Committee for Risk Assessment

RAC

Annex 3

Records

of the targeted experts consultations

5-19 November 2012 on

Cycloxydim

EC number: 405-230-9
CAS number: 101205-02-1

ECHA/RAC/CLH-O-0000003157-76-01/A3

26 November 2012

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Records of the targeted experts consultations

5-19 November 2012

on Cycloxydim

From 5 till 19 November 2012 ECHA conducted the targeted expert consultation on harmonised classification of cycloxydim (EC No 405-230-9, CAS No 101205-02-1) with regard to reproductive toxicity.

The Committee for Risk Assessment (RAC) is currently developing an opinion on a proposal for the harmonised classification of cycloxydim for flammability (according to criteria in Annex VI, Dir. 67/548/EEC). The proposal was submitted to ECHA in August 2011 and has been subject of a public consultation ending on 1 October 2011.

RAC is currently discussing the potential reproductive toxicity of cycloxydim. Therefore, this targeted expert consultation, aimed to provide the parties concerned with an opportunity to comment on the questions presented below related to the reproductive toxicity of cycloxydim.

1. How can the maternal effects in the two-generation study (Hellwig et al., 1988) and developmental toxicity studies (Hellwig and Hildebrand 1987 (a)(b) and (c)) in the rat be interpreted in relation to the developmental toxicity of cycloxydim?

2. The pup effects in rats that may be critical for classification are outlined below (Hellwig and Hildebrand 1987(a)).

A. Dumb-bell shaped ossification centres in the thoracic region, were observed at a foetal incidence of 30% vs. 2.5% in the high dose (400 mg/kg bw/day) and control group, respectively.
Dumb-bell shaped or bipartite ossification centres of vertebral body/bodies with involvement of cartilage are assessed as a variation in the CLH report.
However, the expert group on harmonisation of rat foetal skeletal terminology (Solecki et al., 2001, p. 718) concluded "The term dumb-bell implies that the bone precursor is affected as well as the ossification site and the change is likely to be permanent. Therefore, the condition would classify it as a malformation."

B. Incomplete ossification sternebrae was observed at a foetal incidence of 33% vs. 5.1% in the high dose (400 mg/kgbw/day) and control group, respectively.
Incomplete ossification of the sternebrae (cartilage present) is assessed as a retardation in the CLH report.
The expert group (Solecki et al. 2001) judged incomplete or unossified structures usually (except cranium effects) as a variation because of its transient nature.
Incidences of both effects remained increased by post-natal day 21 (see post-natal segment findings in the study of Hellwig and Hildebrand, 1987b, page 118-119 in the CLH report)

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2.1 Do you interpret these skeletal effects, i.e. dumb-bell shaped ossification centres in the thoracic region and incomplete ossification of the sternebrae, as anomalies/variations/retardations?

2.2 Do you assess these skeletal findings as relevant for classification and why?

The experts were requested to submit their input to the key questions in writing by 19 November 2012 at the latest. ECHA has received comments from 2 interested parties:

- the Spanish Competent Authority - Appendix I
- Dr Rochelle W. Tyl (RTI International, USA) – Appendix II[1]

[1] Personal data has been deleted form the comment.

Appendix I

Expert consultation of cycloxydim

1- *How can the maternal effects in the two-generation study (Hellwig et al., 1988) and developmental toxicity studies (Hellwig and Hildebrand 1987 (a),(b) and (c)) in the rat be interpreted in relation to the developmental toxicity of cycloxydim?*

According to the *Guidance on the Application on the CLP Criteria, 2012,* with regard to the interpretation of the developmental outcome, the classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring (irreversible effects such as structural malformations).

The two-generation study (*Hellwig et al*, 1988) shows a pattern of dose-related maternal effects (reduction on food, water consumption and body weight), more evident during gestation and lactation periods at the high-dose group. At this dose in pups there were: decrease of litter size, litter weight (day 21 p.p.) and an apparent retard of morphological development, probably due to reduction of lactation index (not for F2a pups).

