the route of degradation in soil is presented in Volume 3 CA B.8 (B.8.1.1.1).

Under **aerobic** soil conditions, dicamba degrades rapidly in soil independent of soil pH with formation of the major metabolite DCSA. The maximum observed levels of DCSA was 58.8%. No other metabolites were observed >5%. High levels of $^{14}CO_2$ (up to 58.3%) indicated mineralisation via ring cleavage.
The proposed metabolic pathway for dicamba in aerobic soil is shown below.

No **anaerobic** soil degradation or soil **photolysis** experiments have been performed due to the fast degradation of Dicamba.

Proposed route of degradation of dicamba in soil under aerobic conditions:



2.8.1.2 Rate of degradation in soil

Data on the rate of degradation in soil is presented in Volume 3 CA B.8 (B.8.1.1.2).

The previously submitted studies for dicamba have been technically reviewed and are all but one considered to be acceptable. The degradation kinetics has been re-evaluated according to current guidance.
The degradation halflifes for DCSA have all been determined from the existing parent studies but has been re-evaluated according to current guidance.

Rate of degradation of dicamba in soil:

Study	Soil	Texture	Ki-netic	$DegT_{50}$ [20°C/pF2] (days)
Figge, 1993	BBA Standard Soil 2.2	Loamy Sand	SFO	3.21
Glänzel, 2000	Gartenacker	Loam	SFO	3.37
	Pappelacker	Sandy Loam	SFO	4.24
	Borstel	Loamy Sand	SFO	4.81
Roohi A. and Cooper J., 2010	Farditch	Clay Loam	SFO	18.23
	Longwoods	Sandy Loam	SFO	24.60
	LUFA 2.4	Clay Loam	SFO	8.88
			Geometric mean	7.06 (n=7)

Rate of degradation of DCSA in soil:

Study	Soil	Texture	Kinetic	DegT50 DCSA [20°C/pF2] (days)
Figge, 1993	BBA Standard Soil 2.2	Loamy Sand	SFO	10.5
Glänzel, 2000	Gartenacker	Loam	SFO	4.01
	Pappelacker	Sandy Loam	SFO	3.74
	Borstel	Loamy Sand	SFO	9.65
			Geometric mean	6.24 (n=4)

2.8.1.3 Adsorption and desorpiton in soil

Data on adsorption and desorption in soil is presented in Volume 3 CA B.8 (B.8.1.2).

A soil adsorption/desorption study on dicamba was available from the last EU review. Except for one of the five soils tested, the study was still considered acceptable. A new evaluation of the study using the OECD 106 evaluators checklist (EFSA, 2017) was performed. The adsorption K_{foc} values found ranged from 1.4 – 23.7 mL/g.

A new acceptable study on adsorption/desorption of dicamba in four soils was also submitted. The resulting adsorption K_{foc} values ranged from 2.0 – 11.8 mL/g.

Overall, the adsorption K_{foc} values found for dicamba ranged from 2.0 to 23.7 mL/g with a geometric mean of 5.28 mL/g (n=8) indicating that dicamba has a very high mobility in soil.

For the metabolite DCSA a soil adsorption/desorption study was available from the previous EU review. This study was still considered acceptable. A new evaluation of the study using the OECD 106 evaluators checklist (EFSA, 2017) was performed. The resulting adsorption K_{foc} values ranged from 241.7 to 1433.9 mL/g with a geometric mean of 649.6 mL/g (n=4).

2.8.1.4 Mobility in soil

Data on mobility in soil is presented in Volume 3 CA B.8 (B.8.1.3).

From the previous EU review three studies on the potential mobility in soil of dicamba and its metabolite DCSA were available: One column leaching study with three soils, one aged residue column leaching study with two soils and an outdoor lysimeter study. All three studies were still considered acceptable.

The column leaching study was conducted using three German soils with an organic carbon content ranging from 0.7-2.3%, and pH values between 5.8-6.6. An application rate of 352 g/ha dicamba was used, and 200 mm artificial rain was delivered to each column within 48 hours. Only <0.2-0.68% of the AR (<0.3-1.2 µg/L) was recovered in the percolated water (sum of dicamba and DCSA) after 48 hours, indicating a negligible transport of dicamba and its metabolite DCSA in the soil columns.

In the aged residue column leaching study, the mobility was studied in one German and one Swiss soil (pH range 6.0-7.4, OC contents of 48-0.96%). Dicamba was aged for 40.5 days before transfer of the soil to the columns and addition of 200 mm artificial rain. A maximum of 0.94% of the AR was recovered as dicamba (1.7 µg/L), whereas a maximum of 0.31% of the AR was recovered as DCSA (0.53 µg/L) in the percolation water, indicating a negligible transport of dicamba and DCSA.

In the outdoor lysimeter study, the mobility was studied in intact soil cores following 2-3 annual applications of dicamba. Maize plants were planted and cultivated in the top soil before application of dicamba. After two years with annual applications of dicamba to maize plants grown in the lysimeters (application rate of 360 g/ha), a maximum of 0.15% of the AR was recovered in the leachates. However, neither dicamba nor DCSA were detected in the leachates. The majority of the AR remained in the top 20 cm of the lysimeter column. Only traces amounting to <0.05% of AR were detected below 60 cm at termination of the study, one year after the last treatment.

Furthermore, in a number of field dissipation studies performed with dicamba in Swiss and German soils, several soil horizons were analysed for the distribution of dicamba and DCSA. Downward movement of dicamba and DCSA were not detected below 40 cm in soils characterised as loamy sand, clay loam and silt loam. In sandy loam, the presence of dicamba and DCSA was detected down to 60 cm.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

1.1.1.1 Rapid degradability of organic substances

Table 66: Summary of relevant information on rapid degradability

Method	Results*	Key or Supportive study	Remarks	Reference
OECD 301 F Ready Biodegradability: Manometric respiration (1992)	The theoretical oxygen demand (ThOD) for dicamba was calculated to be 1.09 g oxygen/g, the measured chemical oxygen demand COD		**Acceptable**	Wallace and Daniel (2001). Determination of 28 day ready biodegrability of

Method	Results*	Key or Supportive study	Remarks	Reference
	value was 1.04 g oxygen/g. The biological oxygen demand BOD value for dicamba did not exceed 5% (<0.06 and 0.05 g oxygen/g after 5 days and 28 days, respectively). This indicates a negligible biodegradation of dicamba under the experimental conditions tested. The measured COD and BOD value for the reference substance fulfills the validity criteria of the test. These results indicate that dicamba is <u>not readily biodegradable</u>.			SAN837A. Syngenta File No SAN837/5987
OECD 301 F Ready Biodegradability: Manometric respiration (1992)	The mean percentage biodegradation at the end of the 28 day exposure period was 9% (ThOD). The biodegradation of the reference substance confirms the suitability of the activated sludge inoculum. The degradation rate of Dicamba did not reach 60% within the 10 day window and after 28 days of incubation. Therefore, Dicamba is considered <u>not to be readily biodegradable</u>.		**Acceptable**	Feil (2010). Ready Biodegradability of RC1176 in a Manometric Respirometry Test. Rotam Report No 56061163

** data on full mineralization should be reported*

2.8.2.1.1 Ready biodegradability

Data on ready biodegradability is presented in Volume 3 CA B.8 (B.8.2.2.1).

A study on ready biodegradability was available from the previous EU review. The study was still considered acceptable. The results indicated that dicamba is not readily biodegradable.

A new acceptable study was also submitted by notifier Rotam. This study confirmed that dicamba is not readily biodegradable.

2.8.2.1.2 BOD5/COD

In a study from the previous EU review a BOD of 0.05 g oxygen/g was found after 28 days.

1.1.1.2 Other convincing scientific evidence

2.8.2.1.3 Aquatic simulation tests

Data on aerobic mineralisation in surface water is presented in Volume 3 CA B.8 (B.8.2.2.1).

Two new studies on the degradation in surface water were submitted. One from each notifier.

Both studies followed the guideline OECD 309: Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test (2004)

The extent of mineralisation and the rate and route of degradation of [^{14}C]-dicamba was investigated in two surface waters (Calwich Abbey + River Alte Leine) at four dicamba application rates (1, 10, 95 and 100 µg/L) following incubation at 20°C under dark conditions for up to 90 days. For non-sterile samples, the degradation rate ($DegT_{50}$) of dicamba was 532 and 1280 days when dosed at 10 and 95 µg/L, respectively ($DegT_{50}$ degradation rates were extrapolated beyond the study duration (59 days)). The metabolite DCSA was identified, reaching maximum values of 0.1% and 0.2% at the 10 µg/L and at the 95 µg/L rate respectively. The total carbon dioxide evolved was 2.6% and 2.1% of applied radioactivity for the 10 and 90 µg/L rates respectively.
For sterile samples, the mean level of parent dicamba at the end of the study was 97.7% AR at 95 µg/L. Metabolite DCSA was not detected in sterile samples.

$DegT_{50}$ values for dicamba in surface water

System	Test concentration (µg/L)	SFO				
		$DegT_{50}$ (days)	**k**	**Chi^2**	**R^2**	**Prob > t**
Calwich Abbey, natural water	10	532	0.0013	1.81	0.4858	0.0031
	95	1280	5.4×10^{-4}	1.01	0.3778	0.0099
River Alte Leine, natural water	1	59.3	0.01168			
	10	-	-	-	-	-

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

No information.

2.8.2.1.5 Inherent and enhanced ready biodegradability tests

No information.

2.8.2.1.6 Soil and sediment degradation data

Water-sediment studies are presented in Volume 3 CA B.8 (B.8.2.2.2).

A water-sediment study was available from the previous EU review. The study was still considered acceptable. A new kinetic evaluation of the study was submitted by the notifier Syngenta.

Test guideline: Dutch Guideline for Registration of Pesticides, Section G 2.1: Details on the Nature of Conversion Products and the Rate at which they are formed.

In the study the route and rate of degradation of radio-labelled dicamba was investigated in two aquatic systems under aerobic conditions. The systems used consisted of natural waters (Rhine-river and pond) and 10% of the corresponding sediment. ^{14}C-labelled dicamba was applied to the systems resulting in an initial concentration of 1.0 mg/L.

In the kinetic re-evaluation the following results were found :

Summary of persistence endpoints

Chemical	Level / compartment	Derivation of value [number of values]	*$DegT_{50}$ / DT_{50} [days]
Dicamba	Level P-I whole system degradation	Geometric mean (2 values)	52.1
		Highest value (2 values)	53.5
	Level P-I water column dissipation	Geometric mean (2 values)	50.9
		Highest value (2 values)	51.7
DCSA	Level M-I whole system degradation	Geometric mean (2 values)	52.3
		Highest value (2 values)	56.8

*Normalised to 20ºC

Summary of modelling endpoints

Chemical	Level / compartment	Derivation of value [number of values]	*$DegT_{50}$ / DT_{50} [days]
Dicamba	Level P-I whole system degradation	Geometric mean (2 values)	38.1
		Highest value (2 values)	53.5
	Level P-I water column dissipation	Geometric mean (2 values)	37.3
		Highest value (2 values)	51.7
DCSA	Level M-I whole system degradation	Geometric mean (2 values)	52.3
		Highest value (2 values)	56.8

*Normalised to 20ºC

2.8.2.1.7 Hydrolysis

Data on hydrolysis is presented in Volume 3 CA B.8 (B.8.2.1.1).

Two studies were available from the previous EU review. The studies were still considered acceptable.
Two new studies submitted by Rotam supported the results of the older studies.

Guidelines:

Studies from the previous EU review:

OECD Guideline for Testing Chemicals, Hydrolysis as a Function of pH, 111 (1981)

US EPA Pesticide Assessment Guidelines, Subdivision N, Series No. 161-1

New studies :
OECD Guideline for Testing Chemicals, Hydrolysis as a Function of pH, 111 (2004)Dicamba and its major metabolite DCSA (NOA414746) were demonstrated to be stable. No significant hydrolysis occurred in sterile buffer solutions of pH 4, 5, 7 and 9 at 50°C in the dark for 6 to 14 days. It is concluded that the hydrolytic half-lives of both compounds at ambient temperature are >1 year.

2.8.2.1.8 Photochemical degradation

Data on photochemical degradation in water is presented in Volume 3 CA B.8 (B.8.2.1.2).

Two studies were available from the previous EU review. The studies were still considered acceptable.
A new study was also submitted by the notifier Rotam.

Guidelines:

Previously evaluated study:
US EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Series No. 161-2.

Previously evaluated study:

OECD Guidelines for Testing of Chemicals; Proposal for a New Guideline Phototransformation of Chemicals in Water – Direct and Indirect Photolysis, Draft Document (2000).

OECD Environmental Health and Safety Publications, Series on Testing and Assessment, No. 7: Guidance Document on Direct Phototransformation Chemicals in Water (1997).

OPPTS 835.2210, 'Direct Photolysis Rate in Water by Sunlight'; Fate, transport and Transformation Test Guidelines, EPA (1998).

OECD 101 : UV-VIS Absorption Spectra (1996).

New study
OECD 316 : Phototransformation of Chemicals in Water – Direct Photolysis (2008).

An aqueous photochemical DT_{50} of 17.0 - 50.3 days at 40°N in spring time and 9.44 days at 30°N in summer time was determined for dicamba.

The quantum yield of direct phototransformation in water was found to be $\Phi = 0.46 - 0.047$.

2.8.2.1.9 Other / Weight of evidence

No information

2.8.3 Summary of fate and behaviour in air

2.8.3.1 Hazardous to the ozone layer

Table 67: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
Atmospheric Oxidation Programme (AOP, ver 1.53 and 1.85) and the Atkinson model	Assuming a constant concentration of $1.5 \times 10^6 \times cm^{-3}$ OH-radical and a 12-hour day, the total rate constant was estimated to range between $2.62 \times 10^{-12} \times cm^3\ sec^{-1} \times mol^{-1}$ and $2.985 \times 10^{-12} \times cm^3\ sec^{-1} \times mol^{-1}$. Thus, the half-life period is calculated to be between 3.6 days and 4.1 days.	Acceptable	Stamm (1998) and Müller (1994)

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

For dicamba an atmospheric DT_{50} of 3.6 – 4.1 days was derived using the Atmospheric Oxidation Programme (AOP, ver 1.53 and 1.85) and the Atkinson model. The atmospheric DT_{50} exceeds the 2 day trigger for long-range transport. However, as dicamba is easily soluble in water rainfall is expected to remove dicamba from the air to a large extent. Furthermore, the volatilization from plant and soil surfaces is negligible (0.12% and 0.07 – 1.15%, respectively). Therefore dicamba is not considered hazardous to the ozone layer.

2.8.3.1.2 Comparison with the CLP criteria

There is no available evidence concerning the properties of dicamba and its predicted or observed environmental fate and behaviour indicating that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.
Dicamba is not listed in Annex I to Regulation (EC) No 1005/2009.
Dicamba should not be classified as hazardous to the ozone layer.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

No classification.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No data submitted

2.8.5 Definition of the residues in the environment requiring further assessment

Compartment	Residues requiring further assessment
Soil	Dicamba and DCSA
Surface water	Dicamba and DCSA
Sediment	Dicamba and DCSA
Ground water	Dicamba and DCSA
Air	Dicamba

2.8.6 Summary of exposure calculations and product assessment

PEC calculations were performed for the two representative formulations:

- **A7254B (Dicamba 480 g/L SL)**

  Summary of worst case intended uses of A7254B

Crop	Application rate (g a.s./ha)	Application method	Number of applications	Minimum application interval (days)	Application timing
*Maize	288	Foliar	1	-	BBCH 12-19
Spring Cereals	120	Foliar	1	-	BBCH 10-32

*Maize used as surrogate crop for sorghum in Focus models

- **OCEAL (FH-048)**

  Summary of intended uses of OCEAL

Crop	Application rate (g a.s./ha)	Application method	Number of applications	Minimum application interval (days)	Application timing
Maize	280	Foliar	1	-	BBCH 10-16

PEC soil

Calculation for A7254B (Dicamba 480 g/L SL)

The calculation for A7254B was based on the critical GAP use of one application of 0.288 kg a.s./ha in maize at BBCH 12 (25% interception).

PEC_{soil} of Dicamba immediately after application was calculated using FOCUS guidance[17] (i.e. current guidance) with the following equation:

$$PEC\ (mg/kg) = \frac{A[g/ha] \times (1 - F)}{100 \times d\ [cm] \times \rho\ [g/cm^3]}$$

Where:
A = Application rate
F = Fraction intercepted by crop
d = Depth of field soil layer (5 cm)
ρ = Dry bulk density (1.5 g/cm^3)

PEC_{soil} of the metabolite DCSA was calculated based on the PEC_{soil} calculated for Dicamba:

$PEC_{metabolite}$ [mg/kg soil] = $PEC_{max,parent}$ x (maximum % metabolite formation/100) x molecular weight ratio

Where:
The molar correction factor for DCSA is 0.937
The maximum occurrence of DCSA in soil is 58.8%

The following initial PEC_{soil} values were calculated:

PECS Dicamba (mg/kg)	PECS DCSA (mg/kg)
0.288	0.159

Calculation for OCEAL (FH-048)

The calculation for OCEAL was based on the GAP use of one application of 0.280 kg a.s./ha in maize at BBCH 10 (25% interception).

PEC_{soil} of Dicamba immediately after the first application was calculated using FOCUS guidance[18] (i.e. current guidance) with the following equation:

$$PEC\ (mg/kg) = \frac{A[g/ha] \times (1 - F)}{100 \times d\ [cm] \times \rho\ [g/cm^3]}$$

[17] FOCUS (1997) Soil persistence models and EU Registration - The Final Report of the Soil Modelling Workgroup of FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use) – 29 February 1997.
[18] FOCUS (1997) Soil persistence models and EU Registration - The Final Report of the Soil Modelling Workgroup of FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use) – 29 February 1997.

Where:
A = Application rate
F = Fraction intercepted by crop
d = Depth of field soil layer (5 cm)
ρ = Dry bulk density (1.5 g/cm^3)

PEC_{soil} of the metabolite DCSA was calculated based on the PEC_{soil} calculated for Dicamba:

$PEC_{metabolite}$ [mg/kg soil] = $PEC_{max,parent}$ x (maximum % metabolite formation/100) x molecular weight ratio

Where:
The molar correction factor for DCSA is 0.937
The maximum occurrence of DCSA in soil was assumed to be 75% (conservative compared to the maximum occurrence of 58.8% observed in studies)

Using the following equations, the instantaneous PECsoil at various time-points was calculated for both Dicamba and DCSA:

$$C = C_0 e^{-kt}$$

A time-weighted average PECs was calculated using the following equation:

$$C = \frac{1 - e^{-kt}}{kt} \times C_0$$

Where: C_0 = PECs initial
C = PECs at time t
k = ln 2/DT_{50}

The following PEC_{soil} values were calculated:

Time after application (days)		**Dicamba Actual (mg/kg)**	**Dicamba Time Weighted Average (mg/kg)**	**DCSA Actual (mg/kg)**	**DCSA Time weighted average**
Initial	**0**	0.280	-	0.197	-
Short term	**1**	0.272	0.276	0.186	0.191
	2	0.265	0.272	0.175	0.186
	4	0.250	0.265	0.156	0.176
Long term	**7**	0.230	0.254	0.132	0.162
	14	0.189	0.231	0.088	0.135
	21	0.155	0.211	0.0591	0.114
	28	0.127	0.194	0.0396	0.098
	50	0.0684	0.150	0.0112	0.0648
	100	0.0167	0.0934	0.000640	0.0342

PEC groundwater

Modelling for A7254B (Dicamba 480 g/L SL)

The potential for dicamba and its metabolite DCSA to reach groundwater was examined using the simulation models FOCUS PEARL (v4.4.4), FOCUS PELMO (v5.5.3) and MACRO (v5.5.4)

The risk envelope use patterns used in the modelling were:
Maize: 288 g a.s/ha at BBCH 12 (25% interception)
Spring cereals: 120 g a.s./ha, at BBCH 10 (0% interception)

The 80th percentile annual average PECgw of dicamba and DCSA at 1 m depth were < 0.1 µg/L for all models and all relevant FOCUS groundwater scenarios.

		PEARL 4.4.4		**PELMO 5.5.3**		**MACRO 5.5.4**	
Maize	**Scenario**	**Parent (µg/L)**	**DCSA (µg/L)**	**Parent (µg/L)**	**DCSA (µg/L)**	**Parent (µg/L)**	**DCSA (µg/L)**
	Chateaudun	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Hamburg	<0.001	<0.001	<0.001	<0.001	-	-
	Kremsmunster	0.001	<0.001	<0.001	<0.001	-	-
	Okehampton	0.018	<0.001	0.016	<0.001	-	-
	Piacenza	<0.001	<0.001	<0.001	<0.001	-	-
	Porto	<0.001	<0.001	<0.001	<0.001	-	-
	Sevilla	<0.001	<0.001	<0.001	<0.001	-	-
	Thiva	<0.001	<0.001	<0.001	<0.001	-	-

		PEARL 4.4.4		**PELMO 5.5.3**		**MACRO 5.5.4**	
Spring cereals	**Scenario**	**Parent (µg/L)**	**DCSA (µg/L)**	**Parent (µg/L)**	**DCSA (µg/L)**	**Parent (µg/L)**	**DCSA (µg/L)**
	Chateaudun	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Hamburg	<0.001	<0.001	<0.001	<0.001	-	-
	Jokioinen	<0.001	<0.001	<0.001	<0.001	-	-
	Kremsmunster	<0.001	<0.001	<0.001	<0.001	-	-
	Okehampton	<0.001	<0.001	<0.001	<0.001	-	-
	Porto	<0.001	<0.001	<0.001	<0.001	-	-

Modelling for OCEAL (FH-048)

The potential for dicamba and its metabolite DCSA to reach groundwater was examined using the simulation models FOCUS PEARL (v4.4.4), FOCUS PELMO (v5.5.3)

The modelled use pattern was:
280 g a.s/ha in maize at BBCH 10-12 (25% interception)

The 80th percentile annual average PECgw of dicamba and DCSA at 1 m depth were < 0.1 µg/L for all models and all relevant FOCUS groundwater scenarios.

		PEARL 4.4.4		PELMO 5.5.3	
Maize	**Scenario**	**Parent (µg/L)**	**DCSA (µg/L)**	**Parent (µg/L)**	**DCSA (µg/L)**
	Chateaudun	0.0000	0.0000	0.000	0.000
	Hamburg	0.0023	0.0004	0.000	0.000
	Kremsmunster	0.0009	0.0000	0.001	0.000
	Okehampton	0.0222	0.0007	0.023	0.000
	Piacenza	0.0000	0.0000	0.000	0.000
	Porto	0.0000	0.0000	0.000	0.000
	Sevilla	0.0000	0.0000	0.000	0.000
	Thiva	0.0000	0.0000	0.000	0.000

PEC surface water and sediment

Modelling for A7254B (Dicamba 480 g/L SL)

PEC_{SW} and PEC_{SED} were predicted using the FOCUS STEPS 1-2 model.

The following application patterns were used in the modelling:
Maize: 288 g a.s/ha at BBCH 12 (minimal interception)
Maize: 210 g a.s/ha at BBCH 12 (minimal interception)
Spring cereals: 120 g a.s./ha, at BBCH 10 (minimal interception)
Spring cereals: 96 g a.s./ha, at BBCH 21 (intermediate interception)

At STEP 2 the following maximum values were found:
Dicamba: PEC_{SW} = 30.56 µg/L PEC_{SED} = 2.69 µg/kg (228 g a.s./ha in maize at BBCH 12)
DCSA: PEC_{SW} = 11.66 µg/L PEC_{SED} = 90.15 µg/kg (228 g a.s./ha in maize at BBCH 12)

Modelling for OCEAL (FH-048)

PEC_{SW} and PEC_{SED} were predicted using the FOCUS STEPS 1-2 model.

The following application pattern was used in the modelling:
Maize: 280 g a.s/ha until BBCH 16 (no interception)

At STEP 2 the following maximum values were found:
Dicamba: PEC_{SW} = 31.6 µg/L PEC_{SED} = 2.01 µg/kg
DCSA: PEC_{SW} = 12.5 µg/L PEC_{SED} = 80.5 µg/kg

PEC air

Dicamba:
Vapour pressure: $1.67 \cdot 10^{-3}$ Pa (25°C)
Volatilisation from plant surfaces: 0.12 % of AR
Volatilisation from soil surfaces: 1.15 % of AR
DT_{50} in air (AOP): 3.58 days (12-hour day, $1.5 10^{6}$ OH cm^{-3})

DT_{50} in air (Atkins calculation): 4.1 days (12-hour day, $1.5 10^6$ OH cm^{-3})

The potential for long range transport of dicamba through the atmosphere is assessed from a consideration of (a) the potential for volatilisation; (b) atmospheric half-life under real-world conditions; (c) fate and potential impact after deposition. It is concluded that (a) volatilisation is negligible; (b) real-world half-life is shorter than the AOP modelled DT_{50} of 3.6 d due to "raining out" from the atmosphere; (c) dicamba is not persistent in soil or water and does not bioaccumulate. It is therefore considered that long-range transport of dicamba is not a critical issue and no further information is required.

Other routes of exposure

Other routes of exposure such as deposition of dust by drift during sowing, indirect exposure of surface water via sewage treatment plant after treatments in storage rooms and amenity use are not expected as the GAP uses for which authorisation is sought are restricted to spray applications in the field.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

2.9.1.1 Birds

Avian toxicity studies have been carried out with technical dicamba and no studies with the representative formulations are available. The endpoints were originally reported as technical a.s. and have been corrected for purity; Table 68.

Table 68: Summary of toxicity of dicamba to birds

Test type (time scale)	**Species**	**Test substance**	**Batch no.; purity**	**Endpoint**	**Toxicity** [a]	**Reference**
Acute oral	Bobwhite quail (*Colinus virginianus*)	Dicamba tech.	52103810 86.93 %	LD_{50}	188 mg a.s. /kg bw	Campbell *et al.*, 1993
	Zebra finch (*Taeniopygia guttata*)	Dicamba tech.	0002B01BA-251 93.9 %	LD_{50}	200 mg a.s. /kg bw	Hubbard and Beavers, 2011
				LD_{50} geometric mean	**194 mg a.s. /kg bw**	
Short-term dietary	Mallard duck *(Anas platyrhynchos)*	Dicamba tech.	52625110 86.8 %	LD_{50} (dietary)	> 1360 mg a.s./kg bw/d	Fink, 1977a
	Bobwhite quail (*Colinus virginianus*)	Dicamba tech.	52625110 86.8 %	LD_{50} (dietary)	>864 mg a.s./kg bw/d	Fink, 1977b
Long-term/ reproductive	Mallard duck *(Anas platyrhynchos)*	Dicamba tech.	52103810 86.9 %	NOEL	77 mg a.s. /kg bw/d	Beavers *et al.*, 1994a
	Bobwhite quail (*Colinus virginianus*)	Dicamba tech.	52103810 86.9 %	NOEL	148 mg a.s. /kg bw/d	Beavers *et al.*, 1994b
				LD_{50}/10 of the geometric mean acute endpoint	**19.4 mg a.s. /kg bw/d**	

[a] All endpoints are corrected for purity of the technical a.s.
Values in **bold** are considered relevant for use in risk assessment.

Metabolite 5-OH dicamba (NOA405873) is a major foliar metabolite, present at >10% of applied parent substance. As acute oral toxicity studies with rats and available genotoxicity studies with parent and 5-OH dicamba indicate that the metabolite is not of higher toxicity than the parent compound, it can be concluded that the risk to birds from this metabolite will be covered by the risk assessment for dicamba. Thus no further testing has been conducted.

2.9.1.2 Mammals

Studies have been carried out with technical dicamba, its major foliar metabolite 5-OH dicamba (NOA405873) and the two representative formulations. The endpoints from the a.s. studies were originally reported as technical dicamba and have been corrected for purity; Table 69.

Table 69: Summary of toxicity of dicamba and relevant metabolites to mammals

Test type (time scale)	Species	Test substance	Batch no.; purity	Endpoint	Toxicity [a]	Reference
Acute oral	Rat	Dicamba tech.	Not reported; 85.8 % presumed	LD_{50}, females LD_{50}, males **LD_{50}, sexes combined** LD_{50}, geom. mean	1356 1612 **1465 mg a.s./ kg bw** 1478 mg a.s./ kg bw	Wazeter & Goldenthal, 1974
	Rat	A7254B	PR910061 484 g a.s./L	LD_{50}, females LD_{50}, males LD_{50}, sexes combined **LD_{50}, geom. mean**	2558 (1058) 2375 (982) 2467 (1021) **2465 mg product/kg bw (1020 mg a.s. /kg bw)**	Sommer, 2001a
	Rat	Dicamba 700SG	176-031 703.8 g a.s./kg	LD_{50}, females[b]	> 2000 mg product/ kg bw (> 1408 mg a.s./kg bw)	Ilamurugan, 2010a
	Rat	5-OH dicamba (NOA 405873)	(KI 6212/1-18 94 ± 2 %)	LD_{50}, both sexes	> 2000 mg/kg bw	Sommer, 2001b
Reproductive	Rabbit [c]	Dicamba tech.	52625110 90.4 %	**NOAEL**	**150 mg a.s./ kg bw/d** [c]	Hoberman, 1992

[a] All a.s. endpoints are corrected for purity of the technical a.s.

[b] Only females tested.

[c] Agreed reproductive endpoint following an expert meeting in the previous evaluation (revised DAR 2010).

Values in **bold** are considered relevant for use in risk assessment.

In cases where separate acute endpoints for males and females are available, the Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2009) proposes that the geometric mean LD_{50} is used unless there is a clear indication of a difference in sensitivity between the sexes (i.e. if the difference in LD_{50} values is > 25 %). For technical dicamba and the representative formulation A7254B the difference is < 25 %, indicating no difference in sensitivity between sexes. Combined LD_{50} values are available from the study reports; RMS proposes that the smaller of these values and the geomeans are used.

The reproductive endpoint was agreed upon in the previous evaluation as a compromise between effects observed at 350 mg/kg bw/d in a 2-generation study in rats and the foetal NOEL of 150 mg/kg bw/d from a teratology study in rabbits. The endpoint has been corrected for purity of the technical a.s. used in the teratology study.

The acute oral toxicity study with the foliar metabolite 5-OH-dicamba indicates that the metabolite is not of higher toxicity than the parent compound.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

Studies have been carried out with technical dicamba, its major metabolite DCSA (NOA414746) and the two representative formulations. The endpoints from some of the a.s. studies were originally reported as technical dicamba and have been corrected for purity; Table 70.

Table 70: Summary of toxicity of dicamba and relevant metabolites to aquatic organisms

Test type (time scale)	**Species**	**Test substance**	**Batch no.; purity**	**Endpoint**	**Toxicity**	**Reference**
96 hours, acute (static)	Common carp (*Cyprinus carpio*)	Dicamba tech.	P.MG2726410 89.8%	96-h LC_{50}	> 100 mg a.s./L (nom)	Volz, 2003a
96 hours, acute (static)	Zebra fish (*Danio rerio)*	Dicamba tech.	RTM/DCMB/03/20090612 988.5g/kg	96h LC_{50}	> 98.85 mg a.s./L (nom)	Gilberg D. and Seck C., 2010a
96 hours, acute (static)	Rainbow trout (*Oncorhynchus mykiss*)	Banvel 480 SL (A7254B)	PFB3HI19 484 g a.s./L	96-h LC_{50}	**> 41.0 mg a.s./L (nom) (equivalent to > 100 mg A7254B/L)**	Bätscher, 2005a
96 hours, acute (static)	Rainbow trout (*Oncorhynchus mykiss*)	Dicamba 700 SG	175-024 72.1 % w/w	96 h LC_{50}	> 100 mg a.s./L (nom)	Gilberg D. and Seck C., 2010b
96 hours, acute (semi-static)	Rainbow trout (*Oncorhynchus mykiss*)	DCSA (NOA414746)	012793 99.51 %	96-h LC_{50}	**> 100 mg/L (nom)**	Macdonald et al., 1993
21 days, chronic (semi-static)	Rainbow trout (*Oncorhynchus mykiss*)	Dicamba tech.	52625110 86.8%	21-d NOEC	180 mg a.s./L (nom)	Scheerbaum, 1990
25 days, chronic (flow-through)	Fathead minnow (*Pimephales promelas)*	Dicamba tech.	COD-001266 92.9%	33-d NOEC	10 mg a.s./L (nom)	Salinas, 2011

Test type (time scale)	**Species**	**Test sub-stance**	**Batch no.; purity**	**Endpoint**	**Toxicity**	**Reference**
34 days, chronic (Flow-through)	Sheepshead minnow (*Cyprinodon variegatus*)	Dicamba tech.	002B01BA-251 93.9%	34-d NOEC	**11 mg a.s./L (mm)**	Minderhout et al , 2012
48 hours, acute (static)	*Daphnia magna*	Banvel 480 SL (A7254B)	PFB3HI19 484 g a.s./L	48-h EC_{50}	**> 41.0 mg a.s./L (equivalent to > 100 mg A7254B/L) (nom)**	Bätscher, 2005b
48 hours, acute (static)	*D. magna*	Dicamba 700SG	175-024 72.1 % w/w	48 h EC_{50}	131.6 mg a.s./L (nom)	Egeler P., Goth M. and Seck C., 2010
48 hours, acute (static)	*Daphnia magna*	DCSA (NOA414746)	012793 99.51 %	48-h EC_{50}	**89 mg/L (mm)**	Douglas et al., 1993a
21 days, chronic (semi-static)	*Daphnia magna*	Dicamba tech.	52204112 88.6%	21-d NOEC	97 mg a.s./L (mm)	Douglas, 1993
35 days, chronic (flow-through)	*Mysid shrimp*	Dicamba tech.	002B01BA-251 93.9%	35-d NOEC	**5.8 mg a.s./L (mm)**	Claude et al., 2012
96 hours, chronic (static)	*Pseudokirch-neriella sub-capitata*	Dicamba tech.	P.MG2726410 90.1%	72-h E_r, E_y and E_bC_{50}	> 87 mg a.s./L (mm)	Eckenstein, 2015
120 hours, chronic (static)	*Anabaena flos-aquae*	Dicamba tech.	P.MG2726410 89.9%	72-h E_bC_{50} 72-h E_rC_{50}	> 32 mg a.s./L (nom) > 32 mg a.s./L (nom)	Smyth et al., 1998
120 hours, chronic (static)	*Navicula pel-liculosa*	Dicamba tech.	52204112 89.5%	72-h E_bC_{50} 72-h E_rC_{50}	> 3.8 mg a.s./L (mm) **> 3.8 mg a.s./L (mm)**	Hoberg, 1992b
120 hours, chronic (static)	*Skeletonema costatum* (marine organ-ism)	Dicamba tech.	52204112 89.5%	72-h E_bC_{50} 72-h E_rC_{50}	1.8 mg a.s./L (mm) > 4.1 mg a.s./L (mm)	Hoberg 1993
96 hours, chronic (static)	*Pseudokirch-neriella sub-capitata*	DCSA (NOA414746)	MLA-21/2 99 % w/w, ± 2 %	72-h E_rC_{50} 72-h E_yC_{50} 72-h E_bC_{50}	**67 mg/L** (mm) 45 mg/L (mm) 46 mg/L (mm)	Eckenstein, 2015a
72 hours, chronic (static)	*Pseudokirch-neriella sub-capitata*, (for-merly *Selenas-trum capricor-nutum)*	Banvel 480 SL (A7254B)	PR910061 484 g a.s./L	72-h E_rC_{50}	**> 42.4 mg a.s./L (mm) (equivalent to > 103 mg A7254B/L)**	Peither, 2001
72 hours, chronic (static)	*P. subcapitata*	Dicamba 700SG	175-024 72.1 % w/w	72 h E_bC_{50} 72 h E_rC_{50}	> 103.8 mg a.s./L (nom) > 103.8 mg a.s./L (nom)	Richter E. and Seck C., 2010

Test type (time scale)	Species	Test sub-stance	Batch no.; purity	Endpoint	Toxicity	Reference
72 hours, chronic (static)	*P. subcapitata*	Dicamba 700SG	20150112002 692 g a.s./kg	72 h E_bC_{50} 72 h E_rC_{50}	> 69.2 mg a.s./L (nom) > 69.2 mg a.s./L (nom)	Kosak, L., Emnet, A, 2016
14 days, chronic (static)	*Myriophyllum spicatum*	Dicamba tech.	P.MG2726410 90.1%	14-d E_yC_{50} 14-d E_rC_{50} 14-d E_yC_{50} 14-d E_rC_{50} 14-d E_yC_{50} 14-d E_rC_{50}	Shoot length 0.58 mg a.s./L **0.94 mg a.s./L (im)** Wet weight 0.97 mg a.s./L 2.1 mg a.s./L (im) Dry weight 6.4 mg a.s./L >9 mg a.s./L (im)	Kirkwood, 2015
14 days, chronic (static)	*Lemna gibba*	Dicamba tech.	52204112 89.5%	14-d E_rC_{50}	> 3.2 mg a.s./L (mm)	Hoberg 1992c
7 days, chronic (static)	*Lemna gibba*	DCSA (NOA414746)	MLA-21/1 99%	7-d E_rC_{50}	**> 65.8 mg/L (mm)**	Grade, 2002
14 days, chronic (static)	*Myriophyllum verticillatum*	Banvel 480 SL (A7254B)	PB008205 490 g a.s./L	14-d E_rC_{50}	Biomass **3.7 mg a.s./L (nom) (equivalent to 8.9 mg A7254B/L)**	Volz, 2003c
14 days, chronic (static)	*Myriophyllum spicatum*	Dicamba 700SG	175-024 72.1 % w/w	14-d E_yC_{50} 14-d E_rC_{50} 14-d E_yC_{50} 14-d E_rC_{50} 14-d E_yC_{50} 14-d E_rC_{50}	Shoot length 4.88 mg a.s./L (nom) 5.17 mg a.s./L (nom) Dry weight: 1.86 mg a.s./L (nom) 3.26 mg a.s./L (nom) Wet weight: 3.15 mg a.s./L (nom) 4.00 mg a.s./L (nom)	Gilberg D. and Seck C., 2010c

Values in **bold** are considered relevant for use in risk assessment.

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

2.9.2.1.1 Estimated bioaccumulation

The experimentally derived Log Kow of dicamba is -0.55 at pH 5.0, -1.8 at pH 6.8 and -1.9 at pH 8.9. As such dicamba is not expected to bioaccumulate in aquatic organisms. For classification and labelling purposes a substance with Log Kow <4 may be considered unlikely to bioaccumulate in aquatic organisms.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

For dicamba and its main metabolite DCSA (NOA414746; surface water and soil) the log P_{ow} values are -1.8 (at pH 6.8) and -0.84 (at pH 6.8) respectively, therefore there are no existing EU endpoints and none are required. No further study is required for this point.
Overall, dicamba is not expected to bioaccumulate in aquatic organisms.

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
OECD 203: (1992) JMAFF 2-7-1, 2001 92/69/EEC, O.J. L383A, Part C.1: (1992)	*Cyprinus carpio* (Common carp)	Dicamba technical (89.8%)	96-h LC_{50} > 100 mg a.s./L (nom)	Key study	Static GLP	Volz (2003) SAN837/6142
OECD 204 (1984)	*Oncorhynchus mykiss* (Rainbow trout)	Dicamba technical (86.6%)	96-h LC_{50} = 177 mg a.s./L (nom)	Key study	Static GLP	Caley *et al.*, 1989 SAN837/5030
OECD 203 (1992)	*Danio rerio* (Zebrafish)	Dicamba technical (988.50 g/kg)	LC_{50} (96 h) > 98.85 mg a.s./L (nom)	Key study	Static GLP	Daniel Gilberg, Corinne Seck, 2010 10AV4FA

2.9.2.2.1 Acute (short-term) toxicity to fish

Three studies are available on the acute toxicity of dicamba to fish. All the studies on dicamba technical demonstrate low short-term (96 hour) toxicity to common carp (*Cyprinus carpio*) (LC_{50} > 100 mg a.s./L), rainbow trout (*Oncorhynchus mykiss*; formerly *Salmo gairdneri*) (LC_{50} 177 mg a.s./L) and zebra fish (*Danio rerio*) (LC_{50} > 98.85 mg a.s./L.

Study 1: Volz (2003; SAN837/6142)

In a 96 hour static toxicity study of SAN837 (purity 89.8%) to common carp (*Cyprinus carpio*), seven fish were exposed to a single nominal test concentration of 100 mg a.s./L and a dilution water control. Specific analysis showed measured test concentrations in the treatment tank to be 111% and 112% of nominal at the start and end of the test, respectively. Measurements of dissolved oxygen, pH and temperature were consistent throughout the term of the experiment. In the control and at the nominal test concentration of 100 mg a.s./L no mortality or other visible abnormalities were determined during the test period of 96 hours. Therefore, the 96 hour NOEC and LC_{50} were determined to be 100 mg a.s./L and >100 mg a.s./L, respectively, based on the nominal test concentration.

Effects of dicamba on the survival of common carp

Nominal concentration (mg a.s./L)	Mortality observed (cumulative number of dead fish) (n = 7)				
	3 hour	24 hours	48 hours	72 hours	96 hours

Dilution water control	0	0	0	0	0
100	0	0	0	0	0

n.d. = not determined

Study 2: Caley, Cameron and Chapleo (1989; SAN837/5330)
In a 96 hour static toxicity test of SAN837 (purity 86.6%) to rainbow trout (*Oncorhynchus mykiss*; formerly *Salmo gairdneri*), %), run alongside a prolonged toxicity test, ten fish were exposed to nominal test concentrations of 62.5, 125, 250, 500 and 1000 mg a.s./L (reported as ppm) and a dilution water control. Analysis showed measured test concentrations in the treatment tank of 62 – 119% of nominal. Apart from mortality no unusual swimming behaviour was observed. Based on nominal concentrations the 96-hour LC_{50} was determined to be 177 mg a.s./L.

Effects of dicamba on the survival of *Salmo gairdneri* (96-hour, static)

Time (h)	Cumulative % mortality observed					
	0 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm	1000 ppm
0	0	0	0	0	0	3
3	0	0	0	0	10	10
6	0	0	0	0	10	10
24	0	0	0	10	10	10
48	0	0	0	10	10	10
72	0	0	0	10	10	10
96	0	0	0	10	10	10

Study 3: Gilberg and Seck (2010; 10AV4FA)
In a 96 hour static toxicity test of dicamba technical (purity 988.50 g/kg) to zebra fish (*Danio rerio*), seven fish were exposed to a nominal test concentration of 100 mg a.s./L and a dilution water control. Analysis showed measured test concentrations in the treatment tank of 62 – 119% of nominal. No mortality and no abnormal behaviour of fish was observed in the 100 mg/L test item concentration during the test period. The LC_{50} was determined to be > 98.85 mg dicamba/L (corrected for purity).

