Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

clomazone (ISO); 2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one

EC Number: -
CAS Number: 81777-89-1

CLH-O-0000006701-78-01/F

Adopted

20 September 2019

20 September 2019

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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: **clomazone (ISO); 2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one**

EC Number: **-**

CAS Number: **81777-89-1**

The proposal was submitted by **Denmark** and received by RAC on **16 November 2018.**

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 **26 November 2018**. Concerned parties and MS Competent Authorities (MSCA) were invited to submit comments and contributions by **8 February 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Julie Séba**

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 **20 September 2019** 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				
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)	Specific Conc. Limits, M-factors and ATE	Notes
Current Annex VI entry	No current Annex VI entry										
DSs proposal	TBD	clomazone (ISO); 2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one	-	81777-89-1	Repr. 1B Acute Tox. 4 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H360D H332 H302 H400 H410	GHS07 GHS08 GHS09 Dgr	H360D H332 H302 H410		inhalation: ATE = 4.3 mg/L (dusts or mists) oral: ATE = 754 mg/kg bw M = 1 M = 1	
RAC opinion	TBD	clomazone (ISO); 2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one	-	81777-89-1	Acute Tox. 4 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H332 H302 H400 H410	GHS07 GHS08 GHS09	H332 H302 H410		inhalation: ATE = 4.85 mg/L (dusts or mists) oral: ATE = 767.5 mg/kg M = 1 M = 1	
Resulting Annex VI entry if agreed by COM	TBD	clomazone (ISO); 2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one	-	81777-89-1	Acute Tox. 4 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H332 H302 H400 H410	GHS07 GHS08 GHS09	H332 H302 H410		inhalation: ATE = 4.85 mg/L (dusts or mists) oral: ATE = 767.5 mg/kg M = 1 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) concluded that none of the reported physical properties of clomazone result in classification using the criteria set out in the CLP Regulation.

Comments received during public consultation

The following endpoints were open for comment during the public consultation: explosives, flammable liquids, flammable solids, self-heating substances or mixtures, oxidising liquids and oxidising solids.

One Member State Competent Authority (MSCA) commented on the physical hazards:

- They requested the IR, NMR and MS fragments characteristics. The DS responded that these spectra and information were not a CLP requirement.
- *Oxidising solids*: They argued that a data gap was identified for oxidising solids, requesting a new test which was to be in accordance with CLP regulation. The DS agreed with their view and added that a test on liquid clomazone did not show oxidising properties.
- *Corrosivity to metals*: They stressed that no test had been provided to demonstrate the absence of corrosivity to metals.

One Company-Manufacturer supported the DS's conclusions on physical hazards.

Assessment and comparison with the classification criteria

Pure clomazone is a solid at room temperature, with a melting point of 33.0-34.7°C. However, technical clomazone, with a purity higher than 95 %, has been reported either as a solid or a liquid. For the application of the CLP criteria, clomazone is considered to be a solid.

Explosives

According to the CLP guidance, there is a structural alert based on the N-O bond. The substance identity experts of ECHA concluded that it was unlikely that the substance has explosive properties considering that the substance is a saturated heterocycle in conjunction with a lactam, which is generally stable. In addition, three EU A.14 tests were available in the CLH report all of which led to the conclusion that clomazone is not an explosive. Overall, RAC is of the opinion that **no classification is warranted for this hazard class.**

Flammable liquids

Three closed cup studies were described in the CLH report. The results indicated that the flash point of liquid clomazone is between 110 and 158°C. Clomazone is considered a solid according to CLP criteria. **This endpoint is therefore not applicable.**

Flammable solids

One EU A.10 study on flammability showed that technical clomazone is not highly flammable. The results were further supported by three negative studies with liquid clomazone.

Overall, despite the absence of a negative UN test N.1 (relevant to this hazard class), in line with ECHA guidance, and the study results above, clomazone is therefore not highly flammable. **No classification is warranted for this hazard class.**

Self-reactive substance or mixture

Clomazone does not contain chemical groups associated with self-reactive properties, and explosive properties have been excluded (see above) therefore RAC considers that it fulfils the criterion in Annex I 2.8.4.1(a) of the CLP Regulation. **No classification is therefore warranted for this hazard class.**

Self-heating substances

Four studies on auto-ignition were provided in the CLH report. The results indicated a self-heating temperature range between 374 and 390°C. The melting point was below 160°C. Clomazone is therefore not considered to be a self-heating substance. **No classification is warranted for this hazard class.**

Oxidising liquids

In a study on oxidising properties of liquids, the mean pressure rise time was higher than for the reference. Clomazone is considered as a solid according to CLP criteria. **This endpoint is therefore not applicable.**

Oxidising solids

Clomazone contains oxygen and chlorine atoms, which are chemically bonded to carbon atoms, with the exception of the oxygen in the saturated heterocycle. According to the CLP criteria, there is a structural alert for oxidising solid. No acceptable study was submitted to evaluate the oxidising properties of solid clomazone. Therefore, RAC is of the opinion that **no classification is warranted due to lack of data.**

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral Route

Two OECD TG 401 studies in male and female rats were made available for the oral route. Under the conditions of the studies, the acute oral $LD_{50\ (female)}$ for clomazone were determined to be 1216 mg/kg bw and 1461 mg/kg bw. In both studies, females were shown to be more sensitive to clomazone than male rats. In an acute oral toxicity up and down procedure (OECD TG 425) in female rats, the LD_{50} was established at 754 mg/kg bw. Finally, in an Acute-Toxicity-Class (ATC) test method (OECD TG 423) in female rats, the LD_{50} was found to be between 290 and 1932 mg/kg bw.

The DS proposed a classification for clomazone as Acute Tox. 4, H302 based on all LD_{50} values reported within the range of 300-2000 mg/kg and proposed an acute toxicity estimate (ATE) of 754 mg/kg bw.

Inhalation Route

One OECD TG 403 study is available in rats for the inhalation route. Based on the results of this study, the acute LC_{50} for clomazone was determined at 4.3 mg/L for female and male rats.

The DS concluded on a classification for clomazone as Acute Tox. 4, H332 and proposed an ATE of 4.3 mg/L.

Dermal Route

One OECD TG 402 study is available in rabbit for the dermal route. Under the conditions of the study, the acute dermal LD_{50} for clomazone was greater than 2000 mg/kg bw for female and male New Zealand White (NZW) rabbit. No mortality occurred.

The DS did not propose a classification for clomazone for acute dermal toxicity.

Comments received during public consultation

Three MSCA supported the DS's proposal on classification and labelling for Acute Tox. 4, H302 (oral) and Acute Tox. 4, H332 (inhalation). One of the commenting MSCA also supported the proposed ATEs for acute oral toxicity and acute inhalation toxicity.

One Company-Manufacturer supported the DS's conclusions on acute toxicity for all routes.

Assessment and comparison with the classification criteria

Oral Route

The Table below summarises the available acute oral toxicity studies in animals.

***Table*:** *Summary table for acute oral toxicity studies in animals with clomazone.*

Method	Test substance	Results	Reference
OECD TG 401 study GLP-compliant Single dose, gavage Sprague-Dawley rat 10/sex/group 20 males for the highest dose	Purity 88.8% *Females:* 381, 704, 864, 1060, 1174, 1300 or 2400 mg/kg bw *Males*: 1300, 1595, 1766, 1956, 2167 or 2400 mg/kg	$LD_{50\ (females)}$: 1369 mg/kg bw $LD_{50\ (males)}$: 2077 mg/kg bw	Anon. 1982, A82-709
OECD TG 401 study GLP-compliant Single dose, gavage Sprague-Dawley rat 10/sex/group	Purity 93.4% *Females:* 1200, 1500, 2000 or 2500 mg/kg bw *Males:* 1500, 2000, 2500 or 3000 mg/kg	$LD_{50\ (females)}$: 1564 mg/kg bw $LD_{50\ (males)}$: 2585 mg/kg bw	Anon. 1984, A84-1270
OECD TG 425 study GLP-compliant Up and down procedure Crl: CD(SD) IGS BR rat 3 females/group	Purity 98.2% 430 and 1370 mg/kg bw	$LD_{50\ (females)}$: 767.5 mg/kg bw	Anon. 2007, 0545/0573

OECD TG 423 study GLP-compliant ATC test method Crl: CD(SD) rat 3 females/group	Purity 96.6% 300 and 2000 mg/kg bw	300 < $LD_{50\ (females)}$ < 2000 mg/kg bw	Anon. 2009, 23497

In an acute oral study similar to OECD TG 401 (1987), clomazone (purity 88.8 %) was administered at doses ranging from 381 to 2400 mg/kg bw to 10 Sprague Dawley rats/sex/group (Anon. 1982, A82-709). The clinical signs included decreased locomotion, ataxia, decumbency, lachrymation, chromodacryorrhea and clear oral discharge. The resulting $LD_{50\ (females)}$ of 1369 mg/kg bw was further recalculated based on the purity and determined to be 1216 mg/kg bw by the DS.

In a second acute oral study similar to OECD TG 401, clomazone (purity 93.4 %) was administered at doses between 1200 to 3000 mg/kg bw to 10 Sprague Dawley rats/sex/group (Anon. 1984, A84-1270). The predominant clinical signs were ataxia, decreased locomotion, recumbency, haematuria, and ocular and nasal discharge. The LD_{50} for female rats of 1564 mg/kg bw was corrected for purity and determined to be 1461 mg/kg bw.

A GLP-compliant acute oral toxicity study was further performed in the rat with the up and down procedure according to the OECD TG 425 guideline (Anon. 2007, 0545/0573). Technical clomazone (purity 98.2 %) was administered at a single dose of 430 or 1370 mg/kg bw by gavage. None of the three females exposed to the 430 mg/kg bw died or demonstrated signs of systemic toxicity within the observation period of 14 days.

In contrast, mortality occurred in all animals receiving a dose of 1370 mg/kg bw. Two out of three animals treated with this dose showed patchy pallor of the liver at necropsy. Signs of toxicity at the high dose were hunched posture, ataxia, lethargy, piloerection, decreased respiratory rate, laboured respiration, splayed gait, prostration, dehydration, hypothermia, chromodacryorrhea, loss of righting reflex, pallor of the extremities, emaciation, increased lachrymation and ptosis. Based on the study results, the acute oral LD_{50} of clomazone technical was estimated to be 754 mg/kg bw after correction based on purity.

Finally, a GLP-compliant OECD TG 423 acute toxicity study was performed on Crl:CD(SD) rats following the Acute Toxic Class (ATC) method (Anon. 2009, 23497). During the first step, all three female rats that received a single dose of 2000 mg/kg bw clomazone (purity 96.6 %) via oral gavage died within 24 hours after administration. The associated clinical signs were slightly to severely reduced motility, slight to severe ataxia, slightly to severely reduced muscle tone, slight to severe dyspnoea and abdominal position. Dorsal position was reported in 2 of 3 animals.

The compound was further investigated by the administration of 300 mg/kg bw to three female rats. This step was performed twice. All six animals survived and did not demonstrate signs of toxicity, effects on body weight or macroscopic abnormalities. Based on the study results, the acute oral $LD_{50\ (rat,\ female)}$ of clomazone was determined to be between 300 and 2000 mg/kg bw.

Overall, female rats were shown to be more sensitive to the test material than male rats. RAC considers it not appropriate to correct the LD_{50} based on the purity of the test compound. However, RAC agrees with the DS's view that the lowest LD_{50} based on a GLP OECD TG 425 acute toxicity study (Anon. 2007) should be retained for the determination of the ATE.

In conclusion, clomazone fulfils the criteria for Acute Tox. 4, H302 (300 < LD_{50} ≤ 2000 mg/kg bw) based on all oral $LD_{50\ (rat,\ female)}$ values available in the CLH report. Therefore, RAC is of the opinion that a **classification as Acute Tox. 4, H302 is warranted for clomazone with an acute toxicity estimate (ATE) of 768 mg/kg bw.**

Inhalation Route

In a GLP compliant OECD TG 403 acute inhalation study, clomazone (purity 88.8 %) was administered via the inhalation route (whole-body exposure system) at concentrations of 0, 1.74, 3.67, 5.15 or 6.15 mg/L to 5 male and 5 female Sprague-Dawley rats/group for a single 4-hour period (Anon. 1982, 420-0939). The mean mass median aerodynamic diameter was 2.20 µm ± 1.72 µm.

Numerous clinical signs were reported in treated animals including ataxia, opacity of the eye and exophthalmos, alopecia, irregular breathing, prostration, nasal discharge and salivation. No control animals died during the study. Mortality rates of the treated animals are presented in the following Table.

Table: *Mortality rates in a rat acute inhalation toxicity study with clomazone***.**

Atmosphere concentration (mg/L air)	Mortality in males	Mortality in females
1.75	1/5	0/5
3.46	1/5	2/5
5.19	2/5	4/5
6.43	3/5	3/5
$LC_{50\ (combined)}$: 4.85 mg/L		

The resulting $LC_{50\ (combined)}$ of 4.85 mg/L was recalculated based on the purity of 88.8 % leading to a corrected $LC_{50\ (combined)}$ of 4.3 mg/L by the DS. RAC considers not appropriate to correct the LC_{50} based on the purity of the test compound.

The inhalation LC_{50} of 4.85 mg/L for rats fulfils the criteria for Acute Tox. 4, H332 for dusts and mists ($1.0 < LD_{50} \leq 5.0$ mg/L). Therefore, RAC supports the DS proposal for **classification as Acute Tox. 4, H322 with an ATE of 4.85 mg/L.**

Dermal Route

In an acute dermal toxicity study comparable to OECD TG 402, undiluted clomazone (purity 88.8 %) was administered as a single dose of 0 or 2000 mg/kg bw directly to the abraded skin of 5 male and 5 NZW rabbits under occluded dressing for 24 hours (Anon. 1982, A82-710). No mortalities occurred during the observation period of 14 days. The animals exhibited no systemic or local clinical signs of toxicity or macroscopic pathological abnormalities and the mean body weights increased throughout the study.

The dermal LD_{50} for rats exceeded 2000 mg/kg bw. Since relevant criteria in the CLP Regulation were not met, RAC agrees with the DS's proposal for **no classification of clomazone for acute dermal toxicity.**

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

Summary of the Dossier Submitter's proposal

The DS did not propose classification for clomazone for specific target organ toxicity after single exposure due to the lack of consistent, severe and/or statistically significant toxicity, organ damage or functional changes indicating such hazardous properties following a single administration by oral, dermal or inhalation route. No studies specifically addressing STOT SE were available.

Comments received during public consultation

One Company-Manufacturer supported the DS's conclusion on no classification for STOT SE.

Assessment and comparison with the classification criteria

In a first acute oral study similar to OECD TG 401, clomazone (purity 88.8 %) was administered at doses ranging from 381 to 2400 mg/kg bw to 10 Sprague Dawley rats/sex/group (Anon. 1982, A82-709). The observation period was 14 days. Clinical signs included decreased locomotion, ataxia, recumbency, lachrymation, chromodacryorrhea and clear oral discharge. All surviving animals returned to normal and gained weight by the second week of the study. The individual data were not available in the study report.

Six hours after treatment, decreased locomotion was observed in all females exposed to 381 or 704 mg/kg bw and in 4 females at 864 mg/kg bw. No deaths were reported up to 14 days at these exposure levels.

Table: *Incidences of "decreased locomotion" in female rats exposed to 381-864 mg/kg bw clomazone (No deaths occurred up to 14 days after exposure).*

Time after treatment	0.5 hours	1-2 hours	3-4 hours	6 hours	14 days
381 mg/kg bw	2	6	7	10	0
704 mg/kg bw	1	7	8	10	0
864 mg/kg bw	0	0	3-4	4	0

Small incidences of ataxia and decreased locomotion at 1060 mg/kg bw appeared between 3 hours and 4 days after treatment. Recumbency also occurred with a slight delay. At this exposure level, one female died after 3 days and a second female after 7 days.

In the 1174 mg/kg bw group, only one female died, on day 4. Decreased locomotion was reported in up to 8/10 animals between 3 hours and one day after treatment. At 1300 mg/kg bw, up to 3 females showed ataxia between 3 and 6 hours after treatment. Decreased locomotion affected up to 10 animals 2 hours after gavage and almost completely disappeared on the following day. Eight animals died between days 2 and 5.

In males, exposure to 1300 mg/kg bw led to decreased locomotion (up to 9 incidences) and ataxia (up to 4 incidences) from half an hour until one day after gavage. Only 2 rats died on day three.

In a second acute oral study similar to OECD TG 401, clomazone (purity 93.4 %) was administered at doses between 1200 to 3000 mg/kg bw to 10 Sprague Dawley rats/sex/group (Anon. 1984, A84-1270).

The clinical signs appeared approximately 3 hours after dosing and were the following: ataxia, decreased locomotion, recumbency, haematuria and oral, ocular and nasal discharges. Most surviving animals returned to normal by day 6 of the study and gained weight. Mortality occurred mostly between days 1 and 3 in a dose-dependent way in males and females. The LD_{50} for female rats of 1564 mg/kg bw was corrected for purity and determined to be 1461 mg/kg bw.

Although considered reliable, the results of this study are difficult to assess as such in order to conclude on target-organ toxicity after single exposure. The doses were higher than in the previous study and with the exception of the lowest-dose male group (1500 mg/kg bw), deaths were reported in all groups. Therefore, the clinical symptoms are only considered on a weight-of-evidence basis.

An up-and-down acute oral toxicity study in rats conducted according to the OECD TG 425 guideline was also available in the CLH report (Anon. 2007, 0545/0573). Clomazone (purity 98.2%) was administered at a single dose of 430 or 1370 mg/kg bw by gavage. No mortalities or signs of systemic toxicity were reported at 430 mg/kg bw. In contrast, signs of toxicity at the high dose were hunched posture, ataxia, lethargy, piloerection, decreased respiratory rate, laboured respiration, splayed gait, prostration, dehydration, hypothermia, chromodacryorrhea, loss of righting reflex, pallor of the extremities, emaciation, increased lachrymation and ptosis. These symptoms were associated with the death of all animals at 1370 mg/kg bw and two out of three animals treated with this dose showed patchy pallor of the liver at necropsy.

A further acute toxicity study was a GLP-compliant OECD TG 423 Acute Toxic Class (ATC) study performed on Crl:CD(SD) rats (Anon. 2009, 23497). No signs of toxicity were reported at the dose level of 300 mg/kg bw. After a single dose of 2000 mg/kg bw clomazone (purity 96.6%), all animals died within 24 hours after administration. The associated clinical signs were slightly to severely reduced motility, slight to severe ataxia, slightly to severely reduced muscle tone, slight to severe dyspnoea and abdominal position. Dorsal position was reported in 2 of 3 animals.

An *in vivo* mouse micronucleus test also reported several clinical signs after acute exposure to clomazone (Anon. 2009a, 23881). In the preliminary test, no mortality was reported at 500 mg/kg bw, one out of two and all animals died after exposure to 1000 mg/kg bw and 2000 mg/kg bw, respectively. The maximum tolerated dose was considered to be 500 mg/kg bw. In the main study, a single dose of 125, 250 or 500 mg clomazone/kg bw (purity 96.6 %) was administered by oral gavage to 5 NMRI mice/sex/group. Clinical signs appeared from 250 mg/kg bw and included slight reduction in motility, slight ataxia and slight dyspnoea. These observations increased in severity in a dose-dependent manner as all animals treated with the highest dose of 500 mg/kg bw revealed slightly to moderately reduced motility, slight to moderate ataxia, tremor and slight to moderate dyspnoea 5 to 30 minutes after administration.

This micronucleus study is considered supportive for the evaluation of target-organ toxicity after single exposure as no information on death or recovery is available more than 48 h after exposure and only limited parameters were investigated due to the main purpose of the study. However, the preliminary test informs on the lethality of clomazone in NMRI mice under the conditions of the study and the main test reported clinical signs without associated deaths up to 48 hours. The observations are therefore taken into consideration in the STOT SE assessment of clomazone to support the results observed in the acute toxicity studies.

Overall, RAC is of the opinion that the key-study for the evaluation of specific organ toxicity after single exposure is the acute toxicity report A82-709 (Anon., 1982). The *in vivo* micronucleus study was also performed at lower doses (due to the nature of the study) and is therefore

supportive of the results in the key acute toxicity study. The clinical signs reported at higher doses in various acute toxicity studies are not sufficient as such to classify for STOT SE, the effects occurring at these exposure levels being covered by the oral acute toxicity classification and might be linked to mortality of the animals.

In the key acute toxicity study in rat, clear decreased locomotion was observed at all dose levels. At doses between 381 and 704 mg/kg bw, all females were transiently affected without associated deaths. Incidences of ataxia were also reported from 1060 mg/kg bw. These effects mostly occurred very early after gavage and seemed to be transient. In some groups, the incidences of animals affected by decreased locomotion and/or ataxia were greater than cumulative deaths.

The main mouse micronucleus study also supports the early observations of slightly reduced mobility and ataxia at non-lethal doses. The supportive observations from other acute toxicity studies included in addition splayed gait, loss of righting reflex, tremor and ptosis.

Taking into consideration the overall pattern of clinical signs, RAC is of the view that some symptoms are suggestive of a potential neurotoxic effect of clomazone. Nevertheless, the clinical signs relevant for a classification as STOT SE are only restricted to decreased locomotion and ataxia at doses below the oral ATE. The cause of the decrease in locomotion remains unclear and this effect can be interpreted as being non-specific. In addition, the relevant observations of ataxia are of limited incidence and magnitude, mainly in a supportive mouse micronucleus study. RAC therefore considers that **the evidence is not sufficient to warrant a classification for STOT SE.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Three OECD TG 404 acute skin irritation studies performed with NZW rabbits were reported in the CLH dossier. At the 24h reading, very slight erythema was observed in all studies. Although the erythema persisted up to day 8 in one report, it had disappeared from all test sites 72 h after application in the two other studies.

The mean Draize scores for erythema were comprise between 0.33 and 0.7 for all studies. No oedema was noted at any point in any study.

The DS proposed no classification for clomazone as a skin corrosive/irritant based on negative results.

Comments received during public consultation

One Company-Manufacturer supported the DS's conclusion on no classification for skin corrosion/irritation.

Assessment and comparison with the classification criteria

In a primary skin irritation study, 0.5 mL clomazone (purity 88.8 %) was applied undiluted to the skin of 4 males and 2 females NZW rabbits during 24 hours under occlusive dressing (Anon. 1982c, A82-712). Although the conduct of the study was similar to OECD TG 404, the study was not GLP-compliant and critical deviations were noted (duration of exposure, no 48 h observations).

Very slight erythema occurred in both abraded (4/12) and intact skin (3/12) sites in the rabbits 24 hours after application. The erythema had disappeared at all test sites 72 hours after application. Differences between abraded and intact skin sites were very small. No oedema was observed at 24 or 72 hours after application.

