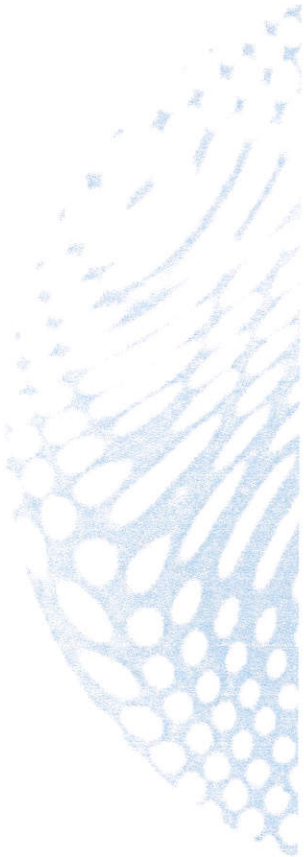
# Evaluation of Reported Findings of Arthrogryposis / Joint Flexures in Fetuses after Clomazone Exposure



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# Introduction

Clomazone (2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one; CAS No.: 81777-89-1) is a selective herbicide from the chemical group of oxazolidinones, inhibiting selective steps during chlorophyll-synthesis.

The European Commission issued their opinion on clomazone in September 2018, stating that two teratogenicity studies in rats and rabbits, respectively, displayed several cases of what was described as arthrogryposis, while several other teratogenicity studies in rats and rabbits and a 2-generation-study in rats did not show these alterations.

In the following, the presented data will be examined and evaluated.

# Arthrogryposis in the Human Population

Medically, arthrogryposis is uniformly defined as the presence of congenital, usually non-progressive joint contractures involving at least two joints in two different body areas. This class of diseases includes the so-called classic arthrogryposis – amyoplasia, with its unique clinical features such as symmetrical, severe contractures, usually involving both the upper and lower limbs [[redacted] 2008; [redacted] 2009; [redacted] 2016]. The finding is not a single disease but a descriptive diagnosis used to describe more than 300 individual diseases with varying etiologies. [redacted] 2016 state that "congenital contracture", the main presentation, denotes a "limitation of the passive and active range of motion in a given joint or joints with coexistent structural and/or functional abnormalities of the surrounding soft tissues – the joint capsule and periarticular ligaments". A rather comprehensive overview of syndromes and genetic defects presenting with arthrogryposis is given by [redacted] 2008.

The pathological mechanism of congenital joint contractures, common to all different types of arthrogryposis, usually involves the absence of active fetal movements (akinesia), normally appearing in the 8th week of human fetal life. Fetal akinesia lasting over 3 weeks may be sufficient to result in absence of normal stretching of muscles and tendons acting on the affected joints, and cause reduced compliance of the joint capsule and periarticular ligaments, consequently leading to fibrosis and contractures of the affected joints determined by the passive position of the limb. The earlier the restriction of active fetal motion occurs, the greater the arthrogryposis severity [[redacted] 2016].

The direct etiological factor causing akinesia in humans remains unknown, but a number of abnormalities can be discerned that can result in disruption of active movement and consequently fetal akinesia.

These abnormalities may origin in the fetus itself and include functional and/or structural pathologies, leading to hypomobility, such as: neurogenic factors (motor center diseases; disorders of the peripheral nerves or neuromuscular junction), myogenic factors (muscular dystrophies, mitochondrial diseases), or diseases of the adjacent tissues and/or articular tissues (diastrophic dysplasia).

Alternatively, the abnormalities may concern the fetal environment and result from maternal diseases (myasthenia gravis, SM, diabetes), mechanical factors (anatomic abnormalities of

the uterus; multiple pregnancy; oligohydramnios, amniotic bands), or vascular and nutritional disorders.

## Available DART Data

1. A primary 2-generation study from 1984 described no significant effects on offspring. The NOAEL for the offspring generations (314 mg/kg/d) corresponded to the highest dose tested and was 2 dose levels higher than the NOAEL for the parental animals (75 mg/kg/d).
2. In two teratogenicity studies in rats and rabbits, 1984 and 1982, respectively, the maternal animals were dosed during the period of organogenesis only, which was guideline compliant at this time. Pronounced maternal toxicity was observed. In the rat study, fetotoxicity was seen, presenting as decreased fetal weight, maturational delay seen as decreased ossification (wrongly described as skeletal malformations). An increased incidence of hydroureter is described for the mid and high dose groups. The correct nomenclature would be dilation of the ureter and this finding is currently not classified as a malformation [see www.devtox.org]. In the rabbit teratogenicity study, no treatment related developmental findings were observed. Both studies displayed pronounced maternal toxicity (e.g. mortality, abortion, decreases in maternal bodyweight, decreased food consumption)