Cumulative survival and mortality of the fish exposed to dicamba

Time (h)	Treatment (mg a.s./L)			
	Treatment (100 mg/L)		Control	
	Survival	Mortality (%)	Survival	Mortality (%)
0	7	0	7	0
3	7	0	7	0
24	7	0	7	0
48	7	0	7	0
72	7	0	7	0
96	7	0	7	0

Summary of acute toxicity to fish
The results of the above studies indicate that dicamba exhibits low acute toxicity to fish. The lowest LC_{50} for dicamba technical was 98.85 mg a.s./L.
Overall, the available data indicates low short term toxicity to fish. For classification purposes a LC_{50} = 98.85 mg a.s./L is used.

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates
No valid data submitted.

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 data submitted.

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	Relevant study*	Remarks	Reference
OECD 204 (1984)	*Oncorhynchus mykiss* (Rainbow trout)	Dicamba (86.8%)	21 day LOEC (mortality) > 1000 mg/L (nom) 21 day NOEC (behaviour) = 180 mg/L (nom)	Supportive	Semi-static GLP	Scheerbaum (1990) SAN837/5331
OECD 210	*Pimephales promelas* (Fathead minnow)	Dicamba (92.9%)	33 day ELS NOEC = 10 mg/L (nom) 33 day ELS LOEC (all endpoints) > 10 mg/L (nom)		Flow-through GLP	Salinas (2011) SAN837_11528
OPPTS 850.1400 Public Draft, (April 1996)	*Cyprinodon variegatus* (sheepshead minnow)	Dicamba (93.9%)	34 day ELS NOEC = 11 mg/L (mm) 34 day ELS LOEC (all endpoints) > 11 mg/L (mm)		Flow-through GLP	Minderhout *et al* (2012) SAN837_11529
OECD 202 Part II	*Daphnia magna*	Dicamba technical (88.6%)	21 day EC_{50} (all endpoints) > 97 mg/L (mm) 21 day NOEC (all endpoints) = 97 mg/L (mm)		Semi-static GLP	Douglas (1993) SAN837/5332
US EPA, OPPRS 850.1350 (1996), ASTM 1191-03a (2008)	*Americamysis bahia* (saltwater mysid)	Dicamba technical (93.9%)	35 day NOEC = 5.8 mg/L (mm) 35 day LOEC = 11.0 mg/L (mm)		Flow-through GLP	Claude *et al* (2012) SAN837_11530
OECD 201 (2006)	*Pseudokirchneriella subcapitata* (green alga)	Dicamba technical (90.1 %)	72 h E_rC_{50}, E_yC_{50} and E_bC_{50} > 87 mg/L (mm) 72-h NOEC (all endpoints) = 43 mg/L (mm) 96 h E_rC_{50} > 87 mg/L (mm)		Static GLP	Eckenstein (2015) SAN837_11464

			96-h E_yC_{50} = 85 mg/L (mm) 96-h E_bC_{50} = 87 mg/L (mm) 96-h NOEC (all endpoints) = 43 mg/L (mm)			
US-EPA FIFRA, J 123-2	*Anabaena flos-aquae* (blue-green alga)	Dicamba technical (89.9%)	72-h E_rC_{50} = 44.85 mg/L (nom) 72-h E_bC_{50} = 43.14 mg/L (nom) 96-h E_rC_{50} = 34.85 mg/L (nom) 96-h E_bC_{50} = 42.01 mg/L (nom) 120-h E_rC_{50} = 40.76 mg/L (nom) 120-h E_bC_{50} = 41.52 mg/L (nom) 96-h NOErC = 32 mg/L (nom)		Static GLP	Smyth *et al* (1998) SAN837/0411
US-EPA FIFRA, J 122-2 and 123-2	*Navicula pelliculosa* (freshwater diatom)	Dicamba technical (89.5%)	72-h ErC_{50} > 3.8 mg/L (mm) 96-h EC_{50} =5.1 mg/L (mm) 120-h EC_{50} = 2.3 mg/L (mm) 120-h NOEC = 0.5 mg/L (mm)		Static GLP	Hoberg (1992a) SAN837/5229
US-EPA FIFRA, J 122-2 and 123-2	*Skeletonema costatum* (marine diatom)	Dicamba technical (89.5%)	72-h ErC_{50} > 4.1 mg/L (mm) 96-h EC_{50} = 1.5 mg/L (mm) 120-h EC_{50} =0.58 mg/L (mm) 120-h NOEC = 0.001 mg/L (mm)	supportive	Static GLP	Hoberg (1993), SAN837/5224
OECD 239 (2014)	*Myriophyllum spicatum* (Eurasian watermilfoil)	Dicamba (90.1%)	14 day E_rC_{50} (shoot length) = 0.94 mg/L (mm) 14 day NOEC		Static GLP (results based on initial measured concentrations)	Kirkwood (2015) SAN837_11580

			(shoot length) = 0.27 mg/L (mm) 14 day LOEC (shoot length) = 0.86 mg/L (mm)			
US-EPA FIFRA, J 122-2 and 123-2	*Lemna gibba* (duckweed)	Dicamba technical (89.5%)	14 day EC_{50} > 3.2 mg a.s./L (mm) 14 day NOEC = 0.19 mg/L (mm)		Static GLP	Hoberg (1992b) SAN837/5223

2.9.2.3.1 Chronic toxicity to fish

Three long term studies on dicamba technical with supporting specific analysis show low long term toxicity to *Oncorhynchus mykiss* (Rainbow trout; formerly *Salmo gairdneri*) (Scheerbaum, 1990), *Pimephales promelas* (fathead minnow) (Salinas, 2011) and *Cyprinodon variegatus* (sheepshead minnow) (Minderhout *et al*, 2012).

Study 1: Scheerbaum (1990; SAN837/5331)

The study is considered acceptable however, long-term toxicity data from OECD TG 204 is not considered adequate under CLP and thus the study is not used for classification. However the data is presented as supportive data.

In a 21 day prolonged semi-static toxicity study of dicamba (purity 86.8%) to *Oncorhynchus mykiss* (rainbow trout), 10 fish were exposed per treatment to nominal test concentrations of 18, 32, 58, 100, 180, 320, 580 and 1000 mg a.s./L and a dilution water control. The mean measured concentrations were in the range 94 to 107% of nominal, adjusting for purity.

Mortality and symptoms of toxicity were recorded throughout the study. Measurements of dissolved oxygen, pH and temperature and salinity were also recorded and remained consistent throughout the study.

With the exception of one fish which died on Day 2 in the 580 mg a.s./L test concentration no mortality was observed in any of the test concentrations. Symptoms of toxicity, including calm behaviour, fish at the top or bottom of the water body, slow flight movement, and low acceptance of food, were observed at concentrations of 320 mg a.s./L and above. No mortality or symptoms of toxicity were observed in the control.

Based on nominal concentrations, the 21 day NOEC was 180 mg a.s./L, and the threshold level of lethal effect was > 1000 mg a.s./L, the highest concentration tested.

Effects of dicamba on the survival of *Salmo gairdneri*

Nominal concentration (mg a.s./L)	Cumulative % mortality observed			
	Day 0	Day 7	Day 14	Day 21
Control	0	0	0	0
18	0	0	0	0
32	0	0	0	0
58	0	0	0	0
100	0	0	0	0
180	0	0	0	0
320	0	0	0	0
580	0	10	10	10
1000	0	0	0	0

Study 2: Salinas (2011; SAN837_11528)

In a 33 day Fish Early Lifestage (OECD 210) flow-through toxicity study of dicamba acid (purity 92.9%) to *Pimephales promelas* (fathead minnow), fish were exposed to nominal test concentrations of 0.1, 0.32, 1.0, 3.2

and 10 mg a.s./L and a dilution water control. The mean measured concentrations were 0.10, 0.331, 1.03, 2.98 and 9.91 mg a.s./L.
Observations for time to hatch, hatching success, stage-specific and overall survival, overall growth and sub-lethal morphological and behavioural effects were made during the pre and post-hatch phases, as appropriate. Measurements of dissolved oxygen, pH and temperature and salinity were recorded and remained consistent throughout the study.
There were no statistically significant treatment related effects for hatching success, survival or growth. Based on nominal concentrations the NOEC was 10 mg a.s./L (the highest concentration tested) and the LOEC was > 10 mg a.s./L.

Effects of dicamba on the survival and growth of fathead minnows

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Quantal responses			Non quantal responses		
		Hatching	Larvae survival (%)	Juvenile survival (%)	Overall survival (%)	Mean length (cm)	Mean wet weight (mg)
Control	Control	98	99	100	97	2.8	229
0.10	0.10	98	97	98	93	2.8	226
0.32	0.331	98	99	100	97	2.9	232
1.0	1.03	97	99	100	96	2.8	232
3.2	2.98	98	100	99	97	2.9	237
10	9.91	98	99	100	97	2.9	231

Study 3: Minderhout *et al* (2012; SAN837_11529)
In a 34 day Fish Early Lifestage (OPPTS 850.1400) flow-through toxicity study of dicamba acid (purity 93.9%) to *Cyprinodon variegatus* (sheepshead minnow), fish were exposed to nominal test concentrations of 0.31, 0.77, 1.9, 4.8 and 12 mg a.s./L, a solvent control and a dilution water control. The mean measured concentrations were 0.28, 0.72, 1.8, 4.5 and 11 mg a.s./L, i.e. 97 to 99.6% of nominal, adjusting for purity.
Observations for time to hatch, hatching success, larval mortality, deformed larvae and other symptoms of toxicity were made daily, as appropriate. At the end of the test, lengths and wet and dry weights of the surviving larvae were measured. Measurements of dissolved oxygen, pH and temperature and salinity were recorded and remained consistent throughout the study.
There were no treatment-related effects on time to hatch, and no statistically significant treatment-related effects on hatching success, survival or growth. Therefore, based on mean measured concentrations, the overall NOEC was 11 mg a.s./L and the LOEC was > 11 mg a.s./L.

Effects of dicamba acid on the survival and growth of sheepshead minnows.

Mean measured concentration (mg a.s./L)	Quantal responses		Non quantal responses		
	Hatching success (%)	Larval survival (%)[1]	Mean length (mm) ± SD	Mean wet weight (mg) ± SD	Mean dry weight (mg) ± SD
Control	95	93	19.7 ± 0.22	95.7 ± 5.4	22.3 ± 1.1
Solvent control	96	99	19.6 ± 0.096	95.6 ± 1.5	21.9 ± 0.34
0.28	96	100	19.1 ± 0.26	86.9 ± 2.6	19.9 ± 0.83
0.72	96	100	19.3 ± 0.14	92.1 ± 3.9	21.3 ± 0.85
1.8	98	97	19.5 ± 0.13	95.6 ± 4.1	21.4 ± 0.80
4.5	93	95	18.7 ± 0.24	86.5 ± 5.4	19.5 ± 1.2
11	98	97	19.3 ± 0.22	97.1 ± 5.2	22.1 ± 0.86

No treatment-related statistically significant effects were observed
[1] The number of surviving larvae at the end of the test (day 32), expressed as a percentage of the number of eggs.

Summary of chronic toxicity to fish
The results of the three available chronic studies indicate that dicamba exhibits low chronic toxicity to fish. For the purpose of classification a NOEC of 10 mg a.s./L is used, based on the data for the fathead minnow.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

Two studies on dicamba technical with supporting specific analysis shows low long term toxicity to *Daphnia Magna* (Douglas, 1993) and *Americamysis bahia* (saltwater mysid) (Claude *et al*, 2012).

Study 1: Douglas (1993; SAN837/5332)

In a 21 day flow-through toxicity study of dicamba acid (purity 88.6%) to *Daphnia Magna*, groups of forty animals (10 x four replicates) were exposed to nominal test concentrations of 1.0, 3.2, 10, 32 and 100 mg a.s./L (mean measured concentrations 0.92, 3.2, 9.7, 32 and 97 mg a.s./L), plus a dilution water control. The temperature remained at a constant 21°C throughout the experiment. The measured pH ranged from 6.6-8.0 at initiation to 6.8-8.2 at termination and the measured O_2 concentration was 8.7 mg/L at initiation and between 8.1 and 8.3 mg/L at termination.

There were no significant effects on survival or reproduction at any of the test concentrations. The EC_{50} and NOEC for all biological endpoints were >97 mg a.s./L and 97 mg a.s./L, respectively.

Effects of dicamba on *Daphnia magna* survival and reproduction

Nominal concentrations (mg a.s./L)	Mean measured concentrations (mg a.s./L)	Mean adult survival (%)	Mean number of juveniles per surviving female at day 21
Control	-	88	43
1.0	0.92	85	42
3.2	3.2	88	40
10	9.7	88	44
32	32	80	36
100	97	83	43

Study 2: Claude *et al* (2012; SAN837_11530)

In a 35 day flow-through toxicity study of dicamba acid (purity 93.9%) to *Americamysis bahia* (saltwater mysid), animals were exposed to nominal test concentrations of 0.75, 1.5, 3.0, 6.0 and 12 mg a.s./L (mean measured concentration: 0.69, 1.4, 2.9, 5.8 and 11 mg a.s./L, adjusted for purity), plus a dilution water and solvent (DMF) control. At the start of the test 60 neonate mysids were exposed to each treatment (15 x 4 replicates). On day 14, five male/female pairs were assigned to reproductive compartments in each replicate test chamber, with one pair per compartment.

Specific analysis showed mean measured test concentrations to be 92 to 97% of nominal.

Measurements of dissolved oxygen, pH and temperature and salinity were recorded throughout the study. The measured temperature ranged from 24.4 – 26ºC, pH ranged from 7.8 – 8.1 and the measured O_2 concentration ranged from 5.7 – 7.4 mg/L (gentle aeration from day 15).

Survival of the parent animals was 82.5 % in the controls. The first brood juveniles were observed on day 16 in the controls and all test concentrations up to and including 11 mg a.s./L. Effects on survival, growth and reproduction are shown in the table below.

Effects of dicamba acid on mysid reproduction, growth and survival

Mean measured concentrations (mg a.s./L)	% survival		Young produced per reproductive day	Number of young per female[1]	Mean body length (mm)		Mean dry weight (mg)	
	Juveniles until pairing Day 14	Adults until test end Day 35	Mean	Mean	Males	Females	Males	Females
Control	88.3	82.5	0.283	6.0	7.90	8.31	1.07	1.26
Solvent control	90.0	82.5	0.710	13.3	7.97	8.41	0.97	1.38

Mean measured concentrations (mg a.s./L)	% survival		Young produced per reproductive day	Number of young per female[1]	Mean body length (mm)		Mean dry weight (mg)	
	Juveniles until pairing Day 14	Adults until test end Day 35	Mean	Mean	Males	Females	Males	Females
Pooled control	89.2	82.5	-	--	7.94	8.36	1.02	1.32
0.69	91.7	85.7	0.287	5.6	7.95	8.14	0.98	1.38
1.4	90.0	80.0	0.342	6.8	7.68	8.30	0.93	1.39
2.9	90.0	69.2	0.517	9.3	7.93	8.10#	0.98	1.15
5.8	90.0	77.5	0.283	5.7	7.86	8.06#	1.02	1.26
11	78.3*	77.3	0.176	3.4	7.74	8.11	1.04	1.41

* Statistically significant decrease in survival in comparison to the pooled control using Fisher's Exact test ($p \leq 0.05$)
# Statistically significant decrease in comparison to the pooled control using Dunnett's test ($p \leq 0.05$)
[1]Statistical analyses were not performed on this parameter

In summary, based on a statistically significant decrease in juvenile survival in the highest test concentration the NOEC was 5.8 mg a.s./L and the LOEC was 11 mg a.s./L.

Summary of chronic toxicity to aquatic invertebrates
Based on the data for *Americamysis bahia* the chronic NOEC for aquatic invertebrates of 5.8 mg a.s./L is taken for the purposes of classification.

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Four studies are available on the acute toxicity of dicamba to algae. In addition two 14 day studies with aquatic macrophytes have been performed using dicamba technical:
Study 1: Eckenstein (2015) - Dicamba Technical: Toxicity to *Pseudokirchneriella subcapitata.*
Study 2: Smyth *et al* (1998) - Dicamba Technical: Toxicity to the blue-green alga *Anabaena flos-aquae*.
Study 3: Hoberg (1992a) - Dicamba Technical: Toxicity to the freshwater diatom, *Navicula pelliculosa.*
Study 4: Hoberg (1993) - Dicamba Technical: Toxicity to the marine diatom, *Skeletonema costatum*.
Study 5: Kirkwood (2015) - Dicamba Technical: Toxicity to *Myriophyllum spicatum* (Eurasian watermilfoil).
Study 6: Hoberg (1992b) - Dicamba Technical: Toxicity to duckweed, *Lemna gibba.*

Study 1: Eckenstein (2015a; SAN837_11464)
The toxicity of technical dicamba (purity 90.1%) to green alga *Pseudokirchneriella subcapitata* was determined (Eckenstein, 2015). Algae were exposed for 120 hours to nominal concentrations 6.25, 12.5, 25, 50 and 100 mg a.s./L, alongside a culture medium control. At the start of the test, the analytically determined concentrations of dicamba were in the range 95 to 97% of the nominal values and at the end of the test were in the range 96 to 98% of nominal values. Mean measured concentrations were 5.5, 11, 22, 43 and 87 mg a.s./L.
Based on mean measured concentrations, the 72-hour E_rC_{50}, E_yC_{50} and E_bC_{50} were > 87 mg a.s./L and the NOEC for all endpoints was 43 mg a.s./L.
The 96-hour E_rC_{50}, E_yC_{50} and E_bC_{50} were > 87 mg a.s./L, 85 mg a.s./L and 87 mg a.s./L. The 96-hour NOEC for all endpoints was 43 mg a.s./L.

Measured parameters over 72 hours

	Growth rate		Yield		Biomass	
Mean measured concentration (mg a.s./L)	Mean (1/day)	% inhibition	Mean (x 10^3 cells/mL)	% inhibition	Mean integral (10^3 * day)	% inhibition

Control	1.640	0.0	89.0	0.0	60.5	0.0
5.5	1.670	-1.8	97.3	-9.3	65.9	-8.8
11	1.663	-1.4	95.6	-7.4	64.7	-7.0
22	1.682	-2.6	100.9	-13.5	68.6	-13.3
43	1.631	0.6	86.4	2.9	59.3	2.0
87	1.423*	13.2	46.2*	48.1	36.6*	39.5

* mean value statistically significantly lower than in the control (according to Williams t-test, one-sided smaller, α = 0.05)

Measured parameters over 96 hours

	Growth rate		**Yield**		**Biomass**	
Mean measured concentration (mg a.s./L)	**Mean (1/day)**	**% inhibition**	**Mean (x 10^3 cells/mL)**	**% inhibition**	**Mean integral (10^3 * day)**	**% inhibition**
Control	1.512	0.0	275.3	0.0	242.6	0.0
5.5	1.520	-0.6	285.1	-3.6	257.1	-5.9
11	1.513	-0.1	278.6	-1.2	251.8	-3.8
22	1.532	-1.3	299.1	-8.7	268.6	-10.7
43	1.547	-2.3	317.0	-15.2	261.0	-7.6
87	1.302*	13.9	118.7*	56.9	119.0#	50.9

* mean value statistically significantly lower than in the control (according to Williams t-test, one-sided smaller, α = 0.05)
# mean value statistically significantly lower than in the control (according to Welch t-test, one-sided smaller, α = 0.05)

Study 2: Smyth *et al* (1998; SAN837/0411)
The toxicity of technical dicamba (purity 89.9%) to blue-green alga *Anabaena flos-aquae* was determined (Smyth *et al*, 1998). Blue-green algae were exposed for 5 days to nominal concentrations 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg a.s./L, alongside a culture medium control. At the start of the test, the analytically determined concentrations of dicamba were in the range 100 to 106% of the nominal values and at the end of the test were in the range 100 to 111% of nominal values. pH values were acceptable at test concentrations up to and including 32 mg a.s./L but are too low at higher concentrations of dicamba. Thus it is not possible to decide whether pH or the test substance caused the effects at concentrations > 32 mg a.s./L. Accordingly the NOEC is determined as 32 mg a.s./L and the EC_{50} as > 32 mg a.s./L.

Mean values at each concentration of dicamba technical for growth rate at 72, 96 and 120 hours for *Anabaena flos-aquae*

Nominal concentrations of dicamba technical (mg a.s./L)	**Mean growth rate (1/day) 0 – 72 hrs**	**Mean growth rate (1/day) 0 – 96 hrs**	**Mean growth rate (1/day) 0 – 120 hrs**
Control	0.062	0.054	0.046
3.2	0.062	0.053	0.046
5.6	0.062	0.053	0.045
10	0.061	0.052	0.045

18	0.063	0.053	0.045
32	0.062	0.053	0.046
56	0.003*	-0.001*	-0.004*
100	0.005*	-0.003*	-0.005*
180	0.004*	-0.001*	-0.002*

*: statistically significantly different from control (according to Dunnett's t-test, p = 0.05)

Mean values at each concentration of dicamba technical for the biomass integral (areas under the growth curve) at 72, 96 and 120 hours for *Anabaena flos-aquae*

Nominal concentrations of dicamba technical (mg a.s./L)	Mean biomass integral (area) 0 – 72 hrs	Mean biomass integral (area)) 0 – 96 hrs	Mean biomass integral (area) 0 – 120 hrs
Control	26.804	73.664	150.388
3.2	26.432	66.524	133.248*
5.6	24.848	65.132	129.536*
10	24.992	64.488	133.552*
18	27.648	69.072	134.016*
32	24.632	66.176	134.680
56	2.020*	2.160*	2.288*
100	1.932*	2.032*	2.080*
180	1.016*	1.096*	1.144*

*: statistically significantly different from control (according to Dunnett's t-test, p = 0.05)

Study 3: Hoberg (1992a; SAN837/5229)

The toxicity of technical dicamba (purity 89.5%) to the freshwater diatom *Navicula pelliculosa* was determined (Hoberg, 1992a). Algae were exposed for 120 hours to nominal concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 mg a.s./L, alongside a culture medium control. At the start of the test, the analytically determined concentrations of dicamba were in the range 96.9 to 109% of the nominal values and at the end of the test were in the range 95.5 to 98.8% of nominal values. Mean measured concentrations were 0.26, 0.5, 1.0, 1.9 and 3.8 mg a.s./L.

Based on mean measured concentrations the 120-hour EC_{50} was 2.3 mg a.s./L and the 120-hour NOEC was 0.5 mg a.s./L. The 72-hour E_bC_{50} and E_rC_{50} values were considered to be >3.8 mg a.s./L and the NOEC = 1.0 mg a.s./L.

Mean values at each concentration of dicamba (SAN837) for the growth rate at 72, 96 and 120 hours for *Navlicula pelliculosa*

Mean measured concentrations (mg/L)	Mean cell density (x 10^4 cells/mL) after 72 hours	Mean cell density (x 10^4 cells/mL) after 96 hours	Mean cell density (x 10^4 cells/mL) after 120 hours
Control	30	39	78
3.8	19*	21*	28
1.9	18	27	41*
1.0	30	33	57*
0.50	32	37	78*
0.26	38	41	79

* Statistically reduced ($p \leq 0.05$) as compared to the control based on Williams' test

Study 4: Hoberg (1993; SAN837/5224)

The toxicity of technical dicamba (purity 89.5%) to the marine diatom *Skeletonema costatum* was determined (Hoberg, 1993). Algae were exposed for 120 hours to nominal concentrations of 0.0097, 0.032, 0.11, 0.36, 1.2 and 4.0 mg a.s./L, alongside a culture medium control. At the start of the test, the analytically determined concentrations of dicamba were in the range 100 to 110% of the nominal values and at the end of the test were in the range 95 to 110% of nominal values. Mean measured concentrations were 0.011, 0.033, 0.11, 0.35, 1.2 and 4.1 mg a.s./L.

Based on mean measured concentrations the 72-hour E_rC_{50} were > 4.1 mg a.s./L, the E_bC_{50} = 1.8 mg a.s./L and NOE_bC = 0.011 mg a.s./L

Mean values at each concentration of dicamba for cell density at 72, 96 and 120 hours for *Skeletonema costatum*

Mean measured concentrations (mg a.s./L)	Mean cell density 72 hrs (cells/mL)	Mean cell density 96 hrs (cells/mL)	Mean cell density 120 hrs (cells/mL)
Control	49 ± 2	82 ± 12	111 ± 7
0.011	42 ±2	81 ± 13	110 ± 5
0.033	41 ± 2	76 ± 8	83 ± 1*
0.11	41 ± 1	65 ± 2	62 ± 7*[a]
0.35	37 ± 1[a]	56 ± 1[a]	58 ± 7*[a]
1.2	26 ± 3[a]	51 ± 6[a]	53 ± 7*[a]
4.1	22 ± 1[a]	24 ± 2[a]	38 ± 5*[a]

Mean values and standard deviation were calculated from the original raw data

*Statistically reduced compared to the control (based on Williams' test, $p \leq 0.05$)

[a] Cell fragments, bloated cells and thin cell walls observed

Study 5: Kirkwood (2015; SAN837_11580)

The toxicity of technical dicamba (purity 90.1%) to *Myriophyllum spicatum* (Eurasian watermilfoil) was determined in a 14 day study with nominal test concentrations of 0.029, 0.092, 0.29, 0.94, 3.0 and 9.6 mg a.s./L) alongside a dilution water control. Corresponding initial measured concentrations were 0.027, 0.083, 0.27, 0.86, 2.8 and 9.0 mg a.s./L. At exposure initiation (day 0) and termination (day 14), concentrations ranged from 90 to 94% and 81 to 93% of nominal concentrations, respectively. Results were reported based on initial measured concentrations.

The pH of test and control solutions ranged from 8.0 to 10 and dissolved oxygen concentrations ranged from 9.3 to 16 mg/L throughout the exposure period. The pH and dissolved oxygen values most likely increased over time as a result of photosynthesis by the plants. The validity criteria for control shoot length, weight and coefficient of variation were met and there were no visual symptoms of chlorosis in the controls throughout the study.

Effect of dicamba on growth rate and yield of *Myriophyllum spicatum* for shoot length

Initial measured concentration (mg a.s./L)	Mean Final total shoot length (cm)	Average specific growth rate		Yield (cm)	
		Mean (days-1)	Percent inhibition (%)	Mean (cm)	Percent inhibition (%)
Control	36.8	0.0899	-	26.5	-
0.027	50.7	0.1047	-16	39.4	-49
0.083	45.0	0.1036	-15	34.4	-30
0.27	34.1	0.0852	5	23.9	10
0.86	21.8	0.0513 [b]	43	11.3 [a]	57
2.8	15.0	0.0235 [b]	74	4.2 [a]	84
9.0	11.1	0.0027 [b]	97	0.6 [a]	98

[a] Significantly reduced compared to the control, based on Wilcoxon's Test with Bonferroni Holm's Adjustment.
[b] Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test.
Negative values indicate an increase relative to the control

Effect of dicamba on growth rate and yield (wet weight) of *Myriophyllum spicatum*

Initial measured concentration (mg a.s./L)	Shoot wet weight (g)	Shoot wet weight			
		Average specific growth rate		Yield (g)	
		Mean (days-1)	Percent inhibition (%)	Mean (g)	Percent inhibition (%)
Control	0.1844	0.0880	-	0.6072	-
0.027	1.0937	0.1064	-21	0.8495	-40
0.083	1.0155	0.1012	-15	0.7713	-27
0.27	0.8393	0.0862	2	0.5951	2
0.86	0.6305	0.0156	24	0.3864	36
2.8	0.4461	0.0429 [a]	51	0.2019 [a]	67
9.0	0.3966	0.0328 [a]	63	0.1524 [a]	75

[a] Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test.
Negative value indicate an increase relative to the control

Effect of Dicamba on growth rate and yield (dry weight) of *Myriophyllum spicatum*

Initial measured concentration (mg a.s./L)	Shoot dry weight (g)	Shoot dry weight			
		Average specific growth rate		Yield (g)	
		Mean (days-1)	Percent inhibition (%)	Mean (g)	Percent inhibition (%)
Control	0.0595	0.0610	-	0.0349	-
0.027	0.0764	0.0810	-33	0.0519	-49
0.083	0.0724	0.0767	-26	0.0479	-37
0.27	0.0679	0.0716	-17	0.0433	-24
0.86	0.0646	0.0686	-13	0.0400	-15
2.8	0.0493	0.0496	19	0.0248	29
9.0	0.0459	0.0431	29	0.0214	39

Negative values indicate an increase relative to the control; n.d. – not determined

The lowest concentration at which effects were observed was 0.86 mg a.s./L for yield and average growth rate based on shoot length (NOEC = 0.27 mg a.s./L, LOEC = 0.86 mg a.s./L).
For classification purposes, the EC_{50} for average growth rate based on shoot length of 0.94 mg a.s./L is considered the most relevant endpoint for acute (short term) toxicity.

Study 6: Hoberg (1992b; SAN837/5223)
The toxicity of dicamba technical (purity 89.5%) to duckweed (*Lemna gibba*) was assessed in a static test design for 14 days with nominal concentrations 0.25, 0.50, 1.0, 2.0 and 4.0 mg a.s./L together with culture medium control. Measured concentrations were 98% and 61% of nominal at the start and end of the test. The results are summarised in the table below:

Toxicity of dicamba technical to *Lemna gibba*

Initial measured concentration (mg a.s./L)	Mean number of fronds 14 days	Standard deviation	Mean frond dry weight (g) 14 days	Standard deviation
Control	418	13	0.0654	0.0100
0.25	421	8.6	0.0836	0.0073
0.51	371*	6.5	0.0639	0.0111
0.99	390*	14	0.0803	0.0053
1.9	360*	11	0.0821	0.0143
3.8	343*	28	0.0651	0.0092

* Significantly different from control ($p < 0.05$).

Frond production in the four highest concentrations (0.51, 0.99, 1.9 and 3.8 mg a.s./L) was significantly different from the controls at 14 days. There were no statistical significant differences in dry weight (biomass) at any of the concentrations tested.
Since no test concentration resulted in a 50% reduction in frond density or biomass as compared to the control, an EC_{50} value was not calculated (effectively EC_{50} >3.2 mg a.s./L based on geometric mean concentrations).
Whilst this may be considered a chronic study, for classification purposes, the EC_{50} is considered a relevant endpoint for acute (short term) toxicity. In summary, the 14 day EC_{50} value was >3.2 mg a.s./L and the 14 day NOEC was 0.19 mg a.s./L.

Table 73: Summary of toxicity data on algae and aquatic plants

Species	Test material	Timepoint for E_rC_{50} & NOEC	Lowest EC_{50} (mg a.s./L)	NOEC (mg a.s./L)	Reference
Pseudokirchneriella sub-capitata	Dicamba technical	72 hour 96 hour	> 87	43	Eckenstein (2015) SAN837_11464
Anabaena flos-aquae	Dicamba technical	72 hour 96 hour	> 32 34.85	32	Smyth *et al* (1998) SAN837/0411
Navicula pelliculosa	Dicamba technical	72 hour 120 hour	3.8 2.3	0.5	Hoberg (1992a) SAN837/5229
Skeletonema costatum	Dicamba technical	72 hour 120 hour	4.1 0.58	0.011	Hoberg (1993) SAN837/5224
Myriophyllum spicatum	Dicamba technical	14 day	0.94	0.27	Kirkwood (2015) SAN837_11580
Lemna gibba	Dicamba technical	14 day	> 3.2	0.19	Hoberg (1992b) SAN837/5223

Based on these data the EC_{50} for *Skeletonema costatum* is the most acutely sensitive endpoint. The EC_{50} is therefore taken as 0.58 mg a.s./L for classification purposes.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

No other information was submitted or required.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 74: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
OECD 203	Fish *(Danio rerio)*	Dicamba tech.	96-h LC_{50} > 98.85 mg a.s./L (nom)		Gilberg & Seck (2010)
US-EPA FIFRA, Subdivision J, Guidelines 122-2 and 123-2	Algae (*Navicula pelliculosa*)	Dicamba tech.	120-h E_rC_{50} > 0.58 mg a.s./L (mm)		Hoberg (1992)
OECD 239	Aquatic plant (*Myriophyllum spicatum*)	Dicamba tech.	14-d E_rC_{50} = 0.94 mg a.s./L		Kirkwood A. (2015)

Based on these data the most sensitive species group to acute (short term) exposure to dicamba is algae; lowest EC_{50} = 0.58 mg a.s./L.

On this basis, the following classification and labelling of dicamba is proposed:
Aquatic Acute 1 H400 (Very toxic to aquatic life); as the lowest $L(E)C_{50}$ is between 0.1 and 1 mg/L; the associated M-factor is 1.

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

Table 75: Summary of information on long-term aquatic toxicity relevant for classification

Species group	Species	Lowest representative NOEC	Reference
Fish	*Pimephales promelas*	10 mg a.s./L	Salinas (2011) SAN837_11528
Aquatic invertebrates	*Americamysis bahia*	5.8 mg a.s./L	Claude *et al* (2012) SAN837_11530
Algae	*Skeletonema costatum*	0.011 mg a.s./L	Hoberg (1993) SAN837/5224
Aquatic plant	*Myriophyllum spicatum*	0.27 mg a.s./L	Kirkwood A. (2015)

Based on these data the most sensitive species group to chronic (long term) exposure to dicamba is algae (marine species); lowest NOEC = 0.011 mg a.s./L. According to the environmental fate data dicamba is classified as not readily biodegradable. Two studies (according to OECD 301F) were performed to determine the biodegradabililty of dicamba and the degradation of dicamba was < 9 % after 10 days and the degradation of the reference substance was > 87 % after 10 days. The results of the studies show that dicamba is considered to be not rapidly degradable (degradation < 60% within 10 days) for purpose of classification and labelling. Dicamba does not have the potential to bioaccumulate, as the log P_{ow} is below 4 and thus should not be classified due to potential for bioaccumulation.

On this basis, the following classification and labelling of dicamba is proposed:
Aquatic Chronic 1 H410 (Very toxic to aquatic life with long lasting effects); as the lowest NOEC is between 0.01 and 0.1 mg/L and the substance is not rapidly degradable; the associated M-factor is 1.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

On the basis of the above information on chronic toxicity, bioaccumulation and rapid degradability, the following classification and labelling of dicamba is proposed:

Acute: Category Acute 1' (H400) with M-factor = 1
Long-term: Category Chronic 1' (H410) with a M-factor = 1

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Dicamba is a systemic herbicide for the control of annual and perennial broadleaf dicotyledonous weed species that mimics auxins, a plant hormone, and causes abnormal growth by affecting cell division.

The dataset presented in the CLH report had been submitted in the context of renewal of pesticide active substances under Regulation n° 1107/2009 concerning the placing of plant protection products on the market. The data are based on tests performed with

the active substance or a technical solution of dicamba with a minimum purity of 980 g/kg (98% w/w) on dry matter.

The DS proposed to classify dicamba as Aquatic Acute 1 (M=1) and Aquatic Chronic 1 (M=1) based on relevant data.

The DS presented reliable acute data for two trophic levels (fish and algae or aquatic plant) and considered an aquatic alga (*Skeletonema costatum*) as the most sensitive species (120h E_rC_{50} = 0.58 mg/L) < 1 mg/L leading to a classification as Aquatic Acute 1 (H400) with an M-factor of 1 ($L(E)C_{50}$ is between 0.1 and 1 mg/L).

Dicamba is not expected to bioaccumulate in aquatic organisms (experimentally derived log K_{ow} of dicamba is -0.55 at pH 5.0, -1.8 at pH 6.8 and -1.9 at pH 8.9) and was considered as non-rapidly degradable.

Experimental chronic toxicity endpoint values were available for all three trophic levels and an aquatic alga (*S. costatum*) was presented as the most sensitive species (NOE_bC = 0.011 mg/L) with this value is used for classification purposes. On this basis, the classification and labelling of dicamba was proposed as Aquatic Chronic 1 (H410); as the NOEC is between 0.01 and 0.1 mg/L and the substance is not rapidly degradable; the M-factor was 1.

Rapid degradability

Hydrolysis

The DS mentioned that two studies were available from the previous EU review. The studies were still considered acceptable. Two new studies submitted by Industry supported the results of the older studies. Dicamba is hydrolytically stable (DT_{50} > 1-year, ambient temperature).

Photochemical degradation

The DS mentions that two studies were available from the previous EU review. The studies were still considered acceptable. A new study (OECD TG 316) was also submitted. An aqueous photochemical DT_{50} of 17.0 - 50.3 days at 40°N in springtime and 9.44 days at 30°N in summertime was determined for dicamba.

The quantum yield of direct phototransformation in water was found between 0.46 and 0.047.

Ready biodegradability

Two reliable valid GLP studies on ready biodegradability (OECD TG 301F) of dicamba are presented by the DS (table 66 of the CLH report). During these tests, low biodegradation levels are reached as less than 10% of biodegradation after 28 days were observed.

Dicamba is considered not to be readily biodegradable.

Water-sediment system

A water-sediment study was available and was considered acceptable. A new kinetic evaluation of the study was submitted by the notifier Industry. In the study, the route

and rate of degradation of radio-labelled dicamba was investigated in two aquatic systems under aerobic conditions. The systems used consisted of natural waters (Rhine-river and pond) and 10% of the corresponding sediment. ^{14}C-labelled dicamba was applied to the systems resulting in an initial concentration of 1.0 mg/L. All presented DT_{50} values were above 16 days.

Degradation in surface water

Two new studies on the degradation in surface water were submitted, both following OECD TG 309. The extent of mineralisation and the rate and route of degradation of [^{14}C]-dicamba was investigated in two surface waters (Calwich Abbey + River Alte Leine) at four dicamba application rates (1, 10, 95 and 100 µg/L) following incubation at 20°C under dark conditions for up to 90 days. For non-sterile samples, the DT_{50} of dicamba was 532 and 1280 days when dosed at 10 and 95 µg/L, respectively (DT_{50} rates were extrapolated beyond the study duration (59 days)). The total carbon dioxide evolved was 2.6% and 2.1% of applied radioactivity for the 10 and 90 µg/L rates respectively. For sterile samples, the mean level of parent dicamba at the end of the study was 97.7% AR at 95 µg/L.

Conclusion on rapid degradability

The DS concluded that on the basis of the available and valid data, dicamba is not rapidly degradable.

Bioaccumulation

No experimental data on fish were available. The DS presented an experimentally derived log K_{ow} for dicamba of -0.55 at pH 5.0, -1.8 at pH 6.8 and -1.9 at pH 8.9. Based on these data, the DS considered that dicamba is not expected to bioaccumulate in aquatic organisms.

Aquatic toxicity

Acute aquatic toxicity

The DS reported acute aquatic data for two trophic levels (fish and algae and aquatic plants) in table 70 of the CLH report. Studies have been carried out with technical dicamba and representative formulations.

Three acute toxicity tests with fish were available, for *Cyprinus carpio* following OECD TG 203 giving a (96) LC_{50} of > 100 mg/L*, Oncorhynchus mykiss* following OECD TG 204 giving a (96) LC_{50} of > 100 mg/L, and *Danio rerio* following OECD TG 203 giving a (96) LC_{50} of > 98.85 mg/L. All studies were considered valid by the DS.

The DS concluded that dicamba exhibits low acute toxicity to fish. The lowest LC_{50} for dicamba in fish was 98.85 mg/L.

No valid Acute toxicity for aquatic invertebrates were available.

Acute and chronic toxicity to algae and aquatic plants are discussed in "chronic toxicity to algae or aquatic plants" section.

Chronic aquatic hazard

Valid studies relevant for the chronic classification of dicamba are reported by the DS are presented in the table 72 of the CLH report.

Three chronic toxicity tests for fish were available for *O. mykiss* following OECD TG 204 giving a NOEC of > 100 mg/L*, Pimephales promelas* following OECD TG 210 giving a NOEC of 10 mg/L, and *Cyprinodon variegatus* following Fish Early Life stage (OPPTS 850.1400) giving a NOEC of 11 mg/L. The DS indicates that the study with *O. mykiss* is considered acceptable. However, long-term toxicity data from OECD TG 204 is not considered adequate under CLP and thus the study is not used for classification.

The DS concluded that dicamba, based on the results of the three available chronic studies, exhibits low chronic toxicity to fish. For the purpose of classification, a NOEC of 10 mg/L is used, based on the data for the *P. promelas*.

Two chronic toxicity tests to aquatic invertebrates were available. A 21-day flow-through toxicity study with *Daphnia magna* following OECD TG 202 (1984 part II) gave a NOEC of > 97 mg/L. In a 35-day flow-through toxicity study with *Americamysis bahia* (salt-water mysid) following a US EPA test guideline, a NOEC of 5.8 mg/L was determined.

The DS concluded that, based on the data for *A. bahia* the chronic NOEC for aquatic invertebrates of 5.8 mg/L is taken for the purposes of classification.

Four studies are available on the toxicity of dicamba to algae (*Pseudokirchneriella subcapitata, Anabaena flos-aquae, Navicula pelliculosa, S. costatum)*. In addition, two 14-day studies with aquatic macrophytes (*Myriophyllum spicatum and Lemna gibba*) have been performed using dicamba technical. The DS concluded that the lowest endpoint values were to be derived from the Hoberg (1993) study with *S. costatum*, which was considered as reliable.

The DS concluded that for dicamba, based on Hoberg (1993), the E_rC_{50} and NOEC for *S. costatum* are the most sensitive endpoints. The (120h) E_rC_{50} is therefore taken as 0.58 mg/L and the (72h) NOE_bC as 0.011 mg/L for classification purposes.

Comments received during consultation

Five MSs, one national authority and one company/manufacturer commented the classification proposal. They agreed that algae and aquatic plants are the most sensitive trophic level.

All commenters emphasized that the key study used for aquatic acute and aquatic chronic classification are not adequate. The acute and chronic classification proposed by the DS are based on the study of Hoberg (1993) with *S. costatum* giving an E_rC_{50} (120h) of 0.58 mg/L and a NOE_bC (72h) of 0.011 µg/L. Further information on the NOEC for *S. costatum* based on growth rate are required as classification should preferably be based on growth rate rather than biomass. The EC_{50} (120 h) of 0.58 mg/L for *S. costatum* (Hoberg, 1993) shown in table 73 of the CLH report is not reported in the summary on page 226. Members states agreed that since the study is not valid, these endpoints should not be used for classification of dicamba. Therefore, the lowest relevant endpoints available are from Kirkwood (2015) on *M. spicatum* with an E_rC_{50} value of 0.94

mg/L and this endpoint resulted in the same classification as previously proposed (Aquatic Acute 1, M=1). The commenting company was of the view that the lowest acute endpoint value was > 1 mg/L and that no classification was warranted for acute hazards. However, the DS agreed with the proposal of the MS and national authority.

All noted that the lowest reliable chronic toxicity endpoints are the *M. spicatum* 14-day NOE_rC of 0.27 mg/L and E_rC_{20} of 0.35 mg/L (initial measured) based on shoot length. Reliable E_rC_{10} values could not be determined for this study. The E_rC_{20} from this study is obtained from the RAR and should be used in preference to the NOE_rC. Finally, the MS noted that the *L. gibba* 14-day NOE_rC of 0.19 mg/L (mean measured) based on frond number is also within this concentration range.