In a second GLP compliant OECD TG 404 skin irritation study, 0.5 mL of undiluted clomazone (purity 89.4 %) was applied undiluted to the skin of three male NZW rabbits during 4 hours under semi-occlusive dressing (Anon. 1999, 18094 TAL). The summary of mean Draize scores for dermal response to intact skin was zero for oedema at all time points. For skin erythema, the mean Draize score were 0.3 after 1 h and 0.7 at both 24 h, 48 h and 72 h time points. Very slight erythema persisted up to day 6 in one rabbit and up to day 8 in another animal. Dryness of the skin was recorded in these two animals from day 7 to 8 and from day 7 to 11, respectively.

Finally, in a GLP-compliant OECD TG 404 skin irritation study, clomazone (purity 98.3 %) was applied as a single 500 mg dermal dose to the skin of three male NZW rabbits (Anon. 2010, C95077). The test substance moistened with water and covered by a semi-occlusive dressing on the skin for 4 hours. At the 1-hour observation, erythema was reported in all tested animals (scores 1 or 2) and persisted as very slight up to the 24 hour or 48 hour readings. The erythema had disappeared at all test sites 72 hours after application and the mean erythema/eschar score was 0.33. No oedema was reported at any time point.

Overall, all the mean scores for erythema/eschar or for oedema were below 2.3 in all studies and no inflammation was reported at the end of any observation period. Since relevant criteria in the CLP Regulation were not met, RAC agrees with the DS's proposal for **no classification of clomazone regarding skin corrosion/irritation.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for clomazone for serious eye damage/irritation based on a GLP compliant OECD TG 405 study in NZW rabbit. The eyes of all animals appeared normal within 24 h. All Draize scores were zero at the 24, 48 or 72 h endpoints.

Comments received during public consultation

One Company-Manufacturer supported the DS's conclusion on no classification for eye irritation.

Assessment and comparison with the classification criteria

In a GLP-compliant OECD TG 405 eye irritation study, undiluted clomazone (purity 88.6%) was applied as a single dose of 0.1 mL into the right eye of 4 male and 5 female NZW rabbits (Anon. 1982, A82-711). The eyes of six rabbits remained unwashed whereas water rinsing was performed on the eye of the three other animals after instillation.

One hour after treatment, redness of the conjunctiva was observed in all animals with unrinsed eyes and in one animal with rinsed eye. The eyes of all animals appeared normal within 24 h. No corneal opacity, iris lesions, conjunctiva redness or conjunctiva oedema were reported in any animal 24, 48 or 72 h after application.

Since the eyes of all animals appeared normal within 24 h and all Draize scores were 0, the CLP criteria for serious eye damage and eye irritation are not met. RAC therefore agrees with the DS's proposal for **no classification of clomazone for serious eye damage/irritation.**

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No data was available addressing respiratory sensitisation. Therefore, no classification was proposed by the DS.

Comments received during public consultation

One Company-Manufacturer supported the DS's conclusion on no classification for respiratory sensitisation.

Assessment and comparison with the classification criteria

RAC agrees with the DS that clomazone **does not warrant classification for respiratory sensitisation due to lack of data.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No classification was proposed for clomazone as a skin sensitiser on the basis of a Buehler test and two Magnusson-Kligman Maximisation studies showing equivocal or negative results in guinea pig. The DS concluded that based on the results of these studies, clomazone could not be presumed to have the potential to produce sensitisation in humans.

Comments received during public consultation

One Company-Manufacturer supported the DS's conclusion on no classification for skin sensitisation.

Assessment and comparison with the classification criteria

A GLP compliant OECD TG 406 Buehler test was performed on two groups of 10 males Hartley guinea pigs using 0.5 mL of undiluted technical clomazone (purity 88.8 %).

The study regimen was determined based on a range finding study using 4 guinea pigs. No irritation at 24 hours was observed in animals treated with 10, 25, 50 or 100 % clomazone diluted in ethanol. In the main study, the induction consisted of ten semi-occlusive dermal applications (6 hours, 3 times per week) with 0.5 mL of undiluted clomazone (88.8 %) or 1-chloro-2,3-dinitrobenzene for the positive control group (Anon. 1982e, A82-713). Animals were challenged with pure clomazone, considered to be a non-irritating concentration. No negative control group was included during this study since clomazone was applied undiluted. Results indicated no skin

reactions and no incidence of erythema or oedema in test group animals neither after induction nor upon challenge. Clear evidence of skin sensitisation was observed in the positive control group.

RAC notes that only 10 instead of 20 animals were treated with the test substance and no irritation was observed after induction with 100 % clomazone. The reliability of the negative results observed in this Buehler test is therefore considered limited.

The dermal sensitisation potential of clomazone (purity 96.5 %) was further evaluated in a GLP OECD TG 406 maximisation test in male and female Hartley albino guinea pigs (Anon. 2006, KZH00137). For induction, ten male and ten female animals received intradermal injections of the test substance (5 % w/v in propylene glycol) along with injections of test substance combined with Freund's Complete Adjuvant (FCA) or FCA only. Ten animals were used for the test vehicle control. One week later, animals received a topical application of 100 % clomazone (after a pre-treatment with sodium lauryl sulphate on day 6).

After a two-week rest period, all animals were topically challenged with 100 % clomazone. Scores of 1 (slight but confluent or moderate patchy erythema) were observed in 5/20 (25 %) of test animals and 3/10 (30 %) in challenge controls at 24 hours. In addition, scores of ½ (slight patchy erythema) were reported in 11/20 (55 %) of test animals.

At the 48 hours scoring time, 3/20 (15 %) test animals and 1/10 (10 %) challenge controls had dermal scores of 1.

Re-challenge was further performed following a seven-day rest period after which animals were topically treated with 50 % and 25 % w/v in propylene glycol. Following re-challenge with 50 % clomazone in propylene glycol, dermal scores of 1 were observed in 15/20 (75 %) of test animals and 8/10 (80 %) in re-challenged controls at 24 hours. Dermal scores of ½ were reported for 5/20 (25 %) test animals. At the 48 hour scoring time point, dermal scores of ½ were reported for 8/20 (40 %) test animals.

Following re-challenge with 25 % clomazone, 8/20 test animals and 6/10 controls demonstrated a dermal score of 1 at 24 hours. In addition, scores of ½ were reported in 5/20 (25 %) of test animals. At the 48 hour scoring time point, 1/20 (5 %) test animals had dermal scores of 1. Dermal scores of ½ were reported for 8/20 (40 %) test animals.

Finally, a last re-challenge was realised after two more weeks with topical application of 10 % or 5 % clomazone (w/v). For both exposure levels, the scores were limited to 0 and ½ in both test animals and challenge control animals. The results were therefore concluded to be negative.

Overall, the results of first challenge with 100 % clomazone and of re-challenges with 50 % and 25 % clomazone in propylene glycol showed positive responses in both the control groups (propylene glycol only) and test animals and are therefore considered to be equivocal. These challenge exposure levels were interpreted to be too irritating and a second re-challenge with 10 % and 5 % clomazone was performed. The results of these two second re-challenges were negative for both test animals and control groups.

The appropriate concentrations for induction and challenge in the main study were chosen based on range-finding studies (two topical and one intradermal). An initial range-finding study indicated that 100 % clomazone was only mildly irritating. The notifier stated that *"the results of re-challenge indicated that 50% and 25% were still too irritating"* (RAR 08 B6, p. 75*).* An additional range-finding study indicated that 10 and 5 % clomazone in propylene glycol as well as the vehicle were non-irritating. Detailed results of the range finding studies were not made available, in particular regarding the irritative potential of the 50 % and 25 % clomazone dilutions.

The OECD TG 406 guideline states that: *"The concentration of test substance used for each induction exposure should be well-tolerated systematically and should be the highest to cause*

mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest non-irritant dose". Based on the available reporting of the range-finding studies, there is no clear evidence that 10 % w/v clomazone in propylene glycol is the highest non-irritant dose. RAC also notes that pre-treatment with SDS was applied before topical induction with 100 % clomazone in order to create a local irritation in the main study. According to the OECD TG 406 guideline (1992), this first step should only be performed if the substance is not a skin irritant.

RAC considers that the results of this maximisation test are highly doubtful because a positive response showing confluent or moderate patchy erythema was observed in up to 80 % of the negative controls exposed to propylene glycol during challenge or re-challenge. In particular, these results are not consistent with the results of the second range-finding study indicating that propylene glycol is non-irritant. The variation in the negative control groups scores for the different challenge and re-challenges also suggest that the validity of the study is questionable.

A second GLP OECD TG 406 Maximisation test using Albino Dunkin Hartley guinea pigs was summarised in the CLH report (Anon. 2010, C95088). Dose regimens were determined based on a pre-test using one male and two males for intradermal and epidermal reactions, respectively. No detailed results were available for the dose-range finding study.

For intradermal induction, ten males received injections of 0.1 mL/site of a solution of 25 % clomazone (purity 98.3 %) w/v in PEG 300 and in an emulsion of Freund's Complete Adjuvant (FCA). One week later, animals received a topical application of 0.2 to 0.3 mL of a 50 % clomazone in PEG 300 on a patch of filter paper for 48 hours (occlusive dressing). Five animals were used for the test vehicle control. Discrete to moderate erythema was reported in all test animals after topical induction with 50 % clomazone. No skin reaction was observed in the control animals treated with PEG 300 alone.

Animals were challenged two weeks after dermal induction by a topical application of 10 % clomazone in PEG 300 and PEG 300 alone under occlusive dressing and cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing. No skin reactions were observed in the control or test animals treated with PEG 300 alone or with the test item at 10 % in PEG 300.

Another GLP OECD TG 406 Maximisation test using Dunkin Hartley guinea pigs was submitted to RAC during the CLH process (Anon. 2009, 23985).

In the preliminary study, clomazone was tested by intracutaneous injections of 0.01, 0.1, 0.5, 1, 5 or 10 % solution on two different animals. Three concentrations of the test item were injected into each animal. No skin reaction was observed at up to 0.5 % clomazone. Discrete or patchy erythema (score 1) was noted after injection of 1 % clomazone. A concentration of 5 % revealed moderate and confluent erythema (score 2) whereas an intracutaneous injection of 10 % clomazone induced intense erythema and swelling (score 3) 24 to 72 hours after administration. The concentration of 1 % was retained for intradermal induction.

For topical administration, 6 animals were used. Six concentrations of the test compound were topically applied (0.5, 1, 5, 10, 25 and 50 %) to the shaved or shaved and depilated flanks of 3 animals. Two concentrations were applied to each animal under an occlusive dressing for 24 (non-depilated) or 48 hours (depilated). No skin reactions were observed up to 25 % clomazone. Discrete or patchy erythema was observed after topical exposure to 50 % clomazone. This concentration was therefore retained for topical induction whereas 25 % was used for the challenge.

In the main study, the intradermal induction of 50 % clomazone (purity 96.6 %) w/v in water revealed discrete or patchy erythema (score 1) in all 10 animals. For topical induction, skins of the animals were first coated with SDS to induce local irritation. The topical application of 50 % clomazone induced discrete or patchy erythema (score 1) in all 10 animals. No skin reaction was observed in the control animals.

Animals were challenged two weeks after dermal induction by a topical application of 25 % clomazone in water. No skin reactions were observed in the control or test animals treated with the solvent or with the test item. Positive control (benzocaine, 2 % intradermal and 5 % topical for induction and challenge) induced discrete or patchy erythema (score 1) in all 20 animals after 48 h.

No animal showed positive reactions in two guinea pig Maximisation test or in a supportive Buehler test. A third guinea pig maximisation test showed inconclusive results. Therefore, since relevant CLP criteria were not met, RAC agrees with the DS's proposal for **no classification of clomazone for skin sensitisation.**

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

Summary of the Dossier Submitter's proposal

The DS described three supportive 28 day studies in rats, dogs and mice. Reduced body weight, body weight gain and food consumption were observed in high-dose rats and mice. Increases in organ weight, hepatic discoloration, enlargement and hepatocellular hypertrophy were also noted at the high dose. Slight effects on haematological parameters suggesting mild anaemia were seen in dogs only.

Two new supportive range-finding 28 day studies were also submitted in rats and mice. In rats, the NOAEL was set at 144.7 mg/kg bw/d in males and females based on reduced body weight gain in males and increase in relative liver weight in males and females. In mice, a LOAEL was proposed at 200 ppm (50-53 mg/kg bw/d) in males and females. Males showed a reduced body weight gain from 200 ppm without a concurrent effect in females. In females, increased relative and absolute liver weight were seen at the mid, high-intermediate and high dose. Hepatocellular hypertrophy was also increased in a dose related manner in the mid, high-intermediate and high dose male and female mice.

Two combined 90 day/24 month repeated toxicity/oncogenicity dietary studies in rats and mice were available (Anon. 1984a and 1984b, respectively; see table below).

In the rat 90-day part of the combined study, body weight and food consumption were reduced at 563 mg/kg bw/d. Liver effects included increased absolute and relative (a/r) liver weight, megalocytosis and altered total cholesterol in the high dose groups (563 mg/kg bw/d). Absolute and relative liver weights were affected from 278 mg/kg bw/d. The NOAEL was established at 138/163 mg/kg bw/d. In the oncogenicity part of the long-term and repeated dose toxicity study in rats, treatment-related findings were limited to increased hepatocytomegaly and liver weight from 0.8-1.1 mg/kg bw/d. Non-significant changes in vacuolative hepatocellular degeneration were also noted at the 12-month sacrifice.

In the 90-day part of the combined study in mice, statistically significant increases in the absolute and relative liver weights and histopathological changes in hepatocytes were observed from 761 mg/kg bw/d. The NOAEL was set at 371/522 mg/kg bw/d in males /females. In the oncogenicity part of the toxicity study in mice, main changes were limited to increases in liver weight and hepatocytomegaly. In addition, persistent thymic glands were seen from 182 mg/kg bw/d in females at final sacrifice.

In a 90-day study in Wistar rats (Anon. 2001, 2839/2000), the NOAEL was determined (m-f) to be 47.7-50.4 mg/kg bw/d based on histopathological effects on the kidney in males starting at 92.0 mg/kg bw/d, accompanied by clinical chemistry effects with higher creatinine effects in

males. Neurobehavioral evaluation showed lower hind limb grip strength in males from 47.7 mg/kg bw/d and significantly higher forelimb grip strength in females from 103.8 mg/kg bw/d.

A 12-month chronic toxicity in dogs showed statistically significant elevated cholesterol levels in both sexes and some inconsistent organ weight changes (absolute and relative liver and ovary weight and relative brain weight) from 67 mg/kg bw/d. Signs of mild but transient anaemia were observed at 147 mg/kg bw/d but were not evident after 12 months. The NOAEL was set at 12 mg/kg bw/d.

Finally, a 28-day dermal repeated toxicity study in rats showed no adverse systemic toxicity resulting in a NOAEL of 927 mg/kg bw/d.

All the NOAEL and LOAEL values proposed by the DS were systematically corrected for purity of the test-material.

Overall, the liver was the main target-organ for rat, mouse and dog after clomazone exposure. For the liver, effects occurring at or below the oral guidance value for STOT RE 2 mainly included increased liver weights and hepatocytomegaly. In the other studies, the DS concluded that the effects occurred at doses greater than the guidance values to assist classification.

In the 90-day rat study, increased creatinine levels and hyaline droplets at the mid and high doses indicated that kidney was a target organ for males. No clear treatment-related effects related to the kidneys were reported in the other available studies.

All the treatment-related effects were interpreted of low severity or occurring above the guidance values for STOT RE. The DS concluded that a STOT RE classification was not warranted for clomazone.

Comments received during public consultation

One Company-Manufacturer supported the DS's conclusion on no classification for STOT RE.

Assessment and comparison with the classification criteria

The Table below summarises the available oral and dermal repeated-dose toxicity studies in animals.

Table: *Summary table for oral repeated dose toxicity studies in animals with clomazone.*

Method	Results	Reference
OECD TG 408 GLP study 90 days Sprague-Dawley Rats 40 animals/sex/dose Oral, diet 0, 20, 100 or 500, 1000, 2000, 4000 or 8000 ppm (equivalent to 0, 1.4, 7, 35, 68, 138, 278 or 563 mg/kg bw/d) Purity 88.8 %	No treatment-related mortality **<u>563 mg/kg bw</u>** (8000 ppm) Decreased bw in males and females *Liver*: increased a/r weight, megalocytosis increased cholesterol **<u>278 mg/kg bw/d</u>** (4000 ppm) Decreased bw in females *Liver*: Increased a/r weight, increased cholesterol **<u>138 mg/kg bw/d</u>** (2000 ppm) *Liver*: increased weight, megalocytosis **<u>≤ 68 mg/kg bw/d</u>** (≤ 1000 ppm) No treatment-related adverse effect	Anon. 1984a (410-0816)

Limit dose level for warranting classification as category 2 ≤ 100 mg/kg bw/d	No histopathology performed in the 4000 ppm, 1000 ppm and 100 ppm dose groups. NOAEL: 2000 ppm LOAEL: 4000 ppm	
OECD TG 408 GLP study 90 days Wistar Rats 10 animals/sex/dose Oral, diet 0, 600, 1200 or 4800 ppm (equivalent to 0, 47.7-50.4, 92.0-103.8 or 377.4-409.0 mg/kg bw/d for males and females, respectively) Purity 94 % ***Limit dose level for warranting classification as category 2 ≤ 100 mg/kg bw/d***	No treatment-related mortality **<u>377.4-409.0 mg/kg bw/d</u>** (4800 ppm) Decreased bw in males *Liver*: increased a/r weight, increased cholesterol, hepatocellular hypertrophy *Kidney*: hyaline droplets in kidney tubular epitheliums (males), increased creatinine. Effects were reversible *Neurological system*: lower grip strength in hind limbs (males); increased grip strength in forelimbs (females). **<u>92.0-103.8 mg/kg bw/d</u>** (1200 ppm) *Liver*: increased a/r weight, increased cholesterol, hepatocellular hypertrophy *Kidney*: hyaline droplets in tubular epithelium (males), increased creatinine levels *Neurological system*: lower grip strength in hindlimbs (males) and increased grip strength in for limbs (females) **<u>47.7-50.4 mg/kg bw/d</u>** (600 ppm) *Neurological system*: lower grip strength in hindlimbs (males) NOAEL = 600/1200 ppm (m/f) LOAEL = 1200/4800 (m/f)	Anon. 2001 (2839/2000)
OECD TG 408 GLP study 90 days CD-1 Mouse 40 animals/sex/dose Oral, diet 0, 20, 100 or 500, 1000, 2000, 4000 or 8000 ppm (equivalent to 0, 3.8, 19.5, 98, 188, 371, 761 or 1766 mg/kg bw/d) Purity 88.8 % ***Limit dose level for warranting classification as category 2 ≤ 100 mg/kg bw/d***	No treatment-related mortality **<u>1766 mg/kg bw/d</u>** (8000 ppm) *Liver*: Increased organ weight (males and females), mild megalocytosis **<u>761 mg/kg bw/d</u>** (4000 ppm) *Liver*: Increased a/r weight (males and females) **<u>371 mg/kg bw/d</u>** (2000 ppm) *Liver*: Increased absolute weight (males and females) **<u>≤ 188 mg/kg bw/d</u>** (≤ 1000 ppm) No treatment-related adverse effect No histopathology performed in the 4000 ppm, 1000 ppm and 100 ppm dose groups. NOAEL: 2000 ppm LOAEL: 4000 ppm	Anon. 1984b (410-0817)
OECD TG 452 GLP study 1 year Beagle Dog 6 animals/sex/dose Oral, diet 0, 100, 500, 2500 or 7500 ppm, highest dose reduced	No mortality **<u>147 mg/kg bw/d</u>** (7500/5000 ppm) - Increased serum cholesterol in both sexes - Organ weight changes (a/r liver weight, a/r ovary and relative brain) - Signs of transient mild anaemia up till 6 months **<u>67 mg/kg bw/d</u>** (2500 ppm) - Increased serum cholesterol in both sexes	Anon. 1984 (6124-101)

to 5000 ppm on day 8 (equivalent to 0, 3, 13, 67 or 147 mg/kg bw/d) Purity 88.8 % ***Limit dose level for warranting classification as category 2 ≤ 25 mg/kg bw/d***	- Organ weight changes (a/r liver weight, a/r ovary and relative brain) **≤ 13 mg/kg bw/d** (≤ 500 ppm and lower) No observed adverse effect NOAEL =500 ppm LOAEL = 2500 ppm	
24-month chronic toxicity and carcinogenicity in rats Sprague-Dawley Rats 0, 20, 100 or 500, 1000, 2000, (equivalent to 0, 0.8-1.1, 4-5, 20-27, 41-55 or 83-110 mg/kg bw/d for males and females, respectively) 80 animals/sex/dose Purity 88.8 % ***Limit dose level for warranting classification as category 2 ≤ 12.5 mg/kg bw/d***	No treatment-related mortality **83-100 mg/kg bw/d (2000 ppm)** *Liver*: increased relative weight, hepatocytomegaly **41-55 mg/kg bw/d (1000 ppm)** *Liver*: hepatocytomegaly **20-27 mg/kg bw/d (500 ppm)** *Liver*: hepatocytomegaly **4-5 mg/kg bw/d (100 ppm)** *Liver*: hepatocytomegaly **0.8-1.1 mg/kg bw/d (20 ppm)** *Liver*: increased relative weight, hepatocytomegaly	Anon. 1984a (410-0816)
24-month chronic toxicity and carcinogenicity in mouse CD-1 Mouse 80 animals/sex/dose 0, 20, 100 or 500, 1000 or 2000ppm (equivalent to 0, 3-4, 15-18, 73-89, 142-182, 290-359 mg/kg bw/d) Purity 88.8 % ***Limit dose level for warranting classification as category 2 ≤ 12.5 mg/kg bw/d***	No treatment-related mortality **290-359 mg/kg bw/d** (2000 ppm) - *Liver*: Increased weight, hepatocytomegaly - *Thymus*: Persistent thymic glands **142-182 mg/kg bw/d** (1000 ppm) - *Liver*: Hepatocytomegaly - *Thymus*: Persistent thymic glands **73-89 mg/kg bw/d** (500 ppm) - *Liver*: hepatocytomegaly **15-18 mg/kg bw/d (100 ppm)** - *Liver*: hepatocytomegaly	Anon. 1984b (410-0817)
Non-guideline GLP range-finding study 28 days CD Sprague-Dawley Rats 10 animals/sex/dose Oral, diet 0, 2000, 4000, 8000, 16000, 24000 or 50000 ppm (equivalent to 0, 255.7-270.0, 513.3-533.4, 994.1-1096.1, 2025.4-2059.9, 2980.3-3123.5 or	No mortality occurred up to and including 24000 ppm **5371.0 - 6199.6 mg/kg bw/d** (50000 ppm) - 3 males and 3 females were found death or moribund - Clinical signs of severe intoxication and weakened debilitated condition - Decreased body weight and food consumption in males and females - *Liver*: diffuse enlargement, focal or multifocal discoloration (males and females) **2980.3 - 3123.5 mg/kg bw/d** (24000 ppm) - Decreased body weight and food consumption in males and females - *Liver:* focal or multifocal discoloration (males and females)	Anon. 1982a (410-0743) *Supportive*