   The NOAEL for development in the rat study was equivalent to the maternal NOAEL (266 mg/kg/d), the developmental NOAEL in the rabbit study (622 mg/kg/d) was one dose group higher than the maternal NOAEL (213 mg/kg/d).
3. In 2002, 2 additional teratogenicity studies (rat/rabbit) were reported, now adjusted to the updated guideline requirements with treatment over the entire period of organogenesis and until the day before Cesarean section. Both of these studies displayed pronounced maternal toxicity (e.g. mortality, decreases on bodyweight, gestational bodyweight gain, food consumption and clinical symptoms, incl. abortions and post-implantation loss). Based on the maternal toxicity, already apparent in the mid dose group, the treatment doses were selected too high, therefore risking a direct impact on fetal development by maternal toxicity.

   Fetal evaluation in the rat study displayed fetotoxicity in form of dilated renal pelvis, a variation (not a malformation!) physiologically caused by growth delay. Two fetuses from 2 litters in the mid dose and 7 fetuses from 4 litters were described as displaying arthrogryposis. This term is not a standard description in experimental animal fetal pathology and the available data give no information on the underlying findings that indicate this diagnosis. Upon external examination, the fetuses from mid dose were not identified as abnormal and only 1 fetus from the high dose group was reported to have forelimb flexure. No additional data of ossification status depending on dose group are reported, while fetal weight was decreased. In many cases, fetuses with decreased bodyweight have also an impaired ossification status as a sign of immaturity. Could it be that not all study findings are listed in the presented data?

   Fetal evaluation in the rabbit study describe 1 fetus with multiple external, visceral and skeletal alterations, including double-sided flexed wrists and inward turned hind limbs

and 1 fetus with isolated forelimb flexure. Unfortunately, the detailed incidence table clearly states that the fetus (marked *c) with the multiple externally visible malformations is the same as the one displaying serious visceral malformations and skeletally visible front- and hindlimb abnormalities and the missing skull bones. In contrast to rat fetal examination, in rabbit fetuses the same animal can be examined externally, viscerally (by fresh visceral dissection and Wilson technique) and skeletally. The data presentation (correction in column 5) in the dose group comparison table suggests that the person assembling this table might not be aware of this particularity.

In contrast to the rat study, these findings resemble the human correlate of arthrogryposis. Based on the given description it can not be discerned, if a malrotation (classified as malformation) was seen or a hyperflexion (not classified as malformation).

In both recent teratogenicity studies, the developmental NOAEL was comparable with the maternal NOAEL (232 mg/kg/d in rat and 333 mg/kg/d in rabbit).

# Inconsistencies in Presented Data and Interpretation

## Presented data

Several inconsistencies are detected by in-depth evaluation of the presented data in the Draft CLH Report.

It is unlikely that findings of limb/joint malrotations or hyperflexures would be present in rat and rabbit teratogenicity studies but not in a 2-generation study [no functional deficits or morphological alterations described in 3 offspring cohorts (F1a, F1b, F2) after complete gestational in-utero exposure] or 2 earlier teratogenicity studies in the 2 species, given the fact that comparable dose ranges were applied and, in all studies, maternal toxicity was obvious.

In all presented studies, the developmental NOAEL was comparable or higher than the maternal NOAEL. This strongly indicates, that maternal toxicity overlapped developmental findings. It is widely accepted, that a teratogenic effect can be clearly labelled as substance-specific if it occurs at concentrations that are below a level causing maternal toxicity.

At least in the rat study, an insufficient description of the findings labelled as "Arthrogryposis" is given. Only 1 fetus (in the high dose group) displayed externally visible findings indicating malpositioning or hyperflexure of the extremities. In contrast, 2 mid dose and 7 high dose fetuses were labelled with "Arthrogryposis" after skeletal examination and only 1 high dose fetus displayed limb abnormalities that might be indicative. The presented data are highly inconsistent, both from a descriptive point of view as well as from a practical point of view, where a careful examiner during Cesarean section would clearly have seen, upon external examination, any malrotation or obvious hyperflexures of fetal limbs. Therefore, the question is raised, if the skeletal findings labelled insufficiently as "Arthrogryposis" are either procedural artefacts during fetal maceration or maybe indication of an unexperienced examiner.

## Data interpretation

The medical literature uniformly describes the pathophysiology of arthrogryposis as a consequence of fetal akinesis, resulting in an insufficient intra-uterine muscle development and stretching of muscles and tendons, leading to contractures and deformity. As little as 3 weeks of reduced fetal movements in human gestation may lead to these long-lasting consequences.

