**Proposal for Harmonised Classification and Labelling**

**Based on Regulation (EC) No. 1272/2008 (CLP Regulation)**

# CLOMAZONE

**(EC/List No. 617-258-0; CAS No. 81777-89-1)**

**Comments on Proposed Classification**

**Submitted by the FMC Corporation**

**(FMC Tracking No. 2019TOX-CLZ4804)**

**8 February 2019**

**Proposed classification based on CLP criteria:**

| | | |
|---|---|---|
| **Current CLH in Annex VI Table 3.1 (CLP)** | Not currently classified | |
| **Dossier submitter's proposal** | Acute Tox 4<br>Acute Tox 4<br>Repro 1B<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H302<br>H332<br>H360D<br>H400; M-factor=1<br>H410; M-factor=1 |
| **Applicant's (FMC) proposal** | Acute Tox 4<br>Acute Tox 4<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H302<br>H332<br>H400; M-factor=1<br>H410; M-factor=1 |

## Executive Summary

FMC submits the following comments for consideration by the Risk Assessment Committee (RAC) regarding the proposed Harmonised Classification and Labelling for Clomazone. FMC agrees with the Dossier Submitter (DS) that it is appropriate to classify Clomazone for acute oral and inhalation toxicity Category 4 (H302, H332) and for aquatic acute and chronic toxicity Category 1 (H400, H410). However, FMC strongly disagrees with the proposal to classify Clomazone for developmental toxicity Repr. 1B, H360D and submits a new guideline compliant (OECD 414 & OPPTS 870.370) rat pre-natal developmental toxicity study ([redacted], 2019) and an in-depth review of the [redacted] (2002), which purported an increase in limb flexures characterised as "arthrogryposis" in high dose foetuses. The observed skeletal findings of "arthrogryposis" in [redacted] (2002) are considered artefacts incurred during foetal processing, and therefore no conclusions can be drawn from this study for purposes of classification.

The reproduction and developmental toxicity data package for Clomazone consists of five pre-natal developmental toxicity studies – three in rats and two in rabbits, as well as a multigeneration reproduction study. On the basis of these studies, the following conclusions regarding the potential of Clomazone to cause adverse effects on the developing foetus can be made:

- There is no evidence of reproductive or developmental toxicity from a two-generation reproduction study in [redacted] CD rats [redacted] 1984).
- There is no evidence of pre-natal developmental toxicity from a study in SD rats [redacted] 1984).
- Highly doubtful evidence of "arthrogryposis" was reported in a deficient, unreliable study in Wistar rats by [redacted] ([redacted] 2002).
    - The disconnect between the recording of external and skeletal findings suggests that the noted skeletal finding of "arthrogryposis" was a result of artefacts induced during foetal handling.
    - Evaluation of foetal morphology should have been conducted without knowledge of treatment group.
    - The lab appears to have been relatively inexperienced when the study was conducted.
- There is no evidence of pre-natal developmental toxicity in a recently conducted, statistically enhanced study in Wistar rats ([redacted] 2019). This is the only study in the pre-natal development dataset conducted according to the current recommended guideline (OECD 414 & OPPTS 870.3700).
- There is no evidence of pre-natal developmental toxicity from a study in New Zealand white rabbits ([redacted], 1982).
- There is no evidence of pre-natal developmental toxicity from a study in New Zealand white rabbits by [redacted], 2002).
    - Incidences of limb flexure at the high dose are well within historical control data range.

    - The occurrence of "arthrogryposis" in the control group in the dose range finding study was 8%, exceeding the incidence observed in high dose rabbit foetuses in the main study.
- A comprehensive review of the updated dataset **cannot establish** that there is *"…**clear** evidence of an adverse effect on…development"* – such that classification as Category 1B would be appropriate.
- Further, the pre-natal developmental toxicity dataset does not support a Category 2 classification: *"substances are classified in Category 2…when there is **some evidence** from humans or experimental animals…of an adverse effect…on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. **If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification**."* No meaningful credibility can be ascribed to the [redacted] (2002) rat study considering incongruent findings and availability of other study data.
- **Therefore, based on the weight of evidence, classification of Clomazone for developmental toxicity is <u>not</u> warranted.**

## Introduction

The DS has proposed harmonised classification and labelling for Clomazone in accordance with the CLP criteria. FMC submits the following comments in response to this proposal.