5371.0-6199.6 mg/kg bw/d for males and females respectively) Purity 87.9 %	**<u>2025.4 – 2059.9 mg/kg bw/d</u>** (16000 ppm) - Decreased body weight and food consumption in males and females - *Glandular stomach*: multifocal depression (females) - *Liver*: focal or multifocal discoloration (males) **<u>994.1 – 1096.1 mg/kg bw/d</u>** (8000 ppm) - *Liver*: focal or multifocal discoloration (males) **<u>≤ 513.3 – 533.4 mg/kg bw/d</u>** (≤ 4000 ppm) No treatment-related adverse effects	
OECD 407 GLP range-finding study 28 days Wistar rat 6 animals/sex/dose Oral, diet 0, 500, 1500, 4500 and 9000 ppm corresponding to 0, 47.7-50.0, 144.7, 421.8-430.9 or 842.8-816.0 mg/kg bw/d for males and females, respectively Purity 92.9 %	No treatment-related mortality **<u>816-842.8 mg/kg bw/d</u>** (9000 ppm) - Decreased body weight - *Liver*: increased absolute/relative organ weight **<u>421.8-430.9 mg/kg bw/d</u>** (4500 ppm) Reduction in bw and increased absolute/relative liver weight. **<u>≤ 144.7 mg/kg bw/d</u>** (≤1500 ppm) No observed adverse effect NOAEL = 1500 ppm LOAEL = 4500 ppm	Anon. 2000 (2838/2000) *Supportive*
Non-guideline GLP range-finding study 28 days CD-1 albino Mouse 10 animals/sex/dose Oral, diet 0, 2000, 4000, 8000, 16000, 24000 or 50000 (equivalent to 0, 432.9-524.7, 921.5-1140.2, 1977.3-2294.2, 3726.8-4764.1 or 5152.8-4589.8 mg/kg bw/d for males and females respectively). Mean test article consumption could not be calculated for the highest dose due to mortality of all animals. Purity 87.9 %	No mortality occurred up to and including 5152.8 mg/kg bw/d **<u>50.000 ppm</u>** All animals died **<u>5152.8 – 4589.8 mg/kg bw/d</u>** (24000 ppm) Lower bw and decreased food consumption - *Liver*: Focal or multifocal areas of hepatic discoloration and increased weights **<u>3726.8 – 4764.1 mg/kg bw/d</u>** (16000 ppm) Lower bw and decreased food consumption - *Liver*: Focal or multifocal areas of hepatic discoloration and increased weights **<u>1977.3– 2294.2 mg/kg bw/d</u>** (8000 ppm) Lower bw and decreased food consumption - *Liver*: Focal or multifocal areas of hepatic discoloration and increased weights **<u>≤ 1140.2 – 921.5 mg/kg bw/d</u>** (≤ 4.000 ppm) No treatment-related adverse effects	Anon. 1982b (410-0744) *Supportive*
OECD 407 GLP range-finding study 28 days Swiss albino mouse	No mortality **<u>2182.4-2363.8 mg/kg bw/d</u>** (9000 ppm) Reduction in bw *Liver*: increased a/r weight and hepatocellular hypertrophy.	Anon. 2004 3693/03 *Supportive*

6 animals/sex/dose Oral, diet 0, 200, 800, 3200 and 9000 ppm corresponding to 0, 52.4-55.8, 211.2-218.6, 816.7-887.7 and 2182.4-2363.8 mg/kg bw/d for males and females, respectively Purity 95.0 %	**<u>816.7-887.7 mg/kg bw/d</u>** (3200 ppm) *Liver*: increased a/r weight (females), hepatocellular hypertrophy **<u>211.2-218.6 mg/kg bw/d</u>** (800 ppm) *Liver*: increased absolute bw (females), hepatocellular hypertrophy **<u>52.4-55.8 mg/kg bw/d</u>** (200 ppm) No treatment-related effects NOAEL = 200 ppm LOAEL = 800 ppm	
Non-guideline GLP range-finding study 28 days Beagle Dog 2 animals/sex/dose Oral, diet 0, 100, 1000, 5000 or 10000/2500 ppm The high dose group level was reduced from 10000 ppm to 2500 ppm on day 9 Purity 88.8 %	**<u>5000 ppm</u>** - *Haematology*:Mild anaemia in both sexes - *Liver*: Increased liver weight in both sexes Hepatocellular swelling - *Kidney*: small interstitial mononuclear cell infiltrations and congestion in the papilla. - *Lung*: Firm, tan or yellow/tan areas in the right caudal lung lobes, chronic granulomatous pneumonitis - *Testis*: lower a/r weights. **<u>10000/2500 ppm</u>** - *Haematology*: Mild anaemia - *Liver*: Increased a/r weight **<u>1000 ppm</u>** No treatment-related effects **<u>100 ppm</u>** No treatment-related effects	Anon. 1983 6124-100 *Supportive*
OECD TG 410 GLP study 28 days Sprague-Dawley CD rat 10 animals/sex/dose Dermal 0 and 1000 mg/kg bw/d 6-hour exposure, 5 days/weeks, semi-occlusive Purity 92.7 %	No mortality No adverse effect after exposure to 1000 mg/kg bw/d	Anon. 2002 A2001-5436 *Supportive*

Five range-finding studies in the rat, mouse or dog and one dermal study in rats were performed using clomazone. These studies are considered supportive and further described in the "Supplemental information - In depth analyses by RAC" section of the present evaluation.

Two earlier combined 90-day/24-month repeated toxicity/oncogenicity dietary studies using clomazone were performed in Sprague Dawley rats and CD-1 mice (Anon. 1984a, 410-0816 and Anon. 1984b, 410-0817). The protocols were similar for the two studies. Groups of 120 animals/sex/dose were exposed to either 0, 20, 100, 500, 1000, 2000, 4000 or 8000 ppm clomazone (purity 88.8 %). Ten animals/sex/group were randomly selected at both 30 and 60 days for examination and subjected to gross necropsy, haematology and urine analysis. At 90

days, 20 animals/sex/group were randomly chosen for sacrifice. After the 90-day sacrifice, the 4000 and 8000 ppm group were terminated following 16 weeks on test and removed from the study. No ophthalmological examination was performed during this first part of the study. Histopathology was performed on untreated control, 20, 500, 2000 and 8000 ppm dose groups on the three-month endpoint only.

For the long-term chronic toxicity and carcinogenicity phase of the study, 10 randomly selected animals/sex/group were sacrificed at 6, 12 and 18 months for blood and histopathological evaluations. Surviving animals, approximately 50/sex/group in the control, 20, 100, 500, 1000 and 2000 ppm groups were continued on the study up to 24 months for chronic toxicity and oncogenicity evaluation. Histopathology performed at the 6-month sacrifice was presented in an additional report that was not available to RAC during the assessment of repeated toxicity of clomazone. No ophthalmological examination was performed at any time-point.

During the 90-day subchronic toxicity part of the rat study, no increased mortality was reported at any dose up to 563 mg/kg bw/d (8000 ppm). Decreased body weight and food consumption were noted from the first week until week 15 at 8000 ppm in males and at 4000 ppm and 8000 ppm in females. In addition, the body weight of females was also statistically significantly decreased on week 15 from the 1000 and 2000 ppm group levels.

No treatment-related effects were noted in haematological parameters or urinalysis at the any timepoint in males or females with the exception of a statistically significant increase in total cholesterol in males and females at the 8000 ppm dose group at all time points and marginally at 1000 ppm and 4000 ppm in females at the three month-endpoint only.

Necropsy did not reveal treatment-related findings at the one-month, two-month or three-month endpoints. Liver weights were statistically significantly increased from 2000 ppm in both males and females at the three-month sacrifice with a dose-response relationship.

Table: *Mean absolute and relative liver weights in male and female rats at the three-month sacrifice in a repeated toxicity study*

Dose (ppm)	**0**	**20**	**100**	**500**	**1000**	**2000**	**4000**	**8000**
Males								
Organ wt. ± S.D. (g)	13.2047 ± 1.7569	14.2614 ± 2.0546	14.2858 ± 1.8073	16.0043** ± 1.8317	14.3759 ± 2.0628	15.6406** ± 2.3450	15.9674** ± 2.1130	16.6553** ± 2.5014
Organ/ BW ± S.D. (g/100g)	2.7476 ± 0.2375	2.8201 ± 0.2051	2.7955 ± 0.1821	3.0345 ± 0.1576	2.9311 ± 0.2175	3.0353 ± 0.2408	3.2867## ± 0.2451	3.6312## ± 0.3406
Organ/ brain wt. ± S.D. (g/g)	6.0405 ± 0.7799	6.5399 ± 0.9103	6.5201 ± 0.8254	7.3387# ± 0.8378	6.6081 ± 0.9253	7.2671 ± 1.2029	7.2684# ± 0.9236	7.7887## ± 1.1549
Females								
Organ wt. ± S.D. (g)	7.5065 ± 0.6509	7.6363 ± 0.6308	7.9608 ± 0.9473	7.6971 ± 0.7553	8.1562 ± 0.7113	8.3038* ± 0.6674	8.5205** ± 0.8507	9.4123** ± 0.7933
Organ/ BW ± S.D. (g/100g)	2.8432 ± 0.1688	2.8445 ± 0.2226	2.8916 ± 0.2463	2.8451 ± 0.1974	2.9786 ± 0.1951	3.0646 ± 0.1744	3.22441## ± 0.1853	3.8484## ± 0.3559
Organ/ brain wt. ± S.D. (g/g)	3.7315 ± 0.3021	3.7222 ± 0.2706	3.9622 ± 0.4593	3.7729 ± 0.3164	4.1093 ± 0.3336	4.0865 ± 0.3460	4.2497# ± 0.4097	4.7404## ± 0.4226

** Statistically significant difference ($p < 0.05$) using Tukey's multiple comparison test

** Statistically significant difference ($p < 0.01$) using Tukey's multiple comparison test
# Statistically significant difference ($p < 0.05$) using Kruskal-Wallis multiple comparison test
Statistically significant difference ($p < 0.01$) using Kruskal-Wallis multiple comparison test

Results of the histopathological examination performed at the three-month endpoint indicate a dose-dependent increase in megalocytosis of centrilobular hepatocytes from 2000 ppm. The frequency of megalocytosis was statistically significant ($p < 0.01$) for the 8000 ppm female rats were compared to control group. The frequency of glomerulonephrosis was slightly increased in the 2000 and 8000 ppm dose groups. No histopathology was performed on the 100, 1000 and 4000 ppm dose groups.

Table: *Histopathological incidences of hepatocellular megalocytosis and glomerulonephrosis at the three-month endpoint in a repeated toxicity study in Sprague-Dawley rats (males/females)*

Dose (ppm)	**0**	**20**	**500**	**2000**	**8000**
Liver: Diffuse centrilobular megalocytosis					
Minimal	0/0	0/0	0/0	2/1*	4/8
Mild	0/0	0/0	0/0	0/0	1/11
Total (%)	**0**	**0**	**0**	**3 (7.5%)**	**24 (60%)**
Kidney: Glomerulonephrosis					
Minimal	9/0	3/0	10/0	13/0	15/1
Mild	0/0	0/0	0/0	0/0	1/0
Total (%)	**9 (22.5%)**	**3 (7.5%)**	**10 (25%)**	**13 (32.5%)**	**17 (42.5%)**

* Finding was reported as questionable for animal AC3735

The observation of increased liver weight in association with megalocytosis at 2000 ppm (corresponding to 138 mg/kg bw/d of clomazone, purity 88.8 %) might be considered relevant for the evaluation of STOT RE. Yet, the absence of histopathology for the 1000 ppm dose group as well as the lack of details on the histopathological findings complicate the overall assessment of the liver effects in rats for this study.

In the long-term chronic toxicity part of the rat study, no statistically significant effect on haematological or clinical chemistry parameters was observed in male or female rats at any time point.

Detailed results of the histopathological examination performed at the six-month endpoint did not indicate treatment-related observations at any dose level. Necropsy did not reveal significant treatment-related effects. Non-statistically significant increases in liver weight were observed at 2000 ppm in males.

Individual histopathology results at the 12-month sacrifice showed several degenerative atypical hepatocytes as well as slight increase in hepatocellular necrosis. In particular, an atypical vacuolative hepatocellular degenerative process was described as a "*localized hepatocellular vacuolation with extraordinary variation in vacuole size and shape and with compression of surrounding normal hepatocytes without indication of malignancy*". No clear dose-dependent relationship was identified for any of these findings. The incidences of relevant hepatocellular observations are presented in the Table below.

Table: *Histopathological incidences of hepatocellular necrosis, degeneration and atypia at the 12-month endpoint in a repeated toxicity study in Sprague-Dawley rats (males/females, N = 20)*

Dose (ppm)	0	20	100	500	1000	2000
Hepatocellular necrosis	1	4	1	2	3	3
Eosinophilic hepatocellular degeneration	2	4	1	1	2	0
Clear cell hepatocellular atypia	2	1	1	1	3	0
Vacuolative hepatocellular degeneration (fatty change)	2	4	7	3	5	3
Atypical vacuolative hepatocellular degeneration	5	6	5	9	7	9

Other non-neoplastic changes comprised adrenal cortical and/or medullary haemorrhage and haemocytes as well as a cortical cytoplasmic atypia described as eosinophilic degeneration. These microscopic findings in the adrenals were considered to be usual changes seen in older rats. Chronic renal disease with tubular and interstitial components was also seen in all study groups.

Increased incidences of enlarged pituitary were reported from 20 ppm without a dose-dependent relationship. This finding is therefore considered incidental. A non-statistically significant increase in liver weight was observed at 1000 ppm and 2000 ppm in females.

Overall, for the 12-month endpoint, the findings observed at 500 ppm (equivalent to 20-27 mg/kg bw/d (m-f)) and lower are considered relevant for the assessment of STOT RE. These observations mainly include vacuolative degeneration. The slight increase in hepatocyte necrosis was reported without a clear dose-dependent relationship and this finding seems to be incidental.

The detailed 18-month histopathological findings were also available in the full study report. Hepatocytomegaly of the liver was slightly more frequent in the test than in the control groups of both males and females without a clear dose relationship to the incidence. No other findings were clearly associated with the test substance. No treatment-related effects were noted during necropsy or organ weight.

At final sacrifice (24 months), statistically significant increases in liver weight were reported in females at the 20 ppm and 2000 ppm. Non-statistically significant increases were also noted at other exposure levels in females and males without a clear dose-response relationship.

Table: *Mean absolute and relative liver weights in female Sprague Dawley rats at the final month sacrifice in a repeated toxicity study.*

Dose (ppm)	0	20	100	500	1000	2000
Organ wt. ± S.D. (g)	11.3001 ± 2.3194	13.6131 ± 4.9910	12.3716 ± 2.5568	13.0741 ± 2.5568	13.4502 ± 2.9709	13.3995 ± 4.4572
Organ/ BW ± S.D. (g/100g)	2.4789 ± 0.3601	3.1749# ± 0.9621	2.6749 ± 0.4606	2.8264 ± 0.6515	2.9767 ± 0.4662	3.1703## ± 05414
Organ/ brain wt. ± S.D. (g/g)	5.3524 ± 1.1617	6.4136 ± 2.3295	5.9006 ± 1.2649	6.2231 ± 1.3072	6.3883 ± 1.3851	6.3342 ± 2.0631

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Histopathology revealed a non-dose related increase in the number of animals with hepatocytomegaly, either focal, multifocal, diffuse or centrilobular. Increased incidences of

necrosis were also noted in males. In the kidney, chronic nephropathy was common in all groups and both sexes. The remaining non-neoplastic findings are considered by RAC to be incidental.

Table: *Incidences of liver hepatocytomegaly and necrosis at final sacrifice in a rat combined chronic toxicity and carcinogenicity study*

Exposure level (ppm)	**0**	**20**	**100**	**500**	**1000**	**2000**
Males						
Liver (No examined)	*37*	*45*	*44*	*43*	*42*	*40*
multifocal hepatocytomegaly	0	4	6	2	5	1
focal hepatocytomegaly	2	2	1	1	1	2
centrilobular hepatocytomegaly	3	7	4	5	2	4
diffuse hepatocytomegaly	4	10	14	12	10	9
multifocal necrosis	0	3	1	0	0	1
centrilobular necrosis	0	1	0	3	0	1
focal necrosis	0	0	1	3	1	3
total hepatocytomegaly	**9**	**23***	**25***	**20***	**18**	**16**
Total necrosis	**0**	**4**	**2**	**6**	**1**	**5**
Females						
Liver (No examined)	*36*	*43*	*42*	*43*	*42*	*42*
multifocal hepatocytomegaly	1	3	5	6	3	3
focal hepatocytomegaly	2	0	2	0	2	2
centrilobular hepatocytomegaly	1	5	7	2	9	3
diffuse hepatocytomegaly	11	18	14	16	13	14
multifocal necrosis	0	0	1	3	2	2
centrilobular necrosis	1	1	0	1	0	1
focal necrosis	4	1	0	0	2	0
total hepatocytomegaly	**15**	**26**	**28***	**24**	**27***	**22**
Total necrosis	**5**	**2**	**1**	**4**	**4**	**3**

** Statistically significant using Fischer's Exact Test with $p < 0.05$*

The findings reported at final sacrifice and considered relevant for the assessment of STOT RE only include relevant observations made at 100 ppm (equivalent to 4-5 mg/kg bw/d) and lower. These effects include increased hepatocytomegaly in males and females. The overall increase in liver weight also might be taken into consideration. Finally, a slight increase in total necrosis is noted in males. This increase was not statistically significant, was without a clear dose-dependent relationship and is therefore of equivocal biological significance.

In the 90-day subchronic toxicity part of the mouse study, no increased mortality was reported at any dose. Slightly decreased body weight was noted for the 8000 ppm males (week 1) and females (week 4). Poor diet acceptance was pointed out in the study report.

No treatment-related effects were noted in haematological parameters or urinalysis at any timepoint in males or females. Necropsy did not reveal treatment-related findings at the one-month, two-month or three-month endpoints. A dose-dependent increase in liver weight was observed in male and female mice at the three-month sacrifice. Statistical significance was, however, only reached from 4000 ppm in both males and females.

Table: *Mean absolute and relative liver weights in male and female mice at the three-month sacrifice in a repeated toxicity study*

Dose (ppm)	0	20	100	500	1000	2000	4000	8000
Males (N = 20)								
Final bw (g)	31.9205 ± 1.9465	32.4490 ± 1.8473	32.7375 ± 2.5608	32.9590 ± 2.2083	32.3840 ± 2.6373	33.0340 ± 3.2288	32.4720 ± 2.5851	31.6530 ± 2.5240
Organ wt. ± S.D. (g)	1.4208 ± 0.1346	1.4547 ± 0.1630	1.5088 ± 0.1349	1.5225 ± 0.1458	1.5330 ± 0.1649	1.6106 ± 0.2000	1.7999 ± 0.2785**	1.9084 ± 0.1540**
Organ/ BW ± S.D. (g/100g)	4.4507 ± 0.3135	4.4820 ±0 .4175	4.6166 ± 0.3271	4.6153 ± 0.2409	4.7392 ± 0.4225	4.8786 ± 0.4031	5.5279 ± 0.5642##	5.7252 ± 0.4061##
Organ/ brain wt. ± S.D. (g/g)	2.5558 ± 0.2972	2.6711 ± 0.2820	2.7460 ± 0.2270	2.7908 ± 0.3136	2.7353 ± 0.3147	2.9589 ± 0.3303	3.2909## ± 0.4952	3.2640## ± 0.2801
Females (N = 20)								
Final bw (g)	25.3476 ± 2.4594	25.0724 ± 1.5456	25.4618 ± 1.9482	25.9253 ± 2.3294	25.4900 ± 2.9827	24.7256 ± 2.2915	26.2238 ± 2.5811	24.6356 ± 1.3709
Organ wt. ± S.D. (g)	1.0896 ± 0.1142	1.0807 ± 0.1597	1.1061 ± 0.1424	1.1355 ± 0.1354	1.1988 ± 0.1974	1.2316 ± 0.1491	1.3490** ± 0.2677	1.4492** ± 0.1495
Organ/ BW ± S.D. (g/100g)	4.2972 ± 0.4316	4.2537 ± 0.4694	4.3861 ± 0.3423	4.3563 ± 0.2697	4.8617 ± 0.5301	4.9808 ± 0.3406	5.1442 ± 0.6556	5.8583## ± 0.4232
Organ/ brain wt. ± S.D. (g/g)	2.0396 ± 0.2006	2.0123 ± 0.3308	2.0130 ± 0.2673	2.0707 ± 0.2552	2.1669 ± 0.3414	2.3240 ± 0.2695	2.4704 ± 0.5061	2.6948## ± 0.3011

** Statistically significant difference ($p < 0.01$) using Tukey's Multiple Comparison test
Statistically significant difference ($p < 0.01$) using Kruskal-Wallis multiple comparison test

Results of the histopathological findings at the three-month sacrifice indicated a dose-dependent increase in hepatocytic megalocytosis in males, generally of a minimal or mild nature, with incidences of 1/20, 2/20 and 9/20* animals at 20, 2000 and 8000 ppm dose levels (*$p < 0.01$, Chi square analysis). In addition, 2/20 females of the high dose group also exhibited the same histopathological finding. All other lesions were not attributable to the test article administration. No histopathology was performed on the 100, 1000 or 4000 ppm dose groups. The observation of hepatocytic megalocytosis from 20 ppm (corresponding to 3.8 mg/kg bw/d) is considered in the evaluation of STOT RE. No clear increase in liver weight was associated to this limited finding at dose levels relevant for STOT RE (500 ppm, corresponding to 98 mg/kg bw/d, and lower).

In the long-term chronic toxicity part of the mouse study, no statistically significant effects on body weight or haematological or clinical chemistry parameters were observed in male or female mice at any time point. Histopathology indicated treatment related morphometric abnormality of hepatocytes (cytomegaly or megalocytosis) at all time intervals, predominantly in males. At the

last three test intervals, the incidences were however not statistically significantly different from controls.

At the six-month sacrifice, relative liver weight was only statistically significantly increased in females in the 2000 ppm group, but non-statistically significant increases in absolute and relative liver weights were observed from 500 ppm. In males, relative and absolute liver weights were slightly increased from 500 ppm, although not statistically significantly and without a clear dose-response relationship.

For this timepoint, histopathology was performed at 0, 20, 100, 500, 1000 and 2000 ppm. A dose-dependent increase in hepatocellular cytomegaly at the two highest doses was reported and described as a localized hepatocellular enlargement of nuclear and cytoplasmic components without indication of malignancy. Hepatocellular clear cell atypia was also observed in treated groups although without a dose-response relationship. Other non-neoplastic findings are considered to be incidental.

Table: *Incidences of relevant liver histopathological findings at final sacrifice in a mouse combined chronic toxicity and carcinogenicity study*

Exposure level (ppm)	0	20	100	500	1000	2000
Hepatocellular cytomegaly	0	0	0	0	2	5
Hepatocellular clear cell atypia (focus)	0	2	3	2	1	1

At the 12-month endpoint, no treatment-related findings were observed during necropsy. There were no statistically significant differences in organ weight. Histopathology showed a variety of findings considered unrelated to the test material. Three males in the 2000 ppm group and one male in the 500 ppm group exhibited a morphometric abnormality of hepatocytes.

