FENPROPIDIN (Ro 12-3094/000)

Expert Opinion of the Toxicological Significance of the Foetal Findings in a Prenatal Developmental Toxicity Study in the Rabbit

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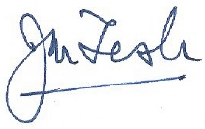
STATEMENT

The following expert opinion of the toxicological significance of the findings from a prenatal developmental toxicity study in the rabbit with Fenpropidin has been prepared by Tesh Consultants International (TCI) based upon the data presented in the original study reports and additional data relating to the developmental toxicity of fenpropidin supplied to TCI by Syngenta Ltd.

It represents the agreed opinions of the undersigned authors.



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Summary

Tesh Consultants International (TCI) have reviewed the data from a rabbit prenatal developmental toxicity (PND) study with fenpropidin performed by WIL Laboratories (WIL), Ashland, Ohio, USA (report no: WIL-639077, 2011). The foetal findings from the WIL PND rabbit study have been evaluated in conjunction with one additional rabbit PND study with fenpropidin, conducted in a different laboratory (Hoffmann-La Roche and Co. Ltd., report no. B-95 154) and two fenpropidin rat PND studies (Hoffmann-La Roche and Co. Ltd., report no. B-90 790, 1981 and Ciba Geigy Ltd., study no. 933117, 1994).

TCI consider that fenpropidin is unlikely to have been involved in the aetiology of 3 foetuses with persistent truncus arteriosus (PTA) or one foetus with severely malaligned sternebrae in the WIL rabbit study at the high dose level (20 mg/kg bwt/day) for the following reasons:

- In neither the second rabbit study, in which the high dose level was 30 mg/kg bwt/day, compared with 20 mg/kg bwt/day in the WIL study, nor the two rat studies, which also had higher top dose levels (125 reducing to 64 mg/kg bwt/day and 90 mg/kg bwt/day respectively) were any cardiovascular or severe sternebral abnormalities reported.

- In the WIL study there were no adverse effects on survival or growth *in utero*, despite evidence of maternal toxicity at the high dose level.

- The Hra:(NZW) SPF strain of rabbit, used in the WIL study has a low background history of PTA in control foetuses and of small isolated clusters (3 foetuses) of PTA in low or intermediate dose treated groups, which were unrelated to treatment. The incidence of PTA in the high dose group of the WIL study (also 3 foetuses) is in accordance with the historical data.

- Having examined photographs of the 3 foetuses that WIL described in the study report as having "severely malaligned sternebrae", TCI consider that only one of the foetuses should be classified as abnormal, since it could have had developmental implications in post-natal life. For the other two foetuses the sternebral findings were of a lesser nature and can be considered as variations/minor anomalies.

- Sternebral malalignment has been recorded in the WIL historical control data at low incidence. The occurrence of one foetus with abnormal sternebrae should not be a cause for concern in terms of compound involvement.

In conclusion, taking all of the above data into consideration, TCI are of the opinion that the occurrence of 3 foetuses with persistent truncus arteriosus and one foetus with an abnormal sternum in the high dose level of the one rabbit PND study is not sufficient evidence to classify fenpropidin as a developmental toxin. Similar findings were not recorded at a higher dose level in the second rabbit study conducted in a different laboratory, nor in either of the two rat studies at higher dose levels of fenpropidin.

1. Introduction

Syngenta Ltd., UK, have contracted TCI to review the data from a rabbit prenatal developmental toxicity (PND) study with fenpropidin performed by WIL Laboratories (WIL), Ashland, Ohio, USA (Number: WIL-639077 report dated 2011) and provide an expert opinion on the toxicological significance of the foetal findings.

Syngenta have provided to TCI the following documents:

Fenpropidin-A Prenatal Developmental Toxicity Study in New Zealand White Rabbits. Report/Study no. WIL-639077; Author: P. Sawhney Coder, 2011

Fenpropidin: Developmental Toxicity Studies in the Rabbit – Historical Control Data.