## Physicochemical Properties and Physical Hazards

FMC agrees based on the physical and chemical properties of Clomazone that classification for physiochemical properties and physical hazards it not required.

## Evaluation of Health Hazards

### *Acute toxicity*

FMC agrees Clomazone does not meet the criteria for classification for acute dermal toxicity, skin or eye irritation or respiratory and skin sensitization. Based on the available data, FMC agrees with the proposal that Clomazone be classified for acute oral and inhalation toxicity (Acute Tox Category 4, H302 and H332, respectively).

### *Phototoxicity*

FMC agrees that Clomazone does not have phototoxicity properties in combination with light.

### *Aspiration hazard*

Clomazone is not an organic solvent and hence, FMC agrees with that no classification is warranted.

***Specific target organ toxicity – single exposure and repeat exposure***

FMC agrees that Clomazone does not meet the criteria for classification for either STOT-SE or STOT-RE.

***Germ cell mutagenicity***

FMC agrees with the conclusion of the DS; the results from guideline genotoxicity studies performed with Clomazone were consistently negative. Thus, Clomazone does not meet the classification criteria for germ cell mutagenicity.

***Carcinogenicity***

FMC agrees that Clomazone is not carcinogenic and thus does not meet the classification criteria for carcinogenicity.

***Reproductive and developmental toxicity***

FMC agrees that no adverse effects on sexual function and fertility were observed in the two-generation reproduction study with Clomazone and that no classification with respect to sexual function and fertility is warranted.

However, it is proposed by the DS to classify Clomazone as Repr. 1B, H360D May damage the unborn child on the basis of two pre-natal developmental toxicity studies [one in rat – [redacted] (2002) and one in rabbit –[redacted] (2002), both conducted at [redacted]. FMC strongly refutes such a classification proposal. Following an in-depth review of the pre-natal developmental toxicity data package by internal and external developmental and reproductive toxicology experts, it is considered that the [redacted] (2002) study, which purported an increase in limb flexures characterized as "arthrogryposis" in high dose foetuses, suffers from significant methodological deficiencies that affect the quality of the study. The observed skeletal findings of "arthrogryposis" are considered artefacts incurred during foetal processing, and therefore the results of the study cannot reasonably be relied upon.

To provide a more robust and conclusive dataset upon which a classification decision can be made – with greater confidence – a new pre-natal developmental toxicity study in the rat has been undertaken [redacted] 2019. FMC Tracking No. 2018TOX-CLZ4337) which is also supported by a toxicokinetic study and a rat dose range finding pre-natal development toxicity study (FMC Tracking No's. 2018MET-CLZ4349 & 2018TOX-CLZ4336 respectively).

The following review provides:

1. An overview of the overall pre-natal developmental toxicity dataset available for clomazone.
2. A review of the [redacted](2002) rabbit pre-natal developmental toxicity study data.
3. A review of the [redacted](2002) rat pre-natal developmental toxicity study data and methodological conduct.
4. A review of the new [redacted](2019) pre-natal developmental toxicity study data.

5. A perspective on additional foetal findings in the pre-natal developmental toxicity data package noted in the CLH dossier.
6. A weight-of-evidence assessment of the overall pre-natal developmental toxicity data package.
7. Consideration of the weight-of-evidence review against the classification criteria for reproductive toxicity (Regulation 1272/2009 and amendments).

## 1. An overview of the overall pre-natal developmental toxicity dataset for Clomazone

The pre-natal developmental toxicity data package for Clomazone, with the inclusion of the latest [redacted] (2019) study, comprises 3 studies in rat (2 with dose range finding phases/studies) and 2 studies in rabbit (1 with a dose range finding study).

**Summary of pre-natal developmental toxicity studies conducted with Clomazone - Rabbit**

| Study phase | Sponsor | Performing laboratory | Dose levels [mg/kg-bw/day] |
|---|---|---|---|
| **[redacted] 1982. A Teratology study in New Zealand rabbits with FMC 57020. Project No.: WIL-81157** | | | |
| Main study | FMC | [redacted] | 0, 30, 240 &1000/700 |
| **[redacted], 2002. Teratogenicity study in New Zealand rabbits with clomazone technical. Report No. 2841/2000, Company (ADM) No. 90004988** | | | |
| Dose range finding phase | Adama | [redacted] | 0, 100, 500, 750 & 1000 |
| Main study phase | | | 0, 150, 350 & 700 |