All commenters concluded that these chronic endpoints are in the range from >0.1 to ≤ 1 mg/L, which result in an Aquatic Chronic 2 classification as dicamba is not rapidly degradable. The DS agreed these comments and concurred with the revised chronic classification proposal.

Assessment and comparison with the classification criteria

Degradation

Dicamba can be considered as hydrolytically stable, based on reliable data from 2 OECD TG 111 studies and one following the US EPA test guideline.

The degradation rate of dicamba did not reach 60% within the 10-day window and after 28 days of incubation (OECD 301F test), so dicamba is considered not to be readily biodegradable.

Dicamba is mineralized in surface water at low concentration of 1 µg/L with a half-life of 59.3 days and low mineralization in surface water observed (approx. 10% at application dose of 10 µg/L after 90 days of exposure) (OECD TG 309).

Dicamba was steadily degraded in the water-sediment systems with DCSA as the only major metabolite. The majority of dicamba was recovered from the water phase, and only minor parts were recovered from the sediments during day 0-90. Dicamba and its major metabolite, 3,6-DCSA, dissipate rapidly in aquatic systems, especially in sediment. Half-lives ($DegT_{50}$) of 35.9 days and 45.5 days for Rhine-river and pond systems, respectively, were determined for dicamba (with a geometric mean whole system half-life of 38.1 days). Dicamba was slowly mineralised. The mean amount of ^{14C}-CO_2 accounted for 2.6, 11.25 and 15.90% in the river system and for 2.37, 6.48 and 10.97% in the pond system after 30, 60 and 90 days, respectively (Regulation (EU) N° 286/2011 - part 4.1.2.9.).

RAC considers the available information reliable and that as dicamba is not readily biodegradable and does not ultimately degrade to a level > 70% within days, RAC agrees with the DS that dicamba it should be considered as not rapidly degradable.

Bioaccumulation

No experimental BCF data are available. The DS reported log K_{ow} values below 0. Nevertheless, RAC noted the inconsistency regarding the experimental log K_{ow} presented in the environment part of CLH report (negative value for log K_{ow}) and the calculated log

K_{ow} presented in the human health sections (log K_{ow} = 2.21). Despite this inconsistency and as all the reported values do not exceed the cut-off value of 4, RAC agrees with the DS that dicamba has a low potential for bioaccumulation.

Aquatic Toxicity

For acute toxicity, data are available for fish, algae, and aquatic plants. A study on *D. magna* is presented in the DRAR but this test is carried out with the formulation dicamba 700SG and was not retained.

For chronic toxicity, data are available for fish, invertebrates, algae, and aquatic plants. For both, acute and chronic toxicity, the primary producers are the most sensitive trophic level.

The initial proposal of the DS was to classify dicamba on the basis of Hoberg (1993) with *S. costatum* giving an E_rC_{50} (120h) of 0.58 mg/L and a NOE_bC (72h) of 0.011 mg/L. This study was assessed in the context of the original guideline. Nevertheless, as highlighted during the consultation, mentioned in the DRAR, this study was reassessed by the DS taking into account the three validity criteria of the current OECD TG 201. In this case, this study only fulfils two of the three validity criteria of the current OECD TG 201. According to the DS, calculations based on raw data (West, 2007) for algal density in the control cultures increased 49 x in 72 h (≥ 16 x required) and the average specific growth rate Coefficient of Variance (CV) was 1.0% (≤ 10% required), but mean section-by-section specific growth rate CV was 48% (≤ 35% required). RAC agrees with the DS's revised view of this study, i.e., that it is not considered suitable for classification.

The study with *L. gibba* (Hoberg, 1992) was not conducted according to a current *Lemna* test guideline (OECD TG 221) and differed from that guideline in several aspects, including study duration (14d instead of 7d) and general test conditions. The *Lemna* test should be performed in semi-static conditions instead of static conditions described in Hoberg (1992). The results of the *Lemna* and *Myriophyllum* studies are quite similar. As already discussed in previous dossiers, *M. spicatum* is considered by RAC as suitable for a classification purpose. Nevertheless, the test duration was 14 days during which multiple generations are not possible which would be a normal prerequisite for chronic aquatic toxicity testing. Similarly, 14 days could also be considered a long period for acute toxicity testing. However, as the substance is a herbicide and had severe effects in the test, RAC concludes that the data is considered suitable for both acute and chronic classification in this case (as previously for quinoclamine). There are multiple effect endpoints reported in the test including growth rate, but RAC is of the opinion that the lowest toxicity value for shoot length should be chosen for classification. Consequently, the study with *M. spicatum* conducted according to OECD TG 238 is considered more robust. *M. spicatum* is the most sensitive species for acute ($E_rC_{50\text{shoot length}}$ (14d) 0.94 mg/L (mm)) and chronic ($NOEC_{\text{shoot length}}$ (14 d) 0.27 mg/L (mm)) exposure.

Conclusion and comparison with Classification criteria

For aquatic acute classification, the E_rC_{50} of the most sensitive species (*M. spicatum*) is below the CLP cut-off value of 1 mg/L. Consequently, RAC agrees with the DS that Dicamba **warrants classification as Aquatic Acute category 1 (H400) with an M-Factor = 1**.

For aquatic chronic classification, the NOEC of the most sensitive species (*M. spicatum*) is ≤ 1 mg/L and since dicamba is considered as non-rapidly degradable, RAC agrees with the DS's revised view on the classification after the consultation that **classification as Aquatic Chronic Category 2 (H411) is warranted**.

2.9.3 Summary of effects on arthropods

2.9.3.1 Bees

Studies have been carried out with technical dicamba and the two representative formulations. No studies of toxicity to bumble bees or solitary bees have been submitted.

The endpoints from the old a.s. studies were originally reported as technical dicamba and have been corrected for purity; Table 75.

Table 76: Summary of toxicity of dicamba to bees

Test type (time scale)	Species	Test substance	Batch no.; purity	Endpoint	Toxicity	Reference
Acute oral	Honey bee	Dicamba tech.	52204112 89.5 %	LD_{50} (72 h)	**> 89.5 µg a.s/bee**[a]	Hillesheim, 1993a
		A7254B	52201602 39.9 % w/w	LD_{50} (72 h)	> 100 µg product/bee (**> 39.9 µg a.s/bee**)	Hillesheim, 1993a
		Dicamba 700SG	20150112002 692 g/kg	LD_{50} (48 h)	> 155.5 µg product/bee (> 107.6 µg a.s/bee)	Schmitzer, 2016
Acute contact	Honey bee	Dicamba tech.	52204112 89.5 %	LD_{50} (72 h)	**> 89.5 µg a.s/bee**[a]	Hillesheim, 1993b
		A7254B	52201602 39.9 % w/w	LD_{50} (72 h)	> 100 µg product/bee (**> 39.9 µg a.s/bee**)	Hillesheim, 1993b
		Dicamba 700SG	20150112002 692 g/kg	LD_{50} (48 h)	> 144.5 µg product/bee (> 100 µg a.s/bee)	Schmitzer, 2016
Adult chronic (10 days)	Honey bee	Dicamba tech.	20140901136 98.46 %	LDD_{50}	**> 61.7 µg a.s./bee/day**	Tanzler & Knebel, 2017
		A7254B	BSN4C1022 41.7 % w/w	LDD_{50}	**> 194.7 µg a.s./bee/day**	Ruhland, 2015
Larval development (8 days)	Honey bee	A7254B	BSN4C1022 41.7 % w/w	NOED	**125 µg a.s./larva/development period**	Kleebaum, 2015
Larval development (10 days)		Dicamba tech.	20140901136 98.46%	NOED	**3.89 µg a.s./larva/development period**	Ortoli, 2017

[a] Endpoint corrected for purity of the technical a.s.

Values in **bold** are considered relevant for use in risk assessment.

2.9.3.2 Non-target arthropods other than bees

Studies have been carried out with the two representative formulations. In addition to standard laboratory tests with the two indicator species (Table 76) extended laboratory test with the standard species and three additional species are available (Table 77).

Table 77: Summary of toxicity of dicamba to non-target arthropods other than bees – standard laboratory tests (Tier 1)

Species	Test type; substrate	Test substance	Batch no.; a.s. content	Endpoint	Toxicity	Reference
Aphidius rhopalosiphi	Tier 1; Glass plate	A7254B	PR910061 484 g/L	LR_{50} (48 h)	**356 g a.s./ha**	Grimm, 2000a
		Dicamba 700SG	175-024 708.6 g/kg	LR_{50} (48 h)	**3412 g a.s./ha**	Sipos, 2010b
Typhlodromus pyri	Tier 1; Glass plate	A7254B	PR910061 484 g/L	LR_{50} (7 d)	**232.6 g a.s./ha**	Grimm, 2000b
		Dicamba 700SG	175-024 708.6 g/kg	LR_{50} (7 d)	**154 g a.s./ha**	Sipos, 2010a

Values in **bold** are considered relevant for use in risk assessment.

Table 78: Summary of toxicity of dicamba to non-target arthropods other than bees – extended laboratory tests and aged residue studies (Tier 2)

Species	Test type; substrate	Test substance	Batch no.; a.s. content	Endpoint	Toxicity	Reference
Aphidius rhopalosiphi	Extended lab.; barley plants (3D)	A7254B	BSN4C1022 487 g/L	Mortality Reproduction	LR_{50} > 2338 g a.s./ha NOER = 2338 g a.s./ha	Stevens, 2014
Typhlodromus pyri	Extended lab.; maize leaves (2D)	A7254B	PB008205 460 g/L	Mortality Reproduction	LR_{50} > 460 g a.s./ha NOER = 57.5 g a.s./ha < 50 % effect at 115 g a.s./ha	Zenz, 2002
	Extended lab.; maize plants (3D)	Dicamba 700SG	175-024 708.6 g/kg	Mortality Reproduction	LR_{50} > 365 g a.s./ha NOER = 365 g a.s./ha	Ythier, 2010a
	Aged residue; maize plants (3D)	A7254B	BSN4C1022 487 g/L	0 and 14 DAT: Mortality Reproduction	LR_{50} > 974 g a.s./ha NOER = 974 g a.s./ha	Fallowfield, 2015
Chrysoperla carnea	Extended lab.; maize leaves (2D)	A7254B	PB008205 460 g/L	Mortality Reproduction	LR_{50} > 960 g a.s./ha NOER = 960 g a.s./ha	Hargreaves & Weyman, 2003
	Extended lab.; maize plants (3D)	Dicamba 700SG	175-024 708.6 g/kg	Mortality Reproduction	LR_{50} > 365 g a.s./ha NOER = 365 g a.s./ha	Ythier, 2010b
Aleochara bilineata	Extended lab.; sand (2D)	A7254B	PR910061 484 g/L	Mortality Reproduction	LR_{50} > 363 g a.s./ha NOER = 363 g a.s./ha	Taruza, 2001
Poecilus cupreus	Extended lab.; sand (2D)	A7254B	5290250 480 g/L	Mortality Predation rate	LR_{50} > 360 g a.s./ha < 50 % effect at 360 g a.s./ha	Rombke, 1990

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Studies have been carried out with the two representative formulations; Table 78.

Table 79: Summary of toxicity of dicamba on non-target soil meso- and macrofauna

Test type (time scale)	Species	Test substance	Batch no.; purity	Endpoint	Toxicity	Reference
56 day chronic	*Eisenia fetida*	A7254B	PFB3HI19; 484 g/L	NOEC	125 mg A7254B/kg dw soil (equivalent to 51.25 mg a.s./kg dw soil) [a]	Friedrich, 2011
56 day chronic	*Eisenia fetida*	Dicamba 700SG	20150112002; 692 g/kg	NOEC	4.15 mg a.s./kg soil	Pavic B., 2016a
28 day chronic	*Folsomia candida*	A7254B	BSN4C1022; 487 g/L	NOEC	62.5 mg A7254B/kg dw soil, (equivalent to 26.1 mg a.s./kg dw soil) [a]	McCormac, 2014
28 day chronic	*Folsomia candida*	Dicamba 700SG	20150112002; 692 g/kg	NOEC	mortality = 100 mg test item/kg soil d.w. eq. to 69.2 mg a.s./kg soil d.w. reproduction = 25.0 mg test item/kg soil eq. to 17.3 mg a.s./kg soil d.w.	Pavic B., 2016b
14 day chronic	*Hypo-aspis aculeifer*	A7254B	BSN4C1022; 487 g/L	NOEC	= 1 000 mg A7254B/kg dw soil, (equivalent to 417 mg a.s./kg dw soil) [a]	Vinall, 2014
14 day chronic	*Hypo-aspis aculeifer*	Dicamba 700SG	20150112002; 692 g/kg	NOEC	mortality = 1 000 mg test item /kg soil d.w. eq. to 692 mg a.s./kg soil d.w. reproduction = 1 000 mg test item/kg soil d.w. eq. to 692 mg a.s./kg soil d.w.	Parsons C., 2016

[a] Based on nominal active substance content of 480 g/L and density of 1170 kg/m^3

2.9.5 Summary of effects on soil nitrogen transformation

Studies have been carried out with technical dicamba and the representative formulation Dicamba 700SG. The endpoints from the a.s. studies were originally reported as technical dicamba and have been corrected for purity; Table 79.

Table 80: Summary of toxicity of dicamba on soil nitrogen transformation

Test type (time scale)	Species	Test substance	Batch no.; purity	Endpoint	Toxicity	Reference
	Soil microflora	Dicamba tech.	P.MG2726410; 89.8%	NOEC	5.75 mg/kg dw soil	Seyfried, 2001
	Soil microflora	Dicamba 700SG	175-024; 72.1%	NOEC	2.45 mg a.s./kg dry soil	Förster, 2010

2.9.6 Summary of effects on terrestrial non-target higher plants

Studies have been carried out with the two representative formulations (Tables 80 and 81).

Table 81: Summary of toxicity of dicamba (A7254B) to terrestrial non-target plants

Species	Family	ER_{50} (g a.s./ha)	
		Seedling emergence	Vegetative vigour
Dicotyledons			
Beta vulgaris (sugar beet) [a]	Chenopodiaceae	**97**	24.4
Daucus carota (carrot) [b]	Apiaceae	318	888
Glycine max (soybean) [a]	Fabaceae	186	590
Helianthus annuus (sunflower) [b]	Asteraceae	290	**15**
Lycopersicon esculentum (tomato) [a]	Solanaceae	507	48.6
Raphanus sativus (radish) [a]	Brassicaceae	> 480	212.5
Monocotyledons			
Allium cepa (onion) [a]	Amaryllidaceae	> 480	>1200
Avena sativa (oat) [a]	Poaceae	> 1200	> 1200
Echinochloa crus-galli (barnyard grass) [b]	Poaceae	533	1315
Zea mays (maize) [b]	Poaceae	> 2945	> 2945

[a] Balluff, 2002 (seedling emergenge) and 2003 (vegetative vigour); batch no. PB008205 (460 g a.s./L).

[b] Bramby-Gunary, 2015 (seedling emergenge) and 2015a (vegetative vigour); batch no. BSN1A1450 (489 g a.s./L).

Lowest endpoint for seedling emergence and vegetative vigour indicated in **bold**.

Table 82: Summary of toxicity of dicamba (Dicamba 700SG) to terrestrial non-target plants

Species	Family	ER_{50} (g a.s./ha)	
		Seedling emergence [a]	Vegetative vigour [b]
Dicotyledons			
Beta vulgaris (red beet)	Chenopodiaceae	-	64.12
Brassica napus (oilseed rape)	Brassicaceae	246.9	> 313
Cucumis sativus (cucumber)	Cucurbitaceae	362.7	-
Lycopersicon esculentum (tomato)	Solanaceae	71.2	**19.43**
Pisum sativum (pea)	Fabaceae	**62.1**	20.21
Monocotyledons			
Allium cepa (onion)	Amaryllidaceae	244.9	426.9
Avena sativa (oat)	Poaceae	942.7	1607

[a] Richter & Seck, 2010; batch no. 175-024 (72.1 % according to certificate of analysis).

[b] Deslandes, 2010; batch no. 175-024 (708.6 g/kg according to certificate of analysis).
Lowest endpoint for seedling emergence and vegetative vigour indicated in **bold**.

In addition, studies from the open litterature indicate that non-target vegetation in general, and certain plant species in particular, may be significantly affected by dicamba at lower treatment rates than indicated by the endpoints derived from the standard studies (0.2 – 2.43 mg a.s./ha).

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No specific information was submitted.

2.9.8 Summary of effects on biological methods for sewage treatment

No inhibition of the activity of activated sludge was recorded at concentrations up to 500 mg a.s./L.

2.9.9 Summary of product exposure and risk assessment

In the following environmental risk assessment, the conclusions are made for the maize use (max 350 g a.s./ha), unless explicity mentioned. The use in sorghum and cereals is considered to be covered by the risk assessment for the maize use unless otherwise stated.

2.9.9.1 Birds

The risk assessment was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12):1438). The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.2.1.

The risk assessment concluded that the acute and long-term risk to birds is acceptable for all representative uses of the formulation A7254B. Concerning the representative uses of the formulation Dicamba 700SG, acceptable acute and long-term risk was concluded for use at 0.280 kg a.s./ha, whereas unacceptable long-term risk was found at 0.350 kg a.s./ha.

The risk to birds from the representative uses of the formulations A7254B and Dicamba 700SG was assessed using the toxicity endpoints for dicamba since the risk can be adequately assessed from the available toxicity data for the active substance. It was considered that the risk from the major foliar metabolite 5-OH dicamba (NOA405873) is covered by the risk assessment for dicamba.

Dietary exposure

Table 83: Assessment of acute risk to birds from dicamba for the representative uses of A7254B – Screening step

GAP use	Application rate (kg a.s./ha)	Indicator species	Geometric mean LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
Maize	0.288	Small omnivorous bird	194	45.7	**4.2**	10
Sorghum	0.210			33.3	**5.8**	
Oat Wheat (BBCH 21–29) Triticale, Rye Barley	0.096			15.2	13	
Wheat (BBCH 10–32)	0.120			19.1	10	

With the exception of maize and sorghum, the TER_A values for all GAP uses are greater than the Commission Regulation (EU) 546/2011 trigger of 10, indicating an acceptable acute dietary risk to birds following the use of A7254B.

Table 84: Assessment of acute risk to birds from dicamba for the representative uses of A7254B – Tier 1

GAP use; application rate (kg a.s./ha)	Tier 1 crop grouping / growth stage	Generic focal species	Geometric mean LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
Maize; 0.288	Maize BBCH 10-19	Small insectivorous bird “wagtail”	194	7.72	25	10
	Maize BBCH 10-29	Medium granivorous bird “gamebird”		1.90	100	
	Maize BBCH 10-29	Medium herbivorous/ granivorous bird “pi-geon”		16.01	12	
	Maize BBCH 10-29	Small omnivorous bird “lark”		6.91	28	
	Maize leaf development BBCH 10-19	Small insectivo-rous/worm feeding species “thrush”		3.02	64	
Sorghum; 0.210	Cereals early (shoots) BBCH 10-29	Large herbivorous bird "goose"		6.41	30	
	Cereals BBCH 10-29	Small omnivorous bird “lark”		5.04	38	

All of the TER_A values are greater than the Commission Regulation (EU) 546/2011 trigger value of 10, indicating an acceptable acute dietary risk to birds for the representative uses of A7254B.

Table 85: Assessment of acute risk to birds from dicamba for the representative uses of Dicamba 700SG – Tier 1*

GAP use; application rate (kg a.s./ha)	Tier 1 crop grouping / growth stage	Generic focal species	Geometric mean LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
Maize; 0.350	Maize BBCH 10-29	Medium granivorous bird “gamebird”	194	2.31	84	10
	Maize leaf development BBCH 10-19	Small insectivo-rous/worm feeding species “thrush”		3.68	53	
	Maize BBCH 10-29	Small omnivorous bird “lark”		8.40	23	
	Maize BBCH 10-29	Medium herbivorous/ granivorous bird “pi-geon”		19.46	10	
	Maize BBCH 10-19	Small insectivorous bird “wagtail”		9.38	21	
Maize; 0.280	Maize BBCH 10-29	Medium granivorous bird “gamebird”		1.85	105	
	Maize leaf development BBCH 10-19	Small insectivo-rous/worm feeding species “thrush”		2.94	66	

GAP use; application rate (kg a.s./ha)	Tier 1 crop grouping / growth stage	Generic focal species	Geometric mean LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
	Maize BBCH 10-29	Small omnivorous bird "lark"		6.72	29	
	Maize BBCH 10-29	Medium herbivorous/ granivorous bird "pi-geon"		15.57	13	
	Maize BBCH 10-19	Small insectivorous bird "wagtail"		7.50	26	

* None of the GAP uses passed the trigger at the screening step.

All of the TER_A values are greater than or equal to the Commission Regulation (EU) 546/2011 trigger value of 10, indicating an acceptable acute dietary risk to birds for the representative uses of Dicamba 700 SG.

Table 86: Assessment of long-term and reproductive risk to birds from dicamba for the representative uses of A7254B – Screening step

GAP use	Application rate (kg a.s./ha)	Indicator species	$LD_{50/10}$ (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_{LT}	Trigger value
Maize	0.288	Small omnivorous bird	19.4	9.89	**2.0**	5
Sorghum	0.210			7.21	**2.7**	
Oat Wheat (BBCH 21–29) Triticale, Rye Barley	0.096			3.30	5.9	
Wheat (BBCH 10–32)	0.120			4.12	**4.7**	

The TER_{LT} values for use of A7254B in oat, wheat (BBCH 21–29), triticale, rye and barley are greater than the Commission Regulation (EU) 546/2011 trigger of 5, indicating an acceptable long-term dietary risk to birds. The TER_{LT} values for use of A7254B in maize, sorghum and wheat (BBCH 10–32) are below the trigger, indicating a need for further assessment.

Table 87: Assessment of long-term and reproductive risk to birds from dicamba for the representative uses of A7254B – Tier 1

GAP use; application rate (kg a.s./ha)	Tier 1 crop grouping / growth stage	Generic focal species	$LD_{50/10}$ (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_{LT}	Trigger value
Maize; 0.288	Maize BBCH 10-19	Small insectivorous bird "wagtail"	19.4	1.72	11	5
	Maize BBCH 10-29	Medium granivorous bird "gamebird"		0.458	42	
	Maize BBCH 10-29	Medium herbivorous/ granivorous bird "pi-geon"		3.46	5.6	
	Maize BBCH 10-29	Small omnivorous bird "lark"		1.66	12	
	Maize leaf development BBCH 10-19	Small insectivo-rous/worm feeding species "thrush"		0.870	22	

GAP use; application rate (kg a.s./ha)	Tier 1 crop grouping / growth stage	Generic focal species	$LD_{50/10}$ (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_{LT}	Trigger value
Sorghum; 0.210	Cereals early (shoots) BBCH 10-29	Large herbivorous bird "goose"		1.80	11	
	Cereals BBCH 10-29	Small omnivorous bird "lark"		1.21	16	
Wheat (BBCH 10–32); 0.120	Cereals early (shoots) BBCH 10-29	Large herbivorous bird "goose"		1.03	19	
	Cereals BBCH 10-29	Small omnivorous bird "lark"		0.693	28	
	Cereals BBCH 30-39	Small omnivorous bird "lark"		0.343	57	

All of the TER_{LT} values are greater than or equal to the Commission Regulation (EU) 546/2011 trigger value of 5, indicating an acceptable long-term dietary risk to birds for the representative uses of A7254B.

Table 88: Assessment of long-term and reproductive risk to birds from dicamba for the representative uses of Dicamba 700SG – Tier 1*

GAP use; application rate (kg a.s./ha)	Tier 1 crop grouping / growth stage	Generic focal species	$LD_{50/10}$ (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_{LT}	Trigger value
Maize; 0.350	Maize BBCH 10-29	Medium granivorous bird "gamebird"	19.4	0.56	35	5
	Maize leaf development BBCH 10-19	Small insectivo-rous/worm feeding species "thrush"		1.06	18	
	Maize BBCH 10-29	Small omnivorous bird "lark"		2.02	9.6	
	Maize BBCH 10-29	Medium herbivorous/ granivorous bird "pi-geon"		4.21	**4.6**	
	Maize BBCH 10-19	Small insectivorous bird "wagtail"		2.10	9.3	
Maize; 0.280	Maize BBCH 10-29	Medium granivorous bird "gamebird"		0.45	44	
	Maize leaf development BBCH 10-19	Small insectivo-rous/worm feeding species "thrush"		0.85	23	
	Maize BBCH 10-29	Small omnivorous bird "lark"		1.62	12	
	Maize BBCH 10-29	Medium herbivorous/ granivorous bird "pi-geon"		3.37	5.8	
	Maize BBCH 10-19	Small insectivorous bird "wagtail"		1.68	12	

* None of the GAP uses passed the trigger at the screening step.

All of the TER_{LT} values for the representative use of Dicamba 700SG at 0.280 kg a.s./ha are above the Commission Regulation (EU) 546/2011 trigger value of 5, indicating an acceptable long-term dietary risk to birds. For the

representative use at 0.350 kg a.s./ha, TER_{LT} for medium herbivorous/ granivorous bird "pigeon" is below the trigger, indicating unacceptable risk. Thus higher tier assessment would be required to support an application rate of 0.350 kg a.s./ha.

Drinking water exposure

The leaf scenario was not considered relevant for the representative uses. For the puddle scenario no specific calculations of exposure and TER were necessary because the ratio of effective application rate (in g/ha) to acute and long-term endpoints (in mg/kg bw/d) does not exceed 50 (K_{OC} < 500 L/kg). The acute and long-term risk to birds from drinking water exposure was considered acceptable for all representative uses of A7254B and Dicamba 700SG.

Secondary poisoning

Dicamba and its major soil and surface water metabolite DCSA have log P_{ow} values < 3, indicating that the risk of secondary poisoning and biomagnification in terrestrial food chains is negligible.

2.9.9.2 Mammals

The risk assessment was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12):1438). The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.2.2.

The risk assessment concluded that the acute and long-term risk to mammals is acceptable for all representative uses of the formulations A7254B and Dicamba 700SG.

Since the toxicity data indicate that the formulation A7254B is more toxic than predicted from the content of active substance, the acute risk from the representative uses of A7254B was assessed using the endpoint for the formulation. For the formulation Dicamba 700SG, the available data indicate a similar toxicity of the formulation and the active substance, so the acute risk was assessed using the endpoint for dicamba. For both formulations the long-term risk can be adequately assessed from the available toxicity data for the active substance.

The available studies indicate that the major foliar metabolite 5-OH dicamba (NOA405873) is not more toxic than the active substance. It was therefore considered that the risk from the metabolite is covered by the risk assessment for dicamba.

Dietary exposure

Table 89: Assessment of acute risk to mammals from dicamba for the representative uses of A7254B – Screening step

GAP use	Application rate (kg a.s./ha)	Indicator species	LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
Maize	0.288	Small herbivorous mammal	1020 [a]	39.3	26	10
Sorghum	0.210			24.9	41	
Oat Wheat (BBCH 21–29) Triticale, Rye Barley	0.096			11.4	89	
Wheat (BBCH 10–32)	0.120			14.2	72	

[a] From study with A7254B

All of the TER_A values are greater than the Commission Regulation (EU) 546/2011 trigger value of 10, indicating an acceptable acute dietary risk to mammals for the representative uses of A7254B.

Table 90: Assessment of acute risk to mammals from dicamba for the representative uses of Dicamba 700SG – Screening step

GAP use	Application rate (kg a.s./ha)	Indicator species	LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
Maize	0.350 [a]	Small herbivorous mammal	1465 [b]	47.7	31	10

[a] Also covers application rate 0.280 kg a.s./ha.

[b] From study with technical dicamba.

TER_A is greater than the Commission Regulation (EU) 546/2011 trigger value of 10, indicating an acceptable acute dietary risk to mammals for the representative uses of Dicamba 700SG.

Table 91: Assessment of long-term and reproductive risk to mammals from dicamba for the representative uses of A7254B – Screening step

GAP use	Application rate (kg a.s./ha)	Indicator species	LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
Maize	0.288	Small herbivorous mammal	136	11.0	12	5
Sorghum	0.210			5.38	25	
Oat Wheat (BBCH 21–29) Triticale, Rye Barley	0.096			2.46	55	
Wheat (BBCH 10–32)	0.120			3.07	44	

All of the TER_{LT} values are greater than or equal to the Commission Regulation (EU) 546/2011 trigger value of 5, indicating an acceptable long-term dietary risk to mammals for the representative uses of A7254B.

Table 92: Assessment of long-term and reproductive risk to mammals from dicamba for the representative uses of Dicamba 700SG – Screening step

GAP use	Application rate (kg a.s./ha)	Indicator species	LD_{50} (mg a.s./ kg bw)	DDD (mg a.s./ kg bw/day)	TER_A	Trigger value
Maize	0.350 [a]	Small herbivorous mammal	136	13.4	10	5

[a] Also covers application rate 0.280 kg a.s./ha.

TER_{LT} is greater than the Commission Regulation (EU) 546/2011 trigger value of 5, indicating an acceptable long-term dietary risk to mammals for the representative uses of Dicamba 700SG.

Drinking water exposure

No specific calculations of exposure and TER were necessary because the ratio of effective application rate (in g/ha) to acute and long-term endpoints (in mg/kg bw/d) does not exceed 50 (K_{OC} < 500 L/kg). The acute and long-term risk to mammals from drinking water exposure was considered acceptable for all representative uses of A7254B and Dicamba 700SG.

Secondary poisoning

Dicamba and its major soil and surface water metabolite DCSA have log P_{ow} values < 3, indicating that the risk of secondary poisoning and biomagnification in terrestrial food chains is negligible.

2.9.9.3 Aquatic organisms

The risk assessments for aquatic organisms (fish, aquatic invertebrates, algae and aquatic plants) were conducted in accordance to the new EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013). The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.4.

Table 93: Derivation of acute RAC values used in the Tier 1 risk assessment for dicamba, metabolite DCSA (NOA414746), A7254B and Dicamba 700SG

Species	**Substance**	**Exposure system**	**Results (µg/L)**	**Assess-ment Safety fac-tor**	**RAC (µg/L)**
Dicamba					
Rainbow trout	Dicamba (tested as A7254B)	96 h, s	LC_{50} > 41 000 µg a.s./L	100	**> 410 (a.s.)**
Daphnia magna	Dicamba (tested as A7254B)	48 h, s	EC_{50} > 41 000 µg a.s./L	100	**> 410 (a.s.)**
DCSA (NOA414746)					
Rainbow trout	DCSA (NOA414746)	96 h, ss	LC_{50} > 100 000 µg/L	100	> 1 000
Daphnia magna	DCSA (NOA414746)	48 h, s	EC_{50} = 89 000 µg/L	100	**890**
A7254B					
Rainbow trout	A7254B	96 h, s	LC_{50} > 100 000 µg A7254B/L LC_{50} > 41 000 µg a.s./L	100	**>1 000 (product)** **> 410 (a.s.)**
Daphnia magna	A7254B	48 h, s	LC_{50} > 100 000 µg A7254B/L LC_{50} > 41 000 µg a.s./L	100	**> 1 000 (product)** **> 410 (a.s.)**
Dicamba 700SG					
Fish	Dicamba 700SG	96 h	LC_{50} > 100 000 µg a.s./L	100	> 1000
Daphina	Dicamba 700SG	48 h	EC_{50} = 131 600 µg a.s./L	100	1 316

s: static; ss: semi-static; f: flow through
RAC values in bold are used for the Tier 1 aquatic risk assessment

Table 94: Derivation of chronic RAC values used in the Tier 1 risk assessment for dicamba, metabolite DCSA (NOA414746), A7254B and Dicamba 700SG

Species	**Substance**	**Exposure system**	**Results (µg/L)**	**Assess-ment Safety fac-tor**	**RAC (µg/L)**
Dicamba					
Sheepshead min-now	Dicamba	34 d, f	NOEC = 11 000 µg a.s./L	10	1 100
Pimephales prome-las	Dicamba	25 d, f	NOEC = 10 000 µg a.s./L	10	**1 000**
Mysid shrimp	Dicamba	35 d, f	NOEC = 5 800 µg a.s./L	10	580
Navicula pellicu-losa	Dicamba	72 h, s	E_rC_{50} > 3 800 µg a.s./L	10	> **380**
Myriophyllum spi-catum	Dicamba	14-d, s	E_rC_{50} = 940 µg a.s./L shoot length	10	**94**
DCSA (NOA414746)					
Pseudokirchneri-ella subcapitata	DCSA (NOA414746)	72 h, s	E_rC_{50} = 67 000 µg/L	10	**6 700**
Lemna gibba	DCSA (NOA414746)	7 d, s	E_rC_{50} > 65 800 µg/L	10	> **6 580**
A7254B					
Pseudokirchneri-ella subcapitata	A7254B	72 h, s	E_rC_{50} > 103 000 µg A7254B/L E_rC_{50} > 42 400 µg a.s./L	10	10 300 (product) 4 240 (a.s.)
Myriophyllum ver-ticillatum	A7254B	14 d, s	E_rC_{50} = 8 900 µg/L E_rC_{50} = 3 700 µg a.s./L	10	**890 (product)** 370 (a.s.)
Dicamba 700SG					
Algae	Dicamba 700SG	72 h, s	72 h E_rC_{50} > 69 200 µg a.s./L	10	> **6920**
Myriophyllum spi-catum	Dicamba 700SG	14 d, s	E_rC_{50} = 3 260 µg a.s./L	10	**326**

s: static; ss: semi-static; f: flow through
RAC values in bold are used for the Tier 1 aquatic risk assessment

Table 95: Comparison of FOCUS Steps 1 and 2 PEC_{sw} to the Tier 1 acute and chronic RAC values for dicamba and DCSA (NOA414746) following application of A7254B in maize to cover all intended crop uses

	Dicamba		**DCSA (NOA414746)**	
Group	**Acute**	**Chronic**	**Acute**	**Chronic**
Tier 1 RAC_{sw} (µg a.s./L)	> 410	94	890	6 700
FOCUS Step 1 PEC_{sw} (µg a.s./L)	97.54	**97.54**	40.58	40.58
FOCUS Step 2 PEC_{sw} (µg a.s./L)	30.56	30.56	-	-

Values in bold indicate an unacceptable risk

The acute Tier 1 RAC_{sw} value is above the FOCUS Step 1 PEC_{SW} value for dicamba, but the chronic Tier 1 RAC value is below the FOCUS Step 1 PEC_{SW} value indicating the need for further refinement. Both the acute Tier 1 RAC_{sw} and the chronic Tier 1 $RAC_{sw;}$ values are above the FOCUS Step 2 PEC_{SW} value for dicamba, indicating an acceptable risk for aquatic organisms following application of A7254B according to the proposed use patterns.

Both of the Tier 1 RAC_{sw} values are above the FOCUS Step 1 PEC_{SW} value for DCSA (NOA414746) indicating an acceptable risk for aquatic organisms following application of A7254B according to the proposed use patterns.

Table 96: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for dicamba for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Dicamba 700SG in maize

Group		**Fish acute**	**Fish chronic**	**Invertebrate acute**	**Invertebrate chronic**	**Algae**	**Aquatic macrophytes**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Navicula pelliculosa***	***Myriophyllum spicatum***
Endpoint (µg/L)		LC_{50} > 41 000	NOEC 10 000	EC_{50} > 41 000	NOEC 97 000	E_rC_{50} > 3 800	E_rC_{50} 3 260
AF		100	10	100	10	10	10
RAC (µg/L)		> 410.0	1000	> 410	970	> 380	326
FOCUS Scenario Step 1	**PEC gl-max (µg/L)**	**PEC/RAC**					
Worst-case Europe/ March-May	97.54 µg/L	< 0.238	0.098	< 0.238	0.101	< 0.257	0.299
FOCUS Scenario Step 2		**PEC/RAC**					

Group		Fish acute	Fish chronic	Invertebrate acute	Invertebrate chronic	Algae	Aquatic macrophytes
Worst-case Europe/ March-May	30.56 µg/L	< 0.075	0.031	< 0.075	0.032	0.080	0.096

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;

Table 97: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for DCSA for each organism group based on FOCUS Step 1 calculations for the use of Dicamba 700SG in maize

Group		Fish acute	Invertebrate acute	Aquatic macrophytes
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Lemna gibba***
Endpoint (µg/L)		LC_{50} > 100 000	EC_{50} > 89 000	E_rC_{50} 65 800
AF		100	100	10
RAC (µg/L)		> 1 000	> 890	6 580
FOCUS Scenario Step 1	**PEC gl-max (µg/L)**	**PEC/RAC**		
Worst-case: S Europe/March-May	40.58 µg/L	< 0.041	< 0.046	0.006

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;

The Tier 1 RAC_{sw} values are above the FOCUS Step 1 PEC_{SW} value for dicamba and DCSA (NOA414746) indicating an acceptable risk for aquatic organisms following application of Dicamba 700 SG according to the proposed use patterns.

The risk assessment concluded that the acute and chronic risk to aquatic organisms is acceptable for all representative uses of the formulation A7254B and Dicamb 700SG.

2.9.9.4 Bees

The risk assessment was carried out according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). In addition, and following EFSA recommendations, the EFSA Guidance Document on the risk assessment of plant protection products on bees (EFSA Journal 2013; 11(7):3295) was used to assess chronic risk to adult honeybees and risk to honeybee larvae. The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.6.1.

The risk assessment concluded that the acute and chronic risk to honeybees is acceptable for all representative uses of the formulation A7254B. For the formulation Dicamba 700SG, acceptable acute and chronic risk to honeybees was concluded for all representative uses.

Acute contact and oral toxicity data for dicamba and formulation A7254B were > 89.5 µg a.s./bee and > 100 µg product/bee (equivalent to > 39.9 µg a.s./bee based on reported content of 39.9% a.s.) respectively. Adjusted endpoints from the formulation studies were used as worst case. For Dicamba 700SG, the available data indicate that the formulation is not more toxic than the active substance, so the acute risk was assessed using the endpoint for dicamba. Assessment of chronic risk was based on the proprietary studies of each notifier.

A risk assessment for bumblebees and solitary bees was not performed since no toxicity data were available.

Table 98: Assessment of acute risk to honeybees from dicamba for the representative uses of A7254B [a]

GAP use	Application rate (g a.s./ha)	Exposure route	LD_{50} (µg a.s./bee)	HQ	Trigger value
Maize [b]	288 [b]	Oral	> 39.9	< 7.2	50
		Contact	> 39.9	< 7.2	50

[a] Assessment according to SANCO/10329/2002.
[b] Also covers other GAP uses with lower application rates.

Table 99: Assessment of acute risk to honeybees from dicamba for the representative uses of Dicamba 700SG [a]

GAP use	Application rate (g a.s./ha)	Exposure route	LD_{50} (µg a.s./bee)	HQ	Trigger value
Maize	350 [b]	Oral	> 89.5	< 3.9	50
		Contact	> 89.5	< 3.9	50

[a] Assessment according to SANCO/10329/2002.
[b] Also covers application rate 280 g a.s./ha.

All of the HQ values are below the trigger of 50, indicating an acceptable acute risk to bees for the representative uses of A7254B and Dicamba 700SG.

Table 100: Assessment of chronic risk to honey bees from dicamba for the representative uses of A7254B – Screening step [a]

GAP use	Application rate (kg a.s./ha)	Life stage	Toxicity end-point	ETR	Trigger value
Maize [b]	0.288 [b]	Adult	LDD_{50} > 194.7 µg a.s./bee/day	< 0.011	0.03
		Larvae	NOED = 125 µg a.s./larva	0.01	0.2

[a] Assessment according to EFSA Journal 2013; 11(7):3295.
[b] Also covers other GAP uses with lower application rates.

The ETR values for adult and larval honeybees are below the respective EFSA (2013) trigger values, indicating an acceptable chronic risk to bees for the representative uses of A7254B.

Table 101: Assessment of chronic risk to honey bees from dicamba for the representative uses of Dicamba 700SG – Screening step [a]

GAP use	Application rate (kg a.s./ha)	Life stage	Toxicity end-point	ETR	Trigger value
Maize	0.350	Adult	LDD_{50} > 61.7 µg a.s./bee/day	**< 0.043**	0.03
	0.280			**< 0.034**	
Maize	0.350	Larvae	NOED = 3.89 µg a.s./larva	0.0428	0.2
	0.280			< 0.034	

[a] Assessment according to EFSA Journal 2013; 11(7):3295.

Both ETR values are above the EFSA (2013) trigger, indicating a need for further assessment.

Table 102: Assessment of chronic risk to honey bees from dicamba for the representative uses of Dicamba 700SG – Tier 1 [a]

GAP use	Application rate (kg a.s./ha)	Scenario	Exposure factor	SV	Toxicity end-point	ETR	Trigger value
Maize	0.350 [b]	Treated crop	1	0.92	LDD_{50} > 61.7 µg a.s./bee/day	< 0.004	0.03
		Weeds in treated field	1	2.9		< 0.012	
		Plants at field margin	0.0092	2.9		< 0.00011	
		Adjacent crop	0.0033	5.8		< 0.00008	
		Succeeding crop	1	0.54		< 0.002	
Maize	0.350 [b]	Treated crop	1	0.15	NOED = 3.89 µg a.s./larva	0.009	0.2
		Weeds in treated field	1	2.2		0.135	
		Plants at field margin	0.0092	2.2		0.001	
		Adjacent crop	0.0033	4.4		0.0009	
		Succeeding crop	1	0.4		0.02	

[a] Assessment according to EFSA Journal 2013; 11(7):3295.
[b] Also covers application rate 0.280 kg a.s./ha.

All ETR values are below the EFSA (2013) trigger, indicating an acceptable chronic risk to honeybees for the representative uses of Dicamba 700SG.

2.9.9.5 Non-target arthropods other than bees

The risk assessment was carried out according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and ESCORT 2 (Candolfi et al. 2001). The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.6.2.

The risk assessment concluded that the in-field and off-field risk to non-target arthropods other than bees is acceptable for all representative uses of the formulations A7254B and Dicamba 700SG.

The assessment was based on the toxicity endpoints for each of the representative products since no studies with technical dicamba were available.

Table 103: Assessment of in-field risk to non-target arthropods other than bees from dicamba for the representative uses of A7254B –Tier 1

Species; study type	LR_{50} (g a.s./ha)	PER in-field (g a.s./ha)	HQ in-field	Trigger value
Typhlodromus pyri Tier 1, 2D exposure scenario	232.6	288 [a]	1.24	2
Aphidius rhopalosiphi Tier 1, 2D exposure scenario	356		0.81	2

[a] Also covers other GAP uses with lower application rates.

The in-field HQ values for both standard species are below the trigger, indicating acceptable risk to non-target arthropods for all representative uses of A7254B. Although not strictly required, higher tier studies are available (Table 77) and support the risk assessment.

