PINOXADEN – SYNGENTA POSITION ON DEVELOPMENTAL TOXICITY

Introduction

Syngenta agrees with the UK HSE that resorptions, post-implantation loss and reduced foetal weights in the rabbit were secondary, unspecific consequences of observed maternal toxicity and that the diaphragmatic malformations seen in one study in the rabbit are unrelated to treatment and are likely to have arisen from mating between siblings.

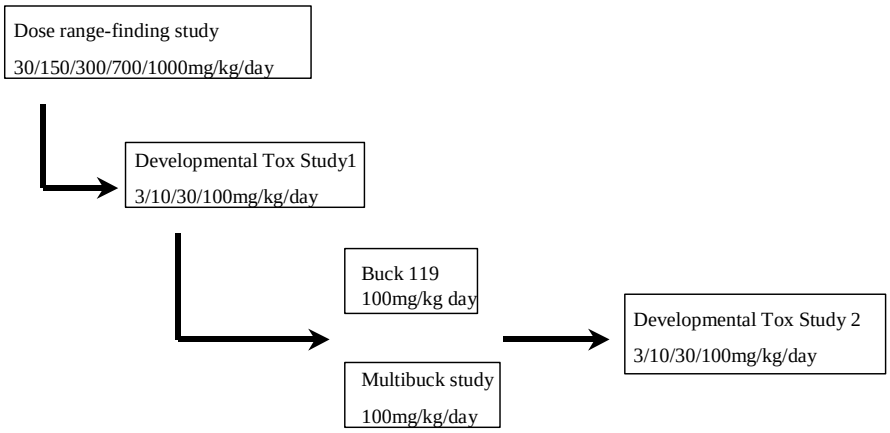
In the EFSA Conclusions on the Pesticide Peer Review, a proposal for classification of H361d: Suspected of damaging the unborn child was made on the basis of diaphragmatic effects in one rabbit study. In their comment on the CLH dossier, EFSA have explained that this was influenced by the results of the second guideline study in the rabbit where '*other effects observed (such as post-implantation loss and early resorptions) could have masked the occurrence of developmental effects*'.

Syngenta believes that the increased incidence of post-implantation loss, which was seen in the presence of maternal toxicity, would not have masked a developmental response as sufficient dams/litters were available for examination at the relevant dose level from the 3 additional developmental studies to ensure that, had a developmental effect occurred, it would have been noted. In addition, Syngenta believes that the weight of evidence points to a genetic aetiology for the findings in the diaphragm. Further details of this position are given below:

Pinoxaden – developmental toxicity studies in the rabbit

Pinoxaden has been evaluated for effects on the developing rabbit foetus in five separate studies (Figure 1). In the initial guideline developmental study 100mg/kg/day was maternally toxic, and single foetal incidences (in separate litters) of defects of the diaphragm were observed, one hernia at 30 mg/kg/day and two hernias and a fissure at 100mg/kg/day. However, semen from the same male (buck 119) had been used to inseminate all the dams in whose offspring these defects were noted, and familial relationships of dams and bucks used in this study were not fully known. Therefore, two investigative studies (at 0 and 100mg/kg/day) were carried out in order to investigate a potential genetic aetiology for the findings and to study the reproducibility of the outcome. The sequence of studies was completed with a full repeat of the first guideline developmental study using the whole range of original dose levels tested.

Figure 1: Overview of rabbit developmental toxicity studies for pinoxaden



Post-implantation loss and early resorptions

A statistically significant increased incidence of post-implantation loss was noted in high dose (100mg/kg) rabbits from the second investigative study and the second guideline study, and a non-significant increase was noted in the preliminary study at 150 mg/kg. No such increase was noted in the first guideline study or the first investigative study. The increased post-implantation loss was attributable to early resorptions, which occurred between days 7 and 16 of gestation, primarily early total litter resorptions.

Table 1: The association of early resorptions to maternal toxicity in high dose groups

Study (100 or 150mg/kg group)	Food consumption Days 7-12 % control	Body weight gain Days 7-12 High dose vs control (g)	Early resorptions (% implanation loss/litter ±SD)	Percent total litter resorptions (%)[1]
Range finder*	38.2	-64 vs. +31	62.0 ± 47.7	57.1
Initial guideline study	64.1	-72 vs. -21	7.2 ± 13.5	0
First investigative study (Buck 119)	70	-32 vs. +44	10.9 ± 26.2	5.6[2]
Second investigative study (Multibuck)	66.8	-31 vs. +38	18.5 ± 35.6	14.3[4]
Second guideline study	57.8	- 63 vs. +20	35.8 ± 48.4	35

*Dose level 150 mg/kg
[1] number of dams with total litter resorptions/number of pregnant dams x100
[2] number of resorptions the same as the control
[4] 2 of the 3 litters with no viable foetuses on GD29 were total litter losses

The two studies in which the greatest reduction in dam's food consumption was noted are the studies showing the highest percentage of total litter resorptions (highlighted in Table 1). In the other 3 studies, food consumption values were less affected. In addition the study with the highest litter loss, the range finding study, also shows the lowest food consumption suggesting a dose related effect.