Fenpropidin-A Prenatal Developmental Toxicity Study in New Zealand White Rabbits. Study No. WIL-639077;WIL Research Laboratories, LLC, Ashland, USA (Syngenta unpublished report no: CGA 114900/10474, dated 23.06.2011)
Malaligned sternebra(e) - A statement on the occurrence, classification criteria and impact on the developing foetus. Author: P. Sawhney Coder, 10.12.19

Embryotoxicity Study in Rabbits with Oral Administration of Ro 12-3094/000. Phase II Teratological Study. Report no. B-95 154; Authors: Dr. Hummler, Mr. McKinney, 1981

Embryotoxicity Study in Rats with Oral Administration of Ro-12-3094/000. Phase II Teratological Study. Report no. B-B-90 790; Author: E. Simon, 1981

CGA 114900 Technical: Rat Oral Teratogenicity. Study no. 933117; Author: S Khalil, 1994

2. Study outine

Four groups of 25 mated Hra:(NZW) SPF rabbits were treated by oral gavage with fenpropidin from gestation day (GD) 7 to GD 28 inclusive, at dose levels of 0, 5, 10 and 20 mg/kg bwt/day (day of mating = GD 0). Clinical condition, body weight performance and food consumption of the dams were monitored throughout gestation. The dams were killed on GD 29, the ovaries were examined for the number of corpora lutea, gravid uteri were weighed and uterine contents were examined for numbers of implantations, early and late resorptions and live and dead foetuses. Viable foetuses were examined macroscopically for external and internal morphological changes, and foetal brains were examined following a single mid-coronal section. Eviscerated foetuses were processed and stained with alizarin red S and alcian blue prior to skeletal evaluation.

3. Results:

Maternal abortions/deaths: One control female was found dead on GD 16 and had 8 normal implantations *in utero*.
At 10 mg/kg bwt/day, one female was found dead on GD 23 and had 11 resorbing foetuses *in utero*, 5 of which had open eye. As development of the foetal eyelids leading to closure of the eye is not completed until approximately GD 23, it is considered that this finding may reflect the developmental age of the foetuses at the time of maternal death, rather than being indicative of abnormal development. On GD 28, a second female was found dead and had 15 apparently normal but dead foetuses *in utero.*
At 20 mg/kg bwt/day one female aborted on GD 24 and was found to have 8 dead and one partially cannibalised foetus. A second female was found dead on GD 29 and had 14 dead foetuses *in utero,* whilst a third female delivered 5 live foetuses prematurely on GD 29 and at necropsy was found to have 3 live and one dead foetuses still within the uterus.
Maternal body weight performance, food consumption and liver weights:
At 20 mg/kg bwt/day both bodyweight gain and food consumption were slightly lower than those of the concurrent controls but at 5 and 10 mg/kg bwt/day there were no effects upon on either parameter. Liver weights were unaffected at any dose level.
Litter parameters: There were no statistically significant intergroup differences in litter parameters at any dose level. Mean foetal weight was essentially similar in all groups.
Foetal examinations: In the study report, the following findings were classed as abnormalities viz.:
At 5 mg/kg bwt/day one foetus had flexed forelimbs.
At 10 mg/kg bwt/day one foetus had microphthalmia.
At 20 mg/kg bwt/day three foetuses from three separate litters had a major cardiovascular abnormality, i.e. persistent truncus arteriosus (PTA), and for two of these foetuses an interventricular septal defect (IVSD) was also recorded.
After skeletal processing three different foetuses from three different litters were recorded as having severely malaligned sternebrae but without any other major skeletal changes.

4. Discussion of study findings

At 5 and 10 mg/kg bwt/day no adverse effects on survival or development *in utero* were recorded that were considered to be related to treatment.