**Summary of pre-natal developmental toxicity studies conducted with Clomazone - Rat**

| Study phase | Sponsor | Performing laboratory | Dose levels [mg/kg-bw/day] |
|---|---|---|---|
| **[redacted] 1984. Teratology study in rats [Sprague Dawley] with FMC 57020 technical. Company (FMC) No. A83-1142** | | | |
| Main study phase | FMC | [redacted] | 0, 100, 300 & 600 |
| **[redacted], 2002. Teratogenicity study in Wistar rats with clomazone technical. Report no. 2840/2000, Company (ADM) No. 90004987** | | | |
| Dose range finding phase | Adama | [redacted] | 0, 250, 500 & 1000 |
| Main study phase | | | 0, 250, 500 & 750 |
| **[redacted] 2018. An Oral (Gavage) Dose Range-Finding Prenatal Developmental Toxicity Study of Clomazone in Wistar Han Rats, Company (FMC) No. 2018TOX-CLZ4336** | | | |
| N/A | FMC & Adama | [redacted] | 0, 250, 500 & 750 |
| **[redacted], 2019. An Oral (Gavage) Prenatal Developmental Toxicity Study of Clomazone in Wistar Han Rats, Company (FMC) No. 2018TOX-CLZ4337** | | | |
| N/A | FMC & Adama | [redacted] | 0, 100, 250, 500 & 750 |

## 2. A review of the [redacted] (2002) rabbit pre-natal developmental toxicity study data.

Following review of the rabbit developmental toxicity study conducted at [redacted] it can be concluded that there were no effects on foetal development as a result of exposure to Clomazone.

**Rabbit Dose Range-finding Study:**
**Foetal Incidences (%) of relevant external major malformations in the [redacted] 2002 study**

| Parameters | Dose level [mg/kg bw/day] | | | | |
|---|---|---|---|---|---|
| | Control | 100 | 500 | 750 | 1000 |
| **Major external malformations** | | | | | |
| **Number of litters examined** | 7 | 7 | 6 | 4 | 2 |
| **Number of foetuses examined** | 49 | 60 | 48 | 30 | 12 |
| **"Arthrogryposis"** | 8.2 (4) | 0 | 0 | 0 | 0 |

Foetal incidence is the percentage of foetuses with a particular observation from the overall foetuses examined from that group.
Numbers in parenthesis are the total number of foetuses noted as having the observations.
"Relevant" in the context of any findings that may be indicative of limb flexure/limb joint contracture.

| **Rabbit Pre-natal Developmental Study:**<br>**Foetal Incidences (%) of relevant external and skeletal major malformations in the [redacted] 2002** | | | | | |
|---|---|---|---|---|---|
| **Parameters** | **Dose level [mg/kg bw/day]** | | | | |
| | Control | 150 | 350 | 700 | Historical control ranges (%) |
| Major external malformations | | | | | |
| Number of litters examined | 22 | 22 | 23 | 21 | - |
| Number of foetuses examined | 157 | 157 | 137 | 135 | - |
| Both forelimbs flexed at wrist | 0 | 0 | 0 | 0.7 (1) | - |
| Both forelimbs "arthrogryposis" | 0 | 0 | 0 | 0.7 (1)@ | - |
| Major skeletal malformations | | | | | |
| Number of litters examined | 22 | 22 | 23 | 21 | - |
| Number of foetuses examined | 157 | 157 | 137 | 135 | - |
| Forelimb flexed at wrist | 0 | 0 | 0 | 0.7 (1)# | 0-3.2 |

Foetal incidence is the percentage of foetuses with a particular observation from the overall foetuses examined from that group.
Numbers in parenthesis are the total number of foetuses noted as having the observations.
"Relevant" in the context of any findings that may be indicative of limb flexure/limb joint contracture
@ - [external examination] multiple malformations that included acephalostomia (lacking most of the head), left ear microtia (under developed pinna), both forelimbs arthrogryposis, ectrodactyly (absence of digits) of 2 digits, small foetus, hind limbs bent inwards.
# -[skeletal examination] isolated incidence of a foetus with multiple malformations of brain agenesis, skull bones absent, hind limb turned inwards and forelimb flexed at the wrist (+++).
Although not conclusively discernible from the study report the foetus noted as having major malformations at external examination (@) and skeletal examination (#) is very likely to be the same animal given the alignment of findings between the examination phases (e.g., acepthalostomia at external examination > brain agenesis and skull bones absent at skeletal examination >; both forelimbs arthrogryposis and hind limbs bent inwards at external examination > hind limb turned inwards and forelimb flexed at the wrist (+++) at skeletal examination).

