Committee for Risk Assessment

RAC

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

Adopted

2 June 2022

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CLH-O-0000007132-84-01/F

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

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

Chemical name: **Dicamba**

EC Number: **217-635-6**

CAS Number: **1918-00-9**

The proposal was submitted by **Denmark** and received by RAC on **13 April 2021.**

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

PROCESS FOR ADOPTION OF THE OPINION

Denmark has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **19 April 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **18 June 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Laure Geoffroy**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **2 June 2022** by **consensus**.

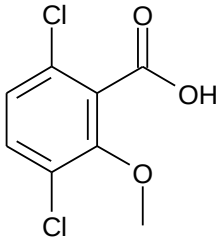
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	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	GHS07 GHS05 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** GHS07 GHS05 Dgr **Add** GHS08 GHS09	**Retain** H318 **Add** H351 H332 H335 H336 **Modify** H302 H410		**Add** inhalation: ATE = 4.46 mg/L oral: ATE = 1581 mg/kg bw M = 1 M = 1	
RAC opinion	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** Acute Tox. 4 STOT SE 3 STOT SE 3 Aquatic Acute 1 **Modify** Acute Tox. 4 Aquatic Chronic 2	**Retain** H318 **Add** H332 H335 H336 H400 **Modify** H302 H411	**Retain** GHS07 GHS05 Dgr **Add** GHS08 GHS09	**Retain** H318 **Add** H332 H335 H336 **Modify** H302 H410		**Add** inhalation: ATE = 4.0 mg/L oral: ATE = 1500 mg/kg bw M = 1	
Resulting Annex VI entry if agreed by COM	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 Acute Tox. 4 Eye Dam. 1 STOT SE 3 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 2	H332 H302 H318 H335 H336 H400 H411	GHS07 GHS05 GHS09 Dgr	H332 H302 H318 H335 H336 H410		inhalation: ATE = 4.0 mg/L oral: ATE = 1500 mg/kg bw M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Dicamba (3,6-dichloro-2-methoxybenzoic acid) (see molecular formula below) is a herbicide with an existing entry in Annex VI of the 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 comprising older data as well as new data generated after the first approval of dicamba.



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.**

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.**

HUMAN HEALTH HAZARD EVALUATION

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 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 14 / 5 5000 / 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	KCA 5.2.1/01
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	KCA 5.2.2/01

		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	
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 Sprague-Dawley derived albino rats 5 animals/sex	Dicamba Purity: 97.8% (w/w) No vehicle 5.14 mg/L Nose-only 4 h MMAD = 2.05 µm GSD = 2.27 µm	All animals survived Following exposure all rats exhibited irregular respiration and hypo activity 2 males had ano-genital staining 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	KCA 5.2.3/01
Acute inhalation toxicity OECD 403 GLP Alpk:APfSD Wistar-derived rats 5 animals/sex/dose	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	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	KCA 5.2.3/02

	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	All animals were symptom free from day 5 Gravimetric concentration (mg/L) / Dead/ Treated / Latency (days) 5.19 ± 0.85 / 3/5 M, 1/5 F / 1 2.68 ± 0.41 / 1/5 M / 1 1.18 ± 0.39 / 0/5 / - 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	
Acute inhalation toxicity OECD 403 GLP CRL:(WI)BR Wistar rats 3 groups of 5 males and 1 group of 5 females	Dicamba Purity: 98.85% No vehicle Mean achieved doses: 5.01, 3.98, 4.50 mg/L 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	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 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	KCA 5.2.3/03

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 following accidental exposures, prospective studies from patients notified from poisons units and retrospective observational studies. These studies described an array of clinical symptoms related mainly to the nervous system (ataxia, depressed mental state, irritability, etc.), to the gastric system (vomiting, abdominal pain, haemorrhagic gastro-duodenitis, etc.). 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 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 H332 (harmful if inhaled)** with an **ATE of 4.0 mg/L.**

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</td><td>KCA 5.7.1/01</td></tr>
</table>

	Signs of neurotoxicity after 14 days No differences from control	
Acute delayed neurotoxicity US-EPA FIFRA GLP Hen *Gallus gallus domesticus*, 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	316 (274 pure) mg/kg bw 9/20 animals died Body weight: weight loss during the first two weeks of the experiment 158 (137 pure) mg/kg bw 1/10 birds found dead day 5 Body weight gain:↓ 67% Food consumption: ↓ days 1 to 3 79 (69 pure) mg/kg bw No mortality Body weight development similar to control Food consumption: ↓ days 1 to 3 Effects at all doses Reversible unsteadiness, inability to walk, collapsing when moved and lying on the pen floor with legs outstretched or lying on one side Does not induce delayed neurotoxicity in hens	KCA 5.7.2 / 01

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).**

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.**

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, 3 rabbits	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