At 20 mg/kg bwt/day the conotruncal septum failed to develop in 3 out of 204 foetuses from 3 out of 23 litters (mean incidence/litter: 1.7%), which resulted in PTA i.e. no separation of the truncus arteriosus into the aortic and pulmonary outflow trunks. In two of these foetuses an IVSD was also identified but this was not recorded for the third foetus. However, since the single outflow trunk overrides the interventricular septum, an IVSD is an integral component of this abnormality. In the third foetus, therefore, the IVSD must have been overlooked. Whilst PTA is compatible with survival *in utero,* untreated it is incompatible with survival after birth. In these foetuses the cardiovascular abnormality occurred in isolation, there were no other visceral or skeletal abnormalities in the affected foetuses.
Historical control data provided by WIL Laboratories, for the Hra: (NZW)SPF strain of rabbit for the four years prior to the fenpropidin study (2006 – 2010), demonstrated the occurrence of sporadic instances of PTA, particularly in 2007 and 2008 (see Text Table 1). Additionally, small clusters of PTA were observed in the low and/or intermediate dose groups of two studies conducted in 2007 (see Text Table 2).
The occurrence, therefore, of three foetuses in three separate litters is not an unprecedented finding in this strain of rabbit in this laboratory, albeit not immediately contemporary with the current study.

Text Table 1: Temporal Distribution of Persistent Truncus Arteriosus in Control Foetuses

Year	**2001 - Aug 2006[a]**	**Aug – Dec 2006[b]**	**2007[b]**	**2008[b]**	**2009[b]**	**2010[b]**
Total Foetuses evaluated	7012	978	5042	3554	2526	1786
Persistent truncus arteriosus						
Foetuses (litters) Affected (No.)	1 (1)	0 (0)	**6 (6)**	**6 (5)**	**1 (1)**	**1 (1)**
Foetuses affected (%)	0.00	0.00	**0.19**	**0.13**	**0.08**	**0.11**

[a] Historical control data presented here represents 40 developmental toxicity studies (2001 – Aug 2006) conducted in Hra: (NZW)SPF rabbits obtained from Covance Research Products (Denver, Pennsylvania, U.S.)
[b] Historical control data presented for Aug 2006 – 2010 represents 69 developmental toxicity studies conducted in Hra; (NZW)SPF rabbits obtained from Covance Research Products (Kalamazoo, Michigan, U.S.)

Text Table 2: Persistent Truncus Arteriosus in Hra:(NZW) SPF Rabbit Developmental Toxicity Studies[a]

Case study group indices						
Groups	Control		Low	Mid		High
Case Study 1 (Apr 2007)						
Foetuses affected/total foetuses evaluated	0/146		3/181	1/180		0/130
Litters affected	0		3	1		0
Total Litters evaluated	17		20	19		17
Mean %/litter affected	0.0		1.8	0.5		0.0
Case Study 2 (Oct 2007)						
Foetuses affected/total foetuses evaluated	0/191		0/184	3/160		0/194
Litters affected	0		0	3		0
Total Litters evaluated	21		20	19		22
Mean %/litter affected	0.0		0.0	1.9		0.0
		Data base 1			Data base 2	
WIL historical control data		1[b]			2[c]	
Foetuses affected/total foetuses evaluated		2/2636			13/12222	
Litter affected		2			12	
Total litters evaluated		298			1394	
Range of HC mean values		0.0 – 2.1%[d]			0.0 – 2.1%[d]	

[a] All studies referenced were conducted at WIL Research Laboratories, LLC
[b] Database 1 is the database existing at the time of initial interpretation of the 2007 case study (14 studies)
[c] Database 2 is the database up to time of the fenpropidin study (69 studies); this database is a continuation of database 1
[d] Maximum mean value of 2.1% attributed to a single study in both databases, where the affected litter had only 2 viable foetuses (i.e. 50% incidence. Range of both data sets without the aforementioned study is 0.0 – 1.1% per litter.

Also in the fenpropidin study, 3 out of 204 foetuses from 3 out of 23 litters at 20 mg/kg bwt/day (mean incidence/litter: 1.6%) were recorded as having severely malaligned sternebrae, which were classified by WIL Laboratories as abnormalities. These occurred in different foetuses/litters from those with PTA. Double staining of the foetuses with alcian blue in addition to alizarin red S enabled cartilaginous structures to be examined and there was no record in the report of malalignment of the costal cartilages.