Table 104: Assessment of in-field risk to non-target arthropods other than bees from dicamba for the representative uses of Dicamba 700SG –Tier 1

Species; study type	LR_{50} (g a.s./ha)	PER in-field (g a.s./ha)	HQ in-field	Trigger value
Typhlodromus pyri Tier 1, 2D exposure scenario	154	350	**2.27**	2
Aphidius rhopalosiphi Tier 1, 2D exposure scenario	3412		0.10 [a]	2
Typhlodromus pyri Tier 1, 2D exposure scenario	154	280	1.82	2

[a] Also covers assessment for application rate 0.280 kg a.s./ha.

The in-field HQ value for *Aphidius rhopalosiphi* is below the trigger, indicating acceptable risk for all representative uses of Dicamba 700SG. HQ for *Typhlodromus pyri* is above the trigger at 350 g a.s./ha, indicating a need for further assessment.

In extended laboratory studies (Tier 2) with *T. pyri* and *Chrysoperla carnea* there were no unacceptable (> 50 %) effects on survival and reproduction at application rates > 350 g a.s./ha, indicating acceptable in-field risk for all representative uses of Dicamba 700SG.

Table 105: Assessment of off-field risk to non-target arthropods other than bees from dicamba for the representative uses of A7254B –Tier 1

Species; study type	LR_{50} (g a.s./ha)	PER off-field (g a.s./ha)	Correction factor	HQ off-field	Trigger value
Typhlodromus pyri Tier 1, 2D exposure scenario	232.6	0.798 [a]	10	0.034	2
Aphidius rhopalosiphi Tier 1, 2D exposure scenario	356			0.022	2

[a] Worst case for the representative GAP uses.

Table 106: Assessment of off-field risk to non-target arthropods other than bees from dicamba for the representative uses of Dicamba 700SG –Tier 1

Species; study type	LR_{50} (g a.s./ha)	PER off-field (g a.s./ha)	Correction factor	HQ off-field	Trigger value
Typhlodromus pyri Tier 1, 2D exposure scenario	154	0.9695 [a]	10	0.063	2
Aphidius rhopalosiphi Tier 1, 2D exposure scenario	3412			0.0028	2

[a] Worst case for the representative GAP uses.

The off-field HQ values are below the trigger, indicating acceptable risk to non-target arthropods other than bees for all representative uses of A7254B and Dicamba 700SG.

2.9.9.6 Non-target soil meso- and macrofauna

The risk assessment was carried out according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.8.

The risk assessment concluded that the chronic risk to non-target soil meso- and macrofauna is acceptable for all representative uses of the formulation A7254B and Dicamba 700SG.

Table 107: Long-term TER values for other soil meso- and macro-fauna

Organism	Test substance	NOEC (mg/kg dw soil)	Maximum instantaneous PEC_S (mg/kg dw soil)	TER_{LT}	Trigger value
Earthworm (*Eisenia fetida)*	Dicamba (tested as A7254B)	51.25 mg a.s./kg dw soil	0.29	180	5
	DCSA	5.13 [a]	0.159	32	
	A7254B	125 (equivalent to 51.25 mg a.s./kg dw soil)	0.70	180	
	Dicamba 700SG	4.15 mg a.s./kg dw soil	0.280	14.8	
Collembola (*Folsomia candida*)	A7254B	62.5 (equivalent to 26.1 mg a.s./kg dw soil)	0.70	89	5
	Dicamba 700SG	17.3 mg a.s./kg dw soil	0.280	61.8	
	Dicamba (tested as A7254B)	26.1	0.29	90	
	DCSA	2.61 [a]	0.159	16	
Soil mite (*Hypoaspis aculeifer*)	A7254B	1 000 (equivalent to 417 mg a.s./kg dw soil)	0.70	1 400	5
	Dicamba 700SG	692 mg a.s./kg dw soil	0.280	2471	
	Dicamba (tested as A7254B)	417	0.29	1 400	
	DCSA	41.7[a]	0.159	262	

[a] In accordance with SANCO/10329/2002 the metabolite was considered to be ten times more toxic than the parent substance

2.9.9.7 Soil nitrogen transformation

The risk assessment was carried out according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.10.

Table 108: Risk assessment for effects on soil micro-organisms

Test substance	NOEC (mg a.s./kg dw soil)	PEC_S (mg/kg dw soil)
Dicamba	5.75	0.29
DCSA	0.575 [a]	0.159
Dicamba 700SG	2.45 mg a.s./kg dry soil	0.280

[a] In accordance with SANCO/10329/2002 the metabolite was considered ten times more toxic than the parent substance

The risk assessment concluded that the risk to non-target soil micro-organisms is acceptable for all representative uses of the formulations A7254B and Dicamba 700 SG (NOEC < PEC_s).

2.9.9.8 Terrestrial non-target higher plants

The risk assessment was carried out according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). The assessment is summarised below and presented in detail in Vol. 3 (PPP), B.9.12.

The risk assessment concluded that the risk to terrestrial non-target plants is acceptable for all representative uses of the formulation A7254B, provided that a 2 m no-spray buffer zone is respected for the representative use of A7254B in maize. For the formulation Dicamba 700SG, acceptable risk was concluded for all representative uses, provided that a 3 m no-spray buffer zone is respected.

The assessment was based on the toxicity endpoints for each of the representative products since no studies with technical dicamba were available.

Table 109: Deterministic assessment of risk to non-target terrestrial plants from dicamba for the representative uses of A7254B

Test type	GAP use	Application rate (g a.s./ha)	Distance (drift)	ER_{50} (g a.s./ha)	PER (g a.s./ha)	TER	Trigger
Seedling emergence	Maize [a]	288	1 m (2.77%)	97	7.98	12	**5**
Vegetative vigour	Maize	288	1 m (2.77%)	15	7.98	**1.9**	**5**
			2 m (1.40%)		4.03	**3.7**	
			3 m (0.94%)		2.71	5.5	
	Sorghum	210	1 m (2.77%)	15	5.82	**2.6**	**5**
			2 m (1.40%)		2.94	5.1	
	Wheat (BBCH 10 – 32)	120	1 m (2.77%)	15	3.32	**4.5**	**5**
			2 m (1.40%)		1.68	8.9	
	Oat, Wheat (BBCH 21 – 29), Triticale, Rye, Barley	96	1 m (2.77%)	15	2.66	5.6	**5**

[a] Also covers other GAP uses with lower application rates.

The deterministic risk assessment indicates acceptable risk to non-target terrestrial plants for all representative uses of A7254B, provided that a 3 m no-spray buffer zone is respected for use in maize and a 2 m no-spray buffer zone is respected for use in sorghum and wheat (120 g a.s./ha).

A probabilistic risk assessment was carried out based on a median HC_5 = 6.90 mg a.s./ha for vegetative vigour. The HC_5 was derived from a Species Sensitivity Distribution for 7 species (excluding the three "greater than" values).

Table 110: Probabilistic assessment of risk to non-target terrestrial plants from dicamba for the representative uses of A7254B

Test type	GAP use	Application rate (g a.s./ha)	Distance (drift)	HC_5 (g a.s./ha)	PER (g a.s./ha)	TER	Trigger
Vegetative vigour	Maize	288	1 m (2.77%)	6.90	7.98	**0.86**	**1**
			2 m (1.40%)		4.03	1.7	
	Sorghum [a]	210	1 m (2.77%)	6.90	5.82	1.2	**1**

[a] Also covers GAP uses with lower application rates.

The probabilistic risk assessment indicates acceptable risk to non-target terrestrial plants for all representative uses of A7254B, provided that a 2 m no-spray buffer zone is respected for the representative use in maize. The

probabilistic approach is considered acceptable but it is unclear to what extent the species included in the SSD are representative for the floral community to be protected, considering the available data from the open literature.

Table 111: Deterministic assessment of risk to non-target terrestrial plants from dicamba for the representative uses of Dicamba 700SG

Test type	GAP use	Application rate (g a.s./ha)	Distance (drift)	ER_{50} (g a.s./ha)	PER (g a.s./ha)	TER	Trigger
Seedling emergence	Maize [a]	350	1 m (2.77%)	62.1	9.70	**6.4**	**5**
Vegetative vigour	Maize	350	1 m (2.77%)	19.43	9.70	**2.0**	**5**
			3 m (0.94%)		3.29	5.9	
	Maize	280	1 m (2.77%)	19.43	7.76	**2.5**	**5**
			3 m (0.94%)		2.63	7.4	

[a] Also covers assessment for application rate 0.280 kg a.s./ha.

The deterministic risk assessment indicates acceptable risk to non-target terrestrial plants for all representative uses of Dicamba 700SG, provided that a 3 m no-spray buffer zone is respected.

A probabilistic risk asssessment was not considered appropriate because toxicity data for Dicamba 700SG were insufficient to construct a reliable SSD.

2.10ENDOCRINE DISRUPTING PROPERTIES

Assessment provided by the applicant. Please also see appendix 1 for the ED assessment including ToxCast plots.

2.10.1 GATHER ALL RELEVANT INFORMATION

2.10.2 Executive Summary

This document summarises and evaluates all of the available evidence on dicamba relevant to the assessment of endocrine disruption, in accordance with EFSA-ECHA (2018) Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. In order to support Applicants and Competent Authorities, EFSA and ECHA have developed guidance on how to identify endocrine disruptors in accordance with the criteria laid down in Regulation (EC) No 1107/2009. The Guidance Document describes how to gather, evaluate and consider all relevant information for the assessment, conduct a mode of action (MoA) analysis, and apply a weight of evidence approach, in order to establish whether the criteria for the identification of endocrine disruptors laid down in Commission Regulation (EU) 2018/605 of 19 April 2018 amended Annex II to Regulation (EC) No 1107/2009 are fulfilled.

The assessment strategy is based on the three conditions stipulated in the ED criteria (adversity, endocrine activity and a biologically plausible link between the two) and the grouping of the parameters described above, as recommended in the EFSA-ECHA guidance document.

All available relevant toxicology and ecotoxicology studies for dicamba are included in this review. The relevant regulatory mammalian toxicology studies for dicamba cover a range of study types including sub-acute, sub-chronic, chronic, developmental and reproductive toxicity studies in a range of mammalian species including rat, mouse, dog and rabbit. The relevant regulatory non-mammalian toxicology studies submitted for dicamba cover a range of study types including chronic and reproductive toxicity studies in birds and fish.

The available data on dicamba do not indicate effects consistent with endocrine disruption. In accordance with the EFSA-ECHA (2018) Guidance, EATS-mediated parameters have been sufficiently investigated and no additional *in vitro* or *in vivo* mammalian data are required to assess the EAS or T modalities. Applying this Guidance

Document, the conclusion can be drawn that dicamba does not meet the criteria for endocrine disruption with respect to humans.

Available ecotoxicology data do not indicate effects consistent with endocrine disruption, however, considering the available data in accordance with the EFSA-ECHA Guidance document (2018), there is not currently a fully adequate dataset to conclude on whether dicamba exhibits endocrine disrupting properties in non-target organisms according to the Endocrine Disruption Criteria (2018/605).

As first steps to make sufficient data available to reach a conclusion, Syngenta proposes to conduct the following studies:

1) 21-day fish screening assay (OECD 230) in the Fathead minnow;
2) Amphibian Metamorphosis Assay (OECD 231).

2.10.3 Introduction

2.10.3.1 Purpose

This document summarises and evaluates all of the available evidence on dicamba relevant to the assessment of endocrine disruption, in accordance with EFSA-ECHA (2018) Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. Following an evaluation of the study reliability, relevance and significance, a weight of evidence assessment is conducted in order to establish whether the criteria are fulfilled.

2.10.3.2 Scientific Criteria in Accordance with Regulation (EC) No 1107/2009

Point 3.6.5 of Annex II to Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC states that, *"An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that 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 point (b) of Article 18(1) of Regulation (EC) No 396/2005.*" Consequently, scientific criteria for the determination of endocrine disrupting properties were developed on the basis of the Weybridge[19] and WHO/IPCS definitions[20].

Commission Regulation (EU) 2018/605 of 19 April 2018 amended Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties. The criteria state that an active substance, safener or synergist is to be considered as having endocrine disrupting properties that may cause adverse effects on humans, or non-target organisms, if all of the following criteria are met, unless it can be demonstrated that the adverse effects are not relevant to humans or (sub)populations for non-target organisms.

Annex II to Regulation (EC) No 1107/2009 (point 3.6.5) was amended to include the following criteria for endocrine disruption considered relevant humans:

(1) it shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.

(2) it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;

(3) the adverse effect is a consequence of the endocrine mode of action

Annex II to Regulation (EC) No 1107/2009 (point 3.8.2) was amended to include the following criteria for endocrine disruption in non-target organisms:

(1) it shows an adverse effect in non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;

(2) it has a an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;

(3) the adverse effect is a consequence of the endocrine mode of action

[19] *"an exogenous substance that causes adverse health effect(s) in an intact organism, or its progeny, secondary to changes in endocrine function"* Weybridge Report (EC 1998)

[20] "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*" WHO/IPCS (2002)

Commission Regulation (EU) 2018/605 stipulates that the identification of endocrine disruptors shall be based on all available relevant scientific data, and that the relevance, quality, consistency and coherence should be considered. Adverse effects that are non-specific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor.

2.10.3.3 EFSA-ECHA (2018) Guidance Document

In order to support Applicants and Competent Authorities, the European Commission asked the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) to develop guidance on how to identify endocrine disruptors in accordance with the criteria laid down in Regulation (EC) No 1107/2009. The Guidance Document describes how to gather, evaluate and consider all relevant information for the assessment, conduct a mode of action (MoA) analysis, and apply a weight of evidence (WoE) approach, in order to establish whether the criteria are fulfilled (EFSA-ECHA 2018).

In order to determine whether a substance causes adverse effect(s) that can be plausibly linked to endocrine activity, all relevant information needs to be collected, assessed and grouped in accordance with the guidance. The rationale for grouping is loosely based on OECD Guidance and the Joint Research Centre (JRC) screening methodology to identify potential disruptors of estrogenic, androgenic, thyroidal and steroidogenic (EATS) modalities (JRC 2016).

The OECD Guidance Document 150 lists the test guidelines and parameters that are considered relevant when investigating the ED properties of a substance (OECD 2018). In the context of this guidance, all the parameters listed by the OECD GD 150 (Table 2.10.1-1 and Table 1.2.3.1-2) are grouped into four groups:

- ***In vitro* mechanistic**: Parameters measured *in vitro* that provide information on the mechanism through which a substance could be considered endocrine active (OECD CF level 2).
- ***In vivo* mechanistic**: Parameters measured *in vivo* that provide information on endocrine activity that are usually not considered adverse (OECD CF level 3).
- **EATS mediated:** Parameters measured *in vivo* that may contribute to the evaluation of adversity, which may also be indicative of an EATS MoA (OECD CF level 4 and 5).
- **Sensitive to, but not diagnostic of EATS:** Parameters measured *in vivo* that may contribute to the evaluation of adversity, however, these effects cannot be considered diagnostic for any one of the EATS modalities.

1.1.1.1 Assessment strategy

The assessment strategy is based on the three conditions stipulated in the ED criteria (adversity, endocrine activity and a biologically plausible link between the two) and the grouping of the parameters described above, as recommended in the EFSA-ECHA (2018) Guidance. The assessment strategy is applicable to both humans and non-target organisms, and is illustrated in Figure 1.2.3.1-1. The remainder of this report is structured as follows:

Section 3: Gather information & assess the evidence
Section 4: Data reviews
Section 5: Integration and assessment of lines of evidence
Section 6: Initial analysis of the evidence (WoE)
Section 7: MoA analysis
Section 8: Conclusion on the ED criteria

Following an outline of the methodology (Section 3), the data reviews in Section 4 are organised around the OECD's Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals (Table 2.10.1-1 and Table 1.2.3.1-2). In accordance with the Guidance (EFSA-ECHA, 2018), data from the various Conceptual Framework levels have differing applications and implications, e.g. providing mechanistic information (Levels 2 and 3) or providing data on adverse effects on endocrine relevant endpoints (Levels 4 and 5). Section 5 integrates and assesses the lines of evidence, whereas Section 6 evaluates all of the available evidence in a weight of evidence assessment, considering the availability of "EATS mediated" parameters. Where EATS mediated parameters are not sufficiently investigated according to the EFSA-ECHA Guidance (2018), potential endocrine modalities and testing strategies are outlined in Section 7. Section 8 provides a conclusion on the ED criteria.

Each Section considers effects relevant to both human health and non-target organisms. It should be noted that non-EATS modalities and potential for endocrine disrupting properties in invertebrate organisms are not currently within the scope of the Guidance (EFSA-ECHA 2018).

Table 2.10.1-1 OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors

Level 1 Existing data and non-test information	• Physical & chemical properties, e.g., MW reactivity, volatility, biodegradability. • All available (eco) toxicological data from standardised or non-standardised tests. • Read-across, chemical categories, QSARs and other *in silico* predictions, and ADME model predictions.
Level 2 *In vitro* assays providing data about selected endocrine mechanism(s)/pathways(s)	• Estrogen (OECD TG493) or androgen receptor binding affinity (US EPA TG OPPTS 890.1150) • Estrogen receptor transactivation (OECD TG 455, ISO 19040-3), yeast estrogen screen (ISO 19040-1&2) • Androgen receptor transactivation (OECD TG 458) • Steroidogenesis *in vitro* (OECD TG 456) • Aromatase assay (US EPA TG OPPTS 890.1200) • Thyroid disruption assays (e.g. thyroperoxidase inhibition, transthyretin binding) • Retinoid receptor transactivation assays • Other hormone receptors assays as appropriate • High-throughput screens
Level 3 – Mammalian Species *In vivo* assays providing data about selected endocrine mechanism(s)/pathway(s)	• Uterotrophic assay (OECD TG 440) • Hershberger assay (OECD TG 441)
Level 4 – Mammalian Species *In vivo* assays providing data on adverse effects on endocrine relevant endpoints	• Repeated dose 28-day study (OECD TG 407) • Repeated dose 90-day study (OECD TG 408) • Pubertal development and thyroid function assay in peripubertal male rats (PP male assay) (US EPA TG OPPTS 890.1500) • Pubertal development and thyroid function assay in peripubertal female rats (PP female assay) (US EPA TG OPPTS 890.1450) • Prenatal development toxicity study (OECD TG 414) • Combined chronic toxicity and carcinogenicity studies (OECD TG 451-453) • Reproduction/developmental toxicity screening test (OECD TG 421) • Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) • Developmental neurotoxicity study (OECD TG 426) • Repeated dose dermal toxicity: 21/28-day study (OEDC TG 410) • Subchronic dermal toxicity: 90-day study (OECD TG 411) • 28-day (subacute) inhalation toxicity study (OECD TG 412) • Subchronic inhalation toxicity: 90-day study (OECD TG 413) • Repeated dose 90-day oral toxicity study in non-rodents (OECD TG 409)

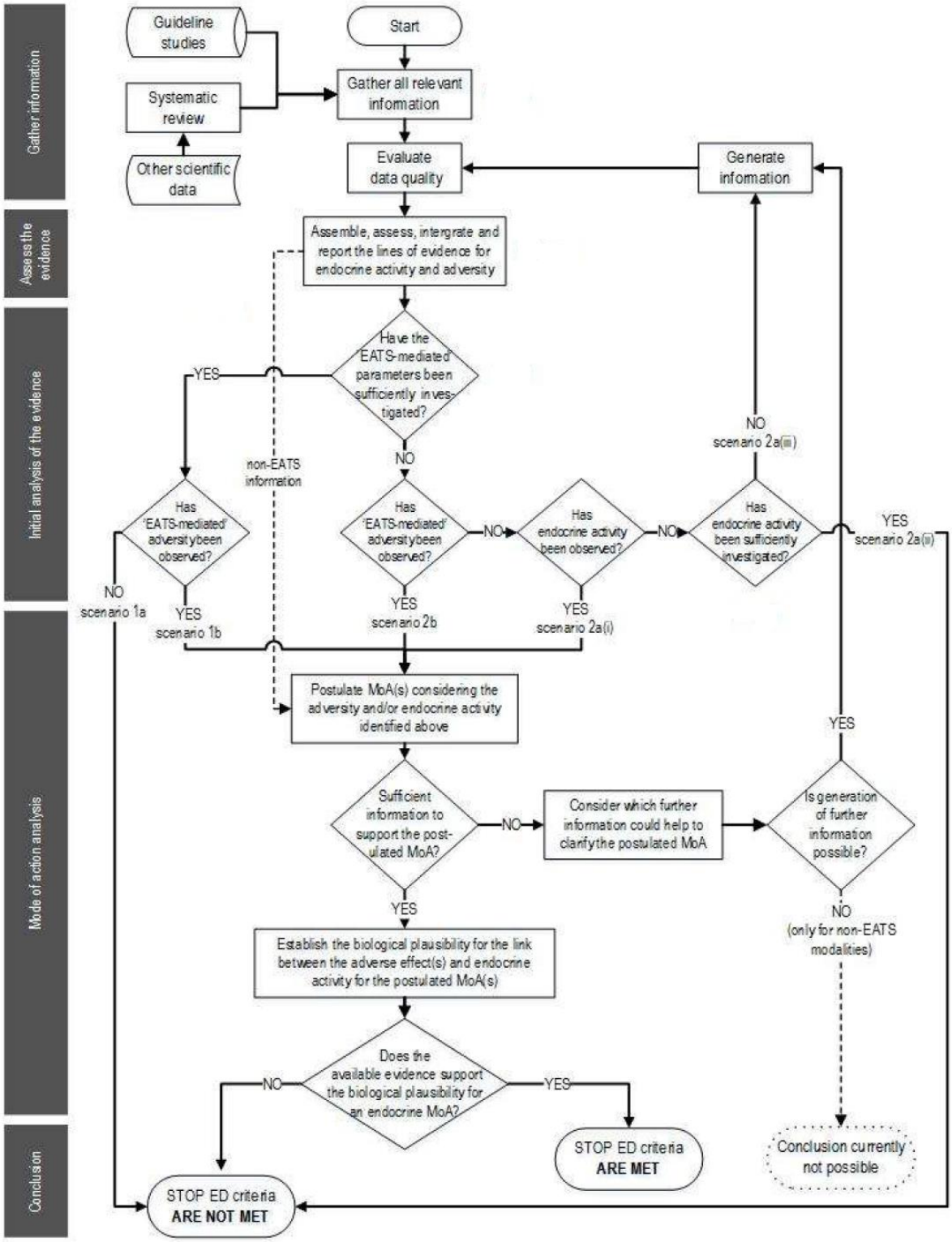
Level 5 – Mammalian Species *In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism	• Extended one-generation reproductive toxicity study (EOGRTS) (OECD TG 443) • Two-generation reproduction toxicity study (OECD TG 416, most recent update)

Table 1.2.3.1-2 OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors (Continued)

Level 3 – Non-Mammalian Species *In vivo* assays providing data about selected endocrine mechanism(s)/pathway(s)	• Amphibian metamorphosis assay (AMA) (OECD TG 231) • Fish short-term reproduction assay (FSTRA) (OECD TG 229) • 21-day fish assay (OECD TG 230) • Androgenised female stickleback screen (AFSS) (OECD GD 148) • EASZY assay. Detection of Substances Activing through Estrogen Receptor using Transgenic cyp19a1b GFP Zebrafish Embryos (When TG is available) • *Xenopus* embryonic thyroid signalling assay (XETA) (When TG is available) • Juvenile medaka anti-androgen screening assay (JMASA) (When GD is available) • Short-term juvenile hormone activity screening assay using *Daphnia magna* (When TG is available) • Rapid androgen disruption adverse outcome reporter (RADAR) assay (When TG is available)
Level 4 – Non-Mammalian Species *In vivo* assays providing data on adverse effects on endocrine relevant endpoints	• Fish sexual development test (FSDT) (OECD TG 234) • Larval amphibian growth & development assay (LAGDA) (OECD TG 241) • Avian reproduction assay (OECD TG 206) • Fish early life stage (FELS) toxicity test (OECD TG 210) • New guidance document on harpacticoid copepod development and reproduction test with *Amphiascus* (OECD GD 201) • *Potamopyrgus antipodarum* reproduction test (OECD TG 242) • *Lymnaea stagnalis* reproduction test (OECD TG 243) • Chironomid toxicity test (OECD TG 218-219) • *Daphnia magna* reproduction test (with male induction) (OECD TG 211) • Earthworm reproduction test (OECD TG 222)* • Enchytraeid reproduction test (OECD TG 220) • Sediment water *Lumbriculus* toxicity test using spiked sediment (OECD TG 225) • Predatory mite reproduction test in soil (OECD TG 226)* • Collembolan reproduction test in soil (TG OECD 232)* *: Studies performed on formulated product
Level 5 – Non-Mammalian Species *In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism	• Fish life cycle toxicity test (FLCTT) (US EPA TG OPPTS 850.1500) • Medaka extended one-generation reproduction test (MEOGRT) (OECD TG 240) • Avian two-generation toxicity test in the Japanese quail (ATGT) (US EPA TG OCSPP 890.2100/740-C-15-003) • Sediment water chironomid life cycle toxicity test (OECD TG 233) • *Daphnia* multigeneration test for assessment of EDCs (When TG is available) • Zebrafish extended one-generation reproduction test (ZEOGRT) (When TG is available)

Note: These lists are not exhaustive.

Figure 1.2.3.1-1 OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors (Continued)



2.10.4 Gather Information & Assess the Evidence

2.10.5 Gather Information

In this step all available relevant information is gathered both in terms of regulatory studies conducted in accordance with internationally agreed study protocols, and peer-reviewed published literature retrieved with systematic review methodology.

1.1.1.2 Regulatory studies

The available relevant regulatory *in vitro* toxicology studies submitted for dicamba are included in this review.

The relevant regulatory mammalian toxicology studies submitted for dicamba cover a range of study types including sub-acute, sub-chronic, chronic, developmental and reproductive toxicity studies in a range of mammalian species including rat, mouse, dog and rabbit.

The relevant regulatory non-mammalian toxicology studies submitted for dicamba cover a range of study types including chronic and reproductive toxicity studies in birds and fish.

1.1.1.3 Open scientific literature

A series of comprehensive searches of the open scientific literature were undertaken for the Annex 1 renewal submission (full details can be found in Section 9 of the MCA). Relevant and reliable data obtained from the published literature, but not identified in the systematic literature search for dicamba, are also included in this review where appropriate.

2.10.6 Assess the Evidence

Information shall be evaluated for its relevance and reliability. Evaluation of each of the relevant studies was based on the framework developed by the European Chemical Industry Council (CEFIC) Endocrine Modulators Steering Group (EMSG) for the weight of the evidence evaluation of potential endocrine disrupting substances (CEFIC, 1999). This framework consists of an independent assessment of a study's reliability and relevance, from which an overall assessment of the study's significance, relative to other studies using the same substance, is then derived**.**

1.1.1.4 Study reliability

Defined as '*the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give clear evidence of the clarity and plausibility of findings'* (Klimisch *et al*. 1997). In accordance with the EFSA-ECHA (2018) Guidance, the reliability of the studies was assessed based on the criteria described by Klimisch *et al*. (1997), Brown *et al*. (2001), and CEFIC (1999). Each study was assigned to one of four categories on the basis of compliance with the criteria, as follows:

1. **Reliable without restrictions** – studies conducted according to testing guidelines (preferably Good Laboratory Practice [GLP]) or in which all of the criteria are fully documented and reported.
2. **Reliable with restrictions** – studies that do not follow broadly accepted testing guidelines, but that document and report compliance with a substantial majority of the criteria.
3. **Not reliable** – studies in which there are notable deficiencies in scientific integrity (e.g. interferences between the measuring system and the test substance) or that document and report compliance with relatively few of the criteria.
4. **Not assignable** – usually reserved for abstracts, secondary literature, subject reviews or book reviews.

Klimisch reliability codes 1 and 2 are equivalent to CEFIC EMSG "High" and "Medium" confidence of repeatability. Klimisch reliability code 3 is equivalent to CEFIC EMSG "Low" confidence of repeatability.

1.1.1.5 Study relevance

Data relevance refers to the appropriateness of the data for the intended purpose of the assessment (EFSA 2015; Vermeire *et al*. 2013). Relevance assessment differentiates between the various endpoints reported to be influenced by endocrine disrupting substances on the basis of mechanistic evidence and observed effects. Some reported endpoints are more explicitly the consequence of an endocrine disrupting mechanism than others. Using the criteria developed by CEFIC EMSG it is possible to establish a hierarchy of endpoint relevance as follows:

- Observed adverse health effects with mechanistic support to establish causal linkage.
- Observed health effects with limited understanding of mechanism.
- Biomarker of exposure.
- Mechanistic potential with no observed effect.

CEFIC EMSG assigns the relevance of *in vitro* and *in vivo* studies as High, Medium or Low according to the criteria detailed in Table and Table 1.3.2.2-3, respectively. Note that these criteria are not exhaustive and in some cases (e.g. unusual study designs), relevance may be assigned according to different criteria.

Table 1.3.2.2-1 Relevance of *In Vitro* Assays According to CEFIC EMSG

Relevance	Description
High	• Endpoint is based upon receptor binding potential coupled with transcriptional activation in a whole cell or subcellular assay. • Endpoint is based on receptor binding potential in a whole cell assay. • Endpoint of steroid metabolism in a whole cell assay.
Medium	• Endpoint is based on receptor binding activity in a subcellular assay. • Endpoint is based on cell growth or other endpoint, not a direct measurement of receptor mediated activity. • Endpoint of steroid metabolism in a subcellular assay.
Low	• Not applicable; all *in vitro* assays are relevant to at least some extent by definition.

Table 1.3.2.2-3 Relevance of *In Vivo* Assays/Endpoints According to CEFIC EMSG

Relevance	Description
High	• Endpoint(s) in a multi-generational test or other repeat dose toxicity test that is specifically controlled by the endocrine system. • Parallel dose-response changes in hormone levels in the presence of consequent toxicological effects (mammalian only). • Negative data from a short term/screening assay specifically controlled by the endocrine system.
Medium	• Endpoint(s) in a multi-generation test or other repeat dose standard toxicity test, which may be influenced by the endocrine system, but is also known to be affected by other factors, e.g. toxicity.. • Positive endpoint data from a short-term/screening assay specifically controlled by the endocrine system. • Changes in hormone levels in the absence of any toxicological changes (mammalian only).
Low	• Evidence indicates that the endpoint is not controlled by the endocrine system.

In accordance with the EFSA-ECHA (2018) guidance, when evaluating the relevance of studies conducted according to outdated guidelines, it is important to consider what parameters relevant for identification of ED properties were included in the study design. Missing parameters with respect to the updated version of the test guidelines are clearly reported.

1.1.1.6 Study significance

According to the CEFIC EMSG framework, the "weight" or significance that should be assigned to a study is derived from a combination of its reliability/repeatability and relevance scores. It is a measure of the significance which can be ascribed to a study in reaching a conclusion about endocrine disruption. It is also the parameter which is ultimately used in the evaluation of the endocrine disrupting potential for the combined dataset for a particular substance. CEFIC EMSG assigns the significance of *in vitro* and *in vivo* studies as High, Indicative, Low or Unusable according to the criteria detailed in Table 1.3.2.3--1 and Table 1.3.2.3-, respectively. Note that

these criteria are not exhaustive and in some cases (e.g. unusual study designs), significance may be assigned according to different criteria.

Table 1.3.2.3-1 Significance of *In Vitro* Assays According to CEFIC EMSG

Significance	Description
Indicative[1]	• Studies of high relevance and with reliability scores of 1.
Low	• Studies of medium relevance and with reliability scores of 1 or 2. • Studies of high relevance and with reliability scores of 2.
Unusable	• Data from studies with reliability scores of 3 or 4.

[1] The CEFIC EMSG framework does not allow for *in vitro* studies to be classified as High significance. At best these can only be "indicative" of mechanistic potential. However, a negative result of "Indicative" significance is sufficient to be definitive for the mechanism being investigated.

Table 1.3.2.3-2 Significance of *In Vivo* Assays According to CEFIC EMSG

Significance	Description
High	• Repeat dose studies of high relevance and with reliability scores of 1 or 2.
Indicative	• Screening assay studies of high relevance and with reliability scores of 1 or 2. • Repeat dose studies of medium relevance and with reliability scores of 1 or 2.
Low	• Screening assay studies of medium relevance and with reliability scores of 1 or 2.
Unusable	• Data from studies with reliability scores of 3 or 4.

The final step in the CEFIC EMSG framework, and Section 4 of this document weighs the balance of evidence from the significance assessments of all the studies evaluated. This weight of the evidence evaluation is consistent with the general approach proposed in the EFSA-ECHA (2018) Guidance and OECD Guidance Document No. 150 (OECD, 2018).

2.10.7 DATA REVIEWS

This section assembles all the lines of evidence for endocrine activity and adversity.
Following the OECD Conceptual Framework and the four groupings specified in the EFSA-ECHA (2018) Guidance, the lines of evidence are organised according to their contribution to their assessment. The available data for dicamba has been compiled using the spread sheet recommended by the EFSA-ECHA (2018) Guidance (appendix E in that document) and is supplied alongside this report.

The available studies and references to appendix E Study Matrix IDs are provided in the table below.

Table 1.4. Outline of dataset considered for mammalian toxicology and ecotoxicology assessments

Type of toxicity	**Study type**	**Study ID Matrix**
In vitro mechanistic data (OECD CF level 2)	Devillers et al. (2015) QSAR model for assessment of estrogen, androgen, and thyroid hormone receptor binding	14
	Zhang et al. (2015) QSAR and *in vitro* transthyrethrin binding assay	15
	US EPA ToxCast Dashboard	16, 17
	Van Vugt-Lussenburg et al. (2014) CALUX screening for interaction with ERa, ERb, AR, PR, GR and TRb	18
Studies in mammalian species		
Repeated dose toxicity studies in mammals (OECD CF level 4)	Estes et al. (1979) 3-Week dermal toxicity study in the rabbit Equivalent to OECD 410 (1981)	3
	Rattray NJ (2002) 28 Day dermal toxicity study in the rat OECD 410 (1981)	4
	Ma-Hock L (2014) 28 Day inhalation toxicity study in the rat OECD 412 (2009)	5
	Doubovetzky M (1997) 13 Week dietary study in the rat OECD 408 (1981)	6
	Jackson et al. (2003) 13 Week capsule toxicity study in the dog OECD 409 (1998)	7
	Kubaszky R (2010) 13 Week capsule toxicity study in the dog OECD 409 (1998)	8
	Minnema D (1994) Subchronic neurotoxicity study in the rat Equivalent to OECD 424 (1997)	9
	Goldenthal EI (1979) 28 Day dietary toxicity study in the rat No guideline	25
Chronic and carcinogenicity toxicity studies in mammals (OECD CF level 4)	Blair M (1986) One year dietary toxicity study in the dog OECD 452 (1981)	10
	Crome et al. (1988) Dietary carcinogenicity study in the mouse OECD 451 (1981)	11

	Goldenthal EI (1985) Dietary combined chronic toxicity and carcinogenicity study in the rat OECD 453 (1981)	12
Developmental toxicity studies in mammals (OECD CF level 4)	Hobermann A (1992) Developmental toxicity study in the rabbit OECD 414 (1981)	1
	Smith and Page (1981) Developmental toxicity study in the rat OECD 414 (1981)	2
Reproductive toxicity studies in mammals (OECD CF level 5)	Masters RE (1993) Two generation reproductive toxicity study in the rat OECD 416 (1983)	13
Studies in non-mammalian species		
Available ecotoxicology data from standardized or non-standardised tests (OECD CF level 1)	Scheerbaum (1990) Prolonged toxicity test in Rainbow trout (OECD 204)	21
	Salinas (2011) Fish early life stage test in Fathead minnow (OECD 210)	22
	Minderhout et al. (2012) Fish early life stage test in Sheepshead minnow (OPPTS 850.1400)	23
	Zhu et al. (2013) Study on effects of dicamba on adult Chinese rare minnow Published in open scientific literature	24
Reproductive Toxicity in Birds (OECD CF level 4)	Beavers et al. (1994) Avian reproduction test in the Mallard duck (OECD 206)	19
	Beavers et al. (199b) Avian reproduction test in the Bobwhite quail (OECD 206)	20

2.10.8 *In Vitro* and *In Silico* Mechanistic Data

2.10.9 In silico data in OECD Conceptual Framework level 1

Reference: **1:** Judson RS *et al*., 2015. Integrated model of chemical perturbations of a biological pathway using 18 in vitro high-throughput screening assays for the estrogen receptor. *Toxicol. Sci.* **148(1)**: 137–154. File number: NA_14831

2: Browne P *et al*., 2015. Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model. *Environ. Sci. Technol.***49(14)**: 8804–8814. File number: NA_14873

These references are reported together as some data are duplicated across studies.

Guidelines: Not applicable.

GLP: No.

Study design: Results from 18 *in vitro* ER ToxCast™ high-throughput screening assays measuring ER binding, dimerization, chromatin binding, transcriptional activation, and ER-dependent cell proliferation were integrated into a computational model that can discriminate bioactivity from assay-specific interference and cytotoxicity. Model scores range from 0 (no activity) to 1 (bioactivity of 17β-estradiol).

The output from this model was compared to the known *in vivo* ER activity in the Uterotrophic assay for a range of reference compounds. The model output score accuracies exceeded 84% for the prediction of Uterotrophic study outcome.

Table 1.1.1.6-1 Summary of the 18 high-throughput *in vitro* ER Assays included in the ToxCast™ ER Bioactivity Model

Assay name	Biological process target	detection technology	organism	tissue	cell line
NVS_NR_bER	receptor binding	radioligand	bovine	uterus	NA
NVS_NR_hER	receptor binding	radioligand	human	NA	NA
NVS_NR_mERa	receptor binding	radioligand	mouse	NA	NA
OT_ER_ERaERa_0480	protein complementation	fluorescence	human	kidney	HEK293T
OT_ER_ERaERa_1440	protein complementation	fluorescence	human	kidney	HEK293T
OT_ER_ERaERb_0480	protein complementation	fluorescence	human	kidney	HEK293T
OT_ER_ERaERb_1440	protein complementation	fluorescence	human	kidney	HEK293T
OT_ER_ERbERb_0480	protein complementation	fluorescence	human	kidney	HEK293T
OT_ER_ERbERb_1440	protein complementation	fluorescence	human	kidney	HEK293T
OT_ERa_EREGFP_0120	protein production	fluorescence	human	cervix	HeLa
OT_ERa_EREGFP_0480	protein production	fluorescence	human	cervix	HeLa
ATG_ERa_TRANS_up	mRNA induction	fluorescence	human	liver	HepG2
ATG_ERE_CIS_up	mRNA induction	fluorescence	human	liver	HepG2
Tox21_ERa_BLA_Agonist_ratio	protein production	fluorescence	human	kidney	HEK293T
Tox21_ERa_LUC_BG1_Agonist	protein production	bioluminescence	human	ovary	BG1
ACEA_T47D_80h_Positive	cell proliferation	electrical impedance	human	breast	T47D
Tox21_ERa_BLA_Antagonist_ratio	protein production	fluorescence	human	kidney	HEK293T
Tox21_ERa_LUC_BG1_Antagonist	protein production	bioluminescence	human	ovary	BG1

Results: Dicamba had a score of 0 for both agonistic and antagonistic activity and is thus considered to have no ER bioactivity.

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**High/Medium** (Endpoint is based on simulated ER pathway stimulation in an *in silico* model). Note: The CEFIC EMSG does not give criteria for relevance of *in silico* data. Relevance has been assigned in line with the criteria for *in vitro* data.
Overall significance	**Low – No evidence of effects relevant to the assessment of endocrine disruption.**

Reference:	Kleinstreuer NC *et al.*, 2017. Development and validation of a computational model for androgen receptor activity. *Chem. Res. Toxicol*., **30 (4)**: 946–964. File number: NA_14876

Guidelines: Not applicable.

GLP: No.

Study design: Eleven high throughput screening (HTS) ToxCast™/Tox21 *in vitro* assays were integrated into a computational network model to distinguish true AR pathway activity from technology-specific assay interference. The *in vitro* HTS assays probed perturbations of the AR pathway at multiple points (receptor binding, coregulator recruitment, gene transcription, and protein production) and multiple cell types. Confirmatory *in vitro* antagonist assay data and cytotoxicity information were used as additional flags for potential nonspecific activity.

The output from this model was compared to the known *in vivo* AR activity in the Hershberger assay for a range of reference compounds. The model output score showed accuracies of 95.2% for the outcome of Hershberger assays run in agonism mode and 97.5% for Hershberger assays run in antagonism mode.

Table 1.4.1.1-2 Tox21/ToxCast™ *in vitro* assays used in AR Pathway Model

assay name	source	gene	species	type
NVS_NR_hAR	Novascreen	AR	*Homo sapiens*	receptor binding
NVS_NR_cAR	Novascreen	AR	P. troglodytes	receptor binding
NVS_NR_rAR	Novascreen	AR	*Rattus norvegicus*	receptor binding
OT_AR_ARSRC1_0480	Odyssey Thera	AR; SRC	*Homo sapiens*	coregulator recruitment
OT_AR_ARSRC1_0960	Odyssey Thera	AR; SRC	*Homo sapiens*	coregulator recruitment
ATG_AR_TRANS	Attagene	AR	*Homo sapiens*	RNA reporter gene
OT_AR_ARELUC_AG_1440	Odyssey Thera	AR; ARE	*Homo sapiens*	reporter gene
Tox21_AR_BLA_Agonist_ratio	NCATS/NCGC	AR	*Homo sapiens*	reporter gene
Tox21_AR_LUC_MDAKB2_Agonist	NCATS/NCGC	AR	*Homo sapiens*	reporter gene
Tox21_AR_BLA_Antagonist_ratio	NCATS/NCGC	AR	*Homo sapiens*	reporter gene
Tox21_AR_LUC_MDAKB2_Antagonist	NCATS/NCGC	AR	*Homo sapiens*	reporter gene
Tox21_AR_LUC_MDAKB2_Antagonist-confirmation	NCATS/NCGC	AR	*Homo sapiens*	reporter gene

Results: Dicamba was predicted to be inactive as an AR agonist or antagonist with AUC values of 0 for both pathways.

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**High/Medium** (Endpoint is based on simulated AR pathway stimulation in an *in silico* model). Note: The CEFIC EMSG does not give criteria for relevance of *in silico* data. Relevance has been assigned in line with the criteria for *in vitro* data.
Overall significance	**Low – No evidence of effects relevant to the assessment of endocrine disruption.**

Reference: Devillers J, Bro E, Millot F (2015). Prediction of the endocrine disruption profile of pesticides. *SAR and QSAR in Environ. Res., 26:10 831-852*. File number: NA_13813

Guidelines: Not applicable.

GLP: No.

Study design: The ability of dicamba to bind and act as an agonist/antagonist of androgen receptor (AR), oestrogen receptor α (ERα), oestrogen receptor β (ERβ), thyroid hormone receptor α (TRα) and thyroid hormone receptor β (TRβ) was predicted using an *in silico* molecular docking approach. The authors provide limited information on the methodology, protein preparation or protocol generation (i.e. docking target). Predicted binding potentials were scored 1 to 4, with 1 representing a low probability of binding and 4 representing a high probability of binding. The degree of inappropriate penetration into the docking site (i.e. crash score) was not considered, the sensitivity and specificity of the models were not detailed, and bootstrap analysis was not conducted.