At the 18-month endpoint, there were no statistically significant differences in organ weight. During necropsy, 6/10 high dose males (and 3/10 controls) had discoloration of the glandular stomach. Histopathology did not reveal treatment-related findings.

At final sacrifice, necropsy did not show treatment-related lesions. As seen at the 12-month and 18-month endpoints, evaluation of liver weights was confounded by the presence of liver masses. A statistically significant increase in relative liver weight without masses was however reported in 2000 ppm males, which was associated with an apparent dose related trend at lower doses.

A larger proportion of female mice given the high doses had persistent thymic glands than controls, with incidences of 2/20, 12/23 and 10/24 for the control, 1000 ppm and 2000 ppm groups, respectively. This finding was diagnosed as lymphoid hyperplasia and interpreted by the study pathologist as treatment-related, although thymic involution is a normal age-related finding commonly seen in older animals. Chronic renal disease with tubular and interstitial components was seen in all study groups, including controls.

Liver histopathological findings included increased incidences of hepatocellular necrosis, although without a clear dose-response relationship (9.0, 23.8, 13.0, 13.9, 20.8 and 8.3 % at 0, 20, 100, 500, 1000 and 2000 ppm, respectively). A dose-dependent increase in hepatocellular cytomegaly was observed in high dose males but the increase was not statistically significant according to Fischer's exact test ($p > 0.10$). The incidences of cytomegaly in male mice were 0, 0, 1/19, 1/23, 1/23 and 3/22 at 0, 20, 100, 500, 1000 and 2000 ppm, respectively. In addition, a single incidence of hepatocellular cytomegaly was reported in females of the 1000 ppm dose group.

Overall, findings reported from 100 ppm (corresponding to 15-18 mg/kg bw/d, purity 88.8%) and 20 ppm (equivalent to 3-4 mg/kg bw/d) at the final sacrifice could be considered relevant for the evaluation of STOT RE. This includes hepatocellular necrosis although this finding was not

statistically significant and without a dose-response relationship. In addition, cytomegaly was reported in males from 20 ppm.

A recent GLP OECD 408 TG subchronic oral toxicity was also performed in Wistar rats (Anon. 2001, 2839/2000). Clomazone (purity 94 %) was dissolved in acetone and administered via the diet to 10 animals/sex/dose at dose levels of 0, 600, 1200 or 4800 ppm for 90 days (equivalent to 0, 47.7-50.4, 92.0-103.8 or 377.4-409.0 mg/kg bw/d for males and females, respectively). In addition, control and high dose recovery groups were run concurrently for a further 28 day period. Histopathology was performed on control and high dose groups. Additional histopathological examination included, for the low and mid dose groups, kidneys and lungs from males, liver and lungs from females and gross lesions. Neurological examination conducted at the end of the treatment period included motor activity, grip strength and sensory reactivity to stimuli (visual, auditory and proprioceptive).

No animal died during the study period. Body weights and body weight gains were significantly decreased during the treatment period in males exposed to 4800 ppm. However, the males had a significantly higher net weight gain during the recovery period, allowing animals to regain body weights. Food consumption was also slightly reduced in both sexes of this dose group. No treatment-related clinical signs or ophthalmological effects were noted at any dose level.

Neurobehavioral toxicity of the compound was evaluated during this study. The hindlimb grip strength of males receiving the 600, 1200 and 4800 ppm dose levels was statistically significantly lower than in the control group. In contrast, the forelimb grip strength of females receiving the 1200 and 4800 ppm dose levels was increased in a dose-related manner. These effects were however not statistically significant in the 4800 ppm recovery group. The landing footsplay was statistically significantly higher and the grip strength (forelimb) was significantly lower in males in the 4800 ppm recovery group.

Table: *Forelimbs and hindlimbs grip strength and landing footsplay in a 90-day rat study (mean ± SD)*

Exposure level (ppm)		**0**	**600**	**1200**	**4800**	**0 RECOVERY**	**4800 RECOVERY**
Forelimb grip strength (g)	Males	977 ± 97.12	922 ± 124.01	956 ± 114.77	986 ± 102.74	999 ± 94.02	880** ± 111.29
	Females	654 ± 80.29	659 ± 147.55	734* ± 105.00	764**d ± 96.16	809 ± 124.33	741 ± 104.55
Hindlimb grip strength (g)	Males	695 ± 101.69	560** ± 122.87	586** ± 92.42	578** ± 81.65	606 ± 89.68	625 ± 129.01
	Females	504 ± 81.50	534 ± 91.40	461 ± 88.93	552 ± 77.64	512 ± 89.12	519 ± 85.08
Landing footsplay (cm)	Males	5.9 ± 0.54	6.4 ± 0.45	6.0 ± 0.67	5.5 ± 0.78	5.8 ± 0.52	6.4* ± 0.60
	Females	5.2 ± 0.43	5.2 ± 0.48	5.2 ± 0.54	5.1 ± 0.89	4.8 ± 0.44	4.8 ± 0.45

*p < 0.05; **p < 0.01 (pairwise comparison)
d: significant dose-response correlation with p < 0.05

No treatment-related changes in haematological parameters were seen at any dose level. Creatinine values were statistically significantly increased in males receiving the 1200 and 4800 ppm dose levels (respectively 42 ± 4.53 and 42 ± 5.71 vs 33 ± 3.25 µmol/L for the controls). However, the effect on creatinine was found to be reversible in males of the 4800 ppm recovery group. Other clinical chemistry values were increased in males of the high dose group, including total protein, total bilirubin, albumin, calcium, cholesterol and sodium. No clear effects on biochemical parameters were noted among the treated females with the exception of an increase in total protein and cholesterol levels in the 4800 ppm dose group.

Marked increases in absolute and relative liver weight were reported in males and females at the 4800 ppm dose level. In addition, relative liver weight was found to be statistically significantly increased in males of the 1200 ppm dose group and in females of the 600 and 1200 ppm dose groups. Nevertheless, these findings were observed to be reversible in the 4800 ppm recovery group and were therefore interpreted to be a physiological response to the exposure to clomazone.

Histopathology revealed statistically significantly increases in hyaline droplets of the tubular epithelium of the kidney were reported in 9/10 and 10/10 males of the 1200 and 4800 ppm dose groups vs 4/10 animals in the control. In the recovery group, 8/10 animals demonstrated the same effect at 4800 ppm (6/10 in the recovery control group). In addition, 4/10 males showed basophilic tubules in the kidney after either exposure to 1200 or 4800 ppm. Centrilobular hepatocellular hypertrophy was also observed in 9/10 females of the high dose group.

The dose level of 600 ppm, corresponding to 47.7 and 50.4 mg/kg bw/d for males and females, respectively, was considered to be the NOAEL.

A GLP OECD TG 452 dietary one-year chronic toxicity study was performed in the dog (Anon. 1984, 6124-101). Six beagle dogs/sex/dose were exposed to 0, 100, 500, 2500 or 7500 ppm clomazone (purity 88.8 %) diluted in corn oil. On day 8, the highest dose was reduced to 5000 ppm due to unpalatability, decreased defecation and mucous or bloody stools. After 90 days, 2 animals/sex/dose were sacrificed. Haematology, plasma clinical chemistry and urinalysis were performed at initiation, 1, 3 and 6 months and upon study termination.

In the high dose group, decreased body weight was reported in males and females during the two first weeks of the study. At the end of the study, the body weight of high dose group males remained lower than in the controls. Treatment-related elevations in serum cholesterol values were observed in males and females at 2500 and 5000 ppm. Increased absolute and relative live weights were seen in both sexes in the two high dose groups at interim and terminal sacrifices. No gross pathology or histopathological observations were found to be treatment related.

No oral neurotoxicity study with clomazone was available in the CLH report.

Conclusions

Lower food consumption associated with decreased body weights or body weight gains was consistently observed in several oral dietary subchronic and chronic toxicity studies in the rat, mouse and dog. This repeated observation raises questions about the palatability of the test-compound in all dietary studies. In particular, this argument is supported by the studies in dogs where the reported low palatability of the test-compound induced modifications of the testing strategy with a lowering of the highest dose. No gavage study was available in the CLH report for the assessment of clomazone toxicity after repeated exposure.

<u>Liver</u>

Liver was clearly the main target-organ after exposure to clomazone in all three tested species. The liver findings occurring in the exposure range relevant for STOT RE classification are presented below.

In a rat combined subchronic long term repeated toxicity and oncogenicity study (Anon. 1984a, 410-0816), megalocytosis was observed in two males and one female in association with increased liver weight after a 3 month-exposure to 138 mg/kg bw/d of clomazone. In addition, histopathology performed after 12 months of exposure showed increased incidences of vacuolative degeneration and necrosis of hepatocytes at 20-27 mg/kg bw/d and below. The biological relevance of these observations, being non-statistically significant and without a clear dose-response relationship, remains unclear. Finally, the findings reported at final sacrifice

included hepatocytomegaly in males and females. Slight, non-statistically significant increase in necrosis was also noted without a clear dose-response relationship in males.

In the corresponding combined repeated toxicity study in the mouse (Anon. 1984b, 410-0817), a single incidence of megalocytosis was also reported from 98 mg/kg bw/d after three months of exposure. Although not statistically significant, this effect is considered to be treatment-related due to an increased incidence at the highest dose and the similar finding in rats. No clear increase in liver weight was associated at 98 mg/kg bw/d or lower. At the 6-month sacrifice, hepatocellular clear cell atypia was observed at 15-18 mg/kg bw/d without a dose-response relationship or statistical significance. Nevertheless, related observations were absent in the control group. This effect therefore remains of unclear significance. At final sacrifice, cytomegaly was noted in male mice. The incidence was slight and non-statistically significant. However, no comparable finding was noted in the control group and cytomegaly increased in the high dose group. This effect is also in accordance with other morphological alterations reported in the same study. The increase in hepatocellular necrosis from 3-4 mg/kg bw/d was non-statistically significant and without a clear dose-response relationship.

In a more recent 90-day repeated toxicity study in Wistar rats (Anon. 2001, 2839/2000), the relative liver weight was increased at doses from 50.4 mg/kg bw/d in females. This finding was concluded to be reversible in the recovery group.

Finally, in a supportive mouse range-finding 28-day toxicity study, increased liver weight in females and hepatocellular hypertrophy were reported from 211.2-218.6 mg/kg bw/d (males and females). These effects were consistent with previous observations.

Overall, the increase in liver weight observed in rats and mice might be seen as an adaptive response to the exposure to clomazone. This effect was concluded to be reversible, based on the results of a rat 90-day study recovery group. In addition, no clear statistically significant effects on liver weight were observed after long term exposure in the rat or mouse, although marked increases were noted after subchronic exposure in the same studies.

In the meantime, liver effects were not restricted to an organ weight alteration and hypertrophy. Hepatocellular morphological changes (megalocytosis, cytomegaly) were mainly observed in mouse, without a related effect on liver weight. In rats, similar effects were noted after 3 months of exposure but not in long term endpoints. The hepatocellular modifications after long term exposure were limited to hepatocytomegaly. The slight incidence of megalocytosis and the absence of similar findings at final sacrifice in combination with the lack of clear hepatocellular necrosis lower the concern. Therefore, RAC is of the opinion that the effects seen in the liver in the rat and mouse are not sufficient to trigger a STOT RE classification

Kidney

In a 90-day rat study (Anon. 2001, 2839/2000), histopathology revealed statistically significantly increases in hyaline droplets of the tubular epithelium were reported in 9/10 and 10/10 males of the 1200 and 4800 ppm dose groups vs 4/10 animals in the control. In the recovery group, 8/10 animals demonstrated the same effect at 377.4 mg/kg bw/d (6/10 in the recovery control group). In addition, 4/10 males showed basophilic tubules in the kidney after exposure to 92 and 377.4 mg/kg bw/d. Creatinine values were statistically significantly increased in males receiving the 1200 and 4800 ppm dose levels (respectively 42 ± 4.53 and 42 ± 5.71 vs 33 ± 3.25 µmol/L for the controls), indicating an adverse effect on kidney function. The effect on creatinine was found to be reversible in males of the 377.4 mg/kg bw/d recovery group.

Chronic renal diseases were also reported in two combined subchronic-long term toxicity and carcinogenicity studies in mice and rats, most prominently in males. Nevertheless, the findings were observed in the control and test groups and did not reach statistical significance and are therefore not interpreted as treatment-related.

Overall, RAC is of the opinion that the effects on the kidney which were mainly observed in the male rats are not sufficient to trigger classification for STOT RE.

Neurological system

No neurotoxicity study was described in the CLH report. Clinical signs of lethargy, weakness, ptosis, staggering gait or ataxia were noted in dose-range finding studies in the rat and/or mouse. In a 90-day rat study (Anon. 2001, 2839/2000), neurobehavioral parameters were included in the protocol. Results showed decreased hindlimb grip strength in males receiving the 47.7, 92 and 377.4 mg/kg bw/d doses, without a clear dose-response relationship. In contrast, the forelimb grip strength of females receiving the 103.8 and 409 mg/kg bw/d dose levels was increased in a dose-related manner. No similar effect was observed in the recovery groups.

Grip strength parameters are known to represent an important variation. RAC notes the absence of similar findings in both sexes and the contradictory results between forelimb and hindlimb grip strength. The observed decrease in hindlimb grip strength in males might be related to a lower body weight. The results are seen as equivocal and no other study investigating neurobehavioral parameters is available to confirm the results. RAC therefore cannot conclude that the effects on grip strength are treatment-related. No classification for STOT RE (neurological system) is warranted.

Overall, RAC concludes that no classification for STOT RE is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro, clomazone was found to be negative in ten different OECD TG 471 bacterial reverse mutation assays, two mammalian gene mutation tests performed in CHO/HGPRT cells or mouse lymphoma cells (OECD TG 476 and 490, respectively), one OECD TG 473 mammalian chromosome aberration test and one OECD TG 482 unscheduled DNA synthesis assay.

Clomazone was also investigated for genotoxicity *in vivo* in a rat chromosome aberration test, a mouse OECD TG 474 erythrocyte micronucleus test and an OECD TG 486 unscheduled DNA synthesis test with rat liver cells. All study results were concluded to be negative.

No classification for germ cell mutagenicity was therefore proposed by the DS.

Comments received during public consultation

One MSCA commented on this endpoint without concluding on the classification. The comment mainly consisted on clarifications regarding the results of the *in vivo* micronucleus test. The MSCA also considered that further evidence should be considered in order to support exposure to bone marrow for this test.

One Company-Manufacturer supported the DS's conclusion on no classification for germ cell mutagenicity.

Assessment and comparison with the classification criteria

In vitro studies

Ten bacterial reverse mutation tests following the OECD TG 471 are available in the CLH report. Seven GLP-compliant bacterial reverse mutation studies followed the OECD TG 471 without (major) deviations and are considered acceptable (Wagner, 2014; Taylor, 2008; Lauenstein, 2013; Flügge, 2009; Flügge, 2013; Indrani, 2000 and Schreib, 2013). The dose selections ranged from 1.6 to 5000 µg/plate and all study results indicated that clomazone did not induce point mutations in *S. typhimurium* (strains TA98, TA100, TA102, TA1535, TA1537) or *E. coli* (strains WP2 uvrA – pkM 101) with or without metabolic activation.

In contrast, three Ames tests (Haworth, 1980; Haworth, 1982 and Haworth, 1984) are considered only supportive due to the incorporation of only one plate instead of two independent trials. For all three studies, clomazone failed to induce point mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 with or without metabolic activation.

In mammalian cells, the mutagenic potential of clomazone was investigated in two different studies. A GLP-compliant OECD TG 476 HGPRT locus mutation assay using CHO-K1-BH4 cells was performed at doses of 200, 300, 500 or 600 µg/mL with and without metabolic activation (Thilagar, 1984). With the exception of an increase in the number of mutants at the highest dose without previous metabolic activation, there was no indication of a positive response in any of the cultures exposed to clomazone. Without the observation of a positive dose-response relationship, clomazone is considered non-mutagenic in the cultured mammalian cells under the conditions of the study.

A recent GLP-compliant OECD TG 490 gene mutation study using lymphoma L5178Y cells was further submitted (Gilby, 2016). Increases in mean mutant frequency that were associated with a statistically significant linear trend ($p < 0.001$) were observed after 3-hour exposure to 25 to 400 µg/mL without metabolic activation and after 24-hour exposure to 2.5 to 250 µg/mL clomazone without S9. An increase in the mean mutant frequencies was also reported after 3-hour exposure to 25 to 300 µg/mL clomazone with metabolic activation. An additional test with 3-hour exposure to 25 to 500 µg/mL clomazone was negative. Finally, 24-hour treatment in the absence of S9 (concentrations 50 to 250 µg/mL) also demonstrated statistically significant increases in mean mutant frequencies at the highest dose. The relative total growth decreased in a dose-dependent way in the three assays. Overall, in the different assays, the increases in mutant frequencies above the concurrent background did not exceed the global evaluation factor. According to OECD TG 490, the results are therefore concluded to be negative.

The clastogenic potential of clomazone (96.6 % purity) was evaluated in a GLP-compliant OECD TG 473 mammalian chromosome aberration test using human peripheral lymphocytes (Flügge, 2009). Based on the findings in a preliminary cytotoxicity assay, cells were exposed to clomazone at doses ranging from 39.06 to 625 µg/mL for 4-hours with and without metabolic activation and for 24-hours without S9. Slight increases in chromosome aberrations, including or not chromatid gaps, were observed at the dose of 625 µg/mL for all culture conditions (6.0 to 7.7 % including gaps and 3.0 to 3.8 % without gaps). Nevertheless, marked cytotoxicity was observed at the dose of 625 µg/mL and only 55 cells were numerically analysed instead of 200. This dose level should therefore not be considered relevant for assessment. No statistically significant increases in chromosome aberrations were observed at doses up to and including 312.5 µg/mL with or without metabolic activation.

Finally, a GLP OECD TG 482 unscheduled DNA synthesis assay performed in is available in the CLH report (Thilagar, 1983). Cultures of primary rat hepatocytes were exposed to 0.001, 0.005, 0.010, 0.050 or 0.10 µL/mL clomazone. None of the test concentrations resulted in a significant

increase in mean net nuclear counts. However, no independent repeat assay was performed to confirm the results. This study is therefore considered as supportive.

In vivo studies

Three *in vivo* studies evaluating the genotoxic potential of clomazone in somatic cells were made available in the CLH report.

A mammalian chromosome aberration test was performed on bone marrow cells of Sprague-Dawley rats (Anon. 1982, T1839.102). Five males/group were orally exposed via gavage once daily to 200, 667 or 2000 mg/kg bw/d clomazone (purity 88.8 %) in corn oil for five consecutive days. One rat in the high dose group died after the second administration. Two to four hours after the last treatment, animals were injected with colchicine. Femur bone marrow cells were sampled approximately two to four hours later. The positive control group received a single i.p. injection of triethylenemelamine one-day prior to sacrifice.

No increase in chromosomal aberration after clomazone exposure was reported under the conditions of the study. Nevertheless, there were several deviations from the OECD TG 475 guideline in this report, potentially impacting on the reliability of the negative results. The study was terminated shortly after colchicine treatment (colchicine treatment 2-4 hours after last treatment instead of 12-18 hours required), possibly impairing the action of the metaphase-arresting agent. Moreover, based on the absence of effect on mitotic index, it cannot be confirmed that bone marrow was adequately reached by the test compound. In addition, only 50 metaphase cells were evaluated per animal instead of 100. Finally, the exclusive use of males did not take into consideration the increased sensitivity of females observed in acute toxicity studies and no HCD were provided. Hence, RAC is of the opinion that the results of this study are not of sufficient reliability for the assessment of clomazone.

Clastogenicity was also investigated in a GLP-compliant OECD TG 474 micronucleus test performed on bone marrow cells of NMRI mice (Anon. 2009a, 23881). A single dose of 125, 250 or 500 mg clomazone/kg bw (purity 96.6 %) was orally given to 5 mice/sex/group. All animals were sampled 24 hours after administration. After 48 hours, a second sampling was prepared in addition for the negative control and high dose level group.

Clinical signs appeared from 250 mg/kg bw and included reduced motility, ataxia and dyspnoea. These observations increased in severity in a dose-dependent manner and tremors were also reported at the highest dose. No significant decrease in the PCE/NCE ratio was found at any dose level or sampling time, indicating an absence of target cell toxicity. No statistically significant increases in micronucleated PCE frequency were reported at any time point up to 500 mg/kg bw clomazone.

Regarding the demonstration of bone marrow exposure, there was no indication of bone marrow specific exposure in the ADME data for the oral route, although clomazone was measured in the blood. In addition, based on available data, there was no available reporting of measurement of clomazone in the plasma or blood during the micronucleus study. RAC is of the opinion that although the clinical symptoms indicated systemic toxicity, there is no evidence of clinical signs that are specific to bone marrow exposure. RAC also considers that measuring the compound in blood in an ADME study does not ensure with confidence that clomazone reached the bone marrow in the micronucleus study. RAC therefore concludes that without further information, it remains unclear whether the bone marrow was adequately exposed to the test compound or not.

Finally, a GLP-compliant OECD TG 486 unscheduled DNA synthesis test was described in the CLH report (Anon. 2009b, 23987). Clomazone (purity 96.6 %) was administered via oral gavage to 6 male CD/Crl: CD (SD) rats/group as a single dose of 500 and 1000 mg/kg bw. Liver cells were further sampled at two different time points (2 and 16 hours after exposure). Three animals per group were analysed and for each animal, 50 cells/slide were scored in two different slides. The

resulting net nuclear grain (NNG) values were lower than minus 0.08 and no more than 0.67 % of cells found in repair showed a NNG ≥ 5. The NNG values obtained for clomazone were within/below the historical control values. It was therefore concluded that clomazone did not induce unscheduled DNA synthesis in rats under the conditions of the study.

No *in vivo* study in germ cells was available in the CLH report.

Conclusion

There was no indication of genotoxicity or mutagenicity induced by clomazone in any of the available *in vitro* and *in vivo* somatic mutagenicity and genotoxicity studies. Therefore, since the CLP criteria were not met RAC agrees with the DS's proposal for **no classification of clomazone regarding germ cell mutagenicity is warranted.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two OECD TG 453 oral 2-year combined chronic toxicity and carcinogenicity studies were performed in Sprague-Dawley SD rats and CD-1 mice (Anon. 1984a, 410-0816 and Anon. 1984b, 410-0817). The liver was identified as the target organ for the two species with observations of increased liver weight and hepatocytomegaly. There was no indication of neoplastic changes in either species.

The DS did not propose a classification for carcinogenicity.

Comments received during public consultation

One MSCA supported the DS proposal not to classify clomazone for carcinogenicity. In their comment, the MSCA requested more details on the effects seen in the thymus of female mice. In response to the MSCA's request, the DS indicated that *"18 percent of the female mice in the 1000 ppm group and 13 percent in the 2000 ppm group had lymphoid hyperplasia in thymus compared with 4 percent in the control group. There were no other histopathology findings of the thymus different from the control group."*

One Company-Manufacturer also supported the conclusions of the DS on carcinogenicity.