## Summary observations:

- In the dose range finding study there were 4 foetuses in the control group (8% of all foetuses examined in that group) recorded as having "arthrogryposis". There were no findings of "arthrogryposis" in the Clomazone-treated groups up to and including a high dose level of 1000 mg/kg-bw/day.
- In the main study, a single foetus in the high dose group demonstrated several frank developmental abnormalities (i.e., acephalaostomia, microtia and forelimb ectodactyly) which also included limb abnormalities. Given the severity of the abnormalities, it is not unexpected to see contractural abnormalities in the limbs. Therefore, this severely malformed foetus should be excluded from further consideration for classification purposes.
- In the main study for one other foetus in the high dose group with "both forelimbs flexed at wrist" was recorded during external examination; such an observation was not observed during skeletal examination for this foetus.

- Incidences of malformations – including "arthrogryposis" are within historical control ranges. The single incidence of flexed forelimbs noted above should not be considered a treatment related finding, particularly given a much greater incidence in control rabbits in the range-finding study.

## 3. A review of the [redacted] (2002) Wistar rat pre-natal developmental toxicity study data and methodological conduct.

Following a thorough investigation of the conduct and results of the study undertaken at [redacted] it can be concluded that significant doubts exist as to the quality and suitability of the findings in this rat pre-natal developmental toxicity study for use in the classification of Clomazone by competent authorities. Noteworthy methodological deficiencies have been identified calling into question the reliability of the reported study findings.

**Rat Dose Range-Finding Study:**
**Fetal Incidences (%) of relevant external in the 2002 study (Report no. 2840/2000)**

| Parameters | Dose level [mg/kg bw/day] | | | |
|---|---|---|---|---|
| | Control | 250 | 500 | 1000 |
| Number of litters examined | 6 | 7 | 7 | 5 |
| Number of foetuses examined | 62 | 60 | 69 | 47 |
| Forelimbs flexed at wrist | 0 | 0 | 0 | 0 |
| "Relevant" in the context of any findings that may be indicative of limb flexure/limb joint contracture. | | | | |

**Rat Main Pre-natal Developmental Study:**
**Fetal Incidences (%) of relevant external and skeletal findings in the 2002 study (Report no. 2840/2000)**

| Parameters | Dose level [mg/kg bw/day] | | | |
|---|---|---|---|---|
| | Control | 250 | 500 | 750 |
| Major external malformations | | | | |
| Number of litters examined | 25 | 23 | 25 | 20 |
| Number of foetuses examined | 265 | 254 | 260 | 204 |
| Forelimbs flexed at wrist | 0 | 0 | 0 | 0.5 (1) |
| Major skeletal malformations | | | | |
| Number of litters examined | 25 | 23 | 25 | 20 |
| Number of foetuses examined | 132 | 127 | 130 | 102 |
| Arthrogryposis | 0 | 0 | 1.5 (2) | 6.9** (7) |
| ** $p<0.01$: Foetal incidence is the percentage of foetuses with a particular observation from the overall foetuses examined from that group.<br>Numbers in parenthesis are the total number of foetuses noted as having the observation.<br>"Relevant" in the context of any findings that may be indicative of limb flexure/limb joint contracture. | | | | |