Binding affinities with receptors not directly involved with the endocrine system were also estimated. These data are outside the scope of this review and are not discussed further.

Results:

Receptor:	**AR**	**ARa***	**ERα**	**ERαa***	**ERβ**	**ERβa***	**TRα**	**TRβ**
Score:	1	2	1	1	1	1	1	1

*: 'a' denotes antagonist mode

Overall, the results of these *in silico* predictions indicate that dicamba has a low potential to interact with the estrogen (α, β) receptors, androgen receptor and thyroid (α, β) receptors. It is important to note that these scores reflect theoretical binding potential, calculated via *in silico* docking to protein structures and are of questionable relevance to *in vitro* and *in vivo* activity. X-ray crystallography selectively favours the protein confirmations most likely to crystalise. Consequently, most structures are ligand-bound dimers (LBD) with associated cofactors, rather than monomeric ligand binding domains stabilised by heat-shock proteins (HSP). Thus, cofactors and ligands should be removed and the protein structure optimised for physiological pH. The authors also failed to minimise and prepare the database for screening, which can lead to docking performance scores worse than random (Jain 2007; Peng *et al*. 1996).

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**Medium** (Endpoint is based on simulated receptor binding potential in an *in silico* model). Note: The CEFIC EMSG does not give criteria for relevance of *in silico* data. Relevance has been assigned in line with the criteria for *in vitro* data.
Overall significance	**Low – No evidence of effects relevant to the assessment of the A and T pathways.**

2.10.10 In vitro data in OECD Conceptual Framework level 2

Reference:	Zhang J, Kamstra JH, Ghorbanzadeh M, Weiss JM, Hamers T, Andersson PL (2015). In Silico Approach To Identify Potential Thyroid Hormone Disruptors among Currently Known Dust Contaminants and Their Metabolites. *Environ. Sci. Technol., 49:10099−10107,* Syngenta File No. NA_13814

Guidelines: Not applicable

GLP: No

Study design: The potential for dicamba as a thyroid hormone disrupting chemical (THDC) was examined using a computational quantitative structure-activity relationship (QSAR) model and an *in vitro* model, a competitive [^{125}I]-T4- hormone transporter transthyretin (TTR) binding assay.

Results: Dicamba was predicted to bind to TTR in the QSAR Model but subsequently tested negative in the radio-ligand TTR binding assay.

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**Medium** (Endpoints are based on receptor binding/ potential in subcellular assay)
Overall significance	**Indicative – No evidence of effects relevant to the assessment of endocrine disruption**

Reference:	US EPA, Computational Toxicology Dashboard. Accessed online at https://comptox.epa.gov/dashboard in 2019

Guidelines: None cited

GLP: No

Study design: The US EPA Computational Toxicology online Dashboard was queried with the keyword "dicamba". The Computational Toxicology Dashboard contains a large quantity of data ranging from high throughput assays (HTS), summaries of regulatory toxicology studies, and US EPA risk assessment endpoints. In order to extract the relevant OECD conceptual framework level 2 *in vitro* assays for this review "EDSP21" data was selected from the "Bioactivity" module.

Estrogenic activity: Twenty-two HTS assays examining estrogenic activity are available. Dicamba was inactive in all assays in the absence of cytotoxicity, indicating no potential for dicamba to interact with the estrogen receptor (Table 4.1.2-1)

Table 1.4.1.2-1 Summary of US-EPA ToxCast™ estrogenic screening data for dicamba

Assay component endpoint name	Assay type	AC50 (µM)	Cytotoxicity z-score	Flags
ACEA_ER_80hr	real-time cell-growth kinetics	Inactive	NA	NA
ATG_ERE_CIS_dn	mRNA induction	Inactive	NA	NA
ATG_ERE_CIS_up	mRNA induction	Inactive	NA	NA
ATG_ERa_TRANS_dn	mRNA induction	Inactive	NA	NA
ATG_ERa_TRANS_up	mRNA induction	Inactive	NA	NA
NVS_NR_bER	radioligand binding	Inactive	NA	NA
NVS_NR_hER	radioligand binding	Inactive	NA	NA
OT_ER_ERaERa_0480	protein fragment complementation assay	Inactive	NA	NA
OT_ER_ERaERa_1440	protein fragment complementation assay	Inactive	NA	NA
OT_ER_ERaERb_0480	protein fragment complementation assay	Inactive	NA	NA
OT_ER_ERaERb_1440	protein fragment complementation assay	Inactive	NA	NA

Assay component endpoint name	Assay type	AC50 (µM)	Cytotoxicity z-score	Flags
OT_ER_ERbERb_0480	protein fragment complementation assay	Inactive	NA	NA
OT_ER_ERbERb_1440	protein fragment complementation assay	Inactive	NA	NA
OT_ERa_EREGFP_0120	fluorescent protein induction	Inactive	NA	NA
OT_ERa_EREGFP_0480	fluorescent protein induction	Inactive	NA	NA
TOX21_ERa_BLA_Agonist_ratio	beta lactamase induction	Inactive	NA	NA
TOX21_ERa_BLA_Antagonist_ratio	beta lactamase induction	Inactive	NA	NA
TOX21_ERa_LUC_VM7_Agonist	luciferase induction	Inactive	NA	NA
TOX21_ERa_LUC_VM7_Antagonist_0.1nM_E2	luciferase induction	Inactive	NA	NA
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	luciferase induction	Inactive	NA	NA
TOX21_ERb_BLA_Agonist_ratio	beta lactamase induction	Inactive	NA	NA
TOX21_ERb_BLA_Antagonist_ratio	beta lactamase induction	Inactive	NA	NA

Androgenic activity: Fourteen HTS assays examining androgenic activity are available. Dicamba was inactive in all assays in the absence of cytotoxicity, indicating no potential for dicamba to interact with the androgen receptor (Table 4.1.2-2).

Table 1.4.1.2-2 Summary of US-EPA ToxCast™ androgen receptor screening data for dicamba

Assay component endpoint name	Assay type	AC50 (µM)	Cytotoxicity z-score	Flags
ACEA_AR_agonist_80hr	real-time cell-growth kinetics	Inactive	NA	NA
ATG_AR_TRANS_dn	mRNA induction	Inactive	NA	NA
ATG_AR_TRANS_up	mRNA induction	Inactive	NA	NA
NVS_NR_cAR	radioligand binding	Inactive	NA	NA
NVS_NR_hAR	radioligand binding	Inactive	NA	NA
NVS_NR_rAR	radioligand binding	Inactive	NA	NA
OT_AR_ARELUC_AG_1440	luciferase induction	Inactive	NA	NA
OT_AR_ARSRC1_0480	protein fragment complementation assay	Inactive	NA	NA
OT_AR_ARSRC1_0960	protein fragment complementation assay	Inactive	NA	NA
TOX21_AR_BLA_Agonist_ratio	beta lactamase induction	Inactive	NA	NA
TOX21_AR_BLA_Antagonist_ratio	beta lactamase induction	Inactive	NA	NA
TOX21_AR_LUC_MDAKB2_Agonist	luciferase induction	Inactive	NA	NA

Assay component endpoint name	Assay type	AC50 (µM)	Cytotoxicity z-score	Flags
TOX21_AR_LUC_MDAKB2_ Antagonist_0.5nM_R1881	luciferase induction	Inactive	NA	NA
TOX21_AR_LUC_MDAKB2_ Antagonist_10nM_R1881	luciferase induction	Inactive	NA	NA

Thyroid activity: Ten thyroid HTS assays are available. Dicamba was inactive in all of these assays Dicamba was not determined to interact with the thyroid hormone receptor (Table 4.1.2-3).

Table 1.1.1.6-1 Summary of US-EPA ToxCast™ thyroid screening data for dicamba

Assay component endpoint name	Assay type	AC50 (µM)	Cytotoxicity z-score	Flags
ATG_THRa1_TRANS_dn	mRNA induction	Inactive	NA	NA
ATG_THRa1_TRANS_up	mRNA induction	Inactive	NA	NA
NCCT_TPO_AUR_dn	enzyme activity	Inactive	NA	NA
NIS_RAIU_inhibition	enzyme activity	Inactive	NA	NA
NVS_NR_hTRa_Antagonist	immunoassay: elisa	Inactive	NA	NA
TOX21_TR_LUC_GH3_Ago-nist	luciferase induction	Inactive	NA	NA
TOX21_TR_LUC_GH3_Antag-onist	luciferase induction	Inactive	NA	NA
TOX21_TSHR_Agonist_ratio	cAMP measurement	Inactive	NA	NA
TOX21_TSHR_Antagonist_ra-tio	cAMP measurement	Inactive	NA	NA
TOX21_TSHR_wt_ratio	cAMP measurement	Inactive	NA	NA

Aromatase activity: One aromatase HTS assays are available. Dicamba was inactive in this assay (Table 4.1.2-4).

Table 1.1.1.6-2 Summary of US-EPA ToxCast™ aromatase screening data for dicamba

ToxCast™ Assay Identifier	Result	AC50	Flags
TOX21_Aromatase_Inhibition	Inactive	NA	NA

Reliability score	**2:** Reliable with restrictions
Relevance score	**High** –Whole cell assays **Medium** – Cell free assays
Overall significance	**Indicative – No evidence of an effect relevant to the assessment of endocrine disruption**

Reference:	Van Vugt-Lussenburg BMA, Pieterse B, Middelhof I, Behnisch PA, van der Burg B and Bram Brouwer (2014). The "dirty dozend" Pops & other pollutants: toxicological profiling by CALUX panel, *Organohalogen Compounds.*, **76**:1071−1073. File number: NA_14243

Guidelines: Not applicable

GLP: No

Study design: 150 reference compounds with known toxicological properties were tested in a high trough put screening assay. The pesticides tested were selected from the ToxCast program. The used Chemical Activated LUciferase gene eXpression (CALUX) assay is a stable reporter gene assay using U20S cell lines expressing either different receptors, among those the following endocrine-related receptors: ERa, ERb, AR, PR, GR and TRb. Cells were treated in triplicates with 2% of a test compound dilution series (16 individual concentrations) in DMSO. Positive and negative controls were included on each plate. After 24h exposure, the exposure medium was removed, cells were lysed and the luciferase signal was measured. Results were calculated as PC10 values compared to the reference compound activity.

Results: Of the different cell lines tested, dicamba only showed an effect in ERα expressing cells. The value calculated was -5.5.

The publication has severe deficiencies in the description of the method and the presentation and discussion of the results. The authors do not give any details on the origin of the test material or the concentration tested. The positive and negative controls used are not described. The results presented do not indicate the units of the result calculated. The results for the controls are not presented. The data presented are not discussed in detail. No conclusion on the comparability of the results of the ToxCast program are made.

CONCLUSIONS
This publication was judged as unreliable but included for completeness into this review.

Reliability score	**3:** Unusable
Relevance score	**Medium** (Endpoints are based on receptor binding/ potential in subcellular assay)
Overall significance	**Indicative – No evidence of effects relevant to the assessment of endocrine disruption**

Reference:	Karmaus AL *et al.*, 2016. High-Throughput Screening of Chemical Effects on Steroidogenesis Using H295R Human Adrenocortical Carcinoma Cells. *Toxicol. Sci.* 150(2):323-32, File number: NA_14616

Guidelines: Study adopted from and broadly in compliance with the OECD guidance reference 456.

GLP: No.

Study design: A high-throughput assay using H295R human adrenocortical carcinoma cells was used to evaluate the effect of 2060 chemical samples, including dicamba, on steroidogenesis via high-performance liquid chromatography followed by tandem mass spectrometry quantification of ten steroid hormones, including progestagens, glucocorticoids, androgens, and oestrogens. The study employed a three-stage screening strategy. The first stage established the maximum tolerated concentration (MTC ≥ 70% viability) per sample. The second stage quantified changes in hormone levels at a single concentration at either the MTC or at 100 µM, whichever was lower. For compounds eliciting a change in steroid hormone biosynthesis (defined as >1.5-fold change up or down vs. negative control DMSO values) for more than four hormones, a concentration-response (CR) was determined. At all stages, cells were prestimulated with 10 mM forskolin for 48 hours to induce steroidogenesis followed by chemical treatment for 48 h.

Results: Dicamba was tested up to a concentration of 100µM and called negative for all endpoints tested in the absence of relevant cytotoxicity.

Table 1.1.1.6-5 Summary of steroidogenesis results for dicamba (Karmaus AL *et al.*, 2016)

Assay name	Result	Flag
CEETOX_H295R_11DCORT_dn	Negative	NA
CEETOX_H295R_11DCORT_up	Negative	NA
CEETOX_H295R_ANDR_dn	Negative	NA
CEETOX_H295R_ANDR_up	Negative	NA
CEETOX_H295R_CORTISOL_dn	Negative	NA
CEETOX_H295R_CORTISOL_up	Negative	NA
CEETOX_H295R_DOC_dn	Negative	NA
CEETOX_H295R_DOC_up	Negative	NA
CEETOX_H295R_ESTRADIOL_dn	Negative	NA
CEETOX_H295R_ESTRADIOL_up	Negative	NA
CEETOX_H295R_ESTRONE_dn	Negative	NA
CEETOX_H295R_ESTRONE_up	Negative	NA
CEETOX_H295R_OHPREG_dn	Negative	NA
CEETOX_H295R_OHPREG_up	Negative	NA
CEETOX_H295R_OHPROG_dn	Negative	NA
CEETOX_H295R_OHPROG_up	Negative	NA
CEETOX_H295R_PROG_dn	Negative	NA
CEETOX_H295R_PROG_up	Negative	NA
CEETOX_H295R_TESTO_dn	Negative	NA
CEETOX_H295R_TESTO_up	Negative	NA

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**High** (Steroid metabolism in whole cell assay)
Overall significance	**Low – No evidence of effects relevant for the assessment of the S pathway**

Reference: Paul Friedman K *et al.*, 2016. Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries. *Toxicol. Sci.* **151(1):** 160-180. File number: NA_14874

Guidelines: Not applicable.

GLP: No.

Study design: The ToxCast^{TM} phase I and II chemical libraries, comprised of 1074 unique chemicals and including dicamba, were initially screened using rat thyroid microsomes to identify potential thyroperoxidase (TPO) inhibitors. Chemicals positive in a first single-concentration screen were retested in concentration-response. Due to high false-positive rates typically observed with loss-of-signal assays such as AUR-TPO, two additional assays were employed in parallel to identify possible sources of nonspecific assay signal loss, enabling stratification of roughly 300 putative TPO inhibitors based upon selective AUR-TPO activity. A cell-free luciferase inhibition

assay was used to identify nonspecific enzyme inhibition among the putative TPO inhibitors, and a cytotoxicity assay using a human cell line was used to estimate the cellular tolerance limit. Additionally, the TPO inhibition activities of 150 chemicals were compared between the AUR-TPO and an orthogonal peroxidase oxidation assay using guaiacol as a substrate to confirm the activity profiles of putative TPO inhibitors.

Results: Dicamba was tested at a single concentration and was scored negative based on less than 20% decrease in maximal TPO activity, which was the threshold used to define a positive hit response.

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**Medium** (Enzyme activity in a subcellular assay)
Overall significance	**Low - No evidence of effects relevant for the assessment of endocrine disruption**

Reference: *Wang J et al., 2018. High-Throughput Screening and Quantitative Chemical Ranking for Sodium-Iodide Symporter Inhibitors in ToxCast Phase I Chemical Library. Environ. Sci. Technol. 52* **(9):** 5417–5426, File number: NA_14880

Guidelines: Not applicable.

GLP: No.

Study design: This study applied a previously validated high-throughput approach to screen for sodium-iodide symporter (NIS) inhibitors in the ToxCast™ phase I library, representing 293 important environmental chemicals. 310 blinded samples, including dicamba, were screened in a tiered-approach using an initial single-concentration (100 μM) radioactive-iodide uptake (RAIU) assay in hNIS-HEK293T-EPA cells, followed by 169 samples further evaluated in multi-concentration (0.001 μM–100 μM) testing in parallel RAIU and cell viability assays. A novel chemical ranking system that incorporates multi-concentration RAIU and cytotoxicity responses was also developed as a standardized method for chemical prioritization in current and future screenings.

Results: Dicamba was screened at a single concentration and was scored negative based on a threshold of less than 20% NIS inhibition in the RAIU assay.

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**High** (Enzyme inhibition in a whole cell assay)
Overall significance	**Low - No evidence of effects relevant for the assessment of endocrine disruption**

Reference: *Hornung MW et al., 2018. Screening the ToxCast Phase 1 Chemical Library for Inhibition of Deiodinase Type 1 Activity. Toxicol. Sci. 162 (2): 570–581*, File number: NA_14882

Olker JH *et al.*, 2019. Screening the ToxCast Phase 1, Phase 2, and e1k Chemical Libraries for Inhibitors of Iodothyronine Deiodinases. *Toxicol. Sci. 168(2):430-442.* File number:. NA_14886

Guidelines: Not applicable.

GLP: No.

Study design: Over 1800 unique chemicals, including dicamba, were screened *in vitro* for potential enzyme inhibition using HEK293 cell lysate with adrenoviral expressed DIO1, DIO2 and DIO3, respectively. Compounds were initially tested at a single concentration; chemicals produced enzyme inhibition of 50% or greater were further tested in concentration-response to determine relative potency. These references are reported together, because they are in parts redundant.

Results: Dicamba was tested at a single concentration of 200μM and was inactive in all three DIO assays.

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**Medium** (Enzyme activity in a subcellular assay)
Overall significance	**Low - No evidence of effects relevant for the assessment of endocrine disruption**

2.10.11 *In Vivo* Mechanistic Data – Mammalian Species

2.10.12 Short term mechanistic studies in OECD Conceptual Framework level 3

No *in vivo* mechanistic data in OECD conceptual framework level 3 was identified for inclusion in this review.

2.10.13 *In Vivo* Data – Mammalian Species

1.1.1.7 Short term studies in OECD Conceptual Framework level 4

Report:	Ma-Hock L (2014). BAS 183 H (Dicamba techn.): Repeated dose 28-day inhalation toxicity study in Wistar rats, dust. BASF DocID 2014/1170794. BASF SE Experimental Toxicology and Ecology 67056 Ludwigshafen, Germany. File number: SAN837_11498

Guidelines: 412 (2009)

GLP: Yes

Study design: 10 male and 10 female Crl: WI (Han) rats per group were head-nose exposed to dust atmospheres on 6 hours per day, on 5 consecutive days per week for 4 weeks (20 exposures). The target concentrations were 1, 5 and 50 mg/m^3 test substance in air. A concurrent control group was exposed to conditioned air as air control.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weight: Adrenal glands, epididymides, ovaries, testes, thymus, thyroid glands and uterus
- Histopathological evaluation: Adrenal glands, epididymides, mammary gland, ovaries, pituitary gland, prostate, seminal vesicle, testes, thyroid/parathyroid and uterus with cervix.

Deviations from the current guideline:
OECD 412 guideline was revised in 2018 to accommodate the testing of particle aerosols including nanomaterials. There were no additional endocrine specific endpoints added to the 2018 version, so the update has no impact for endocrine disruption endpoints.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Rattray NJ (2002). Dicamba Tech. (SAN 837 Tech.): 28-Day dermal toxicity study in rats. CTL report number: CTL/LR0594/REG/REPT. Central Toxicology Laboratory Alderley Park, Macclesfield, Cheshire, UK. File number: SAN837/6040

Guidelines: OECD 410 (1981)

GLP: Yes

Study design: 10 male and 10 Alpk: APfSD (Wistar-derived) female rats per dose group received a dermal application of dicamba at 0, 30, 300, or 1000 mg/kg bw/day for 6 hours/day, 21 days in the 28 days period. Dicamba was mixed with deionized water to form a paste and applied to the clipped dorsal skin on at least 10% of body surface of the animals with a secured gauze batch. After 6 hours, application sites were cleaned with warm water.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations

- Organ weight: Adrenal glands, epididymides, ovaries, testes and uterus with cervix
- Histopathological evaluation: Adrenal glands, epididymides, mammary gland, ovaries, pituitary gland, prostate, seminal vesicle, testes, thyroid/parathyroid and uterus with cervix.

Deviations from the current guideline:
None.

Effects on endpoints relevant for assessment of potential for endocrine disruption:
None.

A lesion in the adrenal gland (necrosis /fibrosis/ vacuolation /pigmentation) was recorded in three males in the 1000 mg/kg/day dose group. However, a similar reaction was also seen in one male given 300 mg/kg, one female given 30 mg/kg/day and one female of the control group. These results were not deemed to be related to treatment and were not reproduced in any other short or long-term study in rat or any other species tested. Therefore, these findings are considered to be normal biological variation and do not reflect an interaction of dicamba with the endocrine system.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Estes FL, Dean WP, Blair M, Goldenthal EI (1979). Banvel Technical: 3-Week dermal toxicity study in rabbits. Vesicol chemical cooperation report number: 17666. Vesicol Chemical Corporation, Mattawan, Michigan, U.S.A. File number: SAN837/5078

Guidelines: Broadly equivalent to OECD 410

GLP: Yes

Study design: 4 male and 4 female New Zealand White rabbits per dose group received a dermal application of dicamba tech. at 0, 100, 500, and 2500 mg/kg bw/day for 6 hours/day, 5 days/week. Dosages were adjusted based upon weekly bodyweight. Dicamba tech. was mixed with 0.9% saline solution to form a paste and applied to the clipped dorsal skin on at least 10% of body surface of the animals. After 6 hours, application sites were cleaned. The skin of 2 males and 2 females per group was abraded twice weekly. Body weight and food consumption were recorded weekly.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weights: adrenals, testes, ovaries, thyroid, parathyroid
- Histopathological evaluation (highest dose group only): Adrenal glands, ovaries, pituitary gland, prostate, testes, thyroid/parathyroid, uterus

Deviations from the current guideline:
OECD 410 specifies that 5 animals per sex per dose are used, however this study only used 4 animals per sex per group.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

There was a statistically significant increase in absolute adrenals weight in the female 100 mg/kg/day group; however, in the absence of compound related morphologic lesions in the adrenals or a dose response, this weight variation was not considered toxicologically significant.

CONCLUSIONS

Reliability score	**2**: Reliable with restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Doubovetzky M (1997). Dicamba TC: 13-week feeding study in rats (including 4-week recovery). Novatis Crop Protection report number: 97/059. Novartis Protection AAG, Basel, Switzerland. File number: SAN837/0010

Guidelines: OECD 408 (1981)

GLP: Yes

Study design: Dicamba tech. was administered to groups of 10 male and 10 female HanIbm: WIST (Wistar) rats at dietary concentrations of 0, 500, 3000, 6000 and 12000 ppm (mg/kg) for 13 weeks. 10 additional rats/sex in each of the control and high dose groups were permitted a 28-day recovery period following the 13-week treatment period.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weights: Adrenal glands, ovaries and testes
- Histopathological evaluation: Adrenal glands, epididymides, mammary area, ovaries, pituitary gland, prostate, seminal vesicles, testes, thyroid/parathyroid, uterus and vagina.

Deviations from the current guideline:
OECD test guideline 408 was revised in 2018 to include additional parameters which may be sensitive to perturbation of the endocrine system. The following parameters would be expected in a study conducted to the current OECD test guideline but were not assessed in this study: assessment of the organ weight of the epididymides, the prostate including the seminal vesicles with coagulating glands as a whole complex, uterus, pituitary gland and thyroid gland; vaginal smears (oestrus cycle determination at necropsy); serum/plasma analyses of thyroid hormones (Thyroxine, TSH, T3), LDL and HDL cholesterol.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Minnema D. (1994). Subchronic Neurotoxicity Study of Dietary Technical Dicamba in Rats. HWA laboratory project number: 686-178. Hazelton Washington Inc. 9200 Leesburg Pike, Vienna, Virginia 22182. File number: SAN837/5210

Guidelines: Subchronic neurotoxicity study – equivalent to OECD 424 (1997)

GLP: Yes

Study design: Dicamba was administered orally via diet to SD rats (10/sex/dose) at dose levels of 0, 300, 600 and 12000 ppm for 13 weeks. The mean consumption during the 13-week study was 197.1, 401.5 and 767.9 mg/kg/day for males and 253.4, 472.0 and 1028.9 mg/kg/day for females.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Histopathological evaluation: Pituitary gland

Deviations from the current guideline:
None.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Jackson AM, Hansen R, Chevalier H-J (2003). SAN 837 tech.; 13-Week oral (capsule) toxicity study in the dog. RCC study report number: 826795. RCC Ltd, Toxicology division, CH-4452 Itingen, Switzerland. File number: SAN837/6130

Guidelines: OECD 409 (1998)

GLP: Yes

Study design: Dicamba was administered orally (capsule) to groups of Beagle dogs at dose levels of 0, 10, 50 or 300 mg/kg bw/day for 13 weeks followed by a four week recovery period in some dogs. The four groups contained 4 male and 4 female dogs and the control and 300 mg/kg group also contained an additional 4 males and 4 females which were retained after the treatment period for the 4-week recovery period. The test substance was weighed

directly into gelatine capsules in accordance the most recently recorded body weight for each animal. The control animals received empty capsules.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weights: Adrenal glands, ovaries, testes with epididymides, thyroid gland with parathyroid and uterus
- Histopathological evaluation: Adrenal glands, epididymides, mammary gland area, ovaries, pituitary gland, prostate gland, testes, thyroid/parathyroid, uterus with vagina.

Deviations from the current guideline:
None.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Raluca Kubaszky (2010). RC1176: 90-Day Oral Capsule Toxicity Study in Beagle Dogs. LAB Research Ltd study code: 10/037-101K. LAB Research Ltd, 8200 Veszprém, Szabadságpuszta, Hungary.

Guidelines: OECD 409 (1998)

GLP: Yes

Study design: Dicamba was administered orally (capsule) to groups of dogs at dose levels of 0, 10, 50 or 300 mg/kg bw/day for 90 days. The four groups contained 4 male and 4 female dogs. Capsule filling was performed shortly prior to treatment and stored at room temperature pending administration to animals. The test item used to fill the capsule was calculated and adjusted based on the animal's most recent body weight. The control animals received empty capsules.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weights: Adrenal glands, ovaries, testes, thyroid gland with parathyroid, pituitary, prostate and uterus
- Histopathological evaluation: Adrenal glands, epididymides, mammary gland (inguinal), ovaries, pituitary gland, prostate, testes, thyroid/parathyroid, uterus and vagina.

Deviations from the current guideline:
None.

Effects on endpoints relevant for assessment of potential for endocrine disruption

None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Goldenthal EI (1979), Banvel: 4-Week range-finding study in rats. International Research and Development Corporation, Mattawan Michigan, USA. Syngenta Unpublished Report No 163-670. Syngenta File No. SAN837/5088

Guidelines: None.

GLP: No (study performed before the implementation of GLP)

Study design: Dicamba technical was administered in the diet for 28 days at levels of 0, 5000, 7500, 10000, 12500, or 15000 ppm to groups of 5 rats/sex. Weekly recordings were made of detailed clinical observations, individual body weights and food consumption. Mortality and overt toxicity was recorded twice daily.

Endpoints relevant for assessment of potential for endocrine disruption

- None

Deviations from the current guideline:
Not applicable.**Effects on endpoints relevant for assessment of potential for endocrine disruption**
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Low** (Standard repeat dose toxicity test, with no relevant endpoints for assessment of endocrine disruption)
Overall significance	**Low** – No evidence of an effect relevant to the assessment of endocrine disruption

1.1.1.8 Chronic and carcinogenicity studies in OECD Conceptual Framework level 4

Report:	Crome SJ, Stuart V, Anderson A, Crook D, Gibson WA, Fish E, Lewis DJ, Gopinath C (1988). Dicamba, potential tumorigenic effects in prolonged administration to mice. Huntington Life Science report No. VCL 72/871205. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, PE18 6ES, England. File No. SAN837/5075.

Guidelines: OECD 451 (1981)

GLP: Yes

Study design: 52 Crl:CD-1 (ICR) BR (Swiss) mice per sex per group were administered dicamba via the diet at dose levels of 0, 50, 150, 1000 and 3000 ppm. In addition, 10 male and 10 female mice were assigned to a health check group for haematology check prior to treatment. Male mice were killed following 89 completed weeks of treatment when the survival approached 30% in males administered 150 and 3000 ppm. Females were killed following 104 completed weeks of treatment when the survival was at least 35%.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weights: Testes
- Histopathological evaluation: Adrenal glands, mammary gland, ovaries, pituitary gland, prostate, seminal vesicles, testes with epididymides, thyroid (with parathyroid) and uterus

Deviations from the current guideline
The mice were 7 weeks old at study start (preferably max. 6 weeks, in OECD 451). Clinical observations were not made daily during some parts of the study. In absence of any remarkable clinical observations this is considered not to affect the validity of the study. Survival rate was 30% for males and 35% for females at 89 and 104 weeks respectively, at which time the remaining animals were killed. The deviations are not considered to compromise the scientific validity of the study.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Goldenthal EI (1985). Technical dicamba. Lifetime dietary toxicity and oncogenicity study in rats. International Research and Development Corporation report No. 163-694. International Research and Development Corporation, Mattawa, Michigan, U.S.A. File No. SAN837/5072

Guidelines: OECD 453 (1981)

GLP: Yes

Study design: Dicamba was administered to groups of 60 male and 60 female Sprague-Dawley rats at dietary concentrations of 0, 50, 250, and 2500 ppm for over two years (115 weeks for males and 118 weeks for females) with a scheduled sacrifice at 12 months.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weights: Ovaries and testes
- Histopathological evaluation: Adrenal glands, mammary area, ovaries, pituitary gland, prostate, seminal vesicles, testes with epididymides, thyroid (with parathyroid) and uterus

Deviations from the current guideline:
The terminal necropsy schedule for this study was 27 months, rather than 24 months by current guidelines. Organ weights which were not recorded according to latest guidelines include: epididymides, the thyroid (and parathyroid) and the uterus. Histopathology assessment not recorded according to latest guidelines include: coagulating gland and vagina. No haematological or clinical chemical examinations were performed after 3 months. Survival was (marginally) less than 50 % in all dosed male groups and in mid dose females at 104 weeks.

Effects on endpoints relevant for assessment of potential for endocrine disruption
In males, a higher incidence of C-cell carcinoma was seen in at the top dose level as compared to concurrent controls (8.3% vs. 1.7%). This is considered unrelated to treatment as this was observed in high dose males only and the overall incidence of pre-neoplastic and neoplastic lesions in C-cells did not show a treatment-related effect in males. A more detailed discussion can be found in section 5.1.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Blair M (1986). Dicamba - One year dietary toxicity in dogs. International Research and Development Corporation Report No.163-696. International Research and Development Corporation, Mattawan, Michigan, 49071, U.S.A. File No. SAN837/5083.

Guidelines: OECD 452 (1981)

GLP: Yes

Study design: The test article was administered to groups of 4 male and 4 female Beagle dogs at dietary dose levels of 0, 100, 500 and 2500 ppm for 12 months. All dogs were sacrificed after 12 months and submitted to a complete necropsy.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Organ weights: Adrenal gland, ovaries, pituitary glands, testes and thyroid/parathyroid complex
- Histopathological evaluation: Adrenal glands, mammary gland, ovaries, pituitary gland, prostate, testes with epididymides, thyroid (with parathyroid) and uterus

Deviations from the current guideline:
Compared to OECD guideline 452 haematological examinations are lacking at 3 months after study start. This does not compromise the validity of the study.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

1.1.1.9 Developmental studies in OECD Conceptual Framework level 4

Report:	Smith SH, Page JG (1981). Teratology study in Albino rats with technical dicamba. Toxigenics, Inc. Decatur report No. 450-0460. Toxigenics, Inc, 1800 East Pershing Road Decatur, Il 62526, U.S.A. File No. SAN837/5064.

Guidelines: Equivalent to OECD 414 (1981)

GLP: Yes

Study design: Polygamous cohabitation was employed during mating trials and males were rotated among females on a day-to-day basis until the required number of breedings were obtained. Each male was paired with different females each day of the mating trials. Daily examinations (observation of copulation plug and/or sperm positive results of vaginal smear) were conducted to establish bred females. 25 young, sexually mature, pregnant females were randomly assigned to each dose group. Rats per dose group were administered dicamba via oral gavage in corn oil (1ml/100g) at dose levels of 0, 64, 160, and 400 mg/kg during days 6 to 19 of gestation. Dams were sacrificed on day 20 of gestation and their gravid uterus was excised and weighted, then examined to determine the number of implantation sites, resorption sites and foetuses (live foetuses and intra-uterine deaths).

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Pregnancy parameters (e.g. % pregnant)
- Number of implantations
- Number of abortions/resorptions/intra-uterine deaths
- Foetal abnormalities
- Pup sex ratio

Deviations from the current guideline:
The OECD 414 guideline was updated on 25 June 2018, to include measurement of maternal thyroid hormones (T4, T3 and TSH) and ano-genital distance (AGD) in rats, neither of which were considered in the current study. The volume of test material and vehicle given to the animals were higher (1.0 ml/100 g) than recommended by the guideline (0.4 ml/100g). Only one third of foetuses in each litter were examined for soft tissue alterations. Limited determination of body weight was conducted (day 0, 6 and 20). The number of corpora lutea was not reported. The deviations are not found to compromise the study results as presented. The skeletons were also only singly stained with Alizarin red, rather than double stained with Alcian blue.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

Report:	Hobermann AM (1992). Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of technical dicamba administered orally via capsule to New Zealand white rabbits. Argus Research Laboratories report No. 1819-004. Argus Research Laboratories, Inc., 935 Horsham Road Horsham, Pennsylvania 19044. File No. SAN837/5235.

Guidelines: U.S. Environmental Protection Agency Pesticide Assessment Guidelines Subdivision F, 83-3 (equivalent to OECD 414, 1981)

GLP: Yes

Study design: Groups of 19 (control) or 20 (treated groups) artificially inseminated virgin New Zealand White rabbits (Hra: (NZW) SPF) were administered the test article at dose levels of 0, 30, 150 and 300 mg/kg during days 6 to 18 of gestation by the means of gelatin capsules. Dosages were adjusted to individual body weights recorded on days 6, 9, 12 and 15 of presumed gestation. Dams were sacrificed on day 29 of gestation and their uteri examined for live foetuses and intra-uterine deaths; foetuses were removed.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross macroscopic observations
- Pregnancy parameters (e.g. % pregnant)
- Number of implantations
- Corpora lutea
- Number of abortions/resorptions/intra-uterine deaths
- Foetal abnormalities
- Pup sex ratio

Deviations from the current guideline:
The following parameters would be expected in a study conducted in rabbits to the current OECD test guideline but were not addressed in this study: gravid uterine weight, thyroid weight, skeletal observations used single staining with alizarin red only (did not double stain with Alician blue), cryptorchidism was not examined, dosage did not include the entire period of gestation (organogenesis only). These deviations are not thought to affect the validity of the study.

Effects on endpoints relevant for assessment of potential for endocrine disruption
None.

One abortion was recorded in the 150 mg/kg/day and four abortions were recorded in the 300 mg/kg/day dosage group. The abortions were associated with significant maternal toxicity indicated as clinical observations and reduced body weight gains (300 mg/kg dose group had a 42% reduced weight gain relative to controls). Additionally, reduced relative (-13% compared to control) and absolute (-17% compared to control) food consumption was noted among the 300 mg/kg/day dosage group. 1 dead occurred in the high dose group, however this was the result of an accident (intubation accident) and not considered treatment related. For further details on the relationship between abortions and food consumption and body weight, see table 4.3.3-1 below:

Table 1.4.3.3-1 Deaths and abortions, body weight change, absolute and relative food consumption of dams across day 0-29

Endpoint [Day 0-29]	0 (Vehicle)	30 mg/Kg/Day	150 mg/Kg/Day	300 mg/Kg/Day
Deaths	0	0	0	1a
Abortions	0	0	1	4
Maternal Body Weight Change [kg]	+0.45 ± 0.17	+0.56 ± 0.10	+0.47 ± 0.18 [17]b	+0.26 ± 0.21**[13]b

Maternal Absolute Food Consumption [g/day]	148.0 ± 23.4 [17]c	168.0 ± 12.4*	151.8 ± 18.0 [16]c	121.6 ± 28.2*[13]b
Maternal Relative Food Consumption [g/day]	39.5 ± 4.5 [17]c	44.4 ± 4.5 **	40.7 ± 4.2 [16]c	34.2 ± 5.7 **[13]b

Days = days of gestation
[ ] = Number of values averaged
a. Cause of death was accidental, intubation accident
b. Excludes values for does that aborted or were found dead;
c. Excludes values that were not recorded, as well as those associated with spillage or wet feed.
* Significantly different from vehicle control group [p≤0.05]
** Significantly different from vehicle control group [p≤0.01]

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – No evidence of an effect relevant to the assessment of endocrine disruption

1.1.1.10 Reproductive studies in OECD Conceptual Framework level 5

Report:	Masters RE (1993). Technical Dicamba – A study of the effect on reproductive function of two generations in the rat. Huntingdon Research Centre report No. SNC 140/921437. Syngenta File No. SAN837/5213.

Guidelines: OECD 416 (1983)

GLP: Yes

Study design: Dicamba was administered to groups of 32 male and female albino rats (CrkCD (SD) BR VAF/Plus strain) at dietary dose levels of 0, 500, 1500, and 5000 ppm. Following an acclimation period of 2 weeks treatment started at 6 weeks of age for 10 weeks prior to pairing. Dosing continued until all litters had weaned. From these litters the F1 generation (28/sex/group) was selected on Day 21 post-partum, reared to maturity and paired at 16 and 25 weeks of age. Direct treatment of the F1 generation started at the age of 4 weeks, i.e. 12 weeks before mating and continued until the re-mated females had reared their young (F2 generation) to weanlings. Because of the low pregnancy rate of the F1 generation a second mating was performed in the F1 generation. Following the weaning of F2A pups, F1 males and females were remated employing alternative pairings and, where possible, remating females without litters and males apparently failing to induce pregnancy to animals which were successful at the first mating.

Endpoints relevant for assessment of potential for endocrine disruption

- Gross necropsy (macroscopic) observations
- Reproductive performance: Pre-coital interval, Mating, Fertility, Duration of gestation, Parturition, Litter size and survival (reductions in litter size can be indicative of abortions/resorptions/intra-uterine deaths), Lactation
- Sex ratio
- Sexual maturation (vaginal opening and preputial separation)
- Corpora lutea
- Oestrus cyclicity

- Sperm analysis (number, morphology, motility)
- Foetal abnormalities
- Organ weights: testes, epididymides, prostate, seminal vesicles with coagulating glands, pituitary, thyroid and adrenal glands
- Histopathological examination: vagina, uterus (with cervix), ovaries, testis, epididymis, seminal vesicles, prostate (and coagulating gland)

Deviations from the current guideline
A minimum of 10 males from both P and F1 groups should be used for sperm analysis of homogenisation-resistant spermatids and cauda epididymides sperm reserves. In this study, sperm analysis was performed for 8 (F0) and 7 (F1) males from each group instead of the recommended 10 animals/group. Anogenital distance was not recorded in this experiment. Uterus, spleen and thyroids in parental animals and the spleen of pups were not weighted. The required level of pregnancies was achieved in the F0 population, but low pregnancy rates were achieved in the F1 generation first and second mating. These deviations are not considered to impair the scientific validity of the study.

Effects on endpoints relevant for assessment of potential for endocrine disruption
The mean age of sexual maturation amongst F1 generation males, as determined by cleavage of the balanopreputial skinfold, was significantly ($p \leq 0.01$) delayed in the 5000 ppm dose group compared to the control (45.6 days vs. 43.7 day in control). This slight delay in development was considered to reflect the slower growth rate of these animals prior to weaning rather than indicative of a specific effect on sexual maturation. The slower growth rate and development of the high dose F1 males observed prior to weaning is manifested as consistently lower body weight, food consumption and water consumption throughout the maturation process. This is further discussed in the assessment of lines of evidence in section 5.1.

CONCLUSIONS

Reliability score	**1**: Reliable without restrictions
Relevance score	**Medium** (Standard repeat dose toxicity test, with endpoints that may be influenced by the endocrine system, but are also known to be affected by other factors, e.g. toxicity, etc.)
Overall significance	**Indicative** – Limited evidence of an effect relevant to the assessment of endocrine disruption

2.10.14 *In Vivo* Data – Non-Mammalian Species

1.1.1.11 Existing data in OECD Conceptual Framework level 1

The following studies conducted as part of the regulatory data package for registration of dicamba are not specifically designed for detection of endocrine disrupting properties, but as they cover life stages and endpoints relevant to development, growth or reproduction, have been included in the current evaluation.

Report:	CA 8.2.2/01 Scheerbaum D, 1990, Study of Prolonged Toxicity (21 d) to Fish (Rainbow trout) of Dicamba. Report Number 1554, Dr. U. Noack-Laboratorium Für Angewandte Biologie, Richthofenstrasse 29, D-3200 Hildeshelm, Germany. (Syngenta File No. SAN837/5331)

Guidelines: OECD 204

GLP: Yes

Study design: Rainbow trout (*Onchorhynchus mykiss*) were exposed under semi-static conditions to dicamba at nominal concentrations of 0, 18, 32, 58, 100, 180, 320, 580 and 1000 mg a.i./L for 21 days. Endpoints included survival and growth (length and weight).

Endpoints relevant for assessment of potential for endocrine disruption

- Growth (length and weight)

Effects on endpoints relevant for assessment of potential for endocrine disruption

- None

CONCLUSIONS

Reliability score	**1** - Reliable without restriction
Relevance score	**Medium** - Endpoint in a multi-generation test, or other repeat dose standard toxicity test, which may be influenced by the endocrine system, but is also known to be affected by other factors, e.g. toxicity, etc.
Overall significance	**Indicative for no evidence of effects relevant for the assessment of endocrine disruption** (indicative study/no effects observed)

Report:	CA 8.2.2.1/01: Salinas P. 2011. BAS 183 H (Dicamba Techn.) –Early Life-Stage Toxicity Test on Fathead Minnow (Pimephales promelas) in a Flow through System, Report Number 50F0267/97E002 405803. Experimental Toxicology and Ecology BASF SE 67056 Ludwigshafen, Germany. (Syngenta file No. SAN837_11528)

Guidelines: OECD 210

GLP: Yes

Study design: Fathead minnows (*Pimephales promelas*) were exposed under flow-through conditions to dicamba at nominal concentrations of 0, 0.10, 0.32, 1, 3.2 and 10 mg a.i./L (measured as 0, 0.100, 0.331, 1.03, 2.98, and 9.91 mg a.i./L) for 33 days. Endpoints included hatching success, survival, and growth (length and weight).