Assessment and comparison with the classification criteria

In a 2-year chronic toxicity and carcinogenicity feeding study, clomazone (purity 88.8 %) was administered to approximately 120 Sprague Dawley CD rats/sex/concentration (Anon. 1984a, 410-0816). Concentrations were 0, 20, 100, 500, 1000 and 2000 ppm corresponding, respectively, to 0, 0.8-11, 4-5, 20-27, 41-55 and 83-110 mg/kg bw/d for males and females.

There was no treatment related effect on mortality or body weight at the end of the study. Decreased body weight and food consumption were noted from the first week until week 15 at 8000 ppm in males and at 4000 ppm and 8000 ppm in females. In addition, the body weight of females was also statistically significantly decreased on week 15 in the 1000 and 2000 ppm group levels. The liver was identified as the target-organ, with increased liver weight, hepatocytomegaly and megalocytosis, as discussed in the STOT RE part of this report. At final sacrifice, a slight increase in hepatocellular adenomas was noted in male rats of the 20 ppm and

500 ppm dose groups. In addition, one tumour in the 100 ppm dose-group was reported as a hepatocellular carcinoma. In females, a single incidence of hepatic adenoma was observed in the 100, 500 and 1000 ppm dose groups. Nevertheless, the incidences of hepatocellular adenomas and carcinomas were non-statistically significant, without dose-response relationship and without clear progression to malignancy. This finding is therefore considered non relevant for carcinogenicity classification.

Table: *incidences of hepatocellular adenomas in males and incidences of hepatocytomegaly in males and females (24 month)*

Dose group (ppm)	0	20	100	500	1000	2000
Hepatocellular adenomas in males						
No. of animals / No. of tumours	37 1	45 4	44 2[A]	43 6	42 0	40 2
Hepatocytomegaly						
No. of males / Hepatocytomegaly	37 9	45 23*	44 25*	43 20*	42 18	40 16
No. of females / Hepatocytomegaly	36 15	43 26	42 28*	43 24	42 27*	42 22

A One tumour was a carcinoma
* statistically significant increase when compared to the control (Fischer Exact test, $p < 0.05$)

In the mouse 2-year chronic toxicity and carcinogenicity feeding study, technical clomazone (purity 88.8 %) was administered at doses of 0, 20, 100, 500, 1000 and 2000 ppm (Anon. 1984b, 410-0817). The mean daily intakes for male and female mice were, respectively, 0, 3, 15, 73, 142, and 290 mg/kg bw/d and 0, 4, 18, 89, 182 and 359 mg/kg bw/d.

At final sacrifice, no test-related neoplastic findings were reported. Liver changes were also observed, including increased weight and hepatocytomegaly without associated increase in liver neoplastic findings.

Treatment-related persistent thymic glands were noted only in females of the two high dose groups, with incidences of 2/20, 12/23 and 10/24 for the control, 1000 ppm and 2000 ppm, respectively. This finding was diagnosed as lymphoid hyperplasia by the study pathologist. However, it was further interpreted as delayed thymic involution, a normal age-related finding commonly seen in older animals. Considering the absence of malignant tumours in the thymus, this preneoplastic observation is not seen as relevant in the carcinogenicity classification.

According to the OECD guideline on carcinogenicity study, the highest dose level should be chosen to identify the principal target organs and toxic effects while avoiding suffering, severe toxicity, morbidity, or death. The highest dose level should be normally chosen to elicit evidence of toxicity, as evidenced by, for example, depression of body weight gain (approximately 10 %). However, a top dose lower than the dose providing evidence of toxicity may be chosen, e.g., if a dose elicits an adverse effect of concern, which nonetheless has little impact on lifespan or body weight.

Overall, no effect on mortality and body weight were noted in the rat and the mouse carcinogenicity studies to the highest dose level of 2000 ppm (corresponding to 83-110 mg/kg bw/d in rats and 290-359 mg/kg bw/d in mice). However, clear dose-related effects on the liver were noted until the end of the study, indicating that the selected dose level induced treatment-related effects in mice and rats. These effects were mainly limited to increased liver weight, hepatocellular hypertrophy and related morphological alterations (megalocytosis, cytomegaly),

which are not considered to be adverse effects of concern. **RAC considers that the studies in the rat and mouse may not fully inform on the carcinogenic potential of clomazone.**

In conclusion, no significant treatment-related neoplastic effects were observed in studies conducted with clomazone in the mouse. Slight increases in hepatocellular adenomas were observed in rats, although these were not statistically significant, were without a dose-response relationship and without clear progression to malignancy. Therefore, RAC agrees with the DS that **classification for carcinogenicity is not warranted.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

One dietary OECD TG 416 two-generation study in rats at doses of 0, 100, 1000, 2000 and 4000 ppm was described in the CLH report. Decreased body weight, body weight gain and food consumption were noted in adult rats. At the highest dose, increased relative liver weight was reported in both sexes. Body weights of F1 and F2 generations were affected inconsistently and were therefore interpreted as non-treatment related. The marginally reduced fertility index was limited to an F1 litter in the high dose group without impact on the population data. No other effect on fertility parameters were reported. Therefore, the DS did not propose classification for fertility.

Regarding adverse effects on development, four OECD TG 414 teratogenicity studies were summarised in the CLH report. Two were performed in rats and the other studies used rabbits.

The first developmental toxicity study (Anon. 1984, A83-1143) exposed rats to clomazone on gestation day (GD) 6 to 15. Decreased food consumption and clinical signs were observed in dams at 300 and 600 mg/kg bw/d. Foetuses exhibited a lower body weight (in females of the highest dose), an increased incidence of skeletal anomalies (at the 2 highest doses), including absent metatarsal/metacarpal and partially ossified/absent sternebrae, and an increased incidence of hydroureter (at the highest dose).

In a second developmental toxicity study (Anon. 2002, 2840/2000), rats were exposed to clomazone on GD 6 to 19. Dams exhibited a lower body weight gain (at 500 and 750 mg/kg bw/d), a lower food consumption (in all tested groups) and clinical signs. Furthermore, 4 dams exhibited total resorptions at 750 mg/kg bw/d. In addition, a statistically significant decrease in foetal body weight, a lower litter size and a decrease total number of foetuses were observed. Moreover, external and skeletal examinations revealed forelimbs flexed at wrist, malformed forelimbs and arthrogryposis.

Developmental toxicity studies performed in rabbits were also summarised in the CLH report. In the first study (Anon. 1982, WIL-81157 A81-655), rabbits were exposed to clomazone on GD 6 to 18. Decreased body weight, mortality, abortion, red vaginal discharge and decreased or no defecation were noted at 1000 mg/kg bw/d. The number of foetuses and the incidence of malformations were unaffected by the treatment.

A second rabbit study (Anon. 2002, 2841/2000) exposed animals to clomazone on GD 6 to 28. In this study, dams exhibited abortion, clinical signs, decreased body weight and decreased food consumption at 700 mg/kg bw/d. A slight increase in incidence of malformations was observed at the highest dose, such as forelimbs flexed at wrist and multiple malformations associated with arthrogryposis.

Therefore, the DS proposed to classify clomazone as Repr. 1B, H360D.

Comments received during public consultation

Two MSCAs supported classification of clomazone for Repr. 1B, H360D as proposed by the DS. One MSCA requested more information on arthrogryposis and did not take any position on the classification for developmental toxicity of clomazone.

Four company-manufacturers, one industry/trade association and two individuals disagreed with the proposed classification for Repr. 1B, H360D. The main arguments against classification are summarised below:

- In a developmental toxicity study in rat, the reporting of arthrogryposis as a skeletal malformation did not correlate with observations in the foetal necropsy (Anon. 2002, 2840/2000). This finding is therefore considered to be a misdiagnosis caused by artefactual changes during foetal processing;
- The single incidences of flexed limbs at necropsy in rats in the same study were within the historical control data (HCD) range and should therefore be concluded to be non-treatment related;
- From the same laboratory, two incidences of flexed limbs were noted at the highest dose in one developmental toxicity study in rabbits (Anon. 2002, 2841/2000). However, it was argued that one of the two affected foetuses also presented other gross abnormalities which can be interpreted as the cause of the flexed limbs. The second incidence of flexed limb was within the HCD and it should therefore be concluded that this was non-treatment related;
- No arthrogryposis was reported in the other prenatal developmental toxicity studies in rats or rabbits nor in a two-generation study;
- One new study using similar exposure parameters and rat strain to the 2840/2000 study (Anon. 2002) was performed in 2018 and was found to be negative. The study report was attached to the public comment and was taken into consideration in the RAC assessment of developmental toxicity of clomazone.
- A recent toxicokinetic study in female rats (in 2018) indicated that internal systemic exposure to clomazone reached a plateau at dose levels of 500 mg/kg bw. Any "dose-response" relationship for findings (e.g., "arthrogryposis") at 500 and 750 mg/kg bw is not supported by the toxicokinetic data.

Based on these arguments the DS responded that a Repr. 1B, H360D classification was no longer clear and proposed that Repr. 2, H361d be considered based on the arthrogryposis effects, resorptions, implantation-loss, abortions and malformations/variations.

An *ad hoc* targeted consultation was launched for the study reports from a recently completed developmental toxicity study conducted using Wistar Han rats as well as the relevant dose-range finding study and toxicokinetic study.

One Individual commented that the old developmental toxicity studies were already evaluated and found to provide robust scientific evidence of reproductive toxicity despite the new studies.

One Company-Manufacturer submitted a comment disagreeing with the classification proposal, consistent with that provided during the public consultation of the CLH report.

Assessment and comparison with the classification criteria

The following Table summarises the available two-generation and developmental toxicity studies with animals.

Table: *Summary table for reproductive toxicity studies in animals with clomazone.*

Method	Exposure	NOAELs/LOAELs	Reference
Developmental toxicity study OECD TG 414 GLP Oral, gavage SD rats 25 females/dose	0, 100, 300 and 600 mg/kg bw/d Purity 88.8% GD 6-15	*Maternal*: The NOAEL for maternal toxicity was 100 mg/kg bw/d LOAEL = 300 mg/kg bw/d based on the maternal clinical signs (abdominal staining and reduced locomotion at the highest dose) *Embryotoxicity/teratogenicity*: The NOAEL was 100 mg/kg bw/d LOAEL = 300 mg/kg bw/d based on skeletal malformations and visceral findings.	Anon. 1984 (A83-1142)
Developmental toxicity study OECD TG 414 GLP Oral, gavage NZW rabbits 18 females/dose	0, 30, 240 and 1000 (700) mg/kg bw/d 88.8% GD 6-18	*Maternal*: The NOAEL for maternal toxicity was 240 mg/kg bw/d LOAEL = 700 mg/kg bw/d based on the severely decrease body weight *Embryotoxicity/teratogenicity*: The NOAEL for developmental toxicity was 700 mg/kg bw/d (No LOAEL)	Anon. 1982 (WIL-81157)
Developmental toxicity study OECD TG 414 GLP Oral, gavage Wistar rats 28 females/dose	0, 250, 500 and 750 mg/kg bw/d 92.9% GD 6-19	*Maternal*: The NOAEL for maternal toxicity was 250 mg/kg bw/d LOAEL = 500 mg/kg bw/d based on the lower body weight and food intake *Embryotoxicity/teratogenicity*: The NOAEL for developmental toxicity was 250 mg/kg bw/d LOAEL = 500 mg/kg bw/d based on the increased incidence of arthrogryposis	Anon. 2002 (2840/2000)
Developmental toxicity study OECD TG 414 GLP Oral, gavage NZW rabbits 25 females/dose	0, 150, 350 and 700 mg/kg bw/d 95.0% GD 6-28	*Maternal*: The NOAEL for maternal toxicity was 350 mg/kg bw/d LOAEL = 700 mg/kg bw/d based on the lower body weight *Embryotoxicity/teratogenicity*: The NOAEL for developmental toxicity was 350 mg/kg bw/d LOAEL = 700 mg/kg bw/d based on limb malformations	Anon. 2002 (2841/2000)

Developmental toxicity study OECD TG 414 GLP Oral, gavage Wistar rats 21 females/dose	0, 250, 500 and 750 mg/kg bw/d 96.3% GD 6-20	*Maternal*: The NOAEL for maternal toxicity was 250 mg/kg bw/d LOAEL = 500 mg/kg bw/d based on the lower body weight gain and food intake *Embryotoxicity/teratogenicity*: The NOAEL for developmental toxicity was 750 mg/kg bw/d based on the lack of adverse findings	Anon. 2019 (CLZ4337)
Dose-range finding study GLP Oral, gavage Wistar rats 8 females/dose	0, 250, 500 and 750 mg/kg bw/d 96.3% GD 6-20	*Maternal:* Higher liver weights from 250 mg/kg bw/d. No effect on maternal bw *Embryotoxicity/teratogenicity:* No effect on foetal morphology	Anon. 2018 (CLZ4336) *Supportive*
Two-generation toxicity study OECD TG 416 GLP Oral, diet CD rats 25 sex/dose	0, 100, 1000, 2000 and 4000 ppm (corresponding to 0, 8.8, 84, 158 and 354 mg/kg bw/d) Purity 88.8% 8w of premating period (11w for F1), through mating, gestation and until lactation period for F0 and F1	*Maternal:* The NOAEL for maternal toxicity was 1000 ppm LOAEL = 2000 ppm based on decreased final body weight and food consumption *Offspring*: The NOAEL for offspring was 1000 ppm LOAEL = 2000 ppm based on decreased body weight in F2 progeny, dilated kidneys and increased relative liver weight *Reproduction:* The NOAEL for reproduction was 4000 ppm (No LOAEL)	Anon. 1984 (450-1095)

Fertility

In a two-generation reproduction study (Anon. 1984, 450-1095), conducted according to OECD TG 416 and GLP, groups of 25 male and 25 female Charles-River rats were orally exposed to clomazone. The dietary concentration were 0, 100, 1000, 2000 or 4000 ppm (corresponding to 0, 8.8, 84, 158 and 354 mg/kg bw/d) for the F0 and the F1-generation. Adverse maternal effects were observed at the 2 highest doses, such as decreased body weights, body weight gain and food consumption.

No significant difference in the number of pups, the viability index or the number of stillborn and cannibalised pups were noted between treatment groups and the controls. Fertility index was decreased at 4000 ppm for the F0 generation – F1b litter (65.6 % vs 95.5 % in controls, $p < 0.05$). No similar finding was noted in other generations. Furthermore, pup body weights

inconsistently exhibited statistically significant changes. These findings are considered not to be treatment-related.

Table: *Pup body weight (in g) in a rat two-generation study.*

Dose (ppm)	0	100	1000	2000	4000
F1A litter					
LD0	6.5	6.6	6.4	6.1**	6.2*
LD4	9.	9.9	9.1	8.7**	94
LD7	14.0	15.9**	14.7	13.8	14.9
LD14	25.1	27.7**	26.1	25.7	25.6
LD21 (Males)	41.5	45.7**	42.8	42.0	40.0
LD21 (Females)	39.1	43.0**	39.8	39.3	38.0
F1B litter					
LD0	6.4	6.2	6.3	6.1*	6.4
LD4	9.5	9.9	9.6	9.4	9.9
LD7	15.2	16.1	15.9	15.2	16.4**
LD14	28.4	30.3**	30.4**	29.1	30.8**
LD21 (Males)	47.0	50.0*	51.5**	47.9	48.8
LD21 (Females)	43.9	47.3*	48.2**	45.0	45.7

* Significantly different from control, at 0.05 %
** Significantly different from control, at 0.01 %

In a developmental toxicity study, Wistar rats were exposed to clomazone at doses of 0, 250, 500 or 750 mg/kg bw/d (Anon. 2002, 2840/2000). The highest dose was associated with non-statistically significant increases in mean early resorptions (2.2 vs 0.7 for control) and mean post-implantation loss (2.4 vs 0.7 for control). Four, out of 24 dams, exhibited complete resorptions in the high dose group. The summary of maternal data are described in the table below. HCD were provided for studies performed in Wistar rats between 1998 and 2005 (14 studies, 449 animals). HCD incidences of 0.2-0.8 for mean early resorption and of 0.3-0.9 for mean post-implantation loss were reported for maternal data.

Table: *Summary of maternal data in a Wistar rat developmental toxicity study (Anon. 2002, 2840/2000).*

Dose (mg/kg)	0	250	500	750
Number of corpora lutea (mean ± SD)	13 ± 2.0	13 ± 1.6	12 ± 1.6	13 ± 2.6
Number of implantations (mean ± SD)	11 ± 2.2	11 ± 2.1	11 ± 1.7	11 ± 2.9
Mean early resorptions ± SD (%)	0.2 ± 0.4 (2.1%)	0.3 ± 0.6 (2.7%)	0.6 ± 0.9 (5.1%)	2.2 ± 4.1 (20.3%)
Mean late resorptions ± SD (%)	0.5 ± 0.9 (4.6%)	0.1 ± 0.3 (0.8%)	0.1 ± 0.3 (1.1 %)	0.2 ± 0.4 (1.5%)
Mean pre-implantation loss ± SD (%)	1.4 ± 2.2 (11.3%)	1.3 ± 1.3 (10.5%)	1.3 ± 1.7 (10.4%)	1.7 ± 1.9 (13.6%)
Mean post-implantation loss ± SD (%)	0.7 ± 0.9 (6.0%)	0.4 ± 0.7 (3.4%)	0.7 ± 1.0 (6.1%)	2.4 ± 4.1 (21.8%)
Dams with any resorption (%)	11 (44%)	7 (30%)	11 (44%)	10 (42%)
Dams with all resorptions (%)	0	0	0	4* (17%)
** Significantly higher than control group*				

Individual data showed that most of the dams affected by early resorption only had clinical signs of slight salivation. In addition, very transient lethargy was reported in four of the dams between study days 7 and 11 (a single observation for each animal). Necropsy of the dams did not reveal treatment-related gross pathological findings. However, dams of the high dose group had

statistically significant lower absolute body weight (-15.4 % of controls; uterine weight deducted from day 20 body weight) and food intake (-18.8 % of controls). Body weight gain throughout gestation increased compared to the controls. Decreased maternal body weights suggested that the observed increases in early resorption and post-implantation loss might be related to maternal toxicity. Nevertheless, RAC notes the absence of severe clinical signs and pathological findings in the dams affected by early resorption.

In another OECD TG 414 developmental toxicity study, Sprague-Dawley rats were exposed to 0, 100, 300 or 600 mg/kg bw/d clomazone (Anon. 1984, A83-1142). Dams showed a slight increase in early resorptions as a percentage of implantations at the two highest doses of 300 and 600 mg/kg bw/d (respectively 2.4 and 3.4 vs 1.3 % of implantations in the control group), as presented in the table below. Although lower mean food consumption was noted at these dose levels, the mean maternal body weight and the necropsy remained unaffected. Individual data showed an absence of abdominal staining in the dams affected by foetal resorption at 300 mg/kg bw/d.

Table: *Summary of maternal data in a Sprague-Dawley rat developmental toxicity study.*

Dose (mg/kg bw/d)	0	100	300	600
Implantations (mean ± SD)	11.9 ± 2.50	12.1 ± 1.81	12.7 ± 1.80	11.7 ± 1.61
Corpora lutea-Implantations (mean ± SD)	2.4 ± 2.16	3.2 ± 2.83	2.8 ± 3.06	2.3 ± 2.14
Mean resorptions ± SD	0.2 ± 0.37	0.1 ± 0.33	0.3 ± 0.71	0.4 ± 1.47
Early resorptions N (% implant.)	4 (1.3 %)	3 (1.0 %)	7 (2.4 %)	9 (3.4 %)
Late resorptions N (% implant.)	0	0	1 (0.3 %)	0
Total resorptions N (% implant.)	4 (1.3 %)	3 (1.0 %)	8 (2.7 %)	9 (3.4 %)
Dams with any resorption N (%)	4 (16.0 %)	3 (12.0 %)	5 (21.7 %)	3 (13.0 %)
Dams with all resorptions (%)	0	0	0	1 (4.3 %)

Finally, in a supportive dose-range finding study in Wistar Han rats submitted during the public consultation, the total of mean pre-implantation loss increased non-significantly at the highest dose of 750 mg/kg bw/d (3.0 vs 1.3 in controls). The mean percentage of pre-implantation loss per litter also increased in the same group. No clear maternal toxicity was associated with this limited finding. Dam body weight, body weight gain and food consumption remained similar to the control group. Dilated pupils and increased liver weight were observed at 250 mg/kg bw/d and higher. The subsequent rat developmental toxicity did not produce similar effects.

Table: *Summary of maternal data in a Wistar Han rat dose-range finding study (Anon. 2018).*

Dose (mg/kg bw/d)	0	250	500	750
Number of dams	7	7	8	8
Implantation sites (mean ± SD)	10.4 ± 1.72	9.6 ± 2.82	10.3 ± 0.89	8.4 ± 1.85
Corpora lutea (mean ± SD)	11.7 ± 0.95	11.4 ± 1.81	11.1 ± 0.64	11.4 ± 1.41
Mean early resorptions ± SD (% per litter ± SD)	0.4 ± 0.53 (4.7 ± 5.91)	0.4 ± 0.53 (4.3 ± 5.41)	0.5 ± 0.53 (4.9 ± 5.29)	0
Mean late resorptions ± SD	0	0	0	0
Mean pre implantation loss ± SD (% per litter ± SD)	1.3 ± 1.11 (11.3 ± 9.88)	1.9 ± 2.79 (15.4 ± 23.29)	0.9 ± 0.83 (7.8 ± 7.34)	3.0 ± 1.85 (26.1 ± 15.59)
Mean post implantation loss ± SD (% per litter ± SD)	0.4 ± 0.53 (4.7 ± 5.91)	0.4 ± 0.53 (4.3 ± 5.41)	0.4 ± 0.53 (4.9 ± 5.29)	0

In the rabbit, (Anon. 2002, 2841/2000), a statistically significantly higher number of pre-implantation loss (*p=…) was noted at 150 and 350 mg/kg bw/d but not in the high dose group. The incidences of pre-implantation loss were 0.8 (9.33 %), 1.6* (16.50 %), 2.0* (22.96 %) and 1.5 (17.78 %) for the control, 150, 350 and 700 mg/kg bw/d dose groups, respectively. Two dams aborted in the high dose group (on day 24 and 25 of gestation). A slight reduction in body weight gain was reported at 700 mg/kg bw/d. The corrected body weight (without gravid uterine weight) was significantly lower on day 29 than at the beginning of the study in all test groups. This was associated with a significant reduction in food intake in the high dose group. Marginal findings included diarrhoea (1 in low dose, 2 in each mid and high dose groups), weakness (two in high dose group), dullness (two in high dose group) or wet perineum (one in the high dose group).

Similar effects were not observed in the other rabbit developmental toxicity study (Anon. 1982, WIL-81157). However, three dams in the highest dose group aborted. The severe maternal toxicity led to an adaptation of the dose regimen during the conduction of the study, resulting in decreasing the highest dose from 1000 to 700 mg/kg bw/d. This effect is therefore not considered relevant for a reproductive toxicity classification of clomazone.