**Summary observations:**

- No incidence of limb flexure ("arthrogryposis") was evident in the foetuses from the dose range finding study – up to a high dose level of 1,000 mg/kg-bw/day.
- A single foetus in the high dose group of the main study (750 mg/kg-bw/day) was noted as having "forelimbs flexed at the wrist" during external examination, which was not confirmed on skeletal examination. However, 7 foetuses were noted as having "arthrogryposis" in the same dose group at skeletal examination. True instances of pathological limb flexure should have been evident externally and during skeletal examination. This significant discrepancy between the external and skeletal examination seriously calls into question the reliability of the skeletal findings.
- "Arthrogryposis" represents an interpretation by the laboratory of some degree of joint flexure, a term which was not listed in the protocol for foetal skeletal evaluations in this study (Appendix 15). Based on information provided by the testing laboratory, [redacted] defined arthrogryposis as "persistent flexure or contracture of a joint flexed paw (bent or twist)" which included flexures of even mild severity.
- The term "arthrogryposis" appears to be used in an unconventional manner by [redacted] at the time of study conduct. Arthrogryposis is a diagnostic term that refers to joint contractures that develop before birth, are evident at birth and are characterized by reduced mobility of multiple joints as a result of impaired connective tissue development. Any true case of arthrogryposis seen in a pre-natal developmental toxicity study should have been present at the time of fresh foetal examination and would have been confirmed by gentle pressure to the joint to determine if it was in a genuinely fixed state.
- The logical conclusion is that the recorded findings of "arthrogryposis" by [redacted] are artefacts resulting from improper foetal processing procedures (e.g., limb joint bending resulting from "heavy handed" or incomplete foetal skinning practices). Once placed in fixative, the joints would have been fixed in the position they were at the time of handling or the position they assumed in the storage jar. The fact that none of the incidences of "arthrogryposis" reported on skeletal examination were identified on external examination supports this conclusion.
- It has been reported that artefacts resulting from less than optimal foetal processing procedures can be mis-identified as malformations by inexperienced investigators (Principles and Methods of Toxicology, 5th Edition, edited by Wallace Hayes, page 1681).
- The GLP compliance statement contains a significant GLP deviation noting that the study was conducted in compliance with OECD GLPs and OECD and EPA testing guidelines with the exception that *"…evaluation which was conducted with the knowledge of treatment groups"*. This further suggests that [redacted] did not have much experience with undertaking pre-natal developmental toxicity studies at the time when this study was carried out and evaluator bias may have been a relevant factor in the results of the study.

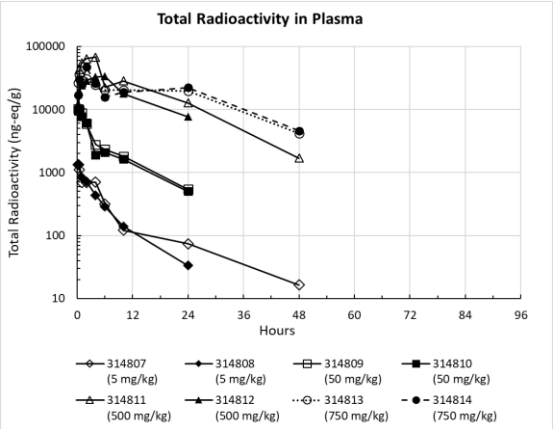
## 4. A review of the new [redacted] (2019) [redacted] rat pre-natal developmental toxicity study.

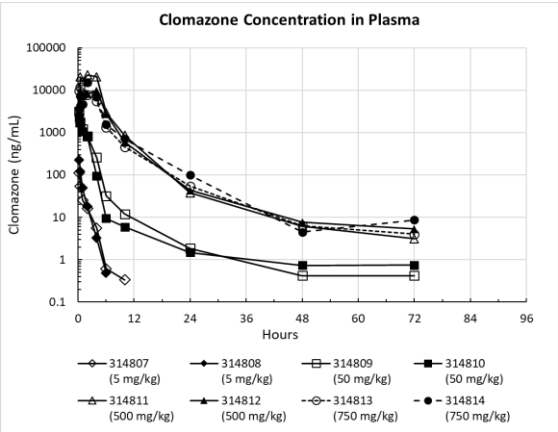
Given the concerns regarding the conduct of the [redacted] (2002) rat pre-natal developmental toxicity study, and the fact that the foetal specimens from the study were no longer available for possible re-evaluation, a third rat pre-natal developmental toxicity study was undertaken (FMC tracking No. 2018TOX-CLZ4337). In preparation for the definitive rat pre-natal developmental toxicity study, a study characterising the toxicokinetic properties of Clomazone over the dose range relevant to the proposed new study was conducted (FMC tracking No. 2018MET-CLZ4349). In addition, a rat pre-natal developmental dose range finding study preceded the definitive study (FMC tracking No. 2018TOX-CLZ4336). The reports for the three studies are being submitted as part of this submission. The purpose of each study is listed in the following table.

| Study title | Study number | Purpose |
|---|---|---|
| Toxicokinetic study in the female Wistar Han rat | FMC tracking No.: 2018MET-CLZ4349 | Determine the maximum kinetic dose<br>Aid in dose level selection for new pre-natal study.<br>Aid in data interpretation (i.e., dose-response relevance) |
| Pre-natal dose range finding study in the Wistar Han rat | FMC tracking No.: 2018TOX-CLZ4336 | Confirm maximum dose for the subsequent main pre-natal developmental toxicity study<br>Provide additional foetal data (e.g., external foetal exams undertaken) |
| Definitive pre-natal toxicity study in the Wistar Han rat (OECD 414) | FMC tracking No.: 2018TOX-CLZ4337 | Provide quality data upon which a more definitive classification assessment can be undertaken |