Endpoints relevant for assessment of potential for endocrine disruption

- Hatching success

- Larval growth (length and weight)

Effects on endpoints relevant for assessment of potential for endocrine disruption

- None

CONCLUSIONS

Reliability score	**1** - Reliable without restriction
Relevance score	**Medium** - Endpoint in a multi-generation test, or other repeat dose standard toxicity test, which may be influenced by the endocrine system, but is also known to be affected by other factors, e.g. toxicity, etc.
Overall significance	**Indicative for no evidence of effects relevant for the assessment of endocrine disruption** (indicative study/no effects observed)

Report:	CA 8.2.2.1/01 Minderhout T, Kendall TZ, Gallagher BS, 2012. Dicamba Acid: An Early Life-Stage Toxicity Test with the Sheepshead Minnow (Cyprinodon variegatus), Report Number 405804, Wildlife International, Ltd. 8598 Commerce Drive, Easton, Maryland 21601 USA. (Syngenta File No. SAN837_11529)

Guidelines: OECD 210

GLP: Yes

Study design: Sheepshead minnows (*Cyprinodon variegatus*) were exposed under flow-through conditions to dicamba at nominal concentrations of 0, 0.31, 0.77, 1.9, 4.8, and 12 mg a.i./L (measured as 0, 0.28, 0.72, 1.8, 4.5, and 11 mg a.i./L) for 34 days. Endpoints included hatching success, survival, and growth (length and weight).

Endpoints relevant for assessment of potential for endocrine disruption

- Hatching success
- Larval growth (length and weight)

Effects on endpoints relevant for assessment of potential for endocrine disruption

- None

CONCLUSIONS

Reliability score	**1** - Reliable without restriction
Relevance score	**Medium** - Endpoint in a multi-generation test, or other repeat dose standard toxicity test, which may be influenced by the endocrine system, but is also known to be affected by other factors, e.g. toxicity, etc.
Overall significance	**Indicative for no evidence of effects relevant for the assessment of endocrine disruption** (indicative study/no effects observed)

Report:	K-CA 8.2.3/02 Zhu et al. (2013). Dicamba Affects Sex Steroid Hormone Level and mRNA Expression of Related Genes in Adult Rare Minnow (*Gobiocypris rarus*) at Environmentally Relevant Concentrations. State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Shuangqing Rd 18, Haidian District, Beijing, 100085, People's Republic of China. Published. Environmental Toxicology 30 (6):693-703 (Syngenta File No. SAN837_11618)

Guidelines: NA

GLP: No

Study design: Adult rare minnows (*Gobiocypris rarus*) were exposed to dicamba under flow-through conditions at nominal concentrations of 0, 0.05, 0.5, and 50 µg a.i./L for 40 days. Test concentrations were not verified by chemical analysis. Endpoints included survival, body length and weight, gonadosomatic index, hepatosomatic index, histological changes, plasma vitellogenin, sex hormone levels, and mRNA transcripts related to endocrine activity.

Endpoints relevant for assessment of potential for endocrine disruption

- Body length and weight
- Gonadosomatic index
- Hepatosomatic index
- Histopathology
- Plasma vitellogenin
- Sex hormone levels
- mRNA transcripts (star, 3β-hsd, cyp17, cyp19a, era, vtg)

Effects on endpoints relevant for assessment of potential for endocrine disruption

- Body length and weight: No effect
- Gonadosomatic index: No effect
- Hepatosomatic index: No effect
- Histopathology: Inhibition of spermatogenesis in male testes and ovarian degeneration in females at 50 µg a.i./L
- Plasma vitellogenin: Increased VTG in males at all test concentrations, no effect on VTG in females
- Sex hormone levels: Increased E2 in males and females at all test concentrations, no effect on 11-KT
- mRNA transcripts:

	Liver				**Gonads**			
Gene	**0.05**	**0.5**	**5**	**50**	**0.05**	**0.5**	**5**	**50**
star (f)	↓	↓	↓	↓	-	-	↓	↓
star (m)	-	-	↓	↓	↑	-	-	-
3β-hsd (f)	-	-	-	-	↑	↑	↑	↑
3β-hsd (m)	-	-	-	-	-	-	↑	-
cyp17 (f)	↑	↑	↑	↑	↑	↑	↑	↑
cyp17(m)	-	↑	↑	↑	↑	-	-	-
cyp19a (f)	↓	↓	-	-	-	↓	↓	↓
cyp19a (m)	↓	↓	↓	↓	-	-	-	-
era (f)	-	-	↑	↑	-	-	-	-
era (m)	-	-	-	-	-	-	-	-
vtg (f)	↑	↑	↑	↑	↑	↑	↑	↑
vtg (m)	↑	↑	↑	↑	↑	↑	↑	↑

Effects on mRNA transcripts and sex hormone levels were not consistent with any specific EAS modality. In the gonads, a decrease in female aromatase (cyp19a) expression was reported in the top 3 treatment levels. However, this effect was not consistent with plasma E2, which increased in all treatment levels. There were no effects on male aromatase expression in the gonads, although an increase in plasma E2 was observed in all treatment levels. No effects were observed on plasma 11-KT. Overall, changes in gene expression were generally not dose-related and did not indicate any consistent effects on steroidogenesis.

Apparent effects on vitellogenesis would likely be secondary to reported increases in plasma E2 levels at all test concentrations in both males and females. While vitellogenin increased at both the transcript (liver mRNA) and protein (plasma) level in males, increases were only reported at the transcript (liver mRNA) level in females.

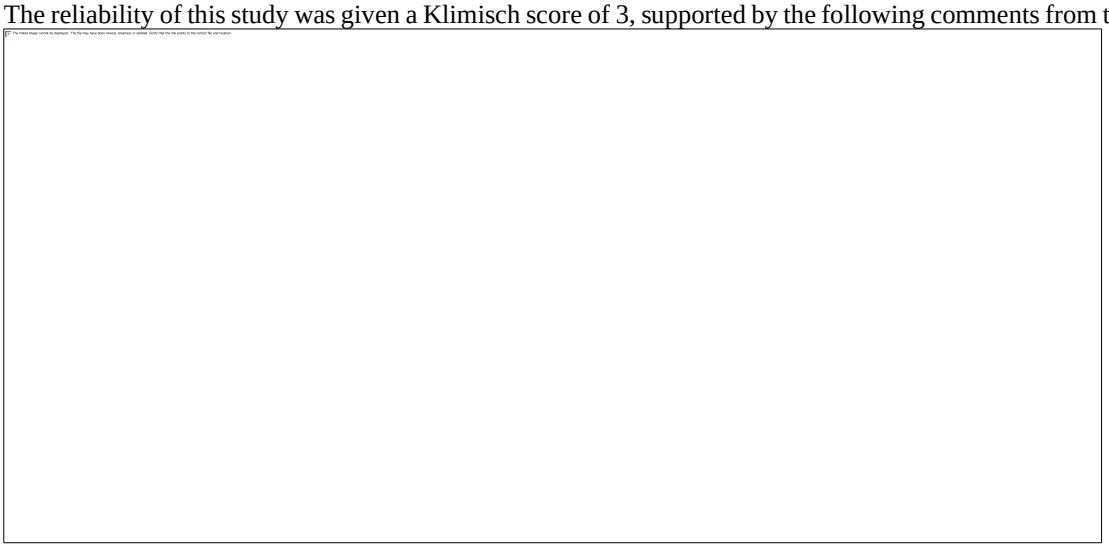
Histological effects were noted in both the liver and gonads. Specifically, inhibition of spermatogenesis in male testes and ovarian degeneration in females was observed at the highest treatment level. The significance of these

effects was not determined as there were no effects on gonadosomatic index, and because reproductive parameters (e.g., fecundity) were not monitored in this study. Additionally, at the highest treatment level, histopathology indicated cytoplasmic degeneration and bile stagnation in the livers of male fish, and enlargement of cell nuclei and bile stagnation in the livers of female fish. These observations may be indicative of hepatotoxicity.

CONCLUSIONS

Reliability score	**3** - Not reliable
Relevance score	**Medium** - Positive endpoint data from a short-term/screening assay specifically controlled by the endocrine system.
Overall significance	**Unusable -** Data from studies with reliability scores of 3 or 4.

The reliability of this study was given a Klimisch score of 3, supported by the following comments from the RMS:



Additionally, the quality of reporting and statistical robustness of this study were questionable, and the study did not examine adverse apical endpoints. Therefore, the significance of the results and overall study was low/unusable.

1.1.1.12 Short term non-mammalian studies in OECD Conceptual Framework level 3

None available

1.1.1.13 Non-mammalian studies in OECD level 4

Report:	CA 8.1.1.3/01, Beavers JB, Haberlein D, Mitchell LR, Jaber M, (1994), Technical Dicamba: A Reproduction Study with the Northern Bobwhite, Report Number 131-182. Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland 21601, USA (Syngenta File No. SAN837/5206)

Guidelines: OECD 206

GLP: Yes

Study design: Northern bobwhites (*Colinus virginianus*) were exposed to dicamba via nominal dietary concentrations of 0, 400, 800, and 1600 ppm (measured as 0, 426, 823, and 1510 ppm) for 21 weeks. Birds were observed for signs of mortality, abnormal behaviour (daily), body weight, egg production, egg shell thickness, egg quality, viability of embryos, hatchability, number and weight of hatchlings, hatchling survival and gross pathology.

Endpoints relevant for assessment of potential for endocrine disruption

- Egg production, egg shell thickness, egg quality
- Viability of embryos
- Hatchability, number and weight of hatchlings
- Gross pathology

Effects on endpoints relevant for assessment of potential for endocrine disruption

- None

CONCLUSIONS

Reliability score	**1** - Reliable without restriction
Relevance score	**Medium** - Endpoint in a multi-generation test, or other repeat dose standard toxicity test, which may be influenced by the endocrine system, but is also known to be affected by other factors, e.g. toxicity, etc.
Overall significance	**Indicative for no evidence of effects relevant for the assessment of endocrine disruption** (indicative study/no effects observed)

Report:	CA 8.1.1.3/01, Beavers JB, Haberlein D, Mitchell LR, Jaber M, (1994), Technical Dicamba: A Reproduction Study with the Mallard, Report Number 131-183. Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland 21601, USA (Syngenta File No. SAN837/5205)

Guidelines: OECD 206

GLP: Yes

Study design: Mallard ducks (*Anas platyrhynchos*) were exposed to dicamba via nominal dietary concentrations of 0, 400, 800, and 1600 ppm (measured as 0, 426, 823, and 1510 ppm) for 21 weeks. Birds were observed for signs of mortality, abnormal behaviour (daily), body weight, egg production, egg shell thickness, egg quality, and viability of embryos, hatchability, number and weight of hatchlings, hatchling survival and gross pathology.

Endpoints relevant for assessment of potential for endocrine disruption

- Egg production, egg shell thickness, egg quality
- Viability of embryos
- Hatchability, number and weight of hatchlings
- Gross pathology
-

Effects on endpoints relevant for assessment of potential for endocrine disruption

- Hatchability: Decrease at 1600 ppm
- Number of 14-day-old survivors: Decrease at 1600 ppm

The effects noted above were only observed at the highest test concentration (1600 ppm) and therefore may be indicative of systemic toxicity.

CONCLUSIONS

Reliability score	**1** - Reliable without restriction

Relevance score	**Medium** - Endpoint in a multi-generation test, or other repeat dose standard toxicity test, which may be influenced by the endocrine system, but is also known to be affected by other factors, e.g. toxicity, etc.
Overall significance	**Indicative -** Repeat dose studies of medium relevance and with reliability scores of 1 or 2

2.10.15 Non-mammalian studies in OECD level 5

None available.

2.10.16 ED ASSESSMENT FOR HUMANS

2.10.17 ED Assessment for T-modality

2.10.18 Have T-mediated parameters been sufficiently investigated?

	Sufficiently investigated
T-mediated parameters	**Yes** based on availability of data in the following studies: Ma-Hock L (2014). BAS 183 H (Dicamba techn.): Repeated dose 28-day inhalation toxicity study in Wistar rats, dust #$ OECD 412 (2009) – ID: 13 Rattray NJ (2002). Dicamba Tech. (SAN 837 Tech.): 28-Day dermal toxicity study in rats $ OECD 410 (1981) – ID: 4 Estes FL *et al*. (1979). Banvel Technical: 3-Week dermal toxicity study in rabbits #$ Equivalent to OECD 410 (1981) – ID: 3 Doubovetzky M (1997). Dicamba TC: 13-week feeding study in rats (including 4-week recovery) $ OECD 408 (1981) – ID: 6 Jackson AM *et al*. (2003). SAN 837 tech.; 13-Week oral (capsule) toxicity study in the dog #$ OECD 409 (1998) – ID: 7 Kubaszky R (2010). RC1176: 90-Day Oral Capsule Toxicity Study in Beagle Dogs #$ OECD 409 (1998) – ID: 8 Crome SJ *et al*. (1988). Dicamba, potential tumorigenic effects in prolonged administration to mice $ OECD 451 (1981) – ID: 11 Goldenthal EI (1985). Technical dicamba. Lifetime dietary toxicity and oncogenicity study in rats $ OECD 453 (1981) – ID: 12 Blair M (1986). Dicamba - One year dietary toxicity in dogs #$ OECD 452 (1981) – ID: 12 Masters RE (1993). Technical Dicamba – A study of the effect on reproductive function of two generations in the rat #$ OECD 416 (1983) – ID: 13

Thyroid weight was measured. $ Thyroid histopathology was measured.

2.10.19 Lines of evidence for adverse effects and endocrine activity related to T-modality

The lines of evidence have been assembled through interrogation of the data assessed in Section 4 of this document:

- Increased thyroid parafollicular (C-cell) carcinoma in rat chronic/carcinogenic study

In a chronic/carcinogenicity study, a statistically significant increase in thyroid parafollicular (c-cell) carcinoma were observed (8.3% vs. 1.7%) in the high dose male group (Table 5.1-2), but not in females (Goldenthal EI. 1985). No concurrent increase in the incidence of hyperplasia or C-cell adenomas was observed (Table 5.1-3). The combined thyroid c-cell tumours (adenomas and carcinomas) are within range of the historical control data from the same laboratory which used the same strain and diet but with a shorter study duration (24 months vs 26.5/27 months in males and females, respectively).

The combined thyroid c-cell tumours (adenomas and carcinomas) were within range of the historical control data from the same laboratory which used the same strain and diet but with a shorter study duration (24 months vs 26.5/27 months in males and females, respectively). Examination of the impact of the length of the in-life phase on thyroid c-cell tumours in Sprague Dawley rats using data from the registry of Industrial Toxicology (RITA) indicates that the incidence of thyroid C-cell adenoma and carcinoma are consistently higher (both sexes) in studies with an in-life phase of 25-26 months vs studies with an in-life phase of 24 months. In males in particular, the mean incidences of thyroid C-cell carcinoma were about twice as high in 25/26-month studies as compared to 24 month studies and these tumours were seen in 78% of the 25/26-month studies vs 52% of 24-month studies. The latter indicates that thyroid C-cell tumours, especially carcinomas in males, have a clear age-related component and exceeding the guideline-recommended 2 year in-life period, as in the dicamba study (27 months), can result in higher incidences of these tumours when compared to 24-month studies. This is further supported by HCD information for Sprague Dawley rats collected from the National Toxicology Program (NTP) in 2008, which observed an incidence range of 17-38% for thyroid C-cell adenomas and 0-8% for thyroid C-cell carcinomas. The information is essentially limited to 24-month studies in female rats but is considered supportive for comparison to the incidences seen for males in the dicamba study

Table 2.1.2-1 Incidences of follicular and parafollicular tumours of the thyroid

	Male				Female			
Dose level [ppm]	0	50	250	2500	0	50	250	2500
No. exam.	60	60	60	60	60	60	60	60
Follicular adenoma	0	1	1	1	-	-	-	-
Parafollicular cell adenoma	2	5	5	3	5	1	3	6
Follicular carcinoma	0	1	0	0	-	-	-	-
Parafollicular cell carcinoma	1	0	2	5	0	1	0	0

Table 2.1.2-2 Thyroid c-cell (parafollicular) findings in male rats in dicamba carcinogenicity study

	Number of males affected animals											
Dosage [ppm]	**0**			**50**			**250**			**2500**		
Time of death	IS	died	TS	IS	died	TS	IS	died	TS	IS	died	TS
No. of animals	10	39	11	10	37	13	10	31	19	10	35	15
Hyperplasia	1	19	9	1	17	10	0	18	19	0	12	14
Mean severity	(2.0)			(1.9)			(1.9)			(2.0)		
Adenoma	0	1	1	0	3	2	0	2	3	0	1	2
Carcinoma	0	0	1	0	0	0	1	1	0	0	5	0
Combined total	1	19	9	1	18	10	1	19	19	0	15	14
Combined total as percentage	29/60 (48%)			29/60 (48%)			39/60 (65%)			29/60 (48%)		

IS = interim sacrifice, TS = terminal sacrifice, died = animals found dead or sacrificed in extremis during the study

No hypertrophy, hyperplasia or other pre-neoplastic lesions were observed in the thryoid, pituitary or hypothalamus in any other repeat dose study with dicamba. Furthermore, data from the literature and ToxCast do not indicate an interaction of dicamba with components of the HPT axis.

Therefore, the apparent increase in the incidence of C-cell carcinoma is therefore considered to have occurred spontaneously, as part of normal biological variability of a very common age-related tumour in a population of aged animals. Table 2.2.1 assembles the lines of evidence for T-mediated adversity in accordance with the ECHA-EFSA (2018) guidance

Table 2.1.2-2 Lines of evidence for thyroid activity and adversity in mammals

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
Evidence for endocrine activity	*In vitro* mechanistic	Thyroid receptor (α / β) transactivation	Rat				No agonism or antagonism of thyroid receptor reporter gene expression in GH3 rat pituitary gland cells	Negative, no evidence for thyroid interaction *in vitro*	Overall negative, no evidence for a consitent pattern of endocrine activity and adversity in the T modality	**T**
		Thyroid receptor (THRa1) transactivation	Human				No up (agonism) or down (antagonism) reporter gene expression in human HepG2 cells			
		Inhibition of TPO (Thyroid peroxidase)	Rat				No inhibition of TPO			
		Inhibition of NIS (Sodium-iodide symporter)	Human				negative based on a threshold of less than 20% inhibition in the RAIU assay			
		Deiodination enzyme inhibition	Human				no inhibition of DIO1, DIO2 and DIO3			
		Thyrotropin releasing hormone (TRH) receptor	Rat				No binding detected			
	T-mediated parameter	Thyroid (weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	Negative, no alteration to thyroid weight		
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			

			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Thyroid (Histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	Increased incidence of c-cell carcinomas in the carcinogenicity study in the absence of an increased incidence of releated histopathological findings. No consistent effect across studies.		
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	Slight increase in parafollicular carcinomas, however not considered toxicologically significant- There were also no accompanying changes to function of thyroid, therefore not considered treatment-related.			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

Evidence of general toxicity	Liver (weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	No effect on organ	No consistent effect on the liver		
		Rat	28 Days	Dermal	1000 mg/kg bw/day	No effect on organ			
		Rat	28 Days	Inhalation	0.05 mg/L	No effect on organ			
		Rat	13 Weeks	Oral	12000 ppm	Statistically significant increase in mean relative liver weight in males and females after treatment at 12000ppm, like control group after recovery			
		Dog	13 Weeks	Oral	300 mg/kg bw/day	No effect on organ			
		Dog	90 Days	Oral	300 mg/kg bw/day	No treatment-related effect on organ			
		Dog	1 Years	Oral	2500 ppm	No effect on organ			
		Mouse	104 Weeks	Oral	3000 ppm	No treatment-related effect on organ			
		Rat	27 Months	Oral	2500 ppm	No effect on organ			
		Rat	2 Gen Adult (F0)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
		Rat	2 Gen Offspring (F1)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
		Rat	2 Gen Adult (F1)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
		Rat	2 Gen Offspring (F2)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
	Liver (histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
		Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			

		Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
		Rat	13 Weeks	Oral	12000 ppm	12000 ppm Minimal/slight hypertrophy in centrilobular hepatocytes in females after treatment at 12000ppm, not observed after recovery			
		Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
		Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
		Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
		Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

1.1.1.14 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Table 1.1.1.13-1 WoE for T-mediated adversity

- Thyroid effects (weight and histopathology) were examined in multiple studies at different dose levels and of different durations in rats and dogs by oral administration of the substance. Thyroid effects were also examined in one study in rabbits via dermal application of the substance.

- No effect on thyroid weight or histopathology was observed in any of the species at any of the dose levels tested

- There was no evidence for the identification of a T-mediated adverse effect

Table 1.1.1.13-2 WoE for T-mediated endocrine activity

- Negative for the following *in vitro* investigations:
  TRbeta binding (antagonism)
  TRbeta binding (agonism)
  InVitroToxCast Thyroid
  Transthyretin (TTR)

- No evidence for identification of T-mediated endocrine activity

1.1.2 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

A dataset is considered to have sufficiently investigated thyroid related adversity in relation to mammals if the parameters investigated in OECD TG 407, 408, 409, 416, and 453 have been assessed. Assessment of the potential for Dicamba in studies spinning a range of durations and exposure levels in the mouse, rat, rabbit and dog. It is therefore determined that the potential for thyroid related adversity in relation to mammals has been sufficiently addressed.

A dataset is considered to have sufficiently investigated thyroid related adversity in relation to mammals if the parameters investigated in OECD TG 407, 408, 409 (and/or the one-year dog study, if available), 416, and 453 have been assessed.

Assessment of the potential for dicamba to alter thyroid related parameters (histology and/or weight) has been conducted in studies spanning a range of durations (from 28 days to 27 months), in the mouse, rat, rabbit and dog, and through multiple exposure routes (see data reviews in Section 4.3). It is therefore determined that the potential for thyroid related adversity in relation to mammals has been sufficiently addressed.

Table 2.1.3-1 Selection of Relevant Scenario for the ED Assessment of T-modality in Mammals

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	

1.1.3 MoA analysis for T-modality

Not applicable.

1.1.4 Conclusion of the assessment of T-modality

Assessment of the potential for dicamba to alter thyroid related parameters (histology and/or weight) has been conducted in studies spanning a range of durations (from 28 days to 27 months), in the mouse, rat, rabbit and dog, and through multiple exposure routes, and no effects on these parameters were observed.

Dicamba therefore occupies scenario 1a for the T modality, and as such the ED criteria are not met for this modality.

2.10.20 ED assessment for EAS-modalities

2.10.21 Have EAS-mediated parameters been sufficiently investigated?

	Sufficiently investigated
EAS-mediated parameters	Yes**,** based on availability of data in the following studies: Masters RE (1993) Technical Dicamba – A study of the effect on reproductive function of two generations in the rat OECD TG 416 (1983) – ID: 13

2.10.22 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

The lines of evidence have been assembled through interrogation of the data assessed in Section 4 of this document:

- Delay in sexual maturation in a two-generation reproductive toxicity study

The mean age of sexual maturation amongst F1 generation males, as determined by cleavage of the balanopreputial skinfold, was significantly ($p \leq 0.01$) delayed in the 5000 ppm dose group compared to the control (45.6 days vs. 43.7 day in control). This slight delay in development was considered to reflect the slower growth rate of these animals prior to weaning rather than indicative of a specific effect on sexual maturation. Cleavage of the balanopreputial skinfold for males at 1500 and 500 ppm and vaginal opening amongst all females of all treated groups were unaffected by treatment. The slower growth rate and development of the high dose F1 males observed prior to weaning is manifested as consistently lower body weight, food consumption and water consumption throughout the maturation process.

In this study body weight at sexual landmark were not recorded, however body weight from week 4-8, food and water consumption from week 5-8 have been calculated (duration up to and during sexual maturation). The mean sexual maturation of F1 males observed in this study was between day 43.3 and 45.6 which is equivalent to 6 to 7 weeks of age. During this time, the high dose body weight, food consumption and water consumption were consistently lower than controls; statistically significant reductions in recorded body weight in week 4 and week 8 and statistically significant reductions in mean food consumption between week 5 and 8 were observed. In addition, although not statistically significant, there was a 9% drop in water consumption in the high dose group relative to control. The table below (Table 5.1-1) has been generated to demonstrate the correlation between body weight, food and water consumption and the observed effect on sexual maturation in the high dose F1 male group:

Table 2.2.2-1 Intergroup comparison of F1 male pup day and age of sexual maturation in a two-generation reproductive toxicity study in rats.

F1 Male						
Observation			**Dose Group (ppm)**			
			0	**500**	**1500**	**5000**
Preputial Separation	Day of age	Mean	43.7	43.3	43.4	45.6**
		N	28	28	28	28
Body weight (mean)		Week 4	95	100	100	80**
		Week 5	151	160	158	129
		Week 6a	216	224	228	191
		Week 7a	282	293	298	254
		Week 8	342	359	362	311**
Food consumption [g/rat/week] week 5-8		mean	673	702	709	629**
		SD	23.4	16.5	40.4	28.0
		% control	-	104	105	93
Water consumption [g/rat/week] week 5-8		mean	338	340	356	308
		SD	43.0	11.6	29.3	18.9
		% control	-	101	105	91

** - Statistically different from control, p<0.01
a. weeks associated with sexual development

Upon recommendation of the RMS, a covariance analysis was done: The aim of the analysis was to compare the developmental landmark (balano-preputial skinfold cleavage) between the treated groups and the control via analysis of covariance (ANCOVA), using bodyweight at 4 weeks as the covariate. There was a strongly significant relationship between bodyweight at 4 weeks and time to balanopreputial separation when parallel linear models were fitted to all four treatment groups (P = 0.001). The ANCOVA comparison of time to balanopreputial separation between the treatment groups, with adjustment for bodyweight at 4 weeks, was not statistically significant: P = 0.117. This suggests that the previously observed difference in the time to balano-preputial skinfold cleavage between the 5000 ppm group and the control group was related to the reduced bodyweight at 4 weeks in the 5000 ppm group.

The delay in sexual maturation is secondary to a reduction in bodyweight, rather than a direct influence of dicamba. Bodyweight and growth rate play a significant role in the onset of puberty (Goldman *et al*. 2000; Glass *et al*. 1976) and pubertal delays are induced by dietary restriction in rats (Wilen & Naftolin 1978; Holehan & Merry 1985). Sexual development is initiated by a shift in the frequency of electrical activity in gonadotropin-releasing hormone expressing (GnRH) neurons of the hypothalamus, which control the release of reproductive hormones from the pituitary. The strongest activators of GnRH neurons are Kisspeptin, Neuropeptide Y, Adiponectin, and white adipose tissue (leptin), which have been demonstrated to positively feedback at the hypothalamus, triggering sexual development in humans and rodents (Pinilla *et al*. 2012). Consequently, the reductions in bodyweight and nutritional status are considered the most plausible mechanism for the apparent delay in sexual development observed in dicamba treated rats. This is supported by the lack of effects on reproduction parameter,

notably mating and fertility indices. Table 5.1-4 assembles the lines of evidence for EAS-mediated adversity in accordance with the ECHA-EFSA (2018) guidance.

Table 2.2.2-2 Lines of evidence for estrogen, androgen, and steroidogenesis activity and adversity in mammals

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
Evidence for endocrine activity	*In vitro* mechanistic	ER binding	Human				Inactive	Negative, no evidence for estrogenicity *in vitro*	Overall negative, no evidence for estrogenic,, androgenic or steroidogenic activity	**E**
			Bovine				Inactive			
		ER dimerization	Human				Inactive (α/α, β/β, α/β)			
		ERE activity	Human				Inactive in HepG2 human liver cell line ERE cis-activation (agonism or antagonism)			
		Estrogen receptor (α / β) transactivation	Human				No up (agonism) or down (antagonism) reporter gene expression in human HepG2, HEK293T, HeLa or BG1 cells			
		AR binding	Chimpanze				Inactive	Negative, no evidence for androgenicity *in vitro*		**A**
			Human				Inactive			
			Rat				Inactive			
		Androgen receptor transactivation	Human				Inactive			
		Aromatase inhibition	Human				Inactive	Negative, no evidence for an effect on steroidogenesis *in vitro*		**S**
		H295R adrenal assay (Ceetox)	Human				No effect on 11-Deoxycortisol and 17-alpha-hydroxyprogesterone, Androstenedione, Cortisol, 11-Deoxycorticosterone, Estradiol, Estrone, 17-alpha-hydroxypregnelone,			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
							testosterone and progesterone levels			
Integrated lines of evidence for adversity	EAS-mediated parameter	Ovary (Weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	Negative, no consistent effects on ovaries	Overall negative, no evidence for a consitent pattern of endocrine adversity	**EAS**
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Ovary (histopatho-logy)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Uterus weight (with cervix)	Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])	Negative, no consistent effects on uterus		
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
		Uterus histo-pathology (with cervix)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Vagina histo-pathology	Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])	Negative, no con-sistent effect on vagina		
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
				Offspring (F1)						
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Oestrus cyclicity	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])	Negative, no alteration to oestrus cyclicity		
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Testis (Weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	Negative, no consistent effects on testis		
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Testis (histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
		Epididymis (Weight)	Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])	No consistent effect on epididymis		
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
		Epididymis (histopatho-logy)	Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Prostate (Weight)	Dog	90 day	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent treatment related effect		
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Prostate histo-pathology (with seminal vesicles and coagulating glands)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
		Sperm Number	Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])	Negative, no alteration to sperm number, sperm motility or sperm morphology		
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Sperm Motility	Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Sperm Morphology	Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])	Decreased pregnancy rate observed in F1 adult rats, evident in all in all groups - associated with higher body weight at pairing in all dose groups (including control). No effects on time of mating or gestation length		
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	Decreased pregnancy rates in F1 generation (all doses)			
		Time to mating	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Gestation length	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Number of implantations, corpora lutea	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent treatment related effects observed		

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	2 Gen adult (F0)	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
		Numbers of embryonic or foetal deaths and viable foetuses	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Rat	14 Days	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
		Post implantation loss	Rabbit	13 Days	Oral	150 mg/kg bw/day	1 abortion at 150 mg/kg day 22 of gestation, 4 abortions at 300 mg/kg on days 19 (1), 21 (1) and 24 (2) of gestation	No consistent effect observed, abortions observed in the presence of systemic toxicity		
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Litter size	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent effect on litter size, viability and weight. In rats, at the second mate (F2B pups), there was a slight, non-significant higher pup loss at 5000ppm during the weaning period (persisting, even after culling on day 4 post-partum), resulting in slightly lower litter size.		
			Rat	14 Days	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	Slight decrease in litter size due to increased pup loss at 5000ppm			
		Litter viability	Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	Slight non-significant increased pup loss at 5000ppm during weaning			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
							period; No effect on loss post-partum			
		Litter/pup weight	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	Decreased mean pup weight at birth at 5000ppm; Decreased litter weight at 5000ppm; decreased pup growth through to weaning at 5000ppm; decreased mean pup weight at weaning at 5000ppm			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	Decreased mean pup weight at birth at 5000ppm; decreased litter weight at 5000ppm; decreased pup growth through to weaning at 1500 and 5000ppm;			
		Fetal development	Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	Delay in preputial separation at 5000ppm	Delay in sexual maturation in males as a result of delayed growth		
		Sex Ratios	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent treatment related effect		
			Rat	14 Days	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
		Presence of anomalies (external, visceral, skeletal	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent treatment related effect observed		
			Rat	2 Gen Adult (F0)	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day]) Increased renal pelvic cavitations at 400 mg/kg, but 3 of 5 affected foetuses were from 1 litter			
		Adrenal gland (Weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	Increased adrenal weight in females in low dose group (100 mg/kg), not observed in any other dose. No histopathological findings.	No consistent treatment related effect on adrenal gland		
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Adrenal gland (Histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	Non treatment-related adrenal lesion in 3 males at 1000 mg/kg- lesion was also seen in 1 male at 300 mg/kg, 1 female at 30 mg/kg, and 1 control female.			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
Evidence of general toxicity		Body weight	Rabbit	13 Days	Oral	300 mg/kg bw/day	Decreased maternal body weight at 150 mg/kg days 6-8 and at 300 mg/kg days 6-19 (all of dosage period),19-29 (post dosage period), and days 6-29 and 0-29 periods; increased body weight gains at 150 and 300 mg/kg days 19-29 of gestation (post dosage period)	Systemic toxicity evident at high dose group – body weight changes	Systemic toxicity evident in doses of 300 mg/kg/day for rabbit and dog, 3000 ppm in mice and 5000 ppm in rat	**EAS**
			Rat	2 Gen adult (F0)	Oral	400 mg/kg bw/day	Statistically significant decrease in maternal body weight gestation day 20			
			Rat	2 Gen Offspring (F1)	Oral	400 mg/kg bw/day	No effect			
			Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	No effect on body weight			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	Slight decrease in body weight in males at 300 and 1000 mg/kg and females at 1000 mg/kg, but not consistently statistically significant			
			Rat	28 Days	Inhalation	0.05 mg/L	Decreased body weight change in males at 0.05 mg/L; No effect on body weight			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	13 Weeks	Oral	12000 ppm	Decreased body weight gain for males and females during treatment at 12000ppm; Increased weight gained in males and females at 12000ppm during recovery period; Decreased weight in males and females at 12000ppm both during treatment and recovery period			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	Decreased mean body weight gain in males and females during treatment at 300 mg/kg, no effect during recovery period			
			Dog	90 Days	Oral	300 mg/kg bw/day	No effect on body weight; no effect on body weight gains			
			Rat	13 Weeks	Oral	12000 ppm	Statistically significant decreased mean body weight at week 4 in males at 12000ppm; decreased overall body weight gain in males and females at 12000ppm			
			Dog	1 Years	Oral	2500 ppm	Decreased mean body weight in male 2500ppm group week 12-5 due to 1 individual; mean body weights dropped week 52 due to fasting for pathology testing			
			Mouse	104 Weeks	Oral	3000 ppm	Decreased body weight gain for females at 3000ppm			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	27 Months	Oral	2500 ppm	No effect on body weight			
			Rat	2 Gen adult (F0)	Oral	5000 ppm	Decreased body weight gain for females during pregnancy at 5000ppm; Increased body weight gain post-partum in females at 5000ppm			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	Decreased mean bodyweight in males and females at 5000ppm; decreased growth rate in males and females' weeks 1-4 at 5000ppm; Decreased body weight gain during pregnancy in females' weeks 1-2 of 1st mating at 1500 and 5000ppm, and full duration of 2nd mating at 1500 and 5000ppm			
		Food Consumption	Rabbit	13 Days	Oral	300 mg/kg bw/day	Decreased absolute maternal feed consumption at 300 mg/kg days 6-19 (entire dosage period); decreased relative maternal feed consumption at 300 mg/kg days 6-19 (entire dosage period)	No consistent treatment related effect on food consumption		
			Rat	2 Gen Adult (F0)	Oral	400 mg/kg bw/day	Statistically significant decreased maternal food consumption at 400 mg/kg			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	Decreased food intake in males and females at 12000ppm during treatment period; Increased food consumption during recovery period in females at 12000ppm, but not in males; Increased food conversion ratio both during treatment and recovery in males and females at 12000ppm			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	Decreased group mean food intake in males and females during treatment at 300 mg/kg, primarily due to lower intake weeks 1-3, no effect during recovery			
			Rat	13 Weeks	Oral	12000 ppm	Slight but not statistically significantly decreased food consumption for males at 12000ppm			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm]) No treatment-related effect on food consumption; initial lack of appetite week 1 in males (2 at 500ppm, 2 at 2500ppm) and females (1 at 2500ppm) recovered week 2 in all except 1 male 500ppm and 1 male			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
							2500ppm, considered due to palatability problems			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	Statistically significant increased food consumption in males' weeks 1-40 at 2500ppm, only occasional after this point			
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	Decreased food consumption weeks 5-8 in males and females at 5000ppm, recovered to control levels week 8-16 in males, marginal reduction in females			

1.1.4.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

The weight of evidence for EAS-mediated adversity is summaries in Table 2.2.2.1-1 and for EAS-mediated endocrine activity in Table 2.2.2.1-2. The overall WoE for EAS-mediated activity is indicative of negative activation of EAS modalities.

Table 1.1.4.1-1 WoE for EAS-mediated adversity

• Evaluation of two generations study that access all the relevant parameters did not show any ED effects.
• EAS parameters were examined in multiple studies at different dose levels and of different durations in rats and dogs by oral administration of the substance. EAS-mediated effects were also examined in one study in rats via dermal application of the substance.
• No EAS-mediated adverse effects were consistently observed in any of the species at any of the dose levels tested.
• There was no evidence for the identification of EAS-mediated adversity.

Table 1.1.4.1-2 WoE for EAS-mediated endocrine activity

• Negative for the following *in vitro* investigations at OECD Conceptual Framework Level 2: ToxCast ER bioactivity (agonism and antagonism) ToxCast AR bioactvity (agonism and antagonism) ToxCast steroidogenesis activity AR binding assay Aromatase assay
• No evidence for identification of EAS-mediated endocrine activity

1.1.5 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

A dataset is considered to have sufficiently investigated EAS related adversity in relation to mammals if the parameters investigated in a two-generation reproductive toxicity study (OECD TG 416) conducted to the 2001 revision of this guideline have been assessed (EFSA-ECHA, 2018).

Although the two-generation study for dicamba was conducted prior to 2001, the current study exceeded requirements of the 1983 revision of the OECD 416 test guideline by including sperm assessment, oestrus cyclicity, corpora lutea counts, full assessment of histopathology and organ weights (with the exception of uterus and thyroid weights).

Table 2.2.3-1 Comparison of the Parameters Sensitive to Perturbation of the Endocrine System required in the 2001 Revision of OECD 416 and the Two-generation Toxicity Study with Dicamba.

Parameter	Assessed in the two-generation study with dicamba
Gross necropsy (macroscopic) observations	Yes
Reproductive performance: • Pre-coital interval • Mating (copulation indices) • Fertility • Gestation index • Duration of gestation • Parturition • Litter size (reductions in litter size can be indicative of abortions/resorptions/intra-uterine deaths) • Number of implantations	Yes
Number of *corpora lutea*	Yes – references the appearance or absence of reduced corpora lutea but does not directly state the number
Sex ratio	Yes
Oestrus cyclicity	Yes – vaginal smears taken daily for 7 days prior to mating for F0 and first mate of F1generation and during the 20 day mating period to detect marked anomalies of the oestrus cycle
Sexual maturation (vaginal opening and preputial separation)	Yes
Ano-genital distance	No
Sperm analysis (number, motility and morphology)	Yes
Organ weights: uterus, ovaries, testes, epididymides, prostate, seminal vesicles with coagulating glands, pituitary, thyroid and adrenal glands	All, except uterus and thyroid were not weighted
Histopathological examination: vagina, uterus (with cervix), ovaries, testis, epididymis, seminal vesicles, prostate (and coagulating gland)	Yes

1.1.6 Data set sufficiency for EATS-related endocrine activity (OECD CF Level 2/3 test)

The potential for dicamba to have endocrine activity *in vitro* was extensively examined as part of the United States Environmental Protection Agency's ToxCast™ programme, which included binding, transactivation and steroidogenic assays equivalent to OECD Conceptual Framework Level 2. Whilst dicamba had no significant effect in any of these assays, the EFSA-ECHA Guidance specifically requests mechanistic studies in OECD Conceptual Framework Level 3, to confirm an absence of activity *in vivo*, following negative *in vitro* assays.

The US EPA ToxCast™ ER and AR Bioactivity Models are able to accurately predict the outcome of Uterotrophic and Hershberger assays, and could therefore be used as alternatives to *in vivo* testing to address E and A modalities. These bioactivity models integrate data from a range of high throughput *in vitro* assays from the US EPA's ToxCast™ and Tox21 programs (18 oestrogen receptor assays and 11 androgen receptor assays) examining pathway perturbations at multiple points along receptor binding, co-regulator recruitment, gene transcription, and protein production axes and across multiple types of cell. The calculations based on *in vitro* assays data have been shown to predict *in vivo* activity in the Uterotrophic and Hershberger assays to a high degree of precision (Browne *et al*., 2015; Judson *et al.* 2015; Kleinstreuer *et al.* 2017) with accuracies of >84% for the prediction of Uterotrophic study outcome, and 95.2% for the outcome of Hershberger assays run in agonism mode and 97.5% for Hershberger assays run in antagonism mode.

In the androgen receptor bioactivity model, dicamba exhibited an agonism score of 0 and an antagonism score of 0, these values do not exceed the 0.001 score specified by the US EPA as the criteria for defining a compound as negative in the bioactivity model (Kleinstreuer *et al.* 2017). On this basis the US EPA androgen receptor bioactivity model prediction is that dicamba will not be positive in a Hershberger assay run in either agonism or antagonism mode. Due to the high degree of predictivity exhibited by this model Syngenta believe that this negative prediction is sufficient to establish that dicamba is not likely to exhibit androgenic or antiandrogenic activity *in vivo* without the need to generate additional data.

Dicamba exhibited a model score of 0 in the US EPA oestrogen receptor bioactivity model, this value indicates no activity against the oestrogen receptor and is below the score of 0.1 defined by the authors as the criteria for considering a compound to be active in this model (Browne *et al*., 2015). This ER bioactivity model was parameterised using the ToxCast™ *in vitro* data described in Section 4.1.2 of this document. This information is sufficient to establish that dicamba is not likely to exhibit estrogenic activity *in vivo* without the need to generate additional data.

Dicamba was tested in 21 ToxCast assay component endpoints related to steroidogenesis, with the majority of these endpoints being assessed in a high-throughput steroidogenesis assay in H295R Human Adrenocortical Carcinoma Cells (Karmaus *et al.*, 2016). There is no indication that dicamba has a specific effect on steroidogenesis in H295R cells. In addition, no effect on aromatase activity were observed. On this basis, sufficient data exist to conclude that dicamba does not inhibit steroidogenesis.

Available *in vitro* mechanistic information indicates that dicamba does not inhibit the activity of thyroid peroxidase, the sodium-iodide symporter, or deiodinase enzymes, and does not interact with the thyroid hormone receptor.

Table 2.2.4-1 Selection of Relevant Scenario for the ED Assessment of EAS-modality in Mammals

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 no "**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	

1.1.7 MoA analysis for EAS-modalities

Not relevant at present time. No consistent effect on any parameter described as "EATS-mediated" in the guidance document was identified in the dicamba mammalian toxicology database.

1.1.8 Conclusion of the assessment of EAS-modalities

Although the two-generation study for dicamba was conducted prior to 2001, the current study exceeded requirements of the 1983 revision of the OECD 416 test guideline by including sperm assessment, oestrus cyclicity, corpora lutea counts, full assessment of histopathology and organ weights (with the exception of uterus and thyroid weights). This study therefore is considered to meet the requirements of the 2001 revision of OECD test guideline. No consistent effects on any EAS parameters were observed for dicamba.

Dicamba therefore occupies scenario 1a for the EAS modalities, and as such the ED criteria are not met for these modalities.

2.10.23 Overall conclusion on the ED assessment for humans

In conclusion, based on the available evidence, the T modality is considered sufficiently investigated and no adversity has been observed. Therefore, the substance does not meet the ED criteria for the T modality.