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).**

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.**

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 a skin sensitiser.**

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 Actual doses corrected for purity correspond, respectively, to 493, 693, 914, 1175 and 1432	<u>15000 ppm</u>			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%	
	<u>12500 ppm</u>			
		Males	Female	
	Impaired mobility in hind extremities	1/5	-	
	Body weight gain (week 4)	↓ 23.7%	↓ 12.8%	
	Food consumption (week 1-4)	↓ 24.9%	↓ 20.7%	
	<u>10000 ppm</u>			

mg/kg bw/day for males and 484, 729, 942, 1184 and 1436 mg/kg bw/day for females Vehicle: diet	<table><tr><th></th><th>Males</th><th>Female</th></tr><tr><td>Impaired mobility in hind extremities</td><td>-</td><td>-</td></tr><tr><td>Body weight gain (week 4)</td><td>↓ 11.2%</td><td>-</td></tr><tr><td>Food consumption (week 1-4)</td><td>↓ 16.9%</td><td>↓ 12%</td></tr></table> <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 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>	KCA 5.3.2 / 01

	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 60/sex (50/sex/group main study, 10/sex/group interim kill after 12 months) Dietary Dicamba (technical material)	<u>NON-NEOPLASTIC FINDINGS</u> <u>2500 ppm</u> *Food consumption:* ↑ 2.6% males during first year Liver necrosis (incidence borderline with top of historical control data): Males 11/50 vs 5/49 control Hydronephrosis of kidney (within historical control data): Males 4/50 vs 1/49 control; Females 3/49 vs 0/49 control Cystic hyperplasia in uterus: Females 20/49 vs 15/49 control	KCA 5.5 / 02

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	<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 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	<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

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. 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	<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) *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>	KCA 5.3.2 / 03

44 mg/kg bw/day for females Vehicle: diet	*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 GLP Crl:WI(Han) Wistar rats 10/sex/group Purity: 93.9% Nose only exposures to dust	<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 *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>	KCA 5.3.3 / 01

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 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 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,	<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 ↓ 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)	KCA 5.6.2 / 02

respectively, to 0, 58, 145 and 362 mg/kg bw/day Vehicle: Mazola corn oil	<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 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,	<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 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)	KCA 5.6.1 / 01

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	↑ 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.**

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 critically, *in vivo* tests for gene mutations were negative, 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 +S9 **Negative Negative** Experiment 2 -S9 +S9 **Positive Negative**	K-CA 5.4.1/01
Mammalian Chromosomal Aberration Test	266, 524, 1039, 2069 µg/mL Purity: 88.8%	No cytotoxicity Top dose = Limit of solubility	KCA 5.4.1 / 02

EC B.10 OECD 473 GLP Chinese hamster ovary cells (CHO)	Solvent: DMSO	**Negative (+ S9)** **Negative (- S9)**	
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 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) Purity: 988.50 g/kg Solvent: DMSO	Positive controls: methyl methane sulfonate and cyclophosphamide **Positive (+ S9)** **Positive (- S9)**	KCA 5.4.1/05
Mammalian cell gene mutation test	Experiment 1: 65.6- 2100 µg/mL (-/+S9)	Positive controls: ethyl methane sulfonate and cyclophosphamide	KCA 5.4.1/06

(forward mutation test) OECD 476 GLP Mouse lymphoma L5178Y cells	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 (+ S9)** **Positive (- S9)**	
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 covers 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 No GLP Male and female Sprague-Dawley rats	208, 416 or 832 mg/kg bw Purity ≥ 99% Vehicle: Water with 20% gum arabic	**Negative**	KCA 5.4.2 / 01
Mammalian Erythrocyte Micronucleus Test EC, B.12 OECD 474/GLP	1300 mg/kg bw Purity = 88.5% Gavage Vehicle: Corn oil	**Negative**	KCA 5.4.2 / 02

Male and female CD-1 mice			
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 assay in rats was 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.**

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 the view 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 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.	McDuffie *et al.*, (2001) McDuffie *et al.*, 2005
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	Lerro *et al.,* 2020

	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)	
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 do not support 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 not to consider these malignant lymphomas as 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.**

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

Fertility and sexual function

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.**

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.

ENVIRONMENTAL HAZARD EVALUATION

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* (saltwater 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 14^{C}-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 ($E_rC_{50shoot\ length}$ (14d) 0.94 mg/L (mm)) and chronic ($NOEC_{shoot\ length}$ (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**.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

For dicamba, the DS considered a valid atmospheric DT_{50} of 3.6 – 4.1 days that 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. As dicamba is easily soluble in water rainfall, the DS expects that dicamba is removed from the air to a large extent. The volatilization from plant and soil surfaces is considered negligible (0.12% and 0.07 – 1.15%, respectively) and dicamba is not considered hazardous to the ozone layer.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

As mentioned by the DS, no available evidence concerning the properties of dicamba and its predicted or observed environmental fate and behaviour indicates that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer and dicamba is not listed in Annex I to Regulation (EC) No 1005/2009. Thus, RAC agrees with the DS that dicamba **does not warrant classification as hazardous to the ozone layer**.

ANNEXES:

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

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