Historical control data collected between 2006 and 2014 confirmed that severely malaligned sternebrae occurred sporadically and at low incidence in this strain of rabbit, viz:

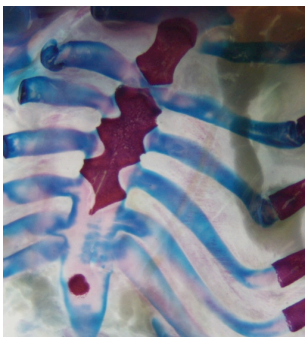
2006 – 2009: No. of control foetuses/litter examined : 12086/1375 (65 studies)
No. of foetuses/litters with severely malaligned sternebrae : 12/12
Mean incidence/litter: 0.10%; range 0.00 – 1.01%

2010 – 2014: No. of control foetuses/litter examined : 11415/1300 (62 studies)
No. of foetuses/litters with severely malaligned sternebrae : 7/7
Mean incidence/litter: 0.07%; range 0.00 – 1.06%

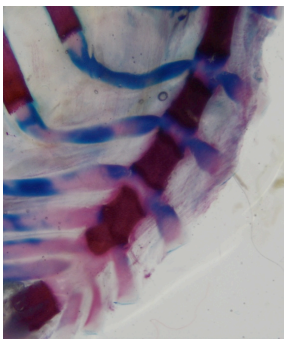
In December 2019, WIL issued a statement entitled:
"Malaligned sternebra(e) – A statement of occurrence, classification criteria and impact on the developing foetus"

In this statement it was explained that "In 2018 a re-evaluation of the justification to classify severely malaligned sternebra(e) as a malformation was undertaken and all instances of malaligned sternebrae are now classified as developmental variations, confirming that this observation is not considered to alter general body conformity, disrupt or interfere with normal bodily function, nor is likely to be incompatible with life".

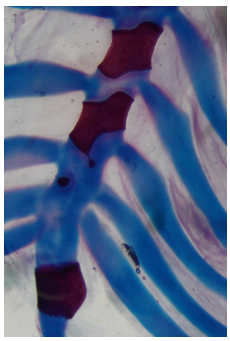
In view of the considerable change in classification, TCI asked Syngenta to obtain photographs of the affected sternebrae. The following images were supplied by WIL:



Foetus 61173/3
WIL description*:
Sternebrae malaigned
2 – 5 severe and fusion



Foetus 61119/2
WIL description*:
Sternebrae malaligned
5 severe; fusion (4-5)



Foetus 61200/5
WIL description*:
Sternebrae malaligned
5 severe, 4 moderate,
2 – 3 slight. Fusion (4-5)

* description in study report before WIL changed the classification

TCI's evaluation from these images is that the sternebral finding in foetus 61173/3 constitutes an abnormality and should **not** be re-classified as a variation, since the extent of the fusion and malalignment of sternebrae 2 – 5 might have given rise to a misshapen ribcage in post-natal life.
Foetus 61119/2 shows minimal malalignment of sternebrae; the fusion between sternebrae 4 and 5 would have had little impact as ossification continued post-natally and, therefore, this finding may be considered to be a variation.
Foetus 61200/5 shows a moderate degree of malalignment of sternebrae 4 and 5 but fusion is limited to a bridge of ossification between 4 and 5. There is an apparent curvature of the sternum between 5 and 6 but this might have been artefactual, due to necropsy procedures. The impact on post-natal life may be minimal and therefore this finding could be re-classified as a minor anomaly.