Overall, increased incidences on early resorption and/or implantation loss were observed in rats and rabbits. The effects were of limited magnitude and no clear pattern on resorption/implantation loss was identified between the studies. Although no causality seems to be clearly established for the various findings, RAC cannot exclude that the more pronounced effects on early resorption and implantation loss observed in the rat and rabbit studies were related to maternal toxicity. No effect on fertility parameters were noted in a two-generation study in rat. **Therefore, RAC is of the opinion that no classification is warranted for fertility.**

Developmental toxicity

In a prenatal developmental toxicity study, conducted according to OECD TG 414, time-mated SD female rats (25 animals/dose) were orally exposed to clomazone on GD 6–15 (Anon. 1984, A83-1142). Gavage doses were prepared in corn oil to achieve 0, 100, 300, and 600 mg/kg bw/d.

During the dosing period, two females of the highest dose died due to an oesophageal perforation. Pregnant females exhibited a lower mean food consumption from day 13-20 and from day 6-13, respectively, at 300 and 600 mg/kg bw/d. Furthermore, at 300 mg/kg bw/d, 4 pregnant females exhibited abdominal staining and among these animals, 3 showed chromorhinorrhea, whereas at 600 mg/kg bw/d, 24 pregnant females exhibited abdominal staining and decreased locomotion. However, the mean maternal body weight and the necropsy findings remained unaffected.

The total number of resorptions was slightly increased at 300 and 600 mg/kg bw/d (4, 3, 8 and 9 at 0, 100, 300 and 600 mg/kg bw/d, respectively). At the highest dose, foetal body weight was statistically significantly lower in females (-7 % compared to control).

Visceral observations included increased incidences of hydroureter from 100 mg/kg bw/d, only reaching statistical significance at the high dose for the foetal incidence. The foetal incidences (litter incidences) were 0 % (0 %), 1.3 % (of 8.0 %), 2.9 % (17.4 %) and 3.8 %* (22.7 %) for the control, 100, 300 and 600 mg/kg bw/d dose groups.

No external malformations were observed. Nevertheless, the skeletal examination revealed a significantly higher incidence of malformations and/or variations. Increases in foetal and litter incidences of absent or partially ossified sternebrae 3-4 were reported at 300 and 600 mg/kg bw/d exposure dose groups. In addition, foetal incidence of absent or partially ossified sternebrae

2 or 5 were also noted at the highest dose. Skeletal anomalies of the limbs included increased foetus and litter incidences of absent metatarsal at 300 and 600 mg/kg bw/d. A statistically significant increase in the foetal incidence of absent metacarpal was also reported at the high dose. The summary incidences of skeletal anomalies from the study report are presented in the Table below:

Table: *Incidences of skeletal anomalies in a prenatal developmental toxicity study in SD rats (Anon. 1984, A83-1142)*

Doses (mg/kg bw/d)		0	100	300	600
Manubrium partially ossified	Litter incidence (%)	0	0	5/23 (21.7)[A]	4/22 (18.2)[A]
	Foetal incidence (%)	0	0	6/144 (4.2)	7/128 (5.5)[B]
Sternebrae 3-4 absent or partially ossified	Litter incidence (%)	0	0	4/23 (17.4)[A]	5/22 (22.7)[A]
	Foetal incidence (%)	0	0	4/144 (2.8)	6/128 (4.7)[B]
Xiphoid absent or partially ossified	Litter incidence (%)	18/25 (72.0)	21/25 (84.0)	20/23 (87.0)	20/22 (90.9)
	Foetal incidence (%)	53/147 (36.1)	58/149 (38.9)	70/144 (48.6)[C]	82/128 (64.1)[D]
Caudal vertebrae absent	Litter incidence (%)	8/25 (32.0)	8/25 (32.0)	14/23 (60.9)[A]	12/22 (54.5)
	Foetal incidence (%)	14/147 (9.5)	14/149 (9.4)	19/144 (13.2)	35/128 (27.3)[B]
Pubis partially ossified or absent	Litter incidence (%)	0	1/25 (4.0)	4/23 (17.4)[A]	2/22 (9.1)
	Foetal incidence (%)	0	1/149 (0.7)	4/144 (2.8)	2/128 (1.6)
Metacarpal(s) absent	Litter incidence (%)	10/25 (40.0)	7/25 (28.0)	12/23 (52.2)	13/22 (59.1)
	Foetal incidence (%)	22/146 (15.1)	12/149 (8.1)	24/144 (16.7)	42/128 (32.8)[B]
Metatarsal(s) absent	Litter incidence (%)	0	0	4/23 (17.4)[a]	1/22 (4.5)
	Foetal incidence (%)	0	0	4/142 (2.8)	1/127 (0.8)
Sternebrae 2 absent or partially ossified	Litter incidence (%)	4/25 (16.0)	9/25 (36.0)	9/23 (39.1)	12/22 (54.5)[B]
	Foetal incidence (%)	6/147 (4.1)	13/149 (8.7)	12/144 (8.3)	29/128 (22.7)[D]
Sternebrae 5 absent or partially ossified	Litter incidence (%)	18/25 (72.0)	20/25 (80.0)	20/23 (87.0)	20/22 (90.9)
	Foetal incidence (%)	40/147 (27.2)	51/149 (34.2)	65/144 (45.1)[C]	73/128 (57.0)[C]

A statistically significant difference relative to control ($p < 0.05$) using Fisher's test
B statistically significant difference relative to control ($p < 0.01$) using Fisher's test
C statistically significant difference relative to control ($p < 0.05$) using Chi² test
D statistically significant difference relative to control ($p < 0.01$) using Chi² test

The summary table of incidences of skeletal anomalies, as available in the original study report, presented partially ossified and absent sternebrae, xiphoid and pubis together. The statistical analysis was also performed on the pooled observations (absent and partially ossified). Based on the individual data available in the study report, an in-depth assessment of these anomalies is presented in the Tables below.

Table: *Foetal and litter incidences of skeletal sternebrae anomalies in a prenatal developmental toxicity study in SD rats (Anon. 1984, A83-1142)*

Doses (mg/kg bw/d)		0	100	300	600
Sternebrae 2					
Partially ossified	Litter incidence (%)	4/25 (16.0%)	9/25 (36%)	8/23 (34.7%)	12/22 (54.5%)
	Foetal incidence (%)	6/147 (4.1%)	12/149 (8.1%)	10/144 (6.9%)	28/128 (21.9%)
Absent	Litter incidence (%)	0	1/25 (4%)	2/23 (8.7%)	1/22 (4.5%)
	Foetal incidence (%)	0	1/149 (0.7%)	2/144 (1.4%)	1/128 (0.8%)
Sternebrae 3					
Partially ossified	Litter incidence (%)	0	0	3/23 (13.0%)	3/22 (13.6%)
	Foetal incidence (%)	0	0	3/144 (2.1%)	3/128 (2.3%)
Absent	Litter incidence (%)	0	0	1/23 (4.3%)	1/22 (4.5%)
	Foetal incidence (%)	0	0	1/144 (0.7%)	1/128 (0.8%)
Sternebrae 4					
Partially ossified	Litter incidence (%)	0	0	2/23 (8.7%)	5/22 (22.7%)
	Foetal incidence (%)	0	0	2/144 (1.4%)	5/128 (3.9%)
Absent	Litter incidence (%)	0	2/25 (8.0%)	2/23 (8.7%)	1/22 (4.5%)
	Foetal incidence (%)	0	2/149 (1.3%)	2/144 (1.4%)	1/128 (0.8%)
Sternebrae 5					
Partially ossified	Litter incidence (%)	18/25 (72%)	20/25 (80%)	20/23 (87%)	19/22 (86%)
	Foetal incidence (%)	37/147 (25%)	46/149 (31%)	52/144 (36%)	53/128 (41%)
Split	Litter incidence (%)	2/25 (8.0%)	0	0	0
	Foetal incidence (%)	2/147 (1.4%)	0	0	0
Absent	Litter incidence (%)	0	1/25 (4.0%)	9/23 (39%)	9/22 (40.9%)
	Foetal incidence (%)	0	3/149 (2.0%)	13/144 (9.0%)	20/128 (15.6%)

The detailed incidence data showed that absent sternebrae 2, 3, 4 or 5 were never observed in control groups. Increased incidences of absent sternebrae 2, 4 and 5 were noted from 100 ppm and from 300 ppm for sternebrae 3. A clear dose-dependent increase in litter and foetal incidences of absent sternebrae 5 was also observed.

Absent or partially ossified pubis and xiphoid were also reported as pooled findings. Individual data showed the following incidences:

Table: *Foetal and litter incidences of skeletal xiphoid and pubis anomalies in a prenatal developmental toxicity study in SD rats (Anon. 1984, A83-1142)*

Doses (mg/kg bw/d)		**0**	**100**	**300**	**600**
Pubis					
Partially ossified	Litter incidence (%)	0	1/25 (4%)	4/23 (17%)	1/22 (4.5%)
	Foetal incidence (%)	0	1/149 (0.7%)	4/144 (2.8%)	1/128 (0.8%)
Absent	Litter incidence (%)	0	0	0	1/22 (4.5%)
	Foetal incidence (%)	0	0	0	1/128 (0.8%)
Xiphoid					
Partially ossified	Litter incidence (%)	18/25 (72%)	21/25 (84%)	19/23 (83%)	19/22 (86%)
	Foetal incidence (%)	53/147 (36%)	57/149 (38%)	64/144 (44%)	76/128 (59%)
Absent	Litter incidence (%)	0	1/25 (4.0%)	6/23 (26%)	4/22 (18%)
	Foetal incidence (%)	0	1/149 (0.7%)	6/144 (4.2%)	6/128 (4.7%)

In a second OECD TG 414 prenatal developmental toxicity study, groups of 18 artificially inseminated NZW female rabbits were orally exposed to clomazone on GD 6–18 (Anon. 1982, WIL-81157). Gavage doses were prepared in 1 % of aqueous methylcellulose to achieve 0, 30, 240 and 1000 (700) mg/kg bw/d. Animals given the highest dose were exposed to 1000 mg/kg bw/d, but due to excessive maternal toxicity, the dose level was reduced to 700 mg/kg bw/d from GD 13.

At the highest dose, four pregnant females died (one each at GD 6, 19, 20 or 21). Their necropsy revealed an intubation error for females which died on GD 6 while the cause of the 3 other deaths was not determined. Furthermore, 1 dam of the control group and 3 dams of the highest dose aborted. An external examination of these aborted foetuses did not reveal anomalies. Clinical signs were observed at 1000/700 mg/kg bw/d, such as anorexia (in 6 animals), ataxia (in 2 dams), red vaginal discharge (in 4 dams of which 3 aborted) and decreased or no defecation. Moreover, statistically significantly lower maternal body weight was observed at the highest dose during gestation. Maternal parameters were examined and did not reveal significant changes.

No test substance-related effects were reported on mean number of viable foetuses, mean number of dead foetuses, corpora lutea or number of implantation sites. Moreover, the foetal weight was not statistically significantly reduced (40.0, 40.4, 37.5 and 37.0 g, respectively at 0, 30, 240 and 1000/700 mg/kg bw/d). No treatment-related foetal malformations were observed (incidence of skeletal malformations: 2, 4, 4 and 0 respectively, at 0, 30, 240 and 1000/700 mg/kg bw/d).

In a more recent OECD TG 414 developmental toxicity study, time-mated female Wistar rats (28/dose) were orally exposed to clomazone on GD 6-19 (Anon. 2002, 2840/2000). Gavage doses were prepared in 0.5 % aqueous solution with Tween 80 to achieve doses of 0, 250, 500 and 750 mg/kg body weight/d.

Twenty-two pregnant females in the highest dose group exhibited slight salivation. Among these animals, 6 exhibited lethargy, 1 wet perineum and 1 died (on GD 11). At the end of the study, 25, 23, 25 and 24 animals were pregnant at 0, 250, 500 and 750 mg/kg bw/d, respectively.

Animals of the mid and high dose groups had statistically significantly lower body weights (see table below) as well as a statistically significantly reduced food intake, which also affected the low dose group (total food intake: GD 0-20: 18.1, 17.2*, 16.2** and 14.7** g/rat/d, respectively, at 0, 100, 250, 500 and 750 mg/kg bw/d). The necropsy of dams did not reveal treatment related gross pathological findings.

Table: *Mean maternal body weight (in g) in a Wistar rat developmental toxicity study (Anon. 2002, 2840/2000)*

Doses (mg/kg bw/d)	0	250	500	750
N animals	25	23	25	24
GD 0	213	215	214	213
GD 6	230	234	230	229
GD 7	233	232	226	227
GD 8	236	232	226*	226**
GD 9	239	237	227*	224**
GD 10	242	241	233	227**
GD 11	246	244	237	231**
GD 12	249	248	241	232**
GD 13	253	251	242*	232**
GD 14	255	254	248	235**
GD 15	264	263	252*	239**
GD 16	270	268	257*	244**
GD 17	281	278	268*	253**
GD 18	294	291	278*	260**
GD 19	304	302	290*	267**
GD 20	316	312	289*	278**

** statistically significant difference relative to control ($p < 0.01$) using Dunnett's test
* statistically significant difference relative to control ($p < 0.05$) using Dunnett's test

At the highest dose, examination of the maternal parameters revealed an increase in the mean early resorptions (0.2, 0.3, 0.6 and 2.2, respectively at 0, 250, 500 and 750 mg/kg bw/d), and an increase in the mean post-implantation loss (0.7, 0.4, 0.7 and 2.4, respectively, at 0, 250, 500 and 750 mg/kg bw/d). Furthermore, four dams exposed to 750 mg/kg bw/d exhibited complete resorptions, which was significant when compared to the control.

The mean litter size was slightly reduced at 750 mg/kg bw/d (11, 11, 10 and 9, respectively, at 0, 250, 500 and 750 mg/kg bw/d). Furthermore, a statistically significantly lower live foetus weight was recorded at this dose level (-8 % of the control group).

Foetal examination also revealed malformations. A statistically significantly higher incidence of arthrogryposis and an additional single incidence of forelimbs malformation were observed at 750 mg/kg bw/d during the skeletal evaluation of the foetuses. One other foetus also demonstrated flexed forelimbs at the wrist during the external evaluation (see table below).

Table: *Incidences of foetal anomalies in a developmental toxicity study in Wistar rats (Anon. 2002, 2840/2000)*

Doses (mg/kg bw/d)	0	250	500	750
External anomalies				
Forelimbs flexed at wrist/arthrogryposis (%)	0	0	0	1 (0.5)
Incidence of anasarca (%)	0	0	1 (0.4)	1 (0.5)
Visceral anomalies				
% of renal pelvis dilatation	0	1.6	0.8	9.8[A]
% of brain 4th ventricle dilatation	0	0	0	1.0

Skeletal anomalies				
% of arthrogryposis	0	0	1.5	6.9[A]
% of malformations: forelimbs	0	0	0	1.0
% of multiple malformations	0	0	0.8	0

[A] statistically significant difference ($p < 0.01$) relative to control using Contingency test

In addition, minor anomalies included an increased incidence of hypoplastic sternebrae 2 from 250 mg/kg bw/d, being only significant at the mid dose. Asymmetrical ossification of sternebrae 3-5 was also observed at the high dose. Finally, normal variant parameters showed a significant increase in some of the bone components. Delayed skeletal ossification of cervical vertebrae 7/7 was statistically significantly increased in all treatment groups. A significant delay in ossification was noted in hindlimb distal phalange 1/5 at the high dose and distal phalange 2/5 at the mid dose. Sternebrae 2 also showed incomplete/poor ossification at high and low doses. The related incidences are presented in the table below.

Table: *Incidences of relevant skeletal normal variants and minor anomalies in a developmental toxicity study in Wistar rats (Anon. 2002, 2840/2000)*

Doses (mg/kg bw/d)	0	250	500	750
Sternebrae				
Hypopl. Sternebrae 2	0.8	2.4	3.8*	2.9
Asy. ossi. Sternebrae 3-5	0.0	0.8	0.0	3.9*
INO/PO Sternebrae 2	18.9	38.6**	30.0	35.3**
Hindlimb distal phalange				
DSO H limb: Dt.Phal 1/5	4.5	3.1	10.8	16.7**
DSO H limb: Dt.Phal 2/5	9.1	14.2	22.3*	11.8
Cervical vertebrae				
DSO CV: centra 7/7	6.8	20.5**	30.0**	31.4**

Hypopl.: Hypoplastic (minor anomaly)
Asy. ossi.: Asymetric ossification (minor anomaly)
DSO: Delayed skeletal ossification (normal variation)
INO/PO: incomplete/poor ossification (normal variation)
* statistically significant difference relative to control ($p \leq 0.05$) using Contingency test
** statistically significant difference relative to control ($p \leq 0.01$) using Contingency test

In a further prenatal developmental toxicity study, time-mated NZW rabbits (25/dose) were given clomazone by gavage on GD 6-28 (Anon. 2002, 2841/2000). Doses were prepared in 0.5 % aqueous carboxymethyl cellulose with Tween 80 to achieve doses of 0, 150, 350 and 700 mg/kg bw/d.

During the study period, 1 female of the control group, 1 of the mid dose and 1 of the highest dose died (respectively, on GD 11, 21 and 21). The necropsy of these animals revealed signs of gavage error for the animals of the control and the mid dose group. Furthermore, at 700 mg/kg bw/d, 2 dams aborted (one on GD 24 and one on GD 25). Therefore, the number of rabbits sacrificed at the end of the study was 24, 25 and 22 animals respectively at 0, 150, 350 and 700 mg/kg bw/d. Among these animals, 2, 3, 1 and 1 were not pregnant, respectively, at 0, 150, 350 and 700 mg/kg bw/d.

Animals were observed daily. Diarrhoea was observed in all treatment groups (1, 2 and 2, respectively at 150, 350 and 700 mg/kg bw/d). Moreover, at 700 mg/kg bw/d, weakness (2 animals), dullness (2) and wet perineum (1) were noted. No statistically significant changes in these parameters were noted during the study. However, a statistically significant lower body weight gain and food intake were observed at 700 mg/kg bw/d.

Table: *Maternal body weight, body weight gain and food intake in a rat developmental toxicity study (Anon. 2002, 2841/2000).*

Doses (mg/kg bw/d)	**0**	**150**	**350**	**700**
No animals	22	22	23	21
Mean body weight (in kg)				
GD 0	3.07	3.18	3.14	3.10
GD 6	3.06	3.23	3.13	3.10
GD 10	3.04	3.21	3.09	3.06
GD 16	3.10	3.28	3.14	3.10
GD 22	3.15	3.34	3.15	3.11
GD 28	3.24	3.45	3.23	3.16
GD 29	3.26	3.46	3.24	3.17
Mean body weight gain (in kg)				
GD 6-29	0.18	0.22	0.10	0.06*
GD 0-29	0.19	0.28	0.10	0.07
Mean absolute body weight (in kg)				
	2.84	3.01	2.86	2.78
Mean food intake (g/rabbit/d)				
GD 6-29	84.5	94.9*	82.5	71.3**
GD 0-29	85.7	96.8**	85.5	76.4*

* statistically significant difference relative to control ($p < 0.05$) using Dunnett's test

** statistically significant difference relative to control ($p < 0.01$) using Dunnett's test

The maternal parameters only exhibited a statistically significant higher number of pre-implantation loss at 150 and 350 mg/kg bw/d. However, the mean number of corpora lutea, the mean number of implantation sites, the early and late resorptions, the post-implantation loss and the number of dams with any resorptions were unaffected.

The mean litter size and the mean live foetuses weight were unaffected by treatment. The necropsy did not reveal significant incidences of malformations (see table below). One foetus of the high dose group revealed external finding of forelimbs flexed at wrist in the high dose group. There was also a foetus with multiple malformations, including absent skull bones, both forelimbs flexed at the wrist and hind limbs turned inwards in the high dose group. In addition, there was a significant increase in hypo-plastic pubis in the high dose group (12.6 % vs 3.2 % in the controls, and above the HCD range 0-5.40 %). This observation was considered to be a minor anomaly.

Table: *Incidence of foetal anomalies in a rabbit developmental toxicity study (Anon. 2002, 2841/2000)*

Doses (mg/kg bw/d)	0	150	350	700
Number of litters	22	22	23	21
Total number of foetuses	157	158	138	137
Mean litter size	7	7	6	7
Number of dead foetuses	0	1	1	2
Mean live foetuses weight (in g)	41.20	43.33	43.11	40.86
External observation				
Number of foetuses examined	157	158	138	137
Forelimbs flexed at wrist (%)	0	0	0	0.7
Visceral observation				
Number of foetuses examined	157	157	137	135
Brain agenesis (%)	0	0	0	0.7
Diaphragmatic hernia (%)	0	0	0.7	0
Hydronephrosis (%)	0	0	0.7	0
Skeletal observation				
Number of foetuses examined	157	157	137	135
Skull bones absent (%)	0	0	0	0.74[A]
Hindlimbs: turn inwards (%)	0	00	0	0.74[A]
Forelimbs flexed at wrist (%)	0	0	0	0.74[A]

[A:] same foetus

An additional range finding study and prenatal developmental toxicity study was submitted during the public consultation (Anon, 2018; CLZ4336). Time-mated Wistar rats (8 females/group) were orally exposed to clomazone on GD 6-20. Gavage doses were prepared in 0.5 % of carboxymethylcellulose with Tween 80 to achieve doses of 0, 250, 500 and 750 mg/kg bw/d.

All dams survived during the study period. One female of the control group and one given the lowest dose were not pregnant at the scheduled necropsy. Dilated pupils were observed in all animals of the treated groups. Body weight and food intake did not exhibit treatment related changes. At necropsy, no statistically significantly higher incidence of macroscopic findings was noted. However, the mean liver weight was statistically significantly increased (*, $p < 0.05$) in all treated groups (10.38, 12.24*, 12.66* and 14.40* g, respectively at 0, 250, 500 and 750 mg/kg bw/d).

The maternal parameters were not affected, apart from the mean pre-implantation loss, which was slightly increased at the highest dose (9, 13, 7 and 24, respectively at 0, 250, 500 and 750 mg/kg bw/d). The foetal examination revealed a statistically significant increased (*, $p < 0.05$) body weight at 250 mg/kg bw/d (5.1, 5.6*, 5.3 and 5.4 g respectively at 0, 250, 500 and 750 mg/kg bw/d). None of the foetuses exhibited malformations.

In the subsequent OECD TG 414 developmental toxicity study, time-mated Wistar female rats (21/dose, except for control: only 20 females) were orally exposed to clomazone on GD 6–20 (Anon. 2019, CLZ4337). Gavage doses were prepared in 0.5 % CMC with 0.1 % Tween 80 to achieve 0, 100, 250, 500 and 750 mg/kg bw/d.