In developmental toxicity studies (*Hellwig and Hildebrand*, 1987 a,b,c), the observed maternal findings in the two-generation study, were confirmed. Besides, affectation of biochemical parameters and red series in blood from the doses of 400 mg/kg bw and even increase levels of copper (ecstasy liver) from the doses of 200 mg/kg bw, were observed (*Hellwig & Hildebreand*, 1987c).

Considering all the aforementioned, it is clear that cycloxydim causes maternal toxicity in reproductive toxicity studies.

However, in these studies toxic effects were observed in the offspring, some of them irreversible malformations that may justify classification to developmental even in presence of maternal toxicity:

a – Variations (*Hellwig and Hildebrand*, 1987a):

- Dumbbell-shaped ossifications centres in the thoracic region were observed at a foetal incidence of 30% vs. 2.5% in the high dose (400 mg/kg bw) and control group respectively.
- Incomplete ossification of the sternebrae (cartilage present) were observed at a foetal incidence of 33% vs. 5.1% in the high dose (400 mg/kg bw) and control group respectively.

b - Malformations (*Hellwig and Hildebrand*, 1987b):

- Dumbbell-shaped of vertebral body/bodies with involvement of cartilage (cartilaginous bone precursor) in the thoracic region was an incidence of 16% in the control group and 67.3% in the treatment group, with litter incidence of 100%.
- Cleft palate (1 case), caudal vertebrae absent (1 case), anasarca (1 case), (Although with very low incidence, 3 litters affected).

2.1 – *Do you interpret these skeletal effects, i.e. dumb-bell shaped ossification centres in the thoracic region and incomplete ossification of the sternebrae, as anomalies/variations/retardations?*

In our opinion, to avoid confusion in terminology, a proper interpretation of the skeletal defect called Dumb-bell shaped ossification centres of vertebral body in the thoracic region, starts with an adequate definition of the following terms:

The term D*umb-bell* implies that the cartilaginous bone precursor is affected as well as the subsequent process of ossification. Therefore, it would be designated as a malformation.

The term *Dumb-bell ossification or Bipartite ossification centres*, does not imply evidence that the cartilaginous bone precursor is affected. In this case, although the process of ossification is abnormal and may be retarded (after post-natal day 21), the end process of ossification is appropriate. Therefore, it would be designated as a variation which can lead to an ossification retardation.

An accurate assessment, case-by-case, of the effect on cartilage (using staining techniques) is necessary to determine skeletal defects.

With regard to the skeletal defect called incomplete ossification of the sternebrae, we consider it a variation of sternal ossification. A malformation is only considered when the cartilaginous bone precursor is affected or when there are fusions of sternebrae, which severity can be judged by the extent of shortening of intersternebral spaces. In the most severe cases, normal sternal growth and respiration might be compromised.

2.2 - *Do you assess these skeletal findings as relevant for classification and why?*

In the supplemental pre-, peri-, postnatal toxicity study in Wistar rats (*Hellwing & Hildebrand, 1987b*), in the prenatal study segment, Dumbbell-shaped of vertebral body/bodies with involvement of cartilage has an incidence of 16% in the control group and 67.3% in the treatment group, with litter incidence of 100%. These disturbers remain in postnatal segment findings in sacrificed pups on day 7 and 21 after birth.

In this study, this finding is described as “variation according to current harmonised criteria”, but in our opinion, it is a malformation because it affects the cartilaginous bone precursor and it can compromise the subsequent vertebral bone development and growth.

Considering Dumbbell-shaped of vertebral body with involvement of cartilage in the thoracic region a malformation and consequently an irreversible effect, there is evidence of developmental toxicity.

Summary

1.1 - According to the *Guidance on the Application on the CLP Criteria,* and considering the findings in reproductive toxicity studies, cycloxydim causes maternal toxicity due to marked metabolic alterations. However, in these studies there are malformations that may justify classification to developmental even in presence of maternal toxicity such as: Dumbbell-shaped of vertebral bodies with involvement of cartilage (cartilaginous bone precursor) in the thoracic region, cleft palate, caudal vertebrae absent, foetal anasarca.

2.1 – The term D*umb-bell* implies that the cartilaginous bone precursor is affected as well as the subsequent process of ossification. Therefore, it would be designated as a malformation.