Based on the available evidence, the EAS modality is considered sufficiently investigated and no adversity has been observed. Therefore, the substance does not meet the ED criteria for the EAS modality.

2.10.24 ED assessment for non-target organisms

According to the Criteria an adverse effect relevant to non-target organisms "is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub) population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences".

Effects on endpoints relevant to survival, growth, development and reproduction in available ecotoxicology studies may therefore be regarded as relevant to establishing evidence for adverse effects. However, as indicated in the Guidance document with respect to validated test guidelines informative for endocrine disrupting properties, such endpoints can only be considered 'Sensitive to, but not diagnostic of, EATS'.

Studies recommended in the guidance document as sufficient for investigation of 'EATS-mediated adversity' in non-target organism are as follows:

- Fish full life study (MEOGRTS, OECD 240, or equivalent);
- Larval amphibian growth and development assay (LAGDA, OECD 241), though a negative AMA is acceptable in lieu of a LAGDA.

Studies recommended in the guidance document as sufficient for investigation of 'endocrine activity' in non-target organism are as follows:

- Fish short-term reproduction assay (FSTRA, OECD 229) or 21-d fish assay (OECD 230);
- Amphibian metamorphosis assay (AMA, OECD 231)

2.10.25 ED assessment for T-modality

2.10.26 Have T-mediated parameters been sufficiently investigated?

Table 3.1.1-1 Assessment of dataset sufficiency for T-modality in non-target organisms

	Sufficiently investigated
T-mediated parameters	**No** Based on non-availability of Studies measuring T-mediated adversity: - LAGDA study (OECD 241) - (negative) AMA (OECD 231) Studies measuring T-mediated activity - AMA (OECD 231)

2.10.26.1 Lines of evidence for adverse effects and endocrine activity related to T-modality

Table 3.1.2-1 Lines of evidence for thyroid activity and adversity in non-target species

	Grouping	Line(s) of Evidence	Species	Exposure	Route of exposure	Effect Concentration	Observed effects	Assessment	Assessment of integrated line of evidence	Modality
Integrated lines of evidence for endocrine activity	***In vitro* mechanistic**	Thyroid transporter transthyretin binding	See section 4.1.2				Inactive in thyroid transporter transthyretin binding assay	No evidence of endocrine activity	Overall not indicative of endocrine activity	T
		ToxCast thyroid assays (10)					Inactive in all ToxCast thyroid assays	No evidence of endocrine activity		
		CALUX nuclear receptor assay (TRb)					Inactive in TRb assay	No evidence of endocrine activity		
		ToxCast thyroid peroxidase inhibition assay					Inactive in ToxCast thyroid peroxidase inhibition assay	No evidence of endocrine activity		
		ToxCast sodium-iodine symporter inhibition assay					Inactive in ToxCast sodium-iodine symporter inhibition assay	No evidence of endocrine activity		
	***In vivo* mechanistic**	n/a								
Integrated lines of evidence for adversity	**EATS-mediated parameters**	n/a								
	Sensitive to, but not diagnostic of, EATS	Length	*Pimephales promelas*	33 days	Water	n/a	No effect on length	No evidence of adversity	Overall not indicative of adverse effects from parameters sensitive to, but not diagnostic of, EATS	N
			Oncorhynchus mykiss	21 days	Water	n/a	No effect on length	No evidence of adversity		
			Cyprinodon variegatus	34 days	Water	n/a	No effect on length	No evidence of adversity		
		Weight	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity		
			Anas platyrhynchos	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity		

			Pimephales promelas	33 days	Water	n/a	No effect on weight	No evidence of adversity		
			Oncorhynchus mykiss	21 days	Water	n/a	No effect on weight	No evidence of adversity		
			Cyprinodon variegatus	34 days	Water	n/a	No effect on weight	No evidence of adversity		
		Development	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on number of hatchlings	No evidence of adversity		
			Anas platyrhynchos	21 weeks	Dietary	1600 ppm	Decrease in hatchlings	Potential evidence of systemic toxicity at highest test concentration		
			Pimephales promelas	33 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
			Cyprinodon variegatus	34 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
		Morphology	*Anas platyrhynchos*	21 weeks	Dietary	n/a	No abnormalities	No evidence of adversity		
			Pimephales promelas	33 days	Water	n/a	No abnormalities	No evidence of adversity		
Evidence of general toxicity	Mortality		*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		
			Anas platyrhynchos	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		
			Oncorhynchus mykiss	21 days	Water	n/a	No effect on mortality	No evidence of adversity		
			Pimephales promelas	33 days	Water	n/a	No effect on mortality	No evidence of adversity		
			Cyprinodon variegatus	34 days	Water	n/a	No effect on mortality	No evidence of adversity		
	Behaviour		*Oncorhynchus mykiss*	21 days	Water	320, 580, 1000 mg/L	Calm behaviour	Consistent with stress due to systemic toxicity		
			Colinus virginianus	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		
			Anas platyrhynchos	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		

3.1.2.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

The weight of evidence for T-mediated adversity is summarized in 3.1.2.5-1 and for T-mediated endocrine activity 3.1.2.5-2. The overall weight of evidence is not indicative of T-mediated adversity or endocrine activity, although not sufficiently investigated.

Table 3.1.2.5-1 WoE for T-mediated adversity

• No endpoints for T-mediated adversity were examined, but several endpoints 'sensitive to, but not diagnostic of, EATS' were considered (e.g., growth, development)
• No effects independent of systemic toxicity were observed for endpoints 'sensitive to, but not diagnostic of, EATS' in any of the species at any of the dose levels tested
• There was no evidence for the identification of a T-mediated adverse effect, although not sufficiently investigated

Table 2.10.26.1-2 WoE for T-mediated endocrine activity

• No data were available for T-mediated '*in vivo* mechanistic' activity in non-mammalian organisms • Several mammalian assays were considered for T-mediated '*in vitro* mechanistic' activity
• Negative for the following '*in vitro* mechanistic' investigations: o Thyroid transporter transthyretin binding o ToxCast thyroid assays (10) o CALUX nuclear receptor assay (TRb) o ToxCast thyroid peroxidase inhibition assay o ToxCast sodium-iodine symporter inhibition assay
• No evidence for identification of T-mediated endocrine activity, although not sufficiently investigated

3.1.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

A Larval Amphibian Growth and Development Assay (LAGDA, OECD 241) is not currently available for dicamba, nor is an amphibian metamorphosis assay (AMA, OECD 231). Therefore, after considering all lines of evidence, T-mediated adversity and endocrine activity are not sufficiently investigated.

Table 3.1.3-1 Selection of Relevant Scenario for the ED Assessment of T-modality in Non-target Organisms

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	
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	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.26.2 MoA analysis for T-modality

T-mediated adversity and T-activity have not been sufficiently investigated for dicamba; the ecotoxicology database only included parameters 'sensitive to, but not diagnostic of, EATS'. Therefore, a MOA analysis for the T modality is not appropriate at this time.

2.10.27 Conclusion of the assessment of T-modality

Based on scenario 2a (iii) applies: No endocrine activity, but not sufficiently investigated.

2.10.28 ED assessment for EAS-modalities

2.10.29 Have EAS-mediated parameters been sufficiently investigated?

Table 3.2.1-1 Assessment of dataset sufficiency for EAS-modalities in non-target organisms

	Sufficiently investigated
EAS-mediated parameters	No, based on non-availability of Studies measuring EAS-mediated adversity: - MEOGRT (OECD 240) or FLCTT measuring all endpoints foreseen to be measured in OECD 240 Studies measuring EAS-mediated activity - FSTRA (OECD 229) or 21 day fish screening study (OECD 230)

2.10.30 3.2.2 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

Table 3.2.2-1 Lines of evidence for estrogen, androgen, and steroidogenesis activity and adversity in non-target species

	Grouping	Line(s) of Evidence	Species	Exposure	Route of exposure	Effect Concentration	Observed effects	Assessment	Assessment of integrated line of evidence	Modality
Integrated line of evidence for endocrine activity	***In vitro* mechanistic**	ToxCast estrogen assays (22) and model	See section 4.1.2				Inactive in all ToxCast estrogen assays and model	No consistent ER bioactivity, for both agonism and antagonism	Overall not indicative of endocrine activity	E
		CALUX nuclear receptor assays (ERα, ERβ)					Active in ERα assay, inactive in ERβ assay			
		ToxCast androgen assays (14) and model					Inactive in all ToxCast androgen assays and model	No AR bioactivity, for both agonism and antagonism		A
		CALUX nuclear receptor assay (AR)					Inactive in AR assay			
		ToxCast H295R assay					Inactive for all steroid hormones	No effects on steroidogenesis		S
		ToxCast aromatase assay					Inactive in ToxCast aromatase assay			
	***In vivo* mechanistic**	n/a								
Integrated line of evidence for adversity	**EATS-mediated parameters**	n/a								
	Sensitive-to-but not diagnostic of EATS	Fecundity	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on egg production	No evidence of adversity	Overall not indicative of adverse effects from parameters sensitive to,	N
			Anas platyrhynchos	21 weeks	Dietary	n/a	No effect on egg production	No evidence of adversity		
		Fertility	*Colinus virginianus*	21 weeks	Dietary	n/a	No effects on egg quality, viable embryos, or number of 14-day-old survivors	No evidence of adversity		

			Anas platyrhynchos	21 weeks	Dietary	1600 ppm	Decrease in number of 14-day-old survivors; no effects on egg quality, viable embryos	Potential evidence of systemic toxicity at highest test concentration	but not diagnostic of, EATS	
		Length	*Pimephales promelas*	33 days	Water	n/a	No effect on length	No evidence of adversity		
			Oncorhynchus mykiss	21 days	Water	n/a	No effect on length	No evidence of adversity		
			Cyprinodon variegatus	34 days	Water	n/a	No effect on length	No evidence of adversity		
		Weight	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity		
			Anas platyrhynchos	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity		
			Pimephales promelas	33 days	Water	n/a	No effect on weight	No evidence of adversity		
			Oncorhynchus mykiss	21 days	Water	n/a	No effect on weight	No evidence of adversity		
			Cyprinodon variegatus	34 days	Water	n/a	No effect on weight	No evidence of adversity		
		Development	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on number of hatchlings	No evidence of adversity		
			Anas platyrhynchos	21 weeks	Dietary	1600 ppm	Decrease in hatchlings	Potential evidence of systemic toxicity at highest test concentration		
			Pimephales promelas	33 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
			Cyprinodon variegatus	34 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
		Morphology	*Anas platyrhynchos*	21 weeks	Dietary	n/a	No abnormalities	No evidence of adversity		
			Pimephales promelas	33 days	Water	n/a	No abnormalities	No evidence of adversity		
	Mortality		*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		

Evidence of general toxicity		*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		
		Oncorhynchus mykiss	21 days	Water	n/a	No effect on mortality	No evidence of adversity		
		Pimephales promelas	33 days	Water	n/a	No effect on mortality	No evidence of adversity		
		Cyprinodon variegatus	34 days	Water	n/a	No effect on mortality	No evidence of adversity		
	Behaviour	*Oncorhynchus mykiss*	21 days	Water	320, 580, 1000 mg/L	Calm behaviour	Consistent with stress due to systemic toxicity		
		Colinus virginianus	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		
		Anas platyrhynchos	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		

3.3.2.1 Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity

The weight of evidence for EAS-mediated adversity is summarized in Table 1.1.4.1-1 and for EAS-mediated endocrine activity in Table **1.1.4.1-2**. The overall weight of evidence is not indicative of EAS-mediated adversity or endocrine activity, although not sufficiently investigated.

Table 2.10.26.2-1 WoE for EAS-mediated adversity

- No endpoints for EAS-mediated adversity were examined, but several endpoints 'sensitive to, but not diagnostic of, EATS' were considered (e.g., fecundity and fertility)

- No effects independent of systemic toxicity were observed for endpoints 'sensitive to, but not diagnostic of, EATS' in any of the species at any of the dose levels tested

- There was no evidence for the identification of an EAS-mediated adverse effect, although not sufficiently investigated

Table 2.10.26.2-2 WoE for EAS-mediated endocrine activity

- No data were available for EAS '*in vivo* mechanistic' activity in non-mammalian organisms
- Several mammalian assays were considered for EAS '*in vitro* mechanistic' activity

- Negative for the following '*in vitro* mechanistic' investigations:
  - ToxCast estrogen assays (22) and model
  - CALUX nuclear receptor assays (ERa and ERb)
  - ToxCast androgen assays (14) and model
  - CALUX nuclear receptor assay (AR)
  - ToxCast H295R assay
  - ToxCast aromatase assay

- No evidence for identification of EAS-mediated endocrine activity, although not sufficiently investigated

2.10.31 3.2.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

A fish full life cycle study (MEOGRTS, OECD 240, or equivalent) is not currently available for dicamba, nor is a fish short-term reproduction assay (FSTRA, OECD 229) or 21-day fish assay (OECD 230). Therefore, after considering all lines of evidence, EAS-mediated adversity and endocrine activity are not sufficiently investigated.

Table 3.2.3-1 Selection of Relevant Scenario for the ED Assessment of EAS-modality in non-target Organisms

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 no "**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	
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	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

3.2.4 MoA analysis for EAS-modalities

EAS-mediated adversity and EAS-activity have not been sufficiently investigated for dicamba; the ecotoxicology database only included parameters 'sensitive to, but not diagnostic of, EATS'. While a published study in rare minnow included 'in vivo mechanistic' and 'EATS-mediated' parameters, the study was not deemed reliable and therefore could not be used to support a MOA analysis. Thus, a MOA analysis for the EAS modality is not appropriate at this time.

3.2.5 Conclusion of the assessment of EAS-modalities

Scenario 2a (iii) applies: No endocrine activity, but not sufficiently investigated.

2.10.32 3.3 Overall conclusion on the ED assessment

In conclusion, for both the T and EAS modalities, adversity has not been sufficiently investigated in non-target organisms, nor has endocrine activity. Therefore, additional information will need to be generated in order to determine whether dicamba exhibits endocrine disrupting properties in non-target organisms.

2.10.33 OVERALL conclusion on the ED assessment

4.0 Human Health

Dicamba has been extensively tested, with the relevant data from literature and regulatory studies covering a wide range of study types in vitro and in vivo. These data fall into the levels 1, 2, 4 and 5 of the OECD Conceptual Framework. Considering the available regulatory study database in accordance with the EFSA-ECHA Guidance (2018) there is sufficient information to conclude that dicamba does not adversely affect the EAS or the T modalities.
In addition, a number of relevant sources of information were identified to evaluate the potential for EAS modalities to be operant for dicamba. Evaluation of the outputs of the US EPA estrogen receptor and androgen receptor bioactivity models indicated a low likelihood that dicamba exhibits E or A activity in vivo. Furthermore, assessments of aromatase activity, and effects on steroidogenesis in H295R cells indicated no overall effect of dicamba on steroidogenesis.

As no further information is required to conclude that E, A, S, and T modalities are not likely to be operant in mammals in vivo it can be concluded that dicamba does not meet the scientific criteria defining a human endocrine disruptor implemented by Commission Regulation (EU) 2018/605.

4.2 Non-Target Organisms

Evaluation of the available data in accordance with the EFSA-ECHA Guidance document (2018) indicates that there is an inadequate ecotoxicology dataset to conclude that dicamba exhibits endocrine disrupting properties in non-target organisms according to the ED Criteria (2018/605). EATS-mediated adversity has not been fully investigated in non-target organisms (e.g., OECD 240, OECD 241), nor has endocrine activity (e.g., OECD 229/230, OECD 231). Consequently, according to the guidance document, additional information will need to be generated in order to determine whether dicamba exhibits endocrine disrupting properties in non-target organisms.

As first steps to make sufficient data available to reach a conclusion, Syngenta proposes to conduct the following studies:

1) 21-day fish screening assay (OECD 230) in the Fathead minnow.
2) Amphibian Metamorphosis Assay (OECD 231).

2.10.34 REFERENCES

NOTE: Only public literature references are listed here.

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2.10.35 RMS comments and ED assessment:

ED assessment for humans

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Lines of evidence for thyroid activity and adversity in mammals

	Grouping	**Line(s) of evidence**	**Species**	**Exposure**	**Route of exposure**	**Effect dose -**	**Observed effects**	**Assessment of each line of evidence**	**Assessment of the integrated lines of evidence**	**Modality**
Evidence for endocrine activity	*In vitro* mechanistic	Thyroid receptor (α / β) transactivation	Rat				No agonism or antagonism of thyroid receptor reporter gene expression in GH3 rat pituitary gland cells	Negative, no evidence for thyroid interaction *in vitro*	Overall negative, no evidence for a consitent pattern of endocrine activity and adversity in the T modality	**T**
		Thyroid receptor (THRa1) transactivation	Human				No up (agonism) or down (antagonism) reporter gene expression in human HepG2 cells			
		Inhibition of TPO (Thyroid peroxidase)	Rat				No inhibition of TPO			
		Inhibition of NIS (Sodium-iodide symporter)	Human				negative based on a threshold of less than 20% inhibition in the RAIU assay			
		Deiodination enzyme inhibition	Human				no inhibition of DIO1, DIO2 and DIO3			
		Thyrotropin releasing hormone (TRH) receptor	Rat				No binding detected			

	T-mediated parameter	Thyroid (weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	Negative, no alteration to thyroid weight		
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	a decrease in thyroid weight in males was noted after 4 weeks of recovery but was not considered biologically plausible because the effect was not present before the recovery period (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Thyroid (Histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	Increased incidence of c-cell carcinomas in the carcinogenicity study in the absence of an increased incidence of releated histopathological findings. No consistent effect across studies.		
			Rat	28 Days	Dermal	1000 mg/kg bw/day	Cysts were observed more frequently in high dose (6/10) female rats than in control (3/10) in the study, but without dose response and the effect was not seen in other studies it was likely spontaneous and not treatment related (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			

			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	In the 4 week recovery group, focal c-cell hyper-plasia was observed in 2 females in control and 4 in high dose, but after 13 weeks here was 1 in each of these groups indicating the finding was likely not related to treatment. No increase was seen in males (Jackson, 2003) (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Mon-ths	Oral	250 ppm	Increase in parafollicular carcinomas, - There were also no accompanying changes to function of thyroid,			

Evidence of general toxicity	Liver (weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	No effect on organ	No consistent effect on the liver		
		Rat	28 Days	Dermal	1000 mg/kg bw/day	No effect on organ			
		Rat	28 Days	Inhalation	0.05 mg/L	No effect on organ			
		Rat	13 Weeks	Oral	12000 ppm	Statistically significant increase in mean relative liver weight in males and females after treatment at 12000ppm, like control group after recovery			
		Dog	13 Weeks	Oral	300 mg/kg bw/day	No effect on organ			
		Dog	90 Days	Oral	300 mg/kg bw/day	No treatment-related effect on organ			
		Dog	1 Years	Oral	2500 ppm	No effect on organ			
		Mouse	104 Weeks	Oral	3000 ppm	No treatment-related effect on organ			
		Rat	27 Months	Oral	2500 ppm	No effect on organ			
		Rat	2 Gen Adult (F0)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
		Rat	2 Gen Offspring (F1)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
		Rat	2 Gen Adult (F1)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
		Rat	2 Gen Offspring (F2)	Oral	5000 ppm	Increased liver weight for males and females at 5000ppm			
	Liver (histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
		Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
		Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
		Rat	13 Weeks	Oral	12000 ppm	12000 ppm Minimal/slight hypertrophy in centrilobular hepatocytes in females after treatment at 12000ppm, not observed after recovery			
		Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
		Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
		Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			

		Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
		Rat	27 Months	Oral	2500 ppm	Increased incidence of liver necrosis (at highest dose tested [2500 ppm])			
		Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

T-mediated endocrine activity:

The level of thyroid hormones or TSH was not measured in any of the above mentioned studies. Dicamba was inactive in 10 of 10 available ToxCast thyroid assays. Based on the data from published literature dicamba was considered to be inactive in the inhibition of deiodinase enzyme 1 (Hornung, 2018), sodium iodide symporter (Wang, 2018), and thyroid peroxidase activity (Friedman, 2017). Dicamba was predicted to bind to Transthyretin in a QSAR Model but subsequently tested negative in a competitive [125I]-T4-Transthyretin ligand binding assay (Zhang,2015). No indication of T-mediated endocrine activity was found in vitro.

T-mediated adversity:

In above mentioned studies, no pattern of adversity relating to the thyroid gland was observed (changes in thyroid weight in rats and dogs; no macroscopic changes in rats, mice, and dogs. Cysts were observed more frequently in high dose (6/10) female rats than in control (3/10) in the Rattray study (2002), but without dose response and the effect was not seen in other studies it was likely spontaneous and not treatment related. In the 90 day dog study (Jackson, 2003) a decrease in thyroid weight in males was noted after 4 weeks of recovery but was not considered biologically plausible because the effect was not present before the recovery period. In the 4 week recovery group, focal c-cell hyperplasia was observed in 2 females in control and 4 in high dose, but after 13 weeks here was 1 in each of these groups indicating the finding was likely not related to treatment. No increase was seen in males (Jackson, 2003).

A study reported a cross-sectional investigation of the association between self-reported history of physician diagnosed thyroid disease (hypothyroidism, hyperthyroidism, and "other" thyroid disease) and exposure to pesticides among 22,246 male pesticide applicators in the Agricultural Health Study (AHS). Statistically significant associations were observed between ever use of dicamba and hypothyroidism (OR=1.37; 95% CI 1.13-1.66, n=289). In exposure–response analyses using the intensity weighted exposure measure, no trend was seen for exposure to dicamba. Limitations included self-reported outcome, the inability to determine whether exposure preceded disease onset, and the possibility of chance associations resulting from the evaluation of 50 different pesticides with three different thyroid outcomes (Goldner et al, 2013). The relevance was therefore considered low but the results should be part of the weight of evidence considerations.

Regarding T-modality, T-mediated adversity has been sufficiently investigated and no T-mediated adversity has been observed across studies and species (rat, mouse and dog).

Conclusion on T-modality: As no treatment related adversity to the thyroid gland was observed and the adversity was sufficiently investigated, it can be concluded that ED criteria for T-modality are not met (i.e. Scenario 1a is applied).

Other MOA:

Only effect observed on the thyroid was the increase in carcinoma of the c-cells in male rats (Goldenthal, 1985). C-cells are not involved in the traditional thyroid hormone production as T3 and T4 but makes calcitonin which is a hormone involved in regulation the calcium level of the blood.

The MOA is unknown, however, the increase in c-cell carcinoma may be hormone related.

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
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	

EAS-modalities

Lines of evidence for estrogen, androgen, and steroidogenesis activity and adversity in mammals

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
Evidence for endocrine activity	*In vitro* mechanistic	ER binding	Human				Inactive	Negative, no evidence for estrogenicity *in vitro*	Overall negative, no evidence for estrogenic,, androgenic or steroidogenic activity	**E**
			Bovine				Inactive			
		ER dimerization	Human				Inactive (α/α, β/β, α/β)			
		ERE activity	Human				Inactive in HepG2 human liver cell line ERE cis-activation (agonism or antagonism)			
		Estrogen receptor (α / β) transactivation	Human				No up (agonism) or down (antagonism) reporter gene expression in human HepG2, HEK293T, HeLa or BG1 cells			
		AR binding	Chimpanze				Inactive	Negative, no evidence for androgenicity *in vitro*		**A**
			Human				Inactive			
			Rat				Inactive			
		Androgen receptor transactivation	Human				Inactive			
		Aromatase inhibition	Human				Inactive	Negative, no evidence for an effect on steroidogenesis *in vitro*		**S**
		H295R adrenal assay (Ceetox)	Human				No effect on 11-Deoxycortisol and 17-alpha-hydroxyprogesterone, Androstenedione, Cortisol, 11-Deoxycorticosterone, Estradiol, Estrone, 17-alpha-hydroxypregnelone, testosterone and progesterone levels			
Integrated lines of evidence for adversity	EAS-mediated para-meter	Ovary (Weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	No consistent effects on ovaries	Overall negative, no evidence for a consitent pattern of endocrine adversity	**EAS**
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhala-tion	0.05 mg/L	decreased (at high-est dose tested [0.05 mg/L]) not statisti-cally significant			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	No treatment related effect [300 mg/kg bw/day]. Not statis-tically significant			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Dog	1 Years	Oral	2500 ppm	Decreased rel and absolute ovary weight (at highest dose tested [2500 ppm]) not statisti-cally significant.			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Ovary (histopa-thology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhala-tion	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Uterus weight (with cer-vix)	Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])	No consistent effects. Some effects were observed in aged animals		
			Rat	28 Days	Inhala-tion	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
		Uterus his-topatho-logy (with cervix)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	28 Days	Inhala-tion	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	endometrial hyper-plasia slight increse in incidenc (at high-est dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	Slightly increased incidence of cystic hyperplasia in Uterus:15/49, 17/49 13/50, 20/49 (at highest dose tested [2500 ppm])			
			Rat	27 Months	Oral	2500 ppm	Increased incidence of polyps (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspri ng (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Vagina histopa-thology	Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])	No consistent effect on va-gina		
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspri ng (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Oestrus cyclicity	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])	No alteration to oestrus cy-clicity		
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
		Testis (Weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])	o consistent effects on testis		
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	Statistically significant increase in rel weight but not abs or rel to brain weight (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	Decreased abs and rel weight (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	Decreased abs and rel (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
		Testis (histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
		Epididymis (Weight)	Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])	No consistent effect on epididymis		
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
		Epididymis (histopathology)	Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Prostate (Weight)	Dog	90 day	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent treatment related effect		
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Prostate histopathology (with seminal vesicles and	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
		coagulating glands)	Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Sperm Number	Rat	2 Gen: Offspring (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])	No alteration to sperm number, sperm motility or sperm morphology		
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Sperm Motility	Rat	2 Gen: Offspring (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Sperm Morphology	Rat	2 Gen: Offspring (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])	Decreased pregnancy rate observed in F1 adult rats, evident in all in all groups - associated with higher body weight at pairing in all dose groups (including control). No effects on		
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	Decreased pregnancy rates in F1 generation (all doses)			
		Time to mating	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Gestation length	Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])	time of mating or gestation length		
		Number of implantations, corpora lutea	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent treatment related effects observed		
			Rat	2 Gen adult (F0)	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
		Numbers of embryonic or foetal deaths and viable foetuses	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Rat	14 Days	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
		Post implantation loss	Rabbit	13 Days	Oral	150 mg/kg bw/day	1 abortion at 150 mg/kg day 22 of gestation, 4 abortions at 300 mg/kg on days 19 (1), 21 (1) and 24 (2) of gestation	No consistent effect observed, abortions observed in the presence of systemic toxicity		
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Litter size	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent effect on litter size, viability and weight. In rats, at the second mate (F2B pups), there was a slight, non-significant higher pup loss at 5000ppm during the weaning period (persisting, even after culling on day 4 post-partum), resulting in slightly lower litter size.		
			Rat	14 Days	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	Slight decrease in litter size due to increased pup loss at 5000ppm			
		Litter viability	Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	Slight non-significant increased pup loss at 5000ppm during weaning period; No effect on loss post-partum			
		Litter/pup weight	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	Decreased mean pup weight at birth at 5000ppm; Decreased litter weight at 5000ppm; decreased pup growth through to weaning at 5000ppm; decreased mean pup			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
							weight at weaning at 5000ppm			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	Decreased mean pup weight at birth at 5000ppm; decreased litter weight at 5000ppm; decreased pup growth through to weaning at 1500 and 5000ppm;			
		Fetal development	Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	Delay in preputial separation at 5000ppm	Delay in sexual maturation in males as a result of delayed growth		
		Sex Ratios	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	No consistent treatment related effect		
			Rat	14 Days	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day])			
			Rat	2 Gen: Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen: Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Presence of anomalies (external, visceral, skeletal	Rabbit	13 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])	Delayed ossification were observed		
			Rabbit	13 Days	Oral	300 mg/kg bw/day	Increased incidence of irregularly ossified internasals at highest dose tested [300 mg/kg bw/day])			
			Rat	14 days	Oral	400 mg/kg bw/day	no effect (at highest dose tested [400 mg/kg bw/day]) Increased renal pelvic cavitations at 400 mg/kg, but 3 of 5 affected foetuses were from 1 litter			
			Rat	14 days	Oral	400 mg/kg bw/day	Increased incidence of incomplete ossification at highest dose tested [400 mg/kg bw/day])			
		Adrenal gland (Weight)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	Increased adrenal weight in females in low dose group (100 mg/kg), not observed in any other dose. No histopathological findings.	No consistent treatment related effect on adrenal gland		
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	10 % increase (at highest dose tested [0.05 mg/L]) not			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
							statistically signifi-cant			
			Rat	13 Weeks	Oral	12000 ppm	Decreased absolute (-30%) and relative to bw weight (-11%) (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Rat	2 Gen Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Adult (F1)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
			Rat	2 Gen Offspring (F2)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			
		Adrenal gland (Histopathology)	Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	no effect (at highest dose tested [2500 mg/kg bw/day])			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	Non treatment-related adrenal lesion in 3 males at 1000 mg/kg- lesion was also seen in 1 male at 300 mg/kg, 1 female at 30 mg/kg, and 1 control female.			
			Rat	28 Days	Inhala-tion	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			
			Rat	13 Weeks	Oral	12000 ppm	no effect (at highest dose tested [12000 ppm])			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	90 Days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm])			
Evidence of general toxicity		Body weight	Rabbit	13 Days	Oral	300 mg/kg bw/day	Decreased maternal body weight at 150 mg/kg days 6-8 and	Systemic toxicity evident at	Systemic toxicity evident in	**EAS**

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
							at 300 mg/kg days 6-19 (all of dosage period),19-29 (post dosage period), and days 6-29 and 0-29 periods; increased body weight gains at 150 and 300 mg/kg days 19-29 of gestation (post dosage period)	high dose group – body weight changes	doses of 160 mg/kg/day for rabbit and dog, 3000 ppm in mice and <500 ppm in rat	
			Rat	14 Days	Oral	160 mg/kg bw/day	Statistically significant decrease in maternal body weight gestation day 20 at 400 mg/kg bw/day and decreased adjusted bw gain at 160 and 400 mg/kg bw/day			
			Rat	2 Gen Offspring (F1)	Oral	400 mg/kg bw/day	No effect			
			Rabbit	3 Weeks	Dermal	2500 mg/kg bw/day	No effect on body weight			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	Slight decrease in body weight in males at 300 and 1000 mg/kg and females at 1000 mg/kg, but not consistently statistically significant			
			Rat	28 Days	Inhalation	0.05 mg/L	Decreased body weight change at 0.05 mg/L;			
			Rat	13 Weeks	Oral	12000 ppm	Decreased body weight gain for males and females during treatment at 12000ppm; Increased weight gained in males and females at 12000ppm during recovery period; Decreased weight in males and females at 12000ppm both during treatment and recovery period			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	Decreased mean body weight gain in males and females during treatment at 300 mg/kg, no effect during recovery period			
			Dog	90 Days	Oral	300 mg/kg bw/day	No effect on body weight; no effect on body weight gains			
			Rat	13 Weeks	Oral	12000 ppm	Statistically significant decreased mean body weight at week			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
							4 in males at 12000ppm; decreased overall body weight gain in males and females at 12000ppm			
			Dog	1 Years	Oral	2500 ppm	Decreased mean body weight in male 2500ppm group week 12-5 due to 1 individual; mean body weights dropped week 52 due to fasting for pathology testing			
			Mouse	104 Weeks	Oral	3000 ppm	Decreased body weight gain for females at 3000ppm			
			Rat	27 Months	Oral	2500 ppm	No effect on body weight			
			Rat	2 Gen adult (F0)	Oral	5000 ppm	Decreased body weight gain for females during pregnancy at 5000ppm; Increased body weight gain postpartum in females at 5000ppm			
			Rat	2 Gen Adult (F1)	Oral	15000 ppm	Decreased mean bodyweight in males and females at 5000ppm; decreased growth rate in males and females' weeks 1-4 at 5000ppm; Decreased body weight gain during pregnancy in females' weeks 1-2 of 1st mating at 1500 and 5000ppm, and 2nd mating at 1500 and 5000ppm.			
		Food Consumption	Rabbit	13 Days	Oral	300 mg/kg bw/day	Decreased absolute maternal feed consumption at 300 mg/kg days 6-19 (entire dosage period); decreased relative maternal feed consumption at 300 mg/kg days 6-19 (entire dosage period)	No consistent treatment related effect on food consumption		
			Rat	2 Gen Adult (F0)	Oral	400 mg/kg bw/day	Statistically significant decreased maternal food consumption at 400 mg/kg			
			Rat	28 Days	Dermal	1000 mg/kg bw/day	no effect (at highest dose tested [1000 mg/kg bw/day])			
			Rat	28 Days	Inhalation	0.05 mg/L	no effect (at highest dose tested [0.05 mg/L])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	13 Weeks	Oral	12000 ppm	Decreased food intake in males and females at 12000ppm during treatment period; Increased food consumption during recovery period in females at 12000ppm, but not in males; Increased food conversion ratio both during treatment and recovery in males and females at 12000ppm			
			Dog	13 Weeks	Oral	300 mg/kg bw/day	Decreased group mean food intake in males and females during treatment at 300 mg/kg, primarily due to lower intake weeks 1-3, no effect during recovery			
			Dog	90 days	Oral	300 mg/kg bw/day	no effect (at highest dose tested [300 mg/kg bw/day])			
			Rat	13 Weeks	Oral	12000 ppm	Slight but not statistically significantly decreased food consumption for males at 12000ppm			
			Dog	1 Years	Oral	2500 ppm	no effect (at highest dose tested [2500 ppm]) No treatment-related effect on food consumption; initial lack of appetite week 1 in males (2 at 500ppm, 2 at 2500ppm) and females (1 at 2500ppm) recovered week 2 in all except 1 male 500ppm and 1 male 2500ppm, considered due to palatability problems			
			Mouse	104 Weeks	Oral	3000 ppm	no effect (at highest dose tested [3000 ppm])			
			Rat	27 Months	Oral	2500 ppm	Statistically significant increased food consumption in males' weeks 1-40 at 2500ppm, only occasional after this point			
			Rat	2 Gen: Adult (F0)	Oral	5000 ppm	no effect (at highest dose tested [5000 ppm])			

	Grouping	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose -	Observed effects	Assessment of each line of evidence	Assessment of the integrated lines of evidence	Modality
			Rat	2 Gen: Adult (F1)	Oral	5000 ppm	Decreased food consumption weeks 5-8 in males and females at 5000ppm, recovered to control levels week 8-16 in males, marginal reduction in females			

EAS mediated endocrine activity

E modality: Dicamba was inactive in Toxcast E R Bioactivity Model, and therefore considered sufficiently investigated for E modality. In a published paper, dicamba showed an effect in ERα expressing cells. The value calculated was -5.5. (Van Vugt-Lussenburg et al , 2014), but the reliability of the study is questionable.

Conclusion on E-mediated endocrine activity: E-mediated endocrine activity was sufficiently investigated and dicamba is likely not an endocrine disruptor via the E receptor.

A modality: dicamba tested negative in 14/14 available ToxCast AR assays. Level 2 (OECD 458) and Level 3 (Hershberger bioassay in rats, i.e. OECD TG 441) tests are not available. Dicamba was not tested in OECD 458 assay.

Conclusion on A-mediated endocrine activity: No indication of A-mediated endocrine activity but A-mediated endocrine activity was not sufficiently investigated.

Steroidogenesis (S): Dicamba was tested in 2 ToxCast assays evaluating the potential of interaction with the human aromatase (hCYP19A1).

Level 2 assays according to guideline (H295R steroidogenesis assay, i.e. OECD TG 456 and aromatase assay, i.e. OPPTS 890.1200) are not available.

Conclusion on S-mediated endocrine activity: No indication of S-mediated endocrine activity but S-mediated endocrine activity was not sufficiently investigated.

EAS-mediated adversity:

Organ weights and histopathology:

Adrenal

No consistent effect was observed on adrenal weight or histopathology. In the combined chronic toxicity study in rats, (Goldenthal, 1987) pheochromocytoma of the adrenal medulla was observed in the incidence: 1/47, 4/48, 3/46 and 5/46 at 0, 50, 250 and 2500 ppm, respectively. No adrenal medulla pheochromocytoma were observed before 12 months of age and therefore RMS considers it appropriate to calculate the incidence out of the number of animals who died after 12 months or were killed at termination. Historical control data were supplied by Syngenta and collected in 1985 (acceptability of HCD are discussed above). Incidence in females was outside HCD range (0-8.3%) in the high dose (11%) but without clear dose-response (not statistically significant trend or by pairwise comparison). Because of the lack of dose-response and lack of increased finding of adrenal medullary hyperplasia, in females, the increased incidence of pheochromocytoma of the adrenal medulla may be considered incidental. Also, if it is considered acceptable to calculate the incidence out of 60 animals, the incidence in high dose group is 8.3% (5/60) which is just inside HCD range. In males, the incidence was also above HCD in some groups, but the highest incidence was found in controls and therefore not considered treatment related.

Uterus: no histopathological changes were observed in dogs. Uterus was weighted in the two 90 days dog studies and seemed increased in high dose in Kubaszky (2010) and decreased in high dose in Jackson (2003). No dose response was observed in either study and was not considered treatment related. In the combined chronic toxicity study in rats (Goldenthal, 1985), a slight increase in cystic hyperplasia in the uterus was observed in the high dose females at termination but not at interim sacrifice. The incidence was 15/49 (31%), 17/49 (35%), 13/50 (26%) and 20/49 (41%) at 0, 50, 250 and 2500ppm, respectively. In females, 4/60 (6.7%), 5/60 (8.3%), 5/60 (8.3%) and 8/60 (13.3%) polyps in the uterus was observed including all animals so the overall incidence of uterine polyps in the high dose group was slightly higher than concurrent and historical control data from the same laboratory (0-8.3%) but did not reach statistical significance. If only animals from 12 months to termination are considered, the incidences are 4/49 (8%), 3/49 (6%), 5/50 (10%), 8/49 (16%).

These effects were not observed in the 90 day rat study (Doubovetsky, 1997). In this study 2 females had hydrometra in high dose (12000ppm) versus none in control, however, hydrometra was also noticed in 1 control animal in the recovery control group and not considered treatment related.

Crome (mice) slight increase in endometrial hyperplasia in uterus in high dose with the incidences: 10/52 (19%), 14/41 (34%), 9/48 (19%), 12/48 (25%), 18/52 (35%) at 0, 50, 150, 1000 and 3000 ppm. There is a lack of clear dose response (although made difficult by different number of animals in groups) and the higher incidence in high dose was not considered treatment related.

In principle, effects on uterus in rats should be considered EATS mediated. The effects were only observed in aged animals and the effects in rats are also considered normal age related changes. However, if the higher incidence of these effects, observed in rats, in the high dose is a sign of treatment induced early reproductive senescence in females, this would be an adverse effect. It is difficult to confirm with the available data as estrus cycle was not investigated in the animals and no effects were observed in histopathology of the ovaries in the chronic studies. Furthermore, even if the aetiology of uterine endometrial stromal polyps is not well defined in rodents, there is no clear evidence that estrogen or estrogen-like compounds are associated with endometrial stromal polyp formation in rats while uterine endometrial polyps are recognised as being hormonal responsive in women (Davis, 2012)[21]. Other ED related MOA could be relevant, though.

Ovaries:
Absolute ovary weights seemed to be decreased in 13 week dog study (Jackson, 2003) but was not considered treatment related as there was no effect after adjusting for body weight. Ovary weight at 300 mg/kg bw/day did seem to be decreased after recovery, though. No changes was found in histopathology of the ovaries either at termination or after recovery.
Absolute and relative ovary weight was decreased in dogs (30 % abs/35 % rel, high dose) in the one year study without effects noted in the histopathological examination of the ovaries (Blair 1986).
No effects on ovary weight or histopathology in 28 day dermal toxicity study in rats (Rattray, 2002).
In the 90 day study in rats absolute ovary weight was 25 % decreased compared with control, but only 4 % relative to body weight probably reflecting the difference in body weight between groups. There were no histopathological findings in the ovaries after 13 weeks, and in recovery groups there was 1 animal with cyst and inflammation in top dose and none in control which were not considered dose related (Doubovetzky (1997).
Non-significant decrease in absolute and relative ovary weight (12-13%) with no histopathologic finding in the ovary was seen in the top dose in the 28 day inhalation toxicity study in rats (Ma-Hock).
In the combined chronic toxicity study in rats, ovaries did not seem affected and no treatment related histopatological differences from control were noted. No dose response was observed in variation of ovary weights (Goldenthal, 1985).
In the 2 generation study in rats, differences from control in high dose groups of absolute ovary weights were sometimes > 10%. However, not when adjusted to body weight or relative to body weight (Masters, 1998).
Ovaries were not weighed in mice in the carcinogenicity study but histopathology did not show effects different from control (Crome, 1988).
In dogs, no clear pattern was obvious of effects on the ovaries since observations were a decrease in the one year dog study (Blair, 1986) and an increase in ovary weight in a 90 day study but not considered treatment related because it was driven by 1 animal with an ovarian cyst in high dose (Kubaszky, 2010) while no clear effect was observed in the other 90 day dog study (Jackson, 2003). No clear pattern was observed in rats or mice either.

Testicles:
Testes weight in the high dose was 17 % (abs) and 11 % (rel) lower than in control without histopathologial changes and the change was not statistically significant (Jackson).

Testes weight seemed to be decreased in the one year dog study (Blair, 1981) (11 % abs/13 % rel, high dose), which was not statistically significant. The standard deviation was a bit high in the control group and considering the low number of test animals, the decreased testes weight may not be considered adverse. No effects were seen in the histopathological examination of the organ.

Kubazski (2012). Testes weight seemed to be slightly decreased in the high dose group but was < 10% absolute, or did not show clear dose response and furthermore no effects were noted histopathologically. Therefore, the effect on testes was not considered adverse.

No effects on testes were seen in rats in 28 days study (Ma-Hock, 2009 ; Rattray, 2002) or in the 2 year study (Goldenthal, 1985). No effects were observed in mice (Crome, 1988). No treatment related effects were observed on testes in rats in the 2 generation study Masters (1993).
In the 90 day rat study (Doubovetski) significantly increased testes weight was seen only relative to bw, but not absolute or relative to brain weight and was attributed to differences in body weight.

Overall, effects on testes weight (decreased weight) were observed in dogs but were generally of small magnitude and no effects were observed on histopathology of the organ. In rats and mice no treatment related effects were observed on testes. Effects were generally not observed on other male reproductive organs.

[21] Davis, B (2012). Endometrial Stromal Polyps in Rodents: Biology, Etiology, and Relevance to Disease in Women. Toxicologic Pathology.

Sexual maturation
Delayed preputial separation in males was observed in the 2 generation study. The observed effect was likely caused by a smaller body weight and may not be a specific effect of treatment as a covariance analysis was done comparing pps between the treated groups and the control via analysis of covariance (ANCOVA), using bodyweight at 4 weeks as the covariate. ANCOVA comparison of time to balanopreputial separation between the treatment groups, with adjustment for bodyweight at 4 weeks, was not statistically significant: P = 0.117. Sexual maturation was not affected in females.