### Rat toxicokinetic study

The rat toxicokinetic study, that measured both total radioactivity and clomazone levels directly (confirmed by separate chemical analysis) following administration of $^{14}C$-labelled test substance, demonstrated a saturation of plasma concentrations in female Wistar rats at ≥ 500 mg/kg-bw. These results indicate that oral doses above 500 mg/kg-bw do not increase the internal systemic exposure. This observation has relevance to any interpretations of possible dose-response relationships above this dose.




## Rat pre-natal developmental toxicity dose range finding study

The dose range finding study confirmed a dose level of 750 mg/kg-bw/day was tolerable, and there were no indications of external malformations – including limb flexure – in the study.

| **Foetal Incidences (%) of external major malformations in the [redacted] 2018 study – Dose Range Finding** | | | | |
|---|---|---|---|---|
| **Parameters** | **Dose level [mg/kg bw/day]** | | | |
| | Control | 250 | 500 | 750 |
| Any external malformations | | | | |
| Number of litters examined | 7 | 7 | 8 | 8 |
| Number of foetuses examined | 70 | 64 | 78 | 67 |
| External malformations / developmental variations | 0 | 0 | 0 | 0 |
| Foetal incidence (%) is the percentage of foetuses with a particular observation from the overall foetuses examined from that group | | | | |

## Definitive rat pre-natal developmental toxicity study

The following is a summary of the main study, which was designed as closely as possible to [redacted] (2002) in order to provide data from a comparable study design.

- A full test guideline compliant (OECD 414 & OPPTS 870.3700) rat pre-natal developmental toxicity study was undertaken at a highly experienced and proficient contract research organisation.
- A comparable strain of rat (Wistar), dosing vehicle (0.5 % carboxymethyl cellulose and 0.1% Tween® 80) and route of administration (oral gavage once daily) were used to permit a more direct comparison to [redacted] (2002).
- Four dose levels (100, 250, 500 and 750 mg/kg-bw/day) were included to characterise any dose-response relationship and provide a data-rich data set. The three highest dose levels (250, 500 and 750 mg/kg-bw/day) matched those used in [redacted] (2002).
- Dose levels were confirmed based on a thorough dose range finding study and using internal dose information (e.g., plateau of internal dose at 500 mg/kg-bw).

- The dosing period (Gestation Days 6-20) was for one additional day not covered in the [redacted] (2002) study (Gestation Days 6-19).
- Parameters and end points evaluated included clinical signs, body weights, body weight gains, gravid uterine weights, food consumption, gross necropsy, liver weights, intrauterine growth and survival, and foetal morphology (external, visceral and skeletal examination).
- All foetuses were subject to foetal morphology (including skeletal) assessment – increasing the statistical power compared to [redacted] (2002) where only half the foetuses were subjected to visceral examination and half to skeletal examination.
- Foetal examinations were conducted without knowledge of treatment group so as to avoid evaluator bias.
- Foetal specimens were handled and processed in compliance with the laboratory standard operating procedures in such a way as to minimize foetal artefacts or mechanically induced alterations. Further, all malformations were verified by a second evaluator. External examination of fresh foetuses included evaluation of the limbs for size, shape and position; feet were examined for carpal/tarsal flexure.
- A conventional and best practice lexicon to record foetal pathology findings was used (i.e., diagnostic terminology such as "arthrogryposis" was not utilised).

**Summary observations:**

- Intermittent instances of dilated pupils in the dams between 2-6 hours post dosing occurred at 500 and 750 mg/kg-bw/day.
- Statistically significant and adverse decreases in mean body weight gain (13%) and mean net (minus the products of conception) body weight gain (25%) occurred in the 750 mg/kg-bw/day group relative to the control group.
- Statistically significant increases in liver weights were 11%, 25%, and 23% in the 250, 500 and 750 mg/kg-bw/day groups, respectively, relative to the control group.
- Foetal intrauterine growth and survival and foetal morphology was unaffected by maternal treatment with Clomazone. There was no impact of treatment on mean litter proportions of pre- and post-implantation loss, early, late and total resorptions, mean number and percentage of viable foetuses, mean foetal weight, and foetal sex ratios. No incidences of limb flexure were observed in any test-substance treated group either on external or skeletal examination.
- This study provides high quality and reliable data upon which reproductive toxicity classification can be determined.