All females survived and no test-substance related clinical signs were observed during the study period. In dams, there was no significant effect on mean terminal body weight at any dose. Effects on body weight gain and net body weight gain were reported only at the top dose (-12.9 % and -25.2%, respectively). There was no significant effect on mean terminal body weight at any

dose. Food consumption was also decreased at the high dose. Increases in mean absolute liver weight were observed from 250 mg/kg bw/d. There was no difference in reproductive performance or developmental effects noted at any dose level in the main study.

Discussion

Overall, five rat or rabbit developmental toxicity studies with clomazone were made available in the CLH report or during the public consultation. Increased resorptions and/or implantation loss were observed after clomazone exposure in rats and rabbits. These effects are considered to be mainly related to fertility and are further discussed in the previous section.

Several external and skeletal malformations affecting the extremities and the limbs of the rat foetuses were reported in two different studies, including forelimbs flexed at the wrist/arthrogryposis, malformed forelimbs, absent metacarpal and metatarsal. One rat study reported in addition increased incidences of absent sternebrae, caudal vertebrae, pubis and xiphoid. Both studies also showed skeletal findings of minor concern affecting e.g. sternebrae and/or extremities of the limbs. In the rabbit, there was also a significant increase in hypoplastic pubis at 700 mg/kg bw/d. Finally, renal effects in foetuses were reported in the rat developmental toxicity studies.

<u>Forelimbs flexed at wrist/arthrogryposis</u>

First, a dose-dependent increase of a foetal skeletal malformation reported as "*arthrogryposis*" was observed in a rat developmental toxicity study at incidences of 1.5 and 6.9**% at the two highest doses of 500 and 750 mg/kg bw/d respectively (Anon. 2002, 2840/2000, ** =$p < 0.01$). HCD related to this developmental toxicity study in Wistar rats were provided for the period between Aug. 1998 and Oct. 2005 (14 studies, 4046 foetuses out of 448 litters for external observations, 2387 foetuses out of 447 litters for skeletal observations). No HCD were available under the description of "*arthrogryposis*", neither for external nor skeletal observations of foetuses. Single incidences of "*forelimbs (Rt/t/B) (+/++) flexed at wrist*" were indicated as a minor skeletal anomaly in 4 different studies. The overall HCD range for this finding was between 0 and 0.9 % with a mean incidence of 0.17 %.

In developmental toxicology, arthrogryposis is a term sometimes used to describe an external observation of excessive flexion or bending of a limb or a joint. The limb cannot be straightened during the external evaluation of the foetus. Arthrogryposis is acknowledged to be caused by a reduction in foetal movements which impacts normal joint development, resulting in excessive contracture of the limb (Staheli *et al.*, 1998).

In study 2840/2000, the increased incidences of arthrogryposis in the foetal *skeletal* examination were not correlated with observations of arthrogryposis during the *external* evaluation of the foetuses. The study report's protocol presented, in Appendix 15 of the 2840/2000 study report, a comprehensive list and classification of malformations for foetal skeletal examination. Only "*bent extremities*" were listed as a major skeletal malformation. Although it is also stated that "*other malformations, if any, recorded during evaluation will also be reported*", there is no mention in this protocol of forelimbs flexed at wrist or arthrogryposis as skeletal malformations.

A letter (Dec. 2017) from the company which conducted the study, was provided to clarify the terminology used in Standard Operating Procedure (SOP) adopted for the conduct of Study No. 2840/2000 and 2841/2000. In this letter, arthrogryposis was described as follows: "*Persistent flexure or contracture of a joint, flexed paw (bent or twist) is the most common form, implies soft tissue alteration*". This definition was based on the SOPs TER-006 Ed. 2/2000 "*Teratogenicity: Examination of rat foetuses for sex, External malformations, foetal weight, Sorting foetuses for*

visceral and skeletal evaluations and preservation of foetuses" and SOP TER-013 Ed. 1/1998 "*Teratogenicity: Examination of rabbit foetuses for external malformations, Evisceration, Skinning and Preservation of foetuses*".

In addition, it was raised during the public consultation that the skeletal observations of arthrogryposis might be caused by *ex utero* procedure during the skeletal examination. RAC acknowledges that it cannot be excluded that the skeletal observation of arthrogryposis might be artefactual but notes that if the reporting of arthrogryposis during the skeletal examination would be an artefact caused by *ex utero* procedure, an increased incidence should have been observed in all groups.

RAC agrees that arthrogryposis is usually reported during the necropsy and not during the skeletal examination and implies soft tissue alteration according to the laboratory's SOPs. The clarification letter did not specify if arthrogryposis is usually classified as an external or skeletal anomaly or both based on the internal SOPs.

Overall, RAC is of the opinion that the observation of arthrogryposis in the 2840/2000 study report (Anon. 2002) is associated with a high degree of uncertainty relating to the reporting of this finding as a skeletal anomaly.

Furthermore, a single incidence of a major malformation related to the limbs was observed at high dose during foetal external examination. This malformation was described as "*forelimbs flexed at wrist*" in the summary of foetal external findings and as "*arthrogryposis*" in the concurrent individual animal data. This single finding was not statistically significant and therefore not considered relevant for a developmental toxicity classification.

According to the available HCD for external observations, forelimb flexed at wrist is uncommon in this strain of rat. One study reported this observation as a minor malformation at an incidence of 0.33 % (corresponding to one foetus out of 302, study 3247/2001). A second study considered forelimb flexed at wrist as a major malformation with an incidence of 2.1 % (study 2723/1999, 141 foetuses out of 18 litters examined). Thus the calculated mean incidence of forelimb flexed at wrist is 0.1 % with a range between 0 and 2.1 % in the HCD.

The single external observation of forelimb flexed at wrist in the clomazone study was not statistically significant and although uncommon, was observed in the HCD. RAC therefore considers this finding not relevant for a developmental toxicity classification.

Finally, a single incidence of skeletal malformation of the limbs was reported in the same study at the highest dose. No detailed description of the observation was available in the report. There was no related HCD and the incidence was not statistically significant. This finding is concluded to be not relevant for a developmental toxicity classification.

Similar malformations affecting the limbs were not observed in an OECD TG 414 developmental toxicity study in Wistar Han rats (Anon. 2019, CLZ4337). This study applied a protocol very closely resembling the 2002 Wistar rat study but used a double staining procedure. Although the maternal effects at the high dose were of lower severity, results showed no difference in reproductive performance or developmental effects at any dose level, in clear contrast with the older Wistar rat study.

In the rabbit, an external observation of forelimbs flexed at wrist was noted in one foetus in a developmental toxicity study (Anon. 2002, 2841/2000). Skeletal observations also revealed one additional foetus with multiple malformations, including hindlimbs turned inwards, forelimbs flexed at the wrist and absent skull bones. For this report, rabbit HCD were provided for the

period between July 1997 and April 2003 (10 studies). The available HCD for external observations were difficult to assess due to unclear reporting. "*Left forelimbs flexed at wrist*" (++) was reported as a minor anomaly and observed in a single study with an incidence of 0.72 % (mean 0.08 %). In contrast, "*right forelimbs flexed at wrist (+++)*" was considered a major malformation and noted in the same single study at an incidence of 0.72 % (mean 0.08 %).The observation of "*forelimbs flexed at wrist*" was also reported as a major malformation with a range of incidences between 0 and 2.6 % (mean 0.4 %). Finally, "*F limb/s – arthrogryposis*" was noted in another study, in which the range of incidences was 0 – 3.2 % with a mean incidence of 0.4 %. Related HCD are available for skeletal observations in the rabbit. "*F limb (+/++) (Rt/Lt/B): flexed at wrist*" was reported as a minor anomaly in a single study at an incidence of 1.47 % (mean for all 10 studies 0.16 %). Major malformations includes "*F limbs flexed at wrist*" observed in one study at an incidence of 1.3 % (mean 0.08 %). "*F limb/s – arthrogryposis*" was also considered a major skeletal malformation in four different studies (range 0 – 2.6 %, mean 0.48 %). Finally, "*H limbs: Feet turned inwards*" was noted in one study at an incidence of 0.9 % (mean 0.08 %).

RAC observes that marginal incidences of arthrogryposis or forelimbs flexed at wrist are not unusual in this strain of rabbit and were reported in both external and skeletal HCD by this laboratory. In addition, there was a higher incidence of arthrogryposis reported in the control group during the related dose-range finding study.

Considering the absence of statistical significance, the related observations in HCD and in the control group, the very low incidences and the multiple malformations in one of the two affected foetuses, these findings are considered non relevant for developmental toxicity classification.

Skeletal anomalies

In one rat developmental toxicity study (Anon. 1984, A83-1142), other skeletal anomalies were reported in the same study, including increased incidences of absent and/or partially ossified sternebrae 2, 3, 4 or 5, pubis, xiphoid, caudal vertebrae, manubrium, metatarsal and metacarpal. No effect on body weight or mortality was associated with clomazone exposure, although decreased food consumption was reported at the two highest doses. Nevertheless, abdominal staining and reduced locomotion were reported from 300 mg/kg bw/d.

Although provided in annex of the study report, the related HCD are considered of limited reliability because only three references were available, including the A83-1142 study report. In addition, the time-range for HCD only covered 2 years.

Single staining with Alizarin red as a standard procedure to detect calcium was used in this study. RAC acknowledges that this technique does not detect cartilage parts and therefore does not enable distinguishing agenesis from absent ossification of a bone. In the main report, findings of absent or partially ossified sternebrae 3 and 4, pubis, caudal vertebrae and xiphoid were classified as indicators of delayed skeletal ossification. Partially ossified or absent sternebrae 2 and 5 were considered in the study as normal variants. RAC is of the view that the increased incidences in partially ossified and/or absent bones observed in the 1984 rat study (Anon. A83-1142) should be interpreted as delays in development and are therefore of lower concern.

RAC notes that according to the study protocol, the animals were not time-mated in the 1984 rat study. In addition, the dose-response relationship of the observed delays in ossification was unclear. Overall, RAC is of the view that the reduction or absence in skeletal ossification observed in the 1984 rat study (Anon. A83-1142) are not sufficient to warrant classification.

In the 2002 rat study (2840/2000), minor anomalies included an increase in the incidence of hypoplastic sternebrae, 2 being only statistically significant at the mid dose. Asymmetrical

ossification of sternebrae 3-5 was also observed at the high dose. Finally, normal variant parameters showed a significant increase in delayed skeletal ossification of the cervical vertebrae 7/7 in all treatment groups (6.8, 20.5*, 30.0* and 31.4*% for the control, low, mid and high dose groups, respectively). Significant delay in ossification was also reported in the hindlimb distal phalange 1/5 at high dose and distal phalange 2/5 at mid dose.

Maternal effects included statistically significantly decreased body weight (at GD20, 9 % and 12 % of controls for the 500 and 750 mg/kg bw/d dose groups, respectively) and food consumption as well as clinical signs of salivation and lethargy. In the same study, one additional foetus showed skeletal malformation of the forelimb.

RAC cannot exclude that these findings were related to maternal toxicity. The pattern of normal variant and delayed ossification was also unclear. Similar skeletal findings were not observed in a recent OECD TG 414 developmental toxicity study in Wistar Han rats (Anon. 2019, CLZ4337) using a double staining procedure. RAC is therefore of the opinion that these findings are not sufficient to warrant a classification for developmental toxicity.

In the rabbit, (Anon. 2002, 2841/2000), there was also a significant increase in the incidence of hypoplastic pubis at 700 mg/kg bw/d (12.6 % vs 3.2 % for controls) which was outside the HCD range (0.00-5.40 %). This observation does not seem to be related to maternal toxicity. Hypoplastic pubis is considered to be a delay in ossification and is therefore considered of minor concern. RAC therefore considers this finding on its own as not sufficient to warrant a classification for developmental toxicity.

Visceral observations

In the 1984 rat study, increased incidences of hydro-ureter were noted from 100 mg/kg bw/d with a statistical significance only reached at the high dose for the foetal incidence. The foetal incidences (litter incidences) were respectively 0 % (0 %), 1.3 % (of 8.0 %), 2.9 % (17.4 %) and 3.8 %* (22.7 %) for the control, 100, 300 and 600 mg/kg bw/d dose groups, respectively. The range of HCD for foetal incidences (litter incidences) of hydroureter was 0–2.2 (0–13.0 %). This information is however considered of limited reliability because available HCD were only based on three different studies, including the report A83-1142 presently described.

In the 2002 rat study, renal pelvis dilatation was significantly increased in the high dose of 750 mg/kg bw/d and classified either as a normal variant or a minor anomaly based on the severity (+ or ++). The percentages of renal pelvis dilatation (+) as a normal variant were 1.5, 4.7, 3.1 and 11.8* % and the incidences of renal pelvis dilatation (++) as a minor anomaly were 0.0, 1.6, 0.8 and 9.8* % for the control, 250, 500 and 750 mg/kg bw/d group level.

The related HCD were 0.00-11.70 % (mean 4.09 %) for the normal variant and 0.00-11.70 % (mean 1.6 %) for the minor anomaly. It should be noted that for the HCD of renal pelvis dilatation (++) as a minor anomaly, the incidences were between 0.00 and 2.80 % (13 studies), one additional study (3369/2002) showed an incidence of 11.70 % (similar to the incidence of the normal variant). This study explains the marked difference between the range and the mean for this observation.

Overall, the renal visceral findings are considered of lower concern. In addition, although statistically significant, the increases in hydro-ureter and renal pelvis dilatation were of low incidence. RAC is of the opinion that these findings are not sufficient to warrant a classification for developmental toxicity.

Conclusion

In conclusion, a dose-dependent increase in arthrogryposis was observed in a rat developmental toxicity study (2840/2000, Anon. 2002). However, taking into consideration the high uncertainties related to the reporting of this finding as a skeletal anomaly and the absence of similar findings in two other developmental toxicity studies in the same species, RAC considers this observation not relevant for classification.

Absent and/or partially ossified sternebrae 2, 3, 4 or 5, pubis, xiphoid, manubrium caudal vertebrae, metatarsal and metacarpal, were reported in a rat developmental toxicity studies (A83-1142, Anon. 1984). Other skeletal anomalies were also noted in a second rat developmental toxicity study (2840/2000, Anon. 2002) and in one rabbit study (Anon. 2002, 2841/2000). Similar observations were not reported in two other rat or rabbit developmental toxicity studies. RAC is of the view that these findings should be interpreted as developmental delays or normal variants and are not sufficient to warrant a classification.

Finally, visceral findings included hydroureter or hydroureter in two rat developmental toxicity studies (A83-1142, Anon. 1984 and 2840/2000, Anon. 2002). RAC is of the opinion that these observations are of limited incidence and lower concern and therefore not sufficient to warrant a classification.

Overall, RAC concludes that based on the available dataset, there was no developmental findings relevant for classification. **No classification for developmental is therefore warranted**.

Lactation

No adverse findings on lactation were reported either in the available 2-generation study in rats or in the developmental toxicity studies in rats or rabbits. No human evidence or toxicokinetic study is available to support a classification for effects on or via lactation. Therefore, RAC is of the opinion that a **classification for lactation is not warranted.**

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

The DS concluded that no classification is needed because clomazone is not considered to be an organic solvent.

Comments received during public consultation

One Company-Manufacturer supported the DS's conclusions on aspiration toxicity.

Assessment and comparison with the classification criteria

According to the CLP legislation, aspiration toxicity category 1 is defined by the following criteria (Table 3.10.1):

"*Substances known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard. A substance is classified in Category 1:*

(a) based on reliable and good quality human evidence or

(b) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm²/s or less, measured at 40°C."

No studies on aspiration hazard were submitted. No measure of viscosity was available but clomazone is not a hydrocarbon. **Therefore, no classification is warranted for aspiration hazard.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Clomazone (CAS No 81777-89-1) is an isoxazolidinone herbicide intended for use against broadleaved weeds and annual grasses in winter oilseed rape and potatoes. The mode of action is to inhibit carotenoids biosynthesis.

The data presented in the CLH report were submitted in the context of renewal of pesticide active substances under Regulation No. 1107/2009 concerning the placing of plant protecting products on the market. The data was evaluated in the Renewal Assessment Report (RAR) Vol. 1-4.

The DS (DS) proposed to classify clomazone as Aquatic Acute 1 (M-factor = 1) and Aquatic Chronic 1 (M-factor = 1) based on this relevant data. There were reliable acute data for all three trophic levels and an aquatic invertebrate (*Americamysis bahia*) is presented as the most sensitive species (EC_{50} 96 h = 0.53 mg a.s./L) < 1mg/L, leading to a classification category 1 for aquatic acute toxicity and an M-factor of 1.

The substance has a low potential for bioaccumulation and was considered as non rapidly degradable. Experimental chronic toxicity endpoints are available for all three trophic levels. The lowest values in the range 0.01 to 0.1 mg/L were observed with *Americamysis bahia*, which had resulted in a classification of Aquatic Chronic 1 for a non rapidly biodegradable substance with an M-factor of 1.

The information on the physicochemical properties represents the information as submitted by two notifiers of the active substance. The water solubility of clomazone has been experimentally determined to be 1.212 g/L and 1.09 g/L at 20°C for all pH. Clomazone is hydrolytically stable and no dissociation in water is observed.

Clomazone is regarded as a surface active with a surface tension value of 52.2 mN/m and 45.98 mN/m and very slightly volatile demonstrate by an arithmetic mean for vapour pressure of 2.76 10^{-2} Pa at 25°C.

Degradation

A summary of reliable valid studies considering the aquatic fate of clomazone and presented by the DS are listed in the table below.

Four experimental studies performed according to OECD TG 301 and GLP are presented by the DS. In each study, a limited mineralisation of clomazone was noticed.

The DS presented data on the aerobic transformation of clomazone in aquatic sediment systems that had been submitted and evaluated in context of the first EU review of clomazone. A study to investigate the dissipation of clomazone in two water/sediment systems (sediment organic carbon content: 0.1 % and 6.7 %) is available from the first EU review, which was performed

with radiolabelled clomazone. Clomazone degraded to concentrations of approximately 18 % AR and 37 % AR, with negligible amounts detected in sediment (< 3.0 % AR). A maximum mineralisation of 7.2 % AR was measured at the end of the study (100 days). One major metabolite was detected in the water phase, identified as CLZ-M01 (also referred to as FMC 65317 in the first EU review) and reaching maximum amounts of 24.9 % AR and 28.1 % AR at day 61. In the sediment phase CLZM01 was measured at levels < 4.5 % AR. A second metabolite, CLZ-M02 (also referred to as FMC 55657 in the first EU review), was shown to occur at maximum concentrations of 11.6-11.8 % AR (100d) in the water phase and < 4 % AR in sediment. In view of the very low amounts of clomazone dissipated to sediment, 1st order DT_{50} were calculated only for the whole systems (40.4 days and 66.9 days). In line with the FOCUS kinetic guidance at that time, the experts agreed on the use of the mean value of 52.5 days for DT_{50} in water and a worst-case half-life of 1000 days for DT_{50} in sediment as input parameters in the modelling.

Based on the ready biodegradability test result, the DS considered clomazone as non rapidly degradable for the purpose of classification.

Table: *Summary of the relevant information on rapid biodegradation*

Method	Results*	Remarks	Reference
OECD TG 301D for testing of chemicals (adopted July 17, 1992)	14% degradation after 21 days (maximum degradation of Clomazone, purity 92.7%)	**Acceptable**	Noack, M., 2002. Clomazone technical: Ready biodegradability closed bottle test.
OECD TG 301D for testing of chemicals (adopted July 17, 1992)	0% degradation after 28 days (clomazone purity 99.7%)	**Acceptable**	Graham, R., 2008. Clomazone: Assessment of ready biodegradability by measurement of oxygen uptake in the closed bottle test.
OECD TG 301F for testing of chemicals (adopted July 17, 1992)	The percentage biodegradation of clomazone (purity 96.6%) at Day 13 was 2%	**Acceptable**	Dickinson, R.A., 2008. Clomazone: Assessment of ready biodegradability by respirometry.
OECD TG 301D for testing of chemicals (adopted July 17, 1992)	Degradation of clomazone (purity 98.3%) was only 1.0% after 28 days.	**Acceptable**	D. Dengler, 2010 Assessment of the ready biodegradability of clomazone TC with the closed bottle test.

Bioaccumulation

The DS presented a log Pow value measured between 2.49 and 2.60 at pH 5, 7 and 9, 20°C, clomazone purity 99.5 %. The partition coefficient for clomazone is not pH dependent. A bioconcentration in fish *Lepomis macrochirus* was considered relevant as supportive study by the DS. After a 28-day exposure period, the whole fish bioconcentration was determined to be 40 L/kg. Based on this experimental data and the logPow measurement, the DS considered that clomazone has **a low potential for bioaccumulation**.

Aquatic toxicity

Acute aquatic hazard

The DS reported acute aquatic data for all three trophic levels for clomazone and all clomazone metabolites as well. Nevertheless, only parent compound data are considered appropriate for classification purposes.

The DS mentioned that, in addition to the data presented in the DAR, new acute toxicity tests with clomazone technical were generated after the first EU review. No critical change of the EU agreed endpoints set in the first EU review is indicated by the results of the newly submitted studies (*supportive information*).

The DS reported an acute toxicity to fish study performed with the rainbow trout (*Oncorhynchus mykiss*) according to OECD TG 203. The concentration range 1, 2, 4, 8, 16, and 32 mg/L was employed in a static system. The test item concentrations and control were analytically verified via HPLC after 0 and 96 h. The recovery of the test substance was 95-102 %, which is within the validity criterion. The test was carried out without any deviation from the guideline and according to GLP. The study is considered reliable by the DS. LC_{50} (96 h) was determined to be 15.5 mg a.s./L. The DS has recalculated the endpoint based on the purity of 92.7 % w/w: LC_{50} (96 h) is 14.4 mg clomazone/L.

Two acute toxicity studies to aquatic invertebrates are available. An acute immobilisation test to *Daphnia magna,* following OECD TG 202 was performed under static conditions. The exposure range was nominally from 6.25 to 100 mg/L and the concentrations were analytically verified. The validity criteria of the study were fulfilled, and test conditions were within the acceptable limit. The 48 h EC_{50} was determined to be 12.7 mg clomazone/L.