Sperm parameters.
Sperm parameters were examined in the 2 generation rat study, however, only in proven males which could create a bias. Sperm analysis was performed for 8 (F0) and 7 (F1) males from each group instead of the recommended 10 animals/group. There were no treatment-related effects on sperm motility, morphology and count of proven males.

Estrus cycle.
Estrus cycle data were not summarised and it was very difficult to assess any patterns in the number of rats with regular/irregular cycles across the groups. Also, according to OECD guideline 416, females of the P generation should be dosed during growth and for several complete oestrus cycles in order to detect any adverse effects on oestrus cycle normality by the test substance. OECD guidance document no 43 states that vaginal smears must be collected daily for at least two weeks for an accurate determination of cycle length. Because the estrus cycle was just assessed for 7 days before mating (and during mating), 14 days was often not reached and normality of the cycles were difficult to evaluate. No differences in estrus cycle or time to mating were reported.

Regarding EAS-modalities, the RMS recommend that the dataset is not sufficiently investigated since the 2 two-generation study was performed before 2001 and several EAS-mediated parameters have not been investigated, or with deviations from guideline:

- Since there were effects on sexual development (delayed preputial separation), AGD should have been determined in F2 pups.
- Sperm parameters were only examined in proven males. Sperm analysis was performed for 8 (F0) and 7 (F1) males from each group instead of the recommended 10 animals/group
- Quantitative evaluation of primordial follicles.
- One randomly selected pup/litter should be selected for examination of thymus, brain and spleen according to OECD TG 416 (2001). In this study, selection was made on the basis of body weight at Day 21 post partum; within each litter, the pup with the median weight for the respective sex was chosen.
- Estrus cycle data were not summarised and it was very difficult/imposible to assess any patterns because vaginal smears were not collected long enough to assess normal cyclicity.

Conclusion on EAS-adversity: The WoE approach is against the EAS-mediated adversity as no clear pattern was observed (but with uncertainties listed above).

Adversity based on EAS-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no "**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	
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	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

RMS's proposed strategy for further ED assessment:

Level 2 studies for A-modality (i.e. OECD 458) and S-modality (H295R steroidogenesis assay, i.e. OECD TG 456 and aromatase assay, i.e. OPPTS 890.1200) should be conducted.

If the above mentioned Level 2 tests are positive (at least for one modality), then MoA should be analysed. If the above mentioned Level 2 tests are negative, then Level 3 (Hershberger bioassay in rats, i.e. OECD TG 441) should be performed.

If Hershberger bioassay in rats is negative, then ED criteria are not met (Scenario 2a (ii)). If Hershberger bioassay in rats is positive, then MoA should be analysed (Scenario 2a (i); additional data might be needed for MoA analysis – extended one-generation reproductive toxicity study as a last step).

ED assessment for non-target organisms

Acc. to the test strategy recommendations provided in the ECHA/EFSA Guidance (2018), further consideration on the potential ED properties on non-target organisms other than mammals is required. The reason for this is that dicamba is likely not endocrine disrupting in mammals with regard to the E- and T-modalities, and that the dataset for the A- and S-modalities was considered not sufficient to address the adversity and endocrine activity of dicamba in mammals. Pending the outcome of requested studies for humans/mammals, further consideration on the potential ED properties of dicamba on non-target organisms other than mammals is required.

See Table ED1 for the studies in non-mammalian species included in the ED assessment of dicamba.

For the ED assessment a total of six ecotoxicity studies are available, comprising two avian reproduction assays (OECD TG 206), two fish early life stage (ELS) toxicity assays (OECD TG 210, or alike) and two additional fish toxicity studies. These bird and fish assays were evaluated in Vol. 3 CA sections B.9.1 and B.9.2, respectively. Noteworthy, these assays are not specifically designed to detect endocrine disruption and therefore the endpoints, though some are endocrine-sensitive, cannot be considered specific to identify an endocrine MoA.

ED assessment for T-modality

To have the T-mediated adversity wrt. other non-target organisms other than mammals sufficiently investigated, the results from an amphibian growth and development assay (LAGDA; OECD TG 241) or alternatively negative test results from an amphibian metamorphosis assay (AMA; OECD TG 231) would be needed. These studies were however not included in the dossier. Based on the available information, the applicant has assembled the lines of evidence table for thyroid adversity and activity.

Table ED1: Lines of evidence for adverse effects and endocrine activity relate to T-modality

	Study ID matrix	Effect classification	Effect target	Species	Duration of exposure	Route of exposure/administration	Effect Concentration	Observed effect	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Integrated lines of evidence for endocrine activity		***In vitro* mechanistic**	Thyroid transporter transthyretin binding	See section 4.1.2				Inactive in thyroid transporter transthyretin binding assay	No evidence of endocrine activity	Overall not indicative of endocrine activity	T
			ToxCast thyroid assays (10)					Inactive in all ToxCast thyroid assays	No evidence of endocrine activity		
			CALUX nuclear receptor assay (TRb)					Inactive in TRb assay	No evidence of endocrine activity		
			ToxCast thyroid peroxidase inhibition assay					Inactive in ToxCast thyroid peroxidase inhibition assay	No evidence of endocrine activity		
			ToxCast sodium-iodine symporter inhibition assay					Inactive in ToxCast sodium-iodine symporter inhibition assay	No evidence of endocrine activity		
		***In vivo* mechanistic**	n/a								
Integrated lines of evidence for adversity		**EATS-mediated parameters**	n/a								
	22	**Sensitive to, but not diagnostic of, EATS**	Length	*Pimephales promelas*	33 days	Water	n/a	No effect on length	No evidence of adversity	Overall not indicative of adverse effects from parameters	No
	21			*Oncorhynchus mykiss*	21 days	Water	n/a	No effect on length	No evidence of adversity		
	23			*Cyprinodon variegatus*	34 days	Water	n/a	No effect on length	No evidence of adversity		

	20		Weight	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity	sensitive to, but not diagnostic of, EATS	
	19			*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity		
	22			*Pimephales promelas*	33 days	Water	n/a	No effect on weight	No evidence of adversity		
	21			*Oncorhynchus mykiss*	21 days	Water	n/a	No effect on weight	No evidence of adversity		
	23			*Cyprinodon variegatus*	34 days	Water	n/a	No effect on weight	No evidence of adversity		
	20		Development	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on number of hatchlings	No evidence of adversity		
	19			*Anas platyrhynchos*	21 weeks	Dietary	1600 ppm	Decrease in hatchlings	Potential evidence of systemic toxicity at highest test concentration		
	22			*Pimephales promelas*	33 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
	23			*Cyprinodon variegatus*	34 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
	19		Morphology	*Anas platyrhynchos*	21 weeks	Dietary	n/a	No abnormalities	No evidence of adversity		
	22			*Pimephales promelas*	33 days	Water	n/a	No abnormalities	No evidence of adversity		
Evidence of general toxicity	20	Mortality		*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		
	19			*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		
	21			*Oncorhynchus mykiss*	21 days	Water	n/a	No effect on mortality	No evidence of adversity		
	22			*Pimephales promelas*	33 days	Water	n/a	No effect on mortality	No evidence of adversity		
	23			*Cyprinodon variegatus*	34 days	Water	n/a	No effect on mortality	No evidence of adversity		
	21	Behaviour		*Oncorhynchus mykiss*	21 days	Water	320, 580, 1000 mg/L	Calm behaviour	Consistent with stress due to systemic toxicity		

	20		*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		
	19		*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		

Assessment of the integrated lines of evidence and weight of evidence

Based on the available information, there was no clear evidence for the identification of T-mediated adverse effects or T-mediated endocrine activity for non-target organisms other than mammals. No endpoints for T-mediated adversity were examined, however, several endpoints "*sensitive to, but not diagnostic of, EATS*" were considered (e.g., growth, development) and these did in general not indicate adverse effects. The overall WoE for non-target organisms other than mammals is not indicative of T-mediated adversity or of T-mediated endocrine activity, although not sufficiently investigated (i.e., LAGDA and/or AMA tests not submitted).

Initial analysis of the evidence and identification of the relevant scenario

Table ED2: 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	
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	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

Conclusion on the ED assessment for T-modality

The available evidence is not sufficient to conclude either on T-mediated endocrine activity or on T-mediated adversity. Based on scenario 2a (iii), the endocrine activity/endocrine adversity was not sufficiently investigated for the T-modality. Therefore, according to the ECHA/EFSA guidance, additional information should be generated (Scenario 2a(iii)). A level 3 study according to OECD TG 231 (AMA) is required. Alternatively, a study acc. to OECD TG 248 (*Xenopus* Eleutheroembryonic thyroid assay; XETA) can be considered acceptable for use instead of the AMA test (agreed by experts at the PREV 14 meeting, September 2019).

Two outcomes are possible:

1) If study OECD TG 231 (or OECD T 248) is negative, scenario 1a applies and the ED criteria are thus not met.
2) If study OECD TG 231 (or OECD T 248) is positive, scenario 2a(i) applies and further data will be needed to support the MoA analysis (i.e., level 4 LAGDA test; OECD TG 241).

ED assessment for EAS-modality

For assessing the ED properties through the EAS-modalities for non-target organisms other than mammals, in this case, six ecotoxicity studies were available. For fish an early life stage study acc. to OECD TG 210 and an alike study (OPPTS 850.1400) were available, and further a prolonged toxicity test (OECD TG 204) and an effect study from the open scientific literature (Zhu *et al*., 2013) were available. In addition, two avian reproduction studies (OECD TG 206) were available. The lines of evidence table for estrogen, androgen, and steroidogenesis adversity and activity has been assembled by the applicant based on the available information.

Table ED3: Lines of evidence for adverse effects and endocrine activity relate to EAS-modalities

	Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Route of exposure/administration	Effect Concentration	Observed effect	Assessment of each line of evidence	Assessment of the integrated line of evidence	Modality
Integrated line of evidence for activity		***In vitro* mechanistic**	ToxCast estrogen assays (22) and model	See section 4.1.2				Inactive in all ToxCast estrogen assays and model	No consistent ER bioactivity, for both agonism and antagonism	Overall not indicative of endocrine activity	E
			CALUX nuclear receptor assays (ER□, ER□)					Active in ER□ assay, inactive in ER□ assay			
			ToxCast androgen assays (14) and model					Inactive in all ToxCast androgen assays and model	No AR bioactivity, for both agonism and antagonism		A
			CALUX nuclear receptor assay (AR)					Inactive in AR assay			
			ToxCast H295R assay					Inactive for all steroid hormones	No effects on steroidogenesis		S
			ToxCast aromatase assay					Inactive in ToxCast aromatase assay			
		***In vivo* mechanistic**	n/a								
Integrated line of evidence for adversity		**EATS-mediated parameters**	n/a								
	20	**Sensitive-to-but not diagnostic of EATS**	Fecundity	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on egg production	No evidence of adversity	Overall not indicative of adverse effects from parameters sensitive to, but not	N
	19			*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on egg production	No evidence of adversity		
	20		Fertility	*Colinus virginianus*	21 weeks	Dietary	n/a	No effects on egg quality, viable embryos, or number of 14-day-old survivors	No evidence of adversity		

	19			*Anas platyrhynchos*	21 weeks	Dietary	1600 ppm	Decrease in number of 14-day-old survivors; no effects on egg quality, viable embryos	Potential evidence of systemic toxicity at highest test concentration	diagnostic of, EATS	
	22		Length	*Pimephales promelas*	33 days	Water	n/a	No effect on length	No evidence of adversity		
	21			*Oncorhynchus mykiss*	21 days	Water	n/a	No effect on length	No evidence of adversity		
	23			*Cyprinodon variegatus*	34 days	Water	n/a	No effect on length	No evidence of adversity		
	20		Weight	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity		
	19			*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on weight	No evidence of adversity		
	22			*Pimephales promelas*	33 days	Water	n/a	No effect on weight	No evidence of adversity		
	21			*Oncorhynchus mykiss*	21 days	Water	n/a	No effect on weight	No evidence of adversity		
	23			*Cyprinodon variegatus*	34 days	Water	n/a	No effect on weight	No evidence of adversity		
	20		Development	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on number of hatchlings	No evidence of adversity		
	19			*Anas platyrhynchos*	21 weeks	Dietary	1600 ppm	Decrease in hatchlings	Potential evidence of systemic toxicity at highest test concentration		
	22			*Pimephales promelas*	33 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
	23			*Cyprinodon variegatus*	34 days	Water	n/a	No effects on hatching time or hatching success	No evidence of adversity		
	19		Morphology	*Anas platyrhynchos*	21 weeks	Dietary	n/a	No abnormalities	No evidence of adversity		
	22			*Pimephales promelas*	33 days	Water	n/a	No abnormalities	No evidence of adversity		

Evidence of general toxicity	20	Mortality	*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		
	19		*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on mortality	No evidence of adversity		
	21		*Oncorhynchus mykiss*	21 days	Water	n/a	No effect on mortality	No evidence of adversity		
	22		*Pimephales promelas*	33 days	Water	n/a	No effect on mortality	No evidence of adversity		
	23		*Cyprinodon variegatus*	34 days	Water	n/a	No effect on mortality	No evidence of adversity		
	21	Behaviour	*Oncorhynchus mykiss*	21 days	Water	320, 580, 1000 mg/L	Calm behaviour	Consistent with stress due to systemic toxicity		
	20		*Colinus virginianus*	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		
	19		*Anas platyrhynchos*	21 weeks	Dietary	n/a	No effect on feed consumption	No evidence of adversity		

Assessment of the integrated lines of evidence and weight of evidence

Based on the available information, there was no clear evidence for the identification of EAS-mediated adverse effects or EAS-mediated endocrine activity for non-target organisms other than mammals. No endpoints for EAS-mediated adversity were examined, however, several endpoints "*sensitive to, but not diagnostic of, EATS*" were considered (e.g., fecundity, fertility). In some fish studies, effects on some parameters were observed, however in general adverse effects were not indicated. The available evidence from fish studies is only considered supportive for the lack of ED related adversity, since those studies provide little information concerning potential ED-related effects. Though the overall WoE for non-target organisms other than mammals is not indicative of EAS-mediated adversity or of EAS-mediated endocrine activity, this is considered to be not sufficiently investigated.

The level 2 dataset (*in vitro* mechanistic) for assessment of A- and S-modalities regarding endocrine activity is considered insufficient following the ECHA/EFSA guidance. It is, however, considered sufficient for the E-modality. Overall, the dataset should thus be regarded incomplete acc. to the ECHA/EFSA guidance. The lines of evidence for EAS-modalities and their evaluations as reported for mammals (see section) is also relevant for non-target organisms other than mammals.

Overall, in line with the ECHA/EFSA guidance the dataset is considered insufficient for the assessment of the E-, A- and S-modalities regarding endocrine activity and endocrine adversity.

Initial analysis of the evidence and identification of the relevant scenario

Table ED4: 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 "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	
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	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

Conclusion on the ED assessment for EAS-modality

The available dataset for non-target organisms other than mammals for dicamba was incomplete since EAS-mediated parameters were not sufficiently investigated.

The available level 2 dataset for the ED assessment for EAS-modalities in mammals was insufficient to conclude on the ED properties of dicamba on human and further data were requested for the A- and S-modalities. This conclusion also applies to wild mammals.

The available information and evidence is not sufficient to conclude either on EAS-mediated endocrine activity or on EAS-mediated adversity.

Based on scenario 2a (iii), the endocrine activity/endocrine adversity was not sufficiently investigated for the EAS-modalities. Therefore, according to the ECHA/EFSA guidance, additional information should be generated (Scenario 2a(iii)). A level 3 study according to OECD TG 229 (FSTRA) is required.

Two outcomes are possible:

1. If OECD TG 229 is negative, scenario 2a(ii) applies and the ED criteria are thus not met.
2. If OECD TG 229 is positive, the scenario 2a(i) applies and further data will be needed to support the MoA analysis (i.e., level 5 MEOGRT test; OECD TG 240).

Overall conclusion on the ED assessment

Based on the available evidence from standard mammalian studies, the E- and T-modalities was considered sufficiently investigated and the data suggest that dicamba is likely not an endocrine disruptor via the E- and/or T-modalities in humans/mammals. However, for the A- and S-modalities the available information was insufficient to draw a conclusion for mammals.

For non-target organisms other than mammals, evaluation of the available data in acc. with the ECHA/EFSA guidance indicates that the ecotoxicological dataset was insufficient to assess the ED properties of dicamba through the EATS-modalities. Awaiting the outcome of requested tests for humans/mammals, tests performed according to OECD TG 229 and OECD TG 231 (or OECD TG 248) could be submitted in order to conclude on the endocrine disruptive properties to non-target organisms other than mammals.

According to the assessment strategy of the guidance for the identification of endocrine disruptors (ECHA/EFSA, 2018), a tiered assessment strategy should be followed. In the case of dicamba, additional tests would be required to complete the current data package:

- Level 2 studies for A-modality (i.e. OECD 458) and S-modality (H295R steroidogenesis assay, i.e. OECD TG 456 and aromatase assay, i.e. OPPTS 890.1200) should be conducted.
- If the above mentioned Level 2 tests are positive (at least for one modality), then MoA should be analysed. If the above mentioned Level 2 tests are negative, then Level 3 (Hershberger bioassay in rats, i.e. OECD TG 441) should be performed.
- If Hershberger bioassay in rats is negative, then ED criteria are not met (Scenario 2a (ii)). If Hershberger bioassay in rats is positive, then MoA should be analysed (Scenario 2a (i); additional data might be needed for MoA analysis – extended one-generation reproductive toxicity study as a last step OECD TG 443).
- A study in line with the OECD TG 231 (AMA), or alternatively OECD TG 248 (XETA) (see section 3.1.4)
- A study in line with the OECD TG 229 (FSTRA) (see section 3.2.4)

The above mentioned tests are relevant to investigate potential EATS-mediated endocrine activity and, if negative, to exclude that dicamba has endocrine properties, acc. to the scientific criteria for the determination of endocrine disrupting properties as set out in point 3.6.5 and point 3.8.2 of Annex II to Regulation (EC) No 1107/2009. However, in case of positive result/s based on the abovementioned tests for at least one modality, additional testing (level 4/5 data, see sections 2.2.5, 3.1.4 and 3.2.4) might be needed in order to further investigate the adversity. In that case the following test/s could be appropriate to test for adversity: a study performed acc. to OECD TG 240 and/or a study performed acc. to OECD TG 241.

After having taking into consideration all the available existing information, taking into account the information on the properties of the substance and the situation summarised in the paragraph above, it is considered that, in order to be able to conclude whether the approval criteria on the endocrine disruption potential in line with Commission Regulation (EU) 2018/605[22] are met for dicamba, the applicant should complete the data package to support a conclusion on absence of EATS-mediated adversity, as explained in section 3.4.1 of the ECHA/EFSA guidance.

In order to meet the objectives of Regulation (EU) No 2018/1659, the data package should be completed within a period not exceeding 30 months.

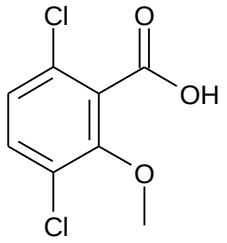
[22] Commission Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties. OJ L 101, 20.4.2018, p. 33–36.

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 [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 112: 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)	3,6-dichloro-2-methoxybenzoic acid
Other names (usual name, trade name, abbreviation)	Dicamba
ISO common name (if available and appropriate)	Dicamba
EC number (if available and appropriate)	217-635-6
EC name (if available and appropriate)	-
CAS number (if available)	1918-00-9
Other identity code (if available)	CIPAC: 85
Molecular formula	$C_8H_6Cl_2O_3$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	221 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum purity: 850 g/kg

2.11.1.2 Composition of the substance

Table 113: 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)**
Dicamba, CAS nr 1918-00-9	Minimum 85% w/w	Acute Tox. 4 * Eye Dam. 1 Aquatic Chronic 3	Acute Tox. 4 Eye Dam. 1 Aquatic Chronic 3

Table 114: 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**

Table 115: 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**
None					

Table 116: 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**

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 117: 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 and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	607-043-00-X	dicamba (ISO); 2,5-dichloro-6-methoxybenzoic acid; 3,6-dichloro-2-methoxybenzoic acid	217-635-6	1918-00-9	Acute Tox. 4* Eye Dam. 1 Aquatic Chronic 3	H302 H318 H412	GHS05 GHS07 Dgr	H302 H318 H412			
Dossier submitters proposal	607-043-00-X	dicamba (ISO); 2,5-dichloro-6-methoxybenzoic acid; 3,6-dichloro-2-methoxybenzoic acid	217-635-6	1918-00-9	**Retain** Eye Dam. 1 **Add** Carc. 2 Acute Tox. 4 STOT SE 3 STOT SE 3 Aquatic Acute 1 **Modify** Acute Tox. 4 Aquatic Chronic 1	**Retain** H318 **Add** H351 H332 H335 H336 H400 **Modify** H302 H410	**Retain** GHS05 GHS07 Dgr **Add** GHS08 GHS09	**Retain** H318 **Add** H351 H332 H335 H336 **Modify** H302 H410		**Add** M=1 M=1 inhalation: ATE = 4.46 mg/L oral: ATE = 1581 mg/kg bw	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	607-043-00-X	dicamba (ISO); 2,5-dichloro-6-methoxybenzoic acid; 3,6-dichloro-2-methoxybenzoic acid	217-635-6	1918-00-9	Carc. 2 Acute tox. 4 Acute Tox. 4 STOT SE 3 STOT SE 3 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H302 H332 H335 H336 H318 H400 H410	GHS05 GHS07 GHS08 GHS09 Dgr	H351 H302 H332 H335 H336 H318 H410		inhalation: ATE = 4.46 mg/L oral: ATE = 1581 mg/kg bw M=1 M=1	

2.11.2.2 Additional hazard statements / labelling

Table 118: 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	hazard class not applicable	No
Flammable solids	data conclusive but not sufficient for classification	Yes
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not applicable	No
Oxidising solids	data conclusive but not sufficient for classification	Yes
Organic peroxides	hazard class not applicable	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	Acute tox 4 H302	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Acute tox 4 H332	Yes
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Eye dam. 1 H318	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	Carc 2 H351	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	STOT SE 3	Yes
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data lacking	No

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	data conclusive but not sufficient for classification	Yes

2.11.3 History of the previous classification and labelling

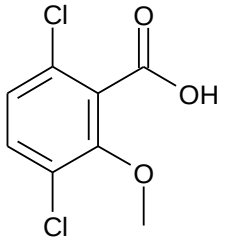
The studies the old/new acute tox classifications are based on are all relatively old (<=2001) and were therefore already evaluated in EU - also for classification purposes. Considering the age of dicamba and how long it is already registered in EU, we believe ECB (European Chemicals Bureau – EchA's predecessor) took a look at the available data when assigning the classification in the past and that these classifications are not just based on voluntarily classification by industry. We assume that - when implementing the new C&L guidance - the old R-phrases were then 'translated' into the new H-phrases.

Concerning toxicity endpoints we think dicamba had been classified as R22 (acute oral tox) and R41 (severe eye irritation) according to the old EU classification scheme. This is based on study data from 1974 which are still considered valid for these endpoints today triggering the respective classifications according to today's C&L scheme.

Older inhalation toxicity studies (e.g. 1974) revealed no relevant inhalation toxic potential but these were not in agreement with current test guidelines (e.g. no monitoring of particle size distribution or actual concentration in the animals breathing zones). The oldest inhalation tox study available to Syngenta with a study design in agreement with current test guidelines is from 2001, was submitted (and evaluated) for the previous EU review and was therefore also available for classification purposes in EU. The reason why no inhalation toxicity classification was considered required at that time may have been the fact that the combined LC50 (both sexes together) in the 2001 study was considered to be >5 mg/L (3/5 males + 1/5 females died at top concentration resulting in 4/10 total deaths). Only in males the LC50 was slightly below 5 mg/L in that study but as dicamba as such did not reveal a relevant sex difference in the available acute toxicity studies, it may have been considered sufficient to base also the classification for inhalation toxicity on the situation in both sexes combined – which then would not trigger a classification for inhalation toxicity. The latter would actually be supported by the newest available study (2015) where the LC50 in both sexes separately was shown to be >5 mg/L.

RAC general comment

Dicamba (3,6-dichloro-2-methoxybenzoic acid) (see molecular formula below) has an existing entry in Annex VI of CLP Regulation. Its representative uses are in maize, sorghum, and small grain cereals for the control of annual and perennial broadleaved weeds. The proposal for classification and labelling of dicamba was included by the Dossier Submitter (DS) in the Renewal Assessment Report which included old data as well as new data generated after the first approval of dicamba.



2.11.4 Identified uses

Dicamba is used as a selective post-emergent broad-leaved herbicide in the EU.

2.11.5 Data sources

The data submitted in the context of renewal of pesticide active substances under Regulation no. 1107/2009 concerning the placing of plant protection products on the market. The data was evaluated in the Renewal Assessment Report (RAR) Vol. 1-4.

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

The soil metabolite DCSA does not exceed 0.1 µg/L in the PECgw modelling performed with PELMO, PEARL and MACRO. Therefore an assessment of relevance of metabolites in groundwater is not needed.

2.12.1 STEP 1: Exclusion of degradation products of no concern

Not relevant

2.12.2 STEP 2: Quantification of potential groundwater contamination

Dicamba and the soil metabolite DCSA does not exceed 0.1 µg/L in the PECgw modelling performed with PELMO, PEARL and MACRO.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.12.3.1 STEP 3, Stage 1: screening for biological activity

Not relevant

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

Not relevant

2.12.3.3 STEP 3, Stage 3: screening for toxicity

Not relevant

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

2.12.5 STEP 5: Refined risk assessment

2.12.6 Overall conclusion

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

The active substance dicamba is not a mixture of isomers. Therefore no information is presented or required.

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: The sum of dicamba and 5-OH dicamba, free and conjugated, expressed as dicamba

Food of animal origin: The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba

Soil: Dicamba and DCSA

Groundwater: Dicamba and DCSA

Surface water: Dicamba and DCSA

Sediment: Dicamba and DCSA

Air: Dicamba

2.14.2 Definition of residues for monitoring

Food of plant origin: The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba

Food of animal origin: The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba

Soil: Dicamba and DCSA

Groundwater: Dicamba and DCSA

Surface water: Dicamba and DCSA

Sediment: None

Air: Dicamba

Level 3

Dicamba

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria - Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	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		Dicamba. There are 2 representative products. Representative product for Syngenta (A7254B). Safe use could be demonstrated without using PPE. Representative product for Rotam (FH-048): Safe use could be demonstrated without using PPE for operatorand also for worker and bystander/residents.
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	x		
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.			
3.1.1.3 Restrictions on approval				
		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	
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	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		

	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.			**For monitoring (residues)** **Food of plant origin:** The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba **Food of animal origin:** The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba **For risk assessment (residues)** **Food of plant origin:** The sum of dicamba and 5-OH-dicamba, free and conjugated expressed as dicamba **Food of animal origin:** The sum of dicamba and its salts and conjugates of dicamba expressed as dicamba
	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		Sufficient information has been submitted.
Efficacy				
		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		See level 2 (section 2.3).
Relevance of metabolites				
		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		
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where rele-		x	For the toxicological studies the specifications are not fully covered in the studies.

	vant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.			
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	x		Dicamba has a FAO specification from 2016 with a dicamba content not less than 850 g/kg. The specifications are in compliance with this.
	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	Explain as necessary
Methods of analysis				
		Yes	No	
	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 synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.			Sufficient information is submitted (with the possible exception of impurities of toxicological concern). The assessment on impurities of toxicological concern is not yet finalised
	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		
	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				
Impact on human health - ADI, AOEL, ARfD				
		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		RMS proposes keeping the acute reference dose from the previous evaluation only corrected for the purity of dicamba tested in the study: The acute oral LD_{50} in the rat was below 2000 mg/kg and the compound is classified as harmful. The acute neurotoxicity study showed neurobehavioral findings upon single treatment of rats. In the rabbit developmental toxicity study clinical signs were observed in dams at $\geq$ 150 mg/kg/day with a NOAEL of 30 mg/kg/day (Hoberman, 1992). Therefore, the criteria may be fulfilled to allocate an ARfD. The proposed ARfD is derived from the NOAEL of 30 (27.1) mg/kg bw/day established in the teratology study in rabbits and a safety factor of 100.

				ARfD = NOAEL/safety factor = 30 mg/kg bw/day/100 = 0.30 mg/kg bw/day ADI was previously based on the multigeneration study in rats by Master et al (1993) as it was the most sensitive study, i.e. the study with the lowest and most relevant NOAEL. Since, at the re-evaluation, a new NOAEL of 10.0 mg/kg bw/day (carcinogenicity) has been proposed at a lower dose in the chronic study in rats (Goldenthal, 1985), it is suggested to use this value for the derivation of the ADI. An UF of 150 is proposed to ensure a margin of safety to the carcinogenic effect of at least 1000 based on the carcinogenic effect (increase in thyroid parafollicular (C-cell) carcinoma) observed in this study. Based on the NOAEL of 10.0 mg/kg bw/day and a safety factor of 150, to achieve a margin of safety above 1000, an ADI can be calculated: ADI = NOAEL/UF = 10 mg/kg bw/day/150 = <u>0.07 mg/kg bw/day</u> (rounded) AOEL was previously based on the Teratology study in rabbits: NOAEL = 30 mg/kg bw/day (Hobermann, 1992). However since during the re-evaluation a NOAEL for Carcinogenicity has been proposed, setting a new AOEL is considered required. At the re-evaluation, a new NOAEL of 10.0 mg/kg bw/day (carcinogenicity) has been proposed at a lower dose in the chronic study in rats (Goldenthal, 1985), it is suggested to use this value for the derivation of the AOEL. An UF of 150 should be used because of the carcinogenic effect (increase in thyroid parafollicular (C-cell) carcinoma) observed in this study. Based on the NOAEL of 10.0 mg/kg bw/day and a safety factor of 150, to achieve a margin of safety above 1000, an AOEL can be calculated: AOEL = NOAEL/UF = 10 mg/kg bw/day/150 = 0.07 mg/kg bw/day (rounded)
Impact on human health – proposed genotoxicity classification				
		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		x	The submitted *in vivo* cytogenetic test with somatic cells was a non GLP study with several deviations from guideline and the acceptability of this study is

	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.**			questionable. The in vivo MN study was. Considering the quality/results of the published and GLP studies in the dossier, the overall conclusion is that, despite some indications of DNA damaging capacity of dicamba, the weight of evidence suggests that dicamba is of no concern regarding chromosomal damage in vivo. The gene mutagenic potential of dicamba was excluded with a negative TGR assay.
Impact on human health – proposed carcinogenicity classification				
		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	Based on the dose-related increased incidence of thyroid parafollicular (C-cell) carcinoma in male rats (although not accompanied by increases in hyperplasia or adenomas), observed above the incidence found in the HCD for mid and high dose group males and a significant trend analysis, RMS considers the increase in these tumors may be treatment related. Since the increase in thyroid parafollicular (C-cell) carcinoma was observed in one species and in one gender, a classification for Carc Cat 2 is suggested by RMS.
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.			*[if no provide a brief explanation of conditions of use and cross refer to the section containing full details to support the contention of negligible exposure]*
Impact on human health – proposed reproductive toxicity classification				
		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	Classification of dicamba as a reproductive toxicant is not 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.			
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		x	
ii)	It is considered that **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 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		x	
iii)	Linked to either i) or ii) immediately above. 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)				
		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 active substance dicamba has a DT_{50} in soil of 3.21 – 24.6 days (geomean DT_{50} = 7.06 days, n = 7). In surface water the DT_{50} of the active substance is 50.0 – 51.7 days and in the whole surface water system (water/sediment) the

				DT_{50} is 50.8 – 53.5 days. Dicamba does therefore not fulfil the persistence criteria for POP. As $logK_{ow}$ = -2.3 (pH 7) dicamba is not expected to bioaccumulate. The DT_{50} of dicamba in air is 3.6 – 4.1 days, but as the volatilisation from plant (0.12%) and soil (0.07 – 1.15%) surfaces is negligible long-range transport of the active substance is not expected. Therefore dicamba is not a POP.
Persistent, bioaccumulative and toxic substance (PBT)				
		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 active substance dicamba has a DT_{50} in soil of 3.21 – 24.6 days (geomean DT_{50} = 7.06 days, n = 7). In surface water the DT_{50} of the active substance is 50.0 – 51.7 days and in the whole surface water system (water/sediment) the DT_{50} is 50.8 – 53.5 days. Dicamba therefore fulfil the P criteria for PBT with regard to the half-life in fresh water. As $logK_{ow}$ = -2.3 (pH 7) dicamba is not expected to bioaccumulate. Dicamba does not fulfil the T criteria. Dicamba is therefore not a PBT substance.
Very persistent and very bioaccumulative substance (vPvB).				
		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 active substance dicamba has a DT_{50} in soil of 3.21 – 24.6 days (geomean DT_{50} = 7.06 days, n = 7). In surface water the DT_{50} of the active substance is 50.0 – 51.7 days and in the whole surface water system (water/sediment) the DT_{50} is 50.8 – 53.5 days. Dicamba does therefore not fulfil the persistence criteria for vPvB. As $logK_{ow}$ = -2.3 (pH 7) dicamba is not expected to bioaccumulate. Dicamba is therefore not a vPvB substance.
Ecotoxicology				
		Yes	No	
	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		In the terrestrial vertebrate risk assessment, all TER_A and TER_{LT} values are in excess of their corresponding trigger values, indicating acceptable acute and long term risks to birds and mammals after application of FH-048 or A7245B at rates up to 288 g a.s./ha in maize, 210 g a.s./ha in sorghum and 96 g a.s./ha in cereals. Based on the FOCUS STEP 1-2 PECsw and PECsed values, the acute and long-term are acceptable to fish, aquatic invertebrates, sediment-dwellers and algae from the use of dicamba to maize, sorghum and cereasl with one

				application per year at rates up to 288 g a.s./ha. No risk mitigation measures beyond 1 m buffer are necessary to protect the aquatic organisms, if the products FH-048 and A7245B are used according to these GAPs. Dicamba is an herbicide with no known insecticidal properties and it exhibits low acute oral and contact toxicity to honey bees. The HQ values for acute oral and contact exposure, calculated in accordance with the guidance of SANCO/10329/202 rev 2 final, are both below the trigger value of 50 for the use of A7245B and FH-048. Additonal risk assessment considering the EFSA Bee guidance (EFSA Journal 2013;11(7):3295) have been performed for adult chronic and larval development for honey bee and the calculated ETE values were below the trigger values. Thus acceptable acute and chronic risk to honey bees for all representative uses of FH-048 and A7245B has beencalculated. The proposed use of dicamba on maize, sorghum and cereals, in accordance with Good Agricultural Practice, will present no unacceptable risk to other non-target arthropods. Acceptable risk of acute and long term toxicity for earthworms and soil macro-organisms at an application rate of 288 g dicamba/ha was calculated. The risk to soil micro-organisms is negligible for applications up to 5.75 mg a.s./kg dw soil. A low risk to terrestrial non-target plants was identified for dicamba after applications of A7245B at rates up to 288 g a.s./ha in maize with the use of a 3 meter buffer zone, 210 g a.s./ha in sorghum and cereals with the use of a 2 meter buffer zone. Follwong application of FH-048 at rates up to 280 g a.s./ha in maize a low risk was identified with the use of a 3 meter buffer zone. Based on these results, the risks to terrestrial plants from A7245B and FH-048 applications to maize, sorghum and cereals are considered acceptable with appropriate risk mitigation measures and if the GAP is assumed.
	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	The available dataset is insufficient to conclude on ED proprerties of dicamba. RMS suggests that tests performed according to OECD TG 229 and OECD TG 231 (or alternatively OECD TG 248) should be submitted in order to conclude on the endocrine disruptive properties to non-target organisms other than mammals.

	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.			Non-target organisms inevitable will be exposed from the intended GAP uses. Any firm conclusion on endocrine properties of dicamba is pending new studies.
	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 HQ values for acute oral and contact exposure, calculated in accordance with the guidance of SANCO/10329/202 rev 2 final, are both below the trigger value of 50 for the use of A7245B and FH-048. Risk assessment considering the EFSA Bee guidance (EFSA Journal 2013;11(7):3295) have been performed for adult chronic and larval development and the calculated ETE values were below the trigger values. Thus acceptable acute and chronic risk to honey bees for all representative uses of FH-048 and A7245B has beencalculated.
Residue definition				
		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		
Fate and behaviour concerning groundwater				
		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		Dicamba and its soil metabolite DCSA does not exceed 0.1 µg/L in the PECgw modelling performed with PELMO, PEARL and MACRO.

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	

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.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bioconcentration factor is lower than 100**.		x	Dicamba does not fulfil the criteria for low risk.

	(f) it is a **semiochemical** and verifies points (a) to (d). Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

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				
-				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
-				
3.1.4.3 Data on uses and efficacy				
-				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
-				
3.1.4.5 Methods of analysis				
-				

3.1.4.6 Toxicology and metabolism				
3.1.4.7 Residue data				
-				
3.1.4.8 Environmental fate and behaviour				
-				
3.1.4.9 Ecotoxicology				
-				

3.1.5 Issues that could not be finalised

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).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
	[specify if measure relates to a specific representative use/use scenario/product or to all uses/products]

3.1.6 Critical areas of concern

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.

Critical area of concern identified	**Relevance in relation to representative use(s)**
The endocrine disrupting potential of dicamba could not be finalised due to lack of sufficient information.	Relevant for all representative uses.

3.1.7 Overview table of the concerns identified for each representative use considered

(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.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Use " A7254B " (X^1)	Use " FH-048" (X^1)
Operator risk	Risk identified		
	Assessment not finalised		
Worker risk	Risk identified		
	Assessment not finalised		
Bystander risk/resident	Risk identified		
	Assessment not finalised		
Consumer risk	Risk identified		
	Assessment not finalised		
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		
Risk to aquatic organisms	Risk identified		
	Assessment not finalised		
Groundwater exposure active substance	Legal parametric value breached		
	Assessment not finalised		
Groundwater exposure metabolites	Legal parametric value breached		
	Parametric value of 10µg/L[(a)] breached		
	Assessment not finalised		
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**
	[specify the reasons why expert consultation is considered necessary]

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 should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
Amyloidosis observed in high dose male mice in the long term study	*This effect is considered adverse and supportive of cancer classification*	*The increase in high dose is slight and might be considered treatment related but RMSis unsure if it can be used to support classification for cancer.*
Classification for Muta 2	Co-RMS considers a positive comet assay as adequate to classify as Muta 2	There was a positive Comet assay available, however, a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays was clearly negative in duodenum up to a dose (924 mg/kg bw/day) a dose near the limit dose of 1000 mg/kg bw/day. Taking into account that a Comet assay detects DNA damage and the TGR Assay detects mutations and the latter was negative, it is not considered likely dicamba causes gene mutations in vivo. On that basis, the criteria of a classification for mutagenicity in category 2 is not considered met.
NOAEL for the 2 year rat study	NOAEL for Carc would have been chosen at 50 ppm	NOAEL was set by RMS at 250ppm

3.2 PROPOSED DECISION

It is proposed that:

Dicamba can be approved under Regulation (EC) No 1107/2009

It is considered that the following is specified in Part A of the Commission Implementing Regulation for the approval of the active substance:

[example] **Only uses as seed treatment may be authorised.**

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):

[example] **the risk to aquatic organisms.**

It is considered that it should be specified that conditions of use shall include risk mitigation measures, where appropriate.

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 THE 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)**
	[specify if measure relates to a specific representative use/use scenario/product or to all uses/products]

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances.

Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products.

Section identity, physical chemical and analytical methods

Section physico chemical properties

ECHA (2017). Guidance on the Application of the CLP Criteria 2017 vers 5.0

UN recommendations on the Transport of Dangerous Goods (2015). Manual of tests and criteria Annex 6 2015 rev 6

Section analytical methods

SANCO/825/00 rev. 8.1, 16 November 2010, Guidance document on pesticide residue analytical methods.

Section Data on application and efficacy

SANCO/10054/2013 - rev. 3 (2013): Guidance document on data requirements on efficacy for the dossier to be submitted for the approval of new active substances contained in plant protection products.

Section Toxicology

EFSA (2012), Guidance on Dermal Absorption, EFSA Panel on Plant Protection Products and their Residues (PPR), EFSA Journal 2012;10(4):2665

EFSA (2014), 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

Section Residue and consumer risk assessment

OECD (2009). Guidance Document on Overview of Residue Chemistry Studies (as revised in 2009). Environment, Health and Safety Publications. Series on Testing and Assessment No. 64 and Series on Pesticides No. 32

OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)

OECD (2008). Guidance document on magnitude of pesticide residues in processed commodities. Environment, Health and Safety Publications. Series on Testing and Assessment No. 96.

OECD (2009). Guidance Document on the Definition of Residues. Environment, Health and Safety Publications. Series on Testing and Assessment No. 63 and Series on Pesticides No. 31

OECD MRL Calculator (2011)

SANCO/7525/VI/95 rev. 10.1 December 2015. Appendix D – Comparability, extrapolation, group tolerance and data requirements

SANCO/11187/2013 rev. 3. 31 January 2013. Appendix J – Nature of pesticide residues in fish

SANCO/3029/99 EU, rev.4, 11 July 2000- Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements

SANCO/825/00 EU, rev. 8.1, November 2010, Guidance document on pesticide residue analytical methods (post-registration monitoring and control)

OECD (2007). Guidance Document on Pesticide Residue Analytical Methods. Environment, Health and Safety Publications. Series on Testing and Assessment No. 7 and Series on Pesticides No. 39

OECD Test Guidelines No. 501, 502, 503, 504, 506, 507, 508, 509

Section fate and behavior in environment

OECD 307 guideline, aerobic and anaerobic transformation in soil (2002).

FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp].

FOCUS (2011) Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration

EFSA (2014) European Food Safety Authority. 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, 38 pp., doi:10.2903/j.efsa.2014.3662

U.S. EPA OPPTS 835.6100 Terrestrial Field Dissipation (October 2008).

EU Commission Working Document 1607/VI/97 Rev. 1 (22/7/1997), Appendix B, Residue Trials, 7029/VI/95 Rev. 5 (22/7/1997).

SETAC – Procedures for Assessing Environmental Fate and Ecotoxicity of Pesticides' (Dr. M. Lynch, March 1995).

SANCO/3029/99/Revision 4, 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 (July 2000).

BBA guideline Part IV, 4-1 (1986)

OECD 106: Adsorption - Desorption Using a Batch Equilibrium Method.

OECD 312 (2004)

OECD 111 guideline on hydrolysis as a function of pH

OECD guideline (draft), Phototransformation of chemicals in water, Part A: Direct phototransformation (1990) prepared by UBA, Germany.

OECD 316 guideline on photodegradation in water.

OECD 301 D for testing of chemicals (adopted July 17, 1992)

OECD 309 (2004)

OECD 308 (2002)

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