| **Foetal Incidences (%) of relevant[1] external and skeletal major malformations in the [redacted] 2018 study** | | | | | |
|---|---|---|---|---|---|
| **Parameters** | **Dose level [mg/kg bw/day]** | | | | |
| | Control | 100 | 250 | 500 | 750 |
| **Major external malformations** | | | | | |
| **Number of litters examined** | 19 | 20 | 20 | 21 | 21 |
| **Number of foetuses examined** | 180 | 199 | 174 | 197 | 205 |
| **Forelimbs flexed at wrist** | 0 | 0 | 0 | 0 | 0 |
| **Carpal and/or Tarsal Flexure** | 0 [0.32] | 0 | 0 | 0 | 0 |
| **Major skeletal malformations** | | | | | |
| **Number of litters examined** | 19 | 20 | 20 | 21 | 21 |
| **Number of foetuses examined** | 180 | 199 | 174 | 197 | 205 |
| **Bent limb bone(s)** | 0.6 (1) [0.32] | 0 | 0 | 0 | 0 |
| Foetal incidence is the percentage of foetuses with a particular observation from the overall foetuses examined from that group.<br>Numbers in parenthesis are the total number of foetuses noted as having the observations.<br>"Relevant" in the context of any findings that may be indicative of limb flexure / limb joint contracture<br>Numbers in square brackets is the max percentage of foetuses from the overall historical control data (n=2082 foetuses) | | | | | |

## 5. Additional observations in the pre-natal developmental toxicity data package.

The following comments are provided relative to the summary of additional potentially relevant observations noted in the CLH dossier (Table 2.6.6.2).

Freeman (1984) rat pre-natal developmental toxicity study:

- Minor decreases in high dose foetal body weight for sexes combined, male foetuses and female foetuses (5%, 3% and 7%, respectively relative to controls) correlated with decreases in high dose maternal body weight and body weight gain (3% and 7%, respectively). Hence, the decreases in foetal weight in the high dose group can be attributed maternal body weight effects.
- The noted increases in the incidence of delayed ossification and hydroureter in the two high dose groups are incorrectly characterised as malformations and anomalies, respectively; normal convention is to categorise these observations as developmental variations. Further, the number of litters affected (considered the experimental unit in studies of this type) did not follow a dose response, the number of affected foetuses in any one litter was similar in range to that observed in control litters, and/or the findings were observed as a single incidence within a litter.
- Further, the Freeman 1984 study was not conducted according to current test guideline, and the gestation period was terminated on day 20, not on day 21, which is a critical developmental period for foetal development (particularly for bone ossification and kidney development). It is recognised ossification is not homogeneous in day 19 and 20 rat foetuses but is sufficiently advance and reliable by

day 21 for evaluating foetal development [redacted] et al., 1979. The extent of foetal ossification as an index of delayed development in teratogenic studies on the rat. Teratology 20:237-42).

- No similar observations on bone or kidney development were noted in the recently conducted rat per-natal developmental toxicity study by [redacted] (2019) in which pregnant female rats were exposed at a higher dose level, were treated for a longer duration and were necropsied one day later on gestation day 21.
- ECHA guidance on the application of CLP Criteria (2017) states that *"Classification is not necessarily the outcome in the case of minor developmental changes when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity."* On this basis, the noted reduction in foetal weight and increases in delayed ossification and hydroureter in [redacted] (1984) should be excluded from further consideration for classification purposes.

[redacted] (2002) rat pre-natal developmental toxicity study:

- Maternal toxicity was evident at the high dose of 750 mg/kg-bw/day where clinical signs of toxicity were present, and one animal died. Statistically significant reductions in body weight and body weight gain were observed throughout most of the dosing period and reduced by 12% and 50%, respectively relative to controls at the end of the dosing period. In addition, absolute body weight (minus uterine weight) in the high dose group was statistically significantly decreased relative to the control group by 9%. [Absolute body weight considers the impact the 4 dams with total resorptions may have had on mean body weight and body weight gain parameters at this dose level.]
- Mean foetal litter weights were reduced (statistically significant) at the high dose relative to controls to a similar degree (reduced 8%) as the reduction in absolute maternal body weight indicating that the decrease in foetal weight was a result of reduced maternal body weight.
- The apparent increase in early resorptions and post-implantation loss in high dose dams was primary due to four dams with total resorptions. Two of these dams had a high degree of pre-implantation loss occurring prior to initiation of treatment suggesting that the reproductive health of these two animals was potentially compromised prior to treatment. More importantly, there was no effect on either early, late or total resorptions and on pre- and post-implantation loss in the recently conducted rat pre-natal developmental toxicity study ([redacted] 2019) using the same strain of rat, route, dose levels and vehicle as in [redacted] (2002).
- Therefore, the noted effects on foetal weight in [redacted] (2002) are attributed to maternal toxicity and should be excluded from further consideration for classification purposes. The apparent trend towards an increase in the number of resorptions and post-implantation loss is likely a function of the reproductive health of the affected dams and/or influenced by maternal toxicity.

## 6. A weight-of-evidence assessment of the overall pre-natal developmental toxicity data

The reproduction and developmental toxicity data package for Clomazone consists of five pre-natal developmental toxicity studies – three in rats and two in rabbits, as well as a multigeneration reproduction study. On the basis of these studies, the following conclusions regarding the potential of Clomazone to cause adverse effects on the developing foetus can be made:

- There is no evidence of reproductive or developmental toxicity from a two-generation reproduction study in [redacted] CD rats ([redacted] 1984).
- There is no evidence of pre-natal developmental toxicity from a study in SD rats [redacted] 1984).
- Highly doubtful evidence of "arthrogryposis" was reported in a deficient, unreliable study in Wistar rats by [redacted] ([redacted] 2002).
    - The disconnect between the recording of external and skeletal findings suggests that the noted skeletal finding of "arthrogryposis" was a result of artefacts induced during foetal handling.
    - Evaluation of foetal morphology should be conducted without knowledge of treatment group.
    - The lab appears to have been relatively inexperienced when the study was conducted.
- There is no evidence of pre-natal developmental toxicity in a recently conducted, statistically enhanced study in Wistar rats ([redacted] 2019).
- There is no evidence of pre-natal developmental toxicity from a study in New Zealand white rabbits ([redacted], 1982).
- There is no evidence of pre-natal developmental toxicity from a study in New Zealand white rabbits by [redacted] ([redacted] 2002).
    - Incidences of limb flexure at the high dose are well within historical control data range.
    - The occurrence of "arthrogryposis" in the control group in the dose range finding study was 8%, exceeding the incidence observed in high dose rabbit foetuses in the main study.

## 7. Conclusion

- The [redacted] (2002) rat pre-natal developmental toxicity study is considered methodologically deficient: robust conclusions **cannot** be drawn from the study.

- Limb flexures that are not apparent during external examination and then "appear" during skeletal examination do not conform to how genuine developmental effects manifest, but rather **reflect inadequate foetal processing and recording practices**. The discrepancy between the external and skeletal findings calls into question the reliability of the study.

- **All other studies demonstrate a lack of developmental effects** that would warrant classification.
- The **weighting** that should be ascribed – within a weight of evidence assessment – to the [redacted] (2019) rat pre-natal developmental toxicity study should be **significant** given the known quality and capability of the laboratory, procedures to avoid artefacts in foetal processing and number of foetuses subject to morphological assessment.
- A comprehensive review of the updated dataset **cannot establish** that there is *"…**clear** evidence of an adverse effect on…development"* – such that classification as Category 1B would be appropriate.
- Further, the pre-natal developmental toxicity dataset does not support a Category 2 classification: *"substances are classified in Category 2…when there is **some evidence** from humans or experimental animals…of an adverse effect…on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. **If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification**."* No meaningful credibility can be ascribed to the [redacted] (2002) rat study considering incongruent findings and availability of other study data.
- **Therefore, based on the weight of evidence, classification of Clomazone for developmental toxicity is <u>not</u> warranted.**

***Effects on Lactation***

FMC agrees that there are no results in the two-generation reproduction study indicating adverse effects on offspring during lactation and as such classification is unnecessary.

**<u>Evaluation of Environmental Hazards</u>**

FMC agrees with the key studies relevant for assessing environmental hazards, the endpoints identified for assessing acute and chronic aquatic toxicity, and the conclusions reached on the classification and labelling of Clomazone for environmental hazards.