Many studies have suggested a link between food restriction, (lower food consumption), and effects on pregnancy in rabbits (Clark et al 1986, Cappon et al 2005, Petrere et al 1993, Matsuzawa et al 1981, Matsuoka et al 2006, 2009, 2012) and have concluded that food deprivation affects the ability of dams to maintain pregnancy. Matsuoka et al 2006 noted that resorptions were increased when food was restricted during early organogenesis (days 6-18 of gestation) which is the same pattern as seen with pinoxaden and supports the conclusion that early resorptions seen in the pinoxaden studies are secondary to significantly lower food consumption/body weight noted in high dose animals at the start of gestation.

An increased incidence of early resorptions was noted in the high dose groups of some rabbit developmental toxicity studies with pinoxaden. This occurred in the presence of significant maternal toxicity, as evidenced by reduced food consumption and body weight gain, and follows a pattern previously noted for food restricted rabbits. Syngenta believes that these early resorptions are secondary to maternal toxicity.

Diaphragmatic hernias/ fissure and early resorptions

In the initial guideline developmental toxicity study in the rabbit, two diaphragmatic hernias and one diaphragmatic fissure were noted in individual foetuses from separate litters at 100 mg/kg and a single instance of diaphragmatic hernia was noted at 30mg/kg. There were no other reported visceral malformations in the affected foetuses and none of the litter mates of those affected showed any

significant developmental delay. Diaphragmatic hernias were not seen in the four other developmental toxicity studies in the rabbit (all dosed up to 100mg/kg or higher) or in the guideline study in the rat. This pattern (single foetus/ single litter, not reproducible) is inconsistent with that expected from a chemical teratogen (Neubert 1980, Manson and Miller, 1983).

A higher number of resorptions were noted in the second investigative and second guideline study. This is consistent with a higher maternal toxicity seen in these studies (Table 1). However, when the total number of fetuses available for examination in the investigative studies and the second guideline study is considered it is clear that there is a sufficient number to reassure that had any developmental effect resulted from dosing it would have been seen (Table 2).

Table 2: Total number of fetuses examined for visceral malformations in studies of pinoxaden

Studies	Doses	
Rabbit studies:	**30 mg/kg/day**	**100mg/kg/day**
Initial guideline study	120	128
First investigative study	-	92
Second investigative study	-	58
Second guideline study	121	72

In addition, the pre-implantation losses occurred early in gestation, prior to the formation of the diaphragm, and this together with the fact that diaphragmatic hernias are not fatal *in utero* leads to the conclusion that fetuses bearing diaphragm abnormalities would not have been preferentially resorbed and potential malformations missed.

Diaphragmatic hernia/fissure – potential genetic aetiology

Diaphragmatic hernias in rabbits are uncommon. Historical control data from the conducting laboratory revealed one instance of diaphragmatic hernia in 3,672 foetuses (664 litters). However, further evaluation of the laboratory's data revealed a total of 5 diaphragmatic hernias in 27 studies where the hernia was judged to be unrelated to test compound administration. (Table3)

Table 3: Incidence of diaphragmatic hernia noted in developmental toxicity studies in Himalayan rabbits

Individual study	Report date	Foetal incidence	Experimental group
911127	7/91	single	Low dose
922822	8/92	single	Control
922847	1/93	single	Mid dose
923154	3/93	single	High dose
942119	8/95	single	Mid dose

Thus, although reportedly rare, in the colony of Himalayan rabbit used by the laboratory conducting the pinoxaden study instances of non-treatment related diaphragmatic hernias are seen sporadically.

The diaphragmatic fissure seen at 100mg/kg was noted on the right hand side of a foetus that was smaller than its littermates. It is likely that this was a tear in the thinnest part of the diaphragm which occurred during dissection and hence does not reflect a common effect on the diaphragm. The diaphragmatic hernias were found on the left side of the foetuses and spontaneous/familial hernias in rabbits and humans have similarly been reported as occurring on the left side (Fox and Crary 1973, Greenholz 1996). In addition, Fox and Crary (1973) reported an increased frequency of spontaneous diaphragmatic hernias occurring in a rabbit colony which were attributable to an autosomal recessive genetic defect. Given the lack of certainty over the relationships of dams and bucks in the initial study, where there was no control over potential sibling matings, a genetic aetiology for the diaphragmatic hernias is possible.

Given the lack of reproducibility of the diaphragmatic hernias in four other studies, the known incidence of spontaneous diaphragmatic hernias in the colony of rabbits used by the conducting laboratory, the pattern of defects being unlike that of a chemical teratogen and the knowledge that diaphragmatic hernias attributable to an autosomal recessive genetic defect have been identified in rabbits, Syngenta concludes that the weight of evidence points to the diaphragmatic hernias in the first pinoxaden developmental toxicity study in the rabbit having a genetic aetiology and being unrelated to treatment with pinoxaden.

In addition, Syngenta believes that a sufficient number of foetuses from the two investigative studies and the repeat guideline study were evaluated at 100mg/kg /day to reassure that a potential treatment-related effect on the diaphragm would not have been missed.

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