5. Toxicological significance of the foetal findings

In 1981 an embryofoetal toxicity (teratology) study in rabbits was conducted by Hoffann-La Roche and Co. Ltd, Basle, Switzerland (report number B-95 154). Groups of 20 mated random inbred Swiss Hare rabbits were treated with Ro 12-3049/000 (fenpropidin) by oral intubation from GD 7 to GD 19 (day of mating = GD 1) at dose levels of 0, 5, 12 and 30 mg/kg bwt/day . Maternal clinical condition and body weight performance were monitored during the in-life phase and the study was terminated on GD 30. Litter parameters, external appearance of foetuses and foetal body weights were recorded at necropsy and the 24-hour viability of live foetuses was assessed by incubation at 34^{o}C . Subsequently, the surviving foetuses were killed, thoracic and abdominal viscera were examined macroscopically, the eviscerated foetuses were x-rayed in the dorso-ventral and lateral positions and the skeletons were examined. Any foetus for which the skeleton could not be definitively examined was processed, stained with alizarin red S and the skeleton was re-examined.
Foetal heads were removed from non-processed foetuses, fixed in 4% formal-glacial acetic acid and examined according to a modified Wilson technique.

Results: At 30 mg/kg bwt/day, slight maternal bodyweight loss was recorded during the treatment period, but lower dose levels were unaffected.
Litter parameters were similar in all groups; mean foetal weight was marginally, but not statistically significantly reduced at 30 mg/kg bwt/day.
By current standards, the data presented in the report for foetal examinations were lacking in detail, but information was provided for visceral and skeletal structures that were considered to differ from the accepted norms for the laboratory.
A small number of foetuses with abnormalities were recorded, viz:
one control foetus with omphalocoele, and a second control foetus with a shortened snout
one foetus at 5 mg/kg bwt/day with omphalocoele and multiple skeletal abnormalities
one foetus at 30 mg/kg bwt/day with ectopic liver, stomach and intestines, missing tail and torsion of the left hind limb and a second foetus with internal hydrocephaly
There were, however, no instances of PTA or any other cardiovascular abnormality nor of abnormal sternebrae at any dose level.

In addition to the rabbit studies, two PND studies have been conducted with fenpropidin in the rat, viz:

In 1981, Hoffann-La Roche and Co. Ltd, Basle, Switzerland performed a PND study, in which a post-natal phase was included (report number B-90 790). Groups of 40 mated Füllinsdorf albino rats were treated with Ro-3049/000 (fenpropidin) via the diet from GD 7 – GD 16 (day of mating = GD 1). Fenpropidin (10%) was bound to fat globules to achieve a good mixture with the diet and the combination was designated Ro-3049/032. Based upon anticipated food intake of control rats, concentrations of Ro 3049/032 in the diet were adjusted to achieve dose levels of 0, 200, 500, and 1250 mg/kg bwt/day of Ro 3049/032,

equating to 0, 20, 50 and 125 mg/kg bwt/day fenpropidin. Maternal clinical condition, bodyweight and food consumption were monitored during the in-life phase and on GD 21 one half of the pregnant females were killed to permit examination of uterine contents. The remaining pregnant females were allowed to litter and rear their young until day 23 *post partum* (*pp*), at which time the dams and litters were killed and examined.

Results: For the females at the high dose level, food consumption progressively decreased during the treatment period such that by GD 15 – GD 17 the achieved dose level was 64 mg/kg bwt/day instead of nominal level of 125 mg/kg bwt/day. High dose level females showed bodyweight loss (10%) during the treatment period but body weight gain was slightly superior to that of the controls during the post-treatment period for all females and during the lactation period for females allowed to litter. Body weight gain of females at the intermediate dose level was slightly reduced during the treatment period, whilst that of low dose level females was similar to the control group.
Despite the marked effects on food consumption and body weight performance at the high dose level, litter parameters and foetal/pup body weights were unaffected by maternal treatment. For foetuses examined on GD 21 there was a dose-related increase in "incised" vertebral neural arches and an increase in other neural arch anomalies at the high dose level, particularly affecting cervical vertebrae. The report author considered these findings to be indicative of reduced ossification, and TCI concur with this opinion.
Two foetuses at the low dose level, one foetus at the intermediate dose level and two foetuses at the high dose level had unilateral or bilateral enlarged renal pelvis. Additionally, one foetus at the intermediate dose level had rudimentary testes, and one foetus at the high dose level had internal hydrocephaly. No macroscopic abnormalities were seen at any dose level when the offspring were necropsied on day 23 *pp*.
No instances of PTA or other cardiovascular abnormalities nor of fused sternebrae were reported at any dose level, despite the marked maternal toxicity recorded at the high dose level.