The term *Dumb-bell ossification or Bipartite ossification centres*, does not imply evidence that the cartilaginous bone precursor is affected. Therefore, it would be designated as a variation which can lead to ossification retardation.

The term Incomplete ossification of the sternebrae, we consider it a variation of sternal ossification.

2.2 – In the pre-, peri-, postnatal toxicity study in Wistar rats (*Hellwing & Hildebrand, 1987b*), Dumbbell-shaped of vertebral bodies with involvement of cartilage has an incidence of 16% in the control group and 67.3% in the treatment group, with litter incidence of 100%.

In our opinion, this is a malformation because it affects the cartilaginous bone precursor and it can compromises the subsequent vertebral bone development and growth.

Therefore, considering Dumbbell-shaped of vertebral body with involvement of cartilage in the thoracic region a malformation, which is an irreversible effect, there is evidence of developmental toxicity that may justify classification to developmental even in presence of maternal toxicity.

Madrid, 19th November 2012

Appendix II

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November 9, 2012

I have read the CLH report and the published developmental and reproductive toxicity studies. My responses to the questions posed are as follows:

1. In my long experience it is very common for fetal delays in the transition from cartilaginous to ossified bone formation accompanied by reduced fetal body weights in rats (and rabbits) at doses where there are treatment- and dose-related decrements in maternal feed (and water) consumption and therefore decreased maternal body weights and weight changes. These effects do not constitute primary fetal toxicity but are clearly secondary to the maternal toxicity (the fetal effects are observed only in the presence of maternal toxicity, with the more profound maternal toxicity causing more profound fetal toxicity). Cycloxydim, in my opinion, is <u>not</u> a primary developmental toxicant.

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2. Pup effects in rats

A. Dumb-bell shaped ossification centers in the thoracic region were observed at much higher incidence in the high dose group (400 mg/kg/g) than in the vehicle control group, associated with the presence of maternal toxicity. Please note that these delayed ossifications and cartilaginous centers are also observed in the control group. It is a normal developmental progression. These reduced thoracic vertebral ossification centers observed in the rat fetuses in these studies can be designated as retardations, or variations but are clearly not malformations. The cartilaginous precursors precede the formation of bone in these areas so that one would expect that central bony ossification sites also to be reduced, due to the reduced cartilaginous structures. In my opinion (and that of others) this is not a malformation.

B. The sternebrae initially in vertebrates are the embryonic precursors to the sternal plate (or sternum) anchoring the ribs in the ventral thoracic region. They are found initially as cartilage and ossify over time to the bony components. In embryos, ossification of vertebrae is not yet complete (this is also observed in the control groups), and less complete in dose groups where the maternal animal exhibits toxicity (reduced feed and/or water intake, reduced body weights and/or body weight gains) and the fetuses almost always exhibit reduced body weights (which in turn result in reduced ossification).

The fact that these incomplete sternal ossifications do not resolve by PND 21 is likely due to continued maternal toxicity (in the presence of continued dosing?) resulting in fetal/pup toxicity associated with reduced ossifications. Also the pups are not yet full sized on PND 21 so cartilaginous structures are useful to allow continued growth of the skeleton.

I concur with the expert group that these incomplete ossifications of the sternebrae (including the continued presence of the precursor cartilage) should be considered variations.

2.1 I interpret the skeletal effects (reduced/incomplete ossification centers in the thoracic region and incomplete sternal ossification) as variations or retardations, typically associated with reduced fetal/pup body weights.

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2.2 I am not as knowledgeable as I should be on the ECHA classification system, but in my opinion, these skeletal findings support developmental delays/toxicity, typically caused by maternal toxicity, NOT as evidence of malformations (teratogenicity) or primary developmental toxicity.

Summary Text (250 weeks or less)

In my expert opinion, these ventral thoracic skeletal ossification delays observed in the thoracic region/sternebrae in term fetuses only at maternally toxic doses in the studies listed are not evidence of fetal malformations or even primary fetal toxicity. Since the maternal animals also exhibit treatment and dose-related toxicity, it is highly likely that the maternal toxicity caused the secondary fetal toxicity, including reduced fetal body weights and therefore delays in ossification in those fetal regions which ossify last.

Sincerely,

Signed

RTI International