Table: *Summary of relevant information on acute aquatic toxicity*

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
Fish	*Oncorhynchus mykiss* (static) OECD TG 203 Clomazone 92.7%	LC_{50} (96h)	14.4 mg a.s./L (nominal, analytical verification)	CA B.9.2.1/01 GLP Valid
Aquatic invertebrates	*Daphnia magna* (static) OECD TG 202 Clomazone 92.7%	EC_{50} (48h)	12.7 mg a.s./L (nominal, analytical verification)	CA B.9.2.4.1/01 GLP Valid
	Americamysis bahia (flow-through) **Adaptation of ASTM Standard Practice No. E729 (1980) and amendment 1 (3-18-86)** **Clomazone 92.94%**	**EC_{50} (96h)**	**0.53 mg a.s./L (nominal, analytical verification)**	**CA B.9.2.4.2/01** **Valid**

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
Algae	*Pseudokirchneriella Subcapitata* OECD TG 201 Clomazone 98.2%	E_rC_{50} (72h) / E_yC_{50} (72h)	> 245.5 / 90.9 mg a.s./L (nominal, analytical verification)	CA B.9.2.6.1/06 GLP Valid
	Pseudokirchneriella subcapitata OECD TG 201 Clomazone 96.6%	E_rC_{50} (72h) / E_yC_{50} (72h)	104.3 / 36.2 mg a.s./L (nominal, analytical verification)	CA B.9.2.6.1/07 GLP Valid
	Navicula pelliculosa OECD TG 201 Clomazone 96.6%	E_rC_{50} (72h) / E_yC_{50} (72h)	102.4 / 61.2 mg a.s./L (nominal, analytical verification)	CA B.9.2.6.2/03 GLP Valid

The second test reported by the DS was carried out with *Americamysis bahia*, former *Misydopsis bahia* (max 24 hours old) according to ASTM Standard Practice No. E729 and amendment 1 (3-18-86). Five test organisms per test vessel (and 4 vessels per concentration) were exposed to each test concentration (nominal, 3.17, 1.58, 0.79, 0.40 and 0.20 mg/L and (measured) 3.339, 1.663, 0.834, 0.419 and 0.215 mg/L, solvent (acetone) solution and control (Duxbury Bay seawater) under flow-through conditions for 96 hours. The test item concentration was analytically verified via HPLC with UV detector-analysis during the test. The overall recovery was in the range of 105-108 %, which is within the validity criteria. The validity criteria of the study were fulfilled and test conditions (pH-value, temperature, and oxygen concentration) were within the ranges prescribed by the protocol. The DS has recalculated the endpoint for *Americamysis bahia* based on the purity of 92.94 % w/w. EC_{50} is 0.566 x 0.9294 = 0.53 mg a.s./L. As indicated by the DS, *Americamysis bahia* is more sensitive than other aquatic invertebrate species, such as the standard test species *Daphnia magna*.

Data on effects of the active substance clomazone on algae were previously submitted by the original notifier and evaluated in the context of the first EU review of this active substance resulting in the approval of clomazone. Tests were carried out on one green algae species (i.e. *Pseudokirchneriella subcapitata*) and, as clomazone exhibits herbicidal activity, on an additional algal species from a different taxonomic group (i.e. *Navicula pelliculosa*) as proposed in the regulation. The data were considered acceptable and resulting endpoints were cited in the EFSA Scientific Report (July 2007) 109, 1-73. Two different studies were reported with *Pseudokirchneriella subcapitata*. The algae were exposed to various concentrations of the test item under static conditions over a period of 72 h. The cell culture density was determined daily. Growth and growth inhibition were quantified from measurements of the algal biomass density (cell counts) as a function of time. For the determination of algal growth three replicates for each concentration and eight replicates for controls (growth medium only) were exposed to nominal concentrations spaced by a factor of 2.2 (10.7, 23.5, 51.7, 113.6 and 250 mg/L) (Wenzel, 2010). The concentrations of Clomazone were chemically analysed in the freshly prepared test media at test start and in the algae cultures after 72 h with LC-MS/MS.

At the two lowest test concentrations no test substance related growth inhibition (growth rate and yield) was observed. There was a concentration dependent inhibition of growth at the next

higher concentrations. All validity criteria, including assessment of variance, were fulfilled. The derived *Pseudokirchneriella subcapitata* 72 hours growth inhibition endpoint based on the purity of 98.2 % w/w had calculated as E_rC_{50} (72 h): > 245.5 mg a.s./L and E_yC_{50} (72 h): 90.9 mg a.s./L.

The other study (Scheerba, 2009) was conducted with an initial cell density of approximately 5× 10^3 - 10^4 cells/mL. Five concentrations were tested in a geometrical series with a dilution factor of 10, nominal: 3.2, 10, 32, 100 and 320 mg a.s./L. Three replicates were tested for each concentration level and 6 replicates for the control. Clomazone concentrations were analytically verified. The derived *Pseudokirchneriella subcapitata* 72 hours growth inhibition endpoint based on the purity of 96.6 % w/w was calculated as E_rC_{50} (72 h): 104.3 mg a.s./L and E_yC_{50} (72 h): 36.2 mg a.s./L.

The effects of clomazone technical on the growth of the freshwater diatom *Navicula pelliculosa* were determined for growth rate and yield over a period of 72 h under static conditions. The study was conducted with an initial cell density of approximately 1× 10^3 - 10^4 cells/mL. Six concentrations were tested in a geometrical series with a dilution factor of 10, nominal: 1.0 - 3.2 - 10.0 - 32.0 - 100 - 320 mg/L. Three replicates were tested for each concentration level and 6 replicates for the control.

The concentrations of the active ingredient clomazone were analysed at all concentration levels at test start and test end via HPLC. At test start, the recovery rates of the active ingredient were in the range of 101 % – 107 %. At the end of the test, clomazone gave recoveries of 102 % – 107 %. Endpoints were determined for growth rate (E_rC_{50}) and yield (E_yC_{50}) over a period of 72 h. All effect values were given based on nominal test item concentrations.

Under the conditions of this study, the 72-hour E_rC_{50} and E_yC_{50} values were established at 102.4 mg a.s./L and 61.2 mg a.s./L.

Long-term aquatic hazard

Valid studies relevant for the classification of clomazone reported by the DS are presented in the following table.

Table: *Summary of relevant information on chronic aquatic toxicity*

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
Fish	*Oncorhynchus mykiss* (ELS study, flow-through) Comparable to OECD TG 210 Clomazone 95.6%	$NOEC_{reproduction}$ (57d)	2.3 mg a.s./L (mean measured)	CA B.9.2.2.1/01 GLP Valid
Invertebrates	*Daphnia magna (semi-static)* Comparable to OECD TG 211 Clomazone 95.6%	$NOEC_{reproduction}$ (21d)	2.2 mg a.s./L (nominal, analytical verification)	CA B.9.2.5.1/01 GLP Valid

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
	Daphnia magna *(semi-static)* OECD TG 211 Clomazone 99.7%	$NOEC_{reproduction}$ (21d) $EC_{10\ reproduction}$ (21d)	1.25 mg a.s./L 2.19 mg a.s./L (nominal, analytical verification)	CA B.9.2.5.1/02 GLP Valid
	Daphnia magna *(semi-static)* OECD TG 211 Clomazone 96.6%	$NOEC_{reproduction}$ (21d) $EC_{10\ reproduction}$ (21d)	4.6 mg a.s./L 8.15 mg a.s./L (nominal, analytical verification)	CA B.9.2.5.1/03 GLP Valid
	Americamysis bahia ***(flow through)*** OCSPP Draft Guideline 850.1350 Clomazone 96.8%	**$NOEC_{reproduction}$ (28d)**	**0.032 mg a.s./L (mean measured)**	**CA B.9.2.5.2/01 GLP Valid**
Algae and aquatic plants	*Lemna gibba* EPA 122-2/123-2 Clomazone 90.4%	E_rC_{50} *Frond number* (7d)	34 mg a.s./L (mean measured)	CA B.9.2.7/02 GLP Valid
	Lemna gibba OECD TG 221 Clomazone 98.2%	E_rC_{50} *Frond number* E_rC_{50} *Biomass increase* (7d)	41.7 mg a.s./L >98.2 mg a.s./L (mean measured)	CA B.9.2.7/03 GLP Valid
	Lemna minor OECD TG 221 Clomazone 96.6%	E_rC_{50} *Frond number** (7d)	49.2 mg a.s./L (mean measured)	CA B.9.2.7/04 GLP Valid
	Myriophyllum spicatum OECD TG 238 Clomazone 96.8%	E_rC_{50} *shoot length* / E_yC_{50} *shoot length* E_rC_{50} *wet weight* / E_yC_{50} *wet weight* E_rC_{50} *dry weight* / E_yC_{50} *dry weight,* (14d)	>32/ 18.4 8.3/ 1.39 27.5/ 5.23 mg a.s./L (mean measured)	CA B.9.2.7/05 GLP Valid

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
	Myriophyllum spicatum OECD TG 238 *Clomazone* 96.92%	E_rC_{50} main *shoot length* /E_yC_{50} main *shoot length*	86.08/ 56.49	CA B.9.2.7/08 GLP Valid
		E_rC_{50} *wet weight* / E_yC_{50} *wet weight*	59.74/ 12.98	
		E_rC_{50} *dry weight* / E_yC_{50} *dry weight*	62.03/ 24.32	
		E_rC_{50} total *shoot length* /E_yC_{50} total *shoot length*	93.81/ 72.94	
		E_rC_{50} number of whorls /E_yC_{50} number of whorls E_rC_{50} total root length	45.36/ 31.59	
		/E_yC_{50} total root length (14d)	4.70/ - mg a.s./L (mean measured)	

For chronic toxicity, the DS reported data for all trophic levels fish, invertebrates and aquatic plants. For fish chronic toxicity, the test with the rainbow trout was initiated with fertilized eggs and continued through 57 days (49 days post-hatch and 30 days post-swim-up). Five test concentrations in duplicates, one control (dilution water), and one solvent control (Dimethyl formamide, 29 µL/L) were included. Observations were done on viability and hatchability of embryos, on survival and growth of larvae. The test concentrations (nominal) were 1.27 mg/L, 2.66 mg/L, 5.31 mg/L, 10.62 mg/L, and 21.24 mg/L, and were analytically verified *via* HPLC. For this early life stage toxicity, the $NOEC_{mortality}$ endpoint for rainbow trout based on measured values, for a 57-day exposure is 2.29 mg as/L.

For invertebrates, four reliable chronic invertebrate studies were reported. Three semi-static *Daphnia magna* are available using clomazone following GLP and the OECD TG 211 (or comparable to). The daphnids were exposed to different ranges of concentrations 1.27, 2.66, 5.31, 10.62 and 21.24 mg/L, 1.25, 2.50, 5.0, 10, and 20 mg/L and 1.92, 4.8, 12, 30 and 75 mg/L clomazone in the first, second and third study respectively. Analytical verification was performed by HPLC in fresh media and old media of all test concentrations. The validity criteria were met, and the studies are considered valid. The measured NOEC for reproduction are respectively 2.2 mg a.s./L; 1.25 mg a.s./L and 4.6 mg a.s./L.

As previously described, a life-cycle toxicity test was performed with the mysid *Americamysis bahia* under flow-through conditions for 28 days. Four replicates with 20 mysids per replicate were exposed to clomazone technical at nominal concentrations of 1.9, 3.8, 7.5, 15, 30 and 60 µg/L (equivalent to 1.7, 3.3, 7.9, 15, 32 and 59 µg/L, mean measured). Mortality, reproduction success, body length and body weight of the first generation (F0) were determined. In addition, the survival of the F1 generation at 96-hours post release following exposure to clomazone was measured. The most sensitive indicator of toxicity for clomazone on *A. bahia* was reproduction. Based on this endpoint and the mean measured concentrations of clomazone, a NOEC was

determined to be 32 µg/L. Since no concentration tested resulted in ≥ 50 % mortality, the 7, 14, 21 and 28-day LC_{50} values were empirically estimated to be > 59 µg/L, the highest mean measured clomazone concentration tested.

The DS reported five toxicity tests for algae and aquatic plants. Two semi-static and a 7-day toxicity to *Lemna sp.* study using clomazone are available following GLP and OECD TG 221. Analytical measurements were included, and results were calculated based on mean measured. All validity criteria were respected, and the studies are considered valid. Because of a general concern regarding to the appropriateness of *Lemna* as test organisms for selective herbicides, toxicity tests with an additional aquatic macrophyte species, i.e. *Myriophyllum spicatum*, was conducted to generate data for a dicotyledonous plant species. The results of these studies indicate that clomazone is more toxic to *M. spicatum* than to *Lemna sp.* The most sensitive endpoint from these *Myriophyllum spicatum* studies is the E_rC_{50} total root length of 4.7 mg a.s./L. DS concluded that the use of this new overall lowest endpoints derived from the study with *M. spicatum* is deemed relevant for the risk assessment on aquatic macrophytes.

Comments received during public consultation

Three MSs and a Manufacturer supported the classification proposed by the DS. Two of them emphasized that the data presented for acute and aquatic chronic classification were not adequate. For the aquatic acute classification, EC_{50} values for growth rate for algae and aquatic macrophytes should be considered. For aquatic chronic classification the NOEC or (preferably) EC_{10} values for growth rate should be considered. Currently, in the CLH report, only the EC_{50} values for algae were presented in the overview tables for acute aquatic hazard and the EC_{50} values for aquatic macrophytes were presented with the data for the long-term aquatic hazard. These MSs requested the DS to provide an overview of all EC_{50} values for algae and aquatic macrophytes for the aquatic acute classification. For aquatic chronic classification, a separate overview of all EC_{10} values for algae and aquatic macrophytes was proposed in order to ensure that the NOECs and EC_{10}s for these species were not lower than the current key endpoint for *Americamysis bahia*.

Further information was also requested by a MS on biodegradation tests and bioaccumulation without changing the DS conclusion.

Assessment and comparison with the classification criteria

Degradation

Since clomazone is hydrolytically stable and no dissociation in water is observed, is poorly degradable in water/sediment systems (mineralisation of 7.2 % after 100 days) and based on the valid ready biodegradability test results showing that the substance is not readily biodegradable, RAC is of the opinion that clomazone should be considered as **non rapidly degradable**, for classification purposes.

Bioaccumulation

Clomazone has a logPow below the CLP threshold of 4 and the experimental BCF was also below the CLP threshold of 500. Nevertheless, as this substance has surface-active properties, the CLH report does not clearly indicate what test method was used to measure the Kow, which introduces uncertainties. Moreover, the reported fish BCF has not been assessed in the CLH report, with additional uncertainties remaining considering the bioaccumulation ability of clomazone. Nevertheless, clomazone could be considered as a substance with a low potential for bioaccumulation.

Aquatic Toxicity

RAC considered that the relevant dataset for classification based on DS and DAR data submitted should be the following:

- For aquatic acute toxicity

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
Fish	*Oncorhynchus mykiss* (static) OECD TG 203 Clomazone 92.7%	LC_{50} (96h)	14.4 mg a.s./L (nominal, analytical verification)	CA B.9.2.1/01 GLP Valid
Aquatic invertebrates	*Daphnia magna* (static) OECD TG 202 Clomazone 92.7%	EC_{50} (48h)	12.7 mg a.s./L (measured)[1]	CA B.9.2.4.1/01 GLP Valid
	Americamysis bahia (flow-through) **Adaptation of ASTM Standard Practice No. E729 (1980) and amendment 1 (3-18-86)** **Clomazone 92.94%**	**EC_{50} (96h)**	**0.53 mg a.s./L (nominal, analytical verification)**	**CA B.9.2.4.2/01** **Valid**
Algae and aquatic plants	*Pseudokirchneriella Subcapitata* OECD TG 201 Clomazone 98.2%	E_rC_{50} (72h) / E_yC_{50} (72 h)	> 245.5 / 90.9 mg a.s./L (nominal, analytical verification))	CA B.9.2.6.1/06 GLP Valid
	Pseudokirchneriella subcapitata OECD TG 201 Clomazone 96.6%	E_rC_{50} (72h) / E_yC_{50} (72h)	104.3 / 36.2 mg a.s./L (nominal, analytical verification)	CA B.9.2.6.1/07 GLP Valid
	Navicula pelliculosa OECD TG 201 Clomazone 96.6%	E_rC_{50} (72h) / E_yC_{50} (72h)	102.4 / 61.2 mg a.s./L (nominal, analytical verification)	CA B.9.2.6.2/03 GLP Valid

[1] The test item concentrations were analytically verified *via* HPLC-analysis at test starts and end of tests. The recovery was less than 80 % so measured concentrations were used to determine the acute toxicity

	Lemna gibba EPA 122-2/123-2 Clomazone 90.4%	E_rC_{50} *Frond number* (7d)	34 mg a.s./L (mean measured)	CA B.9.2.7/02 GLP Valid
	Lemna gibba OECD TG 221 Clomazone 98.2%	E_rC_{50} *Frond number* E_rC_{50} *Biomass increase* (7d)	41.7 mg a.s./L >98.2 mg a.s./L (mean measured)	CA B.9.2.7/03 GLP Valid
	Lemna minor OECD TG 221 Clomazone 96.6%	E_rC_{50} *Frond number** (7d)	49.2 mg a.s./L (mean measured)	CA B.9.2.7/04 GLP Valid
	Myriophyllum spicatum OECD TG 238 Clomazone 96.8%	E_rC_{50} *shoot length* / E_yC_{50} *shoot length* E_rC_{50} *wet weight* / E_yC_{50} *wet weight* E_rC_{50} *dry weight* / E_yC_50 *dry weight,* (14d)	>32/ 18.4 8.3/ 1.39 27.5/ 5.23 mg a.s./L (mean measured)	CA B.9.2.7/05 GLP Valid
	Myriophyllum spicatum OECD TG 238 Clomazone 96.92%	E_rC_{50} main *shoot length* /E_yC_{50} main *shoot length* E_rC_{50} *wet weight* / E_yC_{50} *wet weight* E_rC_{50} *dry weight* / E_yC_{50} *dry weight* E_rC_{50} total *shoot length* /E_yC_{50} total *shoot length* E_rC_{50} number of whorls /E_yC_{50} number of whorls E_rC_{50} total root length /E_yC_{50} total root length (14d)	86.08/ 56.49 59.74/ 12.98 62.03/ 24.32 93.81/ 72.94 45.36/ 31.59 4.70/ - mg a.s./L (mean measured)	CA B.9.2.7/08 GLP Valid

- For aquatic chronic toxicity

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
Fish	*Oncorhynchus mykiss* (ELS study, flow-through) Comparable to OECD TG 210 Clomazone 95.6%	$NOEC_{reproduction}$ (57d)	2.3 mg a.s./L (mean measured)	CA B.9.2.2.1/01 GLP Valid

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
Invertebrates	*Daphnia magna (semi-static)* Comparable to OECD TG 211 Clomazone 95.6%	$NOEC_{reproduction}$ (21d)	2.2 mg a.s./L (nominal, analytical verification)	CA B.9.2.5.1/01 GLP Valid
	Daphnia magna (semi-static) OECD TG 211 Clomazone 99.7%	$NOEC_{reproduction}$ (21d) $EC_{10\ reproduction}$ (21d)	1.25 mg a.s./L 2.19 mg a.s./L (nominal, analytical verification)	CA B.9.2.5.1/02 GLP Valid
	Daphnia magna (semi-static) OECD TG 211 Clomazone 96.6%	$NOEC_{reproduction}$ (21d) $EC_{10\ reproduction}$ (21d)	4.6 mg a.s./L 8.15 mg a.s./L (nominal, analytical verification)	CA B.9.2.5.1/03 GLP Valid
	Americamysis bahia (flow through) OCSPP Draft Guideline 850.1350 Clomazone 96.8%	**$NOEC_{reproduction}$ (28d)**	**0.032 mg a.s./L (mean measured)**	**CA B.9.2.5.2/01 GLP Valid**
Algae and aquatic plants	*Pseudokirchneriella Subcapitata* OECD TG 201 Clomazone 98.2%	**NOEC Growth, Yield** (72h)	23.1 mg a.s./L (nominal, analytical verification))	CA B.9.2.6.1/06 GLP Valid
	Pseudokirchneriella subcapitata OECD TG 201 Clomazone 96.6%	E_rC_{10} (72h) / E_yC_{50} (72h)	35.7 / 12.6 mg a.s./L (nominal, analytical verification)	CA B.9.2.6.1/07 GLP Valid
	Navicula pelliculosa OECD TG 201 Clomazone 96.6%	E_rC_{50} (72h) / E_yC_{50} (72h)	61.1 / 44.4 mg a.s./L (nominal, analytical verification)	CA B.9.2.6.2/03 GLP Valid
	Lemna gibba EPA 122-2/123-2 Clomazone 90.4%	E_rC_{10} *Frond number* (7d)	19 mg a.s./L (mean measured)	CA B.9.2.7/02 GLP Valid
	Lemna gibba OECD TG 221 Clomazone 98.2%	E_rC_{10} *Frond number* E_rC_{10} *Biomass increase* (7d)	8.4 mg a.s./L 5.2 mg a.s./L (mean measured)	CA B.9.2.7/03 GLP Valid

Organisms	Species (exposure conditions)	Endpoint and duration	Result	Study number and validity
	Lemna minor OECD TG 221 Clomazone 96.6%	ErC5 *Frond number** (7 d)	1.4 mg a.s./L (mean measured)	CA B.9.2.7/04 GLP Valid
	Myriophyllum spicatum OECD TG 238 Clomazone 96.8%	ErC10 *shoot length* / EyC10 *shoot length* ErC10 *wet weight* / EyC10 *wet weight* ErC10 *dry weight* / EyC10 *dry weight,* (14 d)	2.33/ 0.572 < 0.1/ < 0.1 < 0.1/ < 0.1mg a.s./L (mean measured)	CA B.9.2.7/05 GLP Valid
	Myriophyllum spicatum OECD TG 238 *Clomazone* 96.92%	ErC10 main *shoot length* /EyC10 main *shoot length* ErC10 *wet weight* / EyC10 *wet weight* ErC10 *dry weight* / EyC10 *dry weight* ErC10 total *shoot length* /EyC10 total *shoot length* ErC10 number of whorls /EyC10 number of whorls ErC10 total root length /EyC10 total root length (14 d)	19.2/ 6.88 23.79/ 7.7 4.79/ 0.94 41.54/ 29.98 4.14/ 2.94 0.48/ - mg a.s./L (mean measured)	CA B.9.2.7/08 GLP Valid

For acute toxicity, data are available for fish, invertebrates, algae and aquatic plants. *Americamysis bahia* is the most sensitive species. This species is considered by RAC as relevant for a classification purpose, as this acute ecotoxicity endpoint, EC_{50} (96 h) 0.53 mg/L, is below the CLP cut-off value of 1 mg/L. Consequently, RAC agrees that based on available and relevant data, **clomazone should be classified as Aquatic Acute category 1 (H400) with an M-factor = 1**.

For chronic toxicity, data are available for fish, invertebrates, algae and aquatic plants. *Americamysis bahia* is the most sensitive species. The $NOEC_{reproduction}$ (28 d) 0.032 mg a.s./L is in the range from 0.01 to 0.1 mg/L and since clomazone is considered as non rapidly biodegradable and to have a low potential for bioaccumulation, RAC considers that the **classification for Aquatic Chronic as Category 1 (H410) with an M-factor = 1 is warranted.**

RAC evaluation of hazards to the ozone layer

Summary of the DS's proposal

Due to the short half-life in air (<2 d) there are no concerns for long range transport in air and hence no concerns for the ozone layer.

Comments received during public consultation

No comments received

Assessment and comparison with the classification criteria

Due to the short half-life in air (<2 d) there are no concerns for long-range transport in air and hence no concerns for the ozone layer. **No classification is, thus, proposed.**

Additional references

Staheli LT, Hall JG, Jaffe KM, Paholke DO (1998). *Arthrogryposis: A Text Atlas.* Cambridge University Press, 178 pages

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 DS; the evaluation performed by RAC is contained in 'RAC boxes'.

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

Annex 3 Records of the targeted public consultation following the submission of additional studies addressing the classification for reproductive toxicity