CLOMAZONE

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

Comments on Proposed Classification – Targeted Public Consultation

Submitted by the FMC Corporation

June 7, 2019

FMC strongly disagrees with the proposal to classify Clomazone for developmental toxicity Repr. 1B, H360D and has submitted a new guideline compliant (OECD 414 & OPPTS 870.370) rat pre-natal developmental toxicity study (2019) along with an in-depth review of one of two earlier studies (2002) in rats that purported an increase in limb flexures characterised as "arthrogryposis" in high dose foetuses – both the subject of this targeted public consultation. The observed skeletal findings of "arthrogryposis" in the earlier 2002 study 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 now 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:

1. There is no evidence of reproductive or developmental toxicity from a two-generation reproduction study in Charles River CD rats (1984).

2. There is no evidence of pre-natal developmental toxicity from a study in Sprague Dawley rats (1984).

3. Highly doubtful evidence of "arthrogryposis" was reported in a deficient, unreliable pre-natal developmental study in Wistar rats (2002).
   a. One of the most significant discrepancies in this study is the disconnect between the recording of external and skeletal findings suggesting that the noted skeletal finding of "arthrogryposis" was a result of artefacts induced during foetal handling. 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 reported 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.
   b. No incidence of limb flexure ("arthrogryposis") was evident in the foetuses from the dose range finding study conducted in advance of the main study – up to a much higher dose level of 1,000 mg/kg-bw/day.
   c. "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 (Study Appendix 15). Based on information provided by the testing laboratory, arthrogryposis was

defined as "persistent flexure or contracture of a joint flexed paw (bent or twist)" which included flexures of even mild severity.

d. The term "arthrogryposis" appears to have been used in an unconventional manner by the laboratory 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.

e. The logical conclusion is that the recorded findings of "arthrogryposis" in the 2002 Wistar rat developmental toxicity study 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). External experts in the field of developmental toxicity with which FMC has consulted have arrived at the same conclusion regarding the purported finding of "arthrogryposis" in the 2002 Wistar rat pre-natal developmental toxicity study (see Public Comments to CLH Proposal; Comments Nos. 9 and 19).

f. Further, 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 the laboratory 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. There is no evidence of pre-natal developmental toxicity in a recently conducted, statistically enhanced study in Wistar rats (2019). This is the only study in the pre-natal development dataset conducted according to the current recommended guideline (OECD 414 & OPPTS 870.3700).

   a. Given the concerns regarding the conduct of the Wistar rat pre-natal developmental toxicity study (2002), 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 by a highly experienced and proficient contract research organisation. 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. In addition, a dose range finding study preceded the definitive study. Summaries of these studies are the subject of this targeted public consultation.

   b. 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 direct comparison to the previous study (2002).

c. 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 the earlier study (2002). Dose levels were confirmed based on a thorough dose range finding study and using internal dose information from the toxicokinetic study (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 2002 study (Gestation Days 6-19).
d. 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).
e. All foetuses were subject to foetal morphology (including skeletal) assessment, thereby increasing the statistical power compared to the earlier study (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 to avoid evaluator bias.
f. 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).
g. The findings in the study included the following:
    - 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.
    - No impact of treatment was observed 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.**

5. Results of the rat toxicokinetic study (2019), measuring both total radioactivity and clomazone levels directly following 14C-labelled test substance, demonstrated saturation of plasma concentrations in female Wistar rats at 500 mg/kg-bw and above indicating oral doses above this dose do not increase internal systemic exposure. This observation has relevance to any interpretation of possible dose-response relationships above this dose.

6. There is no evidence of pre-natal developmental toxicity in a 1982 study in New Zealand white rabbits.

7. There is no evidence of pre-natal developmental toxicity from a second study in New Zealand white rabbits (2002).
   a. In the dose range finding study for this rabbit developmental toxicity 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 treated groups up to and including a high dose level of 1000 mg/kg-bw/day.
   b. 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.
   c. One other foetus in the high dose group of the main study was recorded as having "both forelimbs flexed at wrist" during external examination; such an observation was not observed during skeletal examination for this foetus.
   d. Incidences of malformations – including "arthrogryposis" – in the 2002 rabbit developmental study are within historical control ranges. The single incidence of flexed forelimbs noted above should not be considered as a treatment related finding, particularly given a much greater incidence in control rabbits in general and in the control animals of the accompanying range-finder study.

Conclusions:

1. The 2002 pre-natal developmental toxicity study in Wistar rats is considered methodologically deficient: robust conclusions **cannot** be drawn from the study.

2. 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.

3. **All other studies demonstrate a lack of developmental effects** that would warrant classification.

4. The **weighting** that should be ascribed – within a weight of evidence assessment – to the recent 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. This new study provides high quality, reliable data upon which reproductive toxicity classification can be determined.

5. 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.

6. 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 2002 rat study considering incongruent findings and availability of other study data. Therefore, classification as Category 2 is also not supported.

Therefore, based on the weight of evidence, classification of Clomazone for developmental toxicity is <u>not</u> warranted.