If this timeframe is adjusted to rat or rabbit gestation length (21 or 29 days of gestation), as little as 1.5 or 2.1 days, respectively, might be sufficient to cause arthrogryposis-like limb deformities. In a background of pronounced maternal toxicity and clear signs of impaired fetal growth (fetal weight, delayed ossification, dilated renal pelvis), a reduced fetal intra-uterine activity is possible. The argument (p.89 of the Draft CLH Report), that the timeline and severity of maternal toxicity was not sufficient to potentially cause fetal akinesis can be counterargued by correlation of human data to animal gestation length, see above, and the fact, that typical signs of maternal toxicity (mortality, abortion, lethargy, total litter resorption) and animal distress (reduced food consumption, decreased defecation) were reported, which only become obvious after a prolonged maternal impairment (> 2 days, see above).

In the risk assessment report, the descriptive term arthrogryposis is discussed back and forth. In my opinion, if the human medical term "arthrogryposis" is used, (since this term is not a description in experimental animal fetal pathology), the visual presentation in humans (as depicted in [redacted] 2008) should be used as a reference. Any such visual presentation is clearly discernable at fetal external examination. Therefore, the discrepancy between external and skeletal findings, described in the 2 later teratogenicity studies cannot be explained and the described findings should be questioned in terms of severity and their nature as malformation.

## Summary and Conclusion

The presented data in the 2018 Draft CLH Report by DS Denmark raise doubts in the descriptive quality of 2 teratogenicity studies with skeletal findings described as arthrogryposis.

These findings may be considered as signs of impaired fetal growth since at the dose levels at which these findings were observed maternal toxicity is overlapping with developmental toxicity seen in the fetuses.

It is advised to review the original study data, based on raw data and individual fetal recordings in order to shed light on this issue and in order to avoid any additional requirements for further animal testing.

Furthermore, since the standards of classification of findings in developmental toxicity have changed, the performing laboratory should be contacted and required to give a detailed description of their standards of when the term "arthrogryposis" is used.

Based on the presented data, their inconsistency, the obvious discrepancy between study results and limitations in data quality, there is insufficient evidence to warrant a reproductive toxicity classification for clomazone as reproductive toxicant Category 1B.

## References

European Commission (2018): Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation). Clomazone Vol. 1. 19 p.

[redacted] (2009): ARTHROGRYPOSIS. Causes, Consequences and Clinical Course in Amyoplasia and Distal Arthrogryposis. University of Gothenburg, Institute of Clinical Sciences. ISBN 978-91-628-7928-0

[redacted] (2016): Arthrogryposis: an update on clinical aspects, etiology, and treatment strategies. Arch Med Sci. 12(1): 10–24.

RAR: Risk Assessment Report Clomazone. 282-296.

[redacted] (ed.): Arthrogryposis – A Text Atlas. Cambridge University Press 2008. ISBN 0-521-57106-5

[redacted]

Cases of so-called arthrogryposis were observed only at maternally toxic dose levels. Based on reduced body weight (gain) and reduced feed intake these toxicities were substantial; also, the number of total resorption was increased. At the mid and high dose level the MTD was exceeded and the high dose (750 mg/kg bw/d) was similar to the acute toxic dose level.

General maternal toxicity may well lead to adverse effects in the fetuses and findings observed only at these dose levels should not lead to classification as proposed:[1]

> *Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1.*

While malformations observed in humans and farm animals termed arthrogryposis cover a wide range of findings and are indeed a severe malformation the definition used in the laboratory that conducted both the rat developmental study (2002) and the rabbit developmental study (2002) is unclear. The laboratory was inconsistent in labelling the findings as arthrogryposis: Only one case of arthrogryposis was noted upon external examination of the rat progeny. This one finding would be well within the HCD. It is unusual that the other cases of arthrogryposis were not observed upon external examination. The reliability of the reporting was questionable as this case was not reported in the skeletal findings. Further it has been published that frequently there is a misnaming of findings: Palmer (1968) concluded that oftern temporary flexures of the forelimb in fetuses have been mistaken for the more serious defect arthrogryposis.[2]

No cases of arthrogryposis were reported in earlier studies on developmental toxicity conducted in two other laboratories with Clomazone in rat (1984) or in the rabbit (1982) which administered similar dose levels. Also, no cases were reported in a 2-generation study in rats (1984) conducted in another laboratory.

In conclusion the overall evidence does not support the proposed classification of Clomazone as Reproductive toxicant Category 1B.

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[1] Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017

[2] [redacted] (1968). Spontaneous malformations of the New Zealand White rabbit: the background to safety evaluation tests. *Laboratory Animals*, *2*(2), 195-206.