In 1994, Ciba-Geigy Ltd, Stein, Switzerland investigated the effects of orally administered CGA 114900 Technical (fenpropidin; study number 933117) to groups of 24 mated Tif: RAI.f (SPF) rats at dose levels of 0, 10, 60 and 90 mg/kg bwt/day from GD 6 – GD 15 (day of mating = GD 0). Maternal clinical condition, body weight and food consumption were monitored during the in-life phase of the study and the study was terminated on GD 21. Litter parameters, external appearance of foetuses and foetal body weights were recorded at necropsy. Approximately one half of each litter was fixed in Bouin's fluid and examined for cranial, thoracic and abdominal alterations, the other half of each litter was processed and stained with alizarin red S prior to skeletal evaluation.

Results: Maternal body weight was unaffected at any dose level but at 90mg/kg bwt/day there was a slight reduction in food intake between GD 11 and GD 16. Litter parameters and foetal body weights were similar in all groups.
One control foetus had encephalocoele with protruding tongue and open eye, and a second control foetus had acaudia.

One foetus at 90 mg/kg bwt/day had anal atresia, hydronephrosis, and ureter and bladder atresia.
No foetus at any dose level had PTA nor any other cardiovascular abnormality. Similarly no foetus had severe fusion of sternebrae. Fusion between sternebrae 1 and 2 was noted for 2 foetus at 10 mg/kg bwt/day and 2 foetuses at 60 mg/kg bwt/day, whilst fusion between sternebrae 4 and 5 was noted for one control foetus. No fusions between sternebrae were recorded at 90mg/kg bwt/day.

Having considered the available experimental animal data from the 2 rabbit and 2 rat PND studies with fenpropidin, TCI consider that fenpropidin is unlikely to have been involved in the aetiology of PTA or of "severely malaligned sternebrae" at the high dose level in the WIL rabbit study for the following reasons:

- In neither the Hoffman- La Roche (1981) rabbit study, in which the high dose level was 30 mg/kg bwt/day compared with 20 mg/kg bwt/day in the WIL study, nor the two rat studies, which also had higher top dose levels (125 reducing to 64 mg/kg bwt/day and 90 mg/kg bwt/day respectively) were any cardiovascular or severe sternebral abnormalities reported.

- In the WIL study there were no adverse effects on survival or growth *in utero*, despite evidence of maternal toxicity at the high dose level.

- The Hra:(NZW) SPF strain of rabbit, used in the WIL study has a low background history of PTA in control foetuses and of small isolated clusters (3 foetuses) of PTA in low or intermediate dose treated groups, which were unrelated to treatment. The incidence of PTA in the high dose group of the WIL study (also 3 foetuses) is in accordance with the historical data.

- Having examined photographs of the 3 foetuses that WIL described in the study report as having "severely malaligned sternebrae", TCI consider that only one of the foetuses should be classified as abnormal, since it could have had developmental implications in post-natal life. For the other two foetuses the sternebral findings were of a lesser nature and can be considered as variations/minor anomalies.

- Sternebral malalignment has been recorded in the WIL historical control data at low incidence. The occurrence of one foetus with abnormal sternebrae should not be a cause for concern in terms of compound involvement.

6. Conclusion

Having reviewed the data from two rabbit and two rat PND studies with fenpropidin provided to TCI by Syngenta Ltd., TCI are of the opinion that the occurrence of 3 foetuses with persistent truncus arteriosus and one foetus with an abnormal sternum in the high dose level of one rabbit PND study, conducted at WIL Laboratories, is not sufficient evidence to classify fenpropidin as a developmental toxin.

Similar findings were not recorded at a higher dose level in the second rabbit study conducted in a different laboratory, nor in either of the two rat studies at higher dose levels of fenpropidin.