SYNGENTA POSITION ON DEVELOPMENTAL TOXICITY

A classification for developmental toxicity category 1B is considered not required by Syngenta. Cyproconazole is currently classified for developmental toxicity as Category 2 H361 (Annex of EU Dir 67/548 (26th ATP)) and although it is acknowledged that the second rabbit study (Muller, 1991) may not have been considered as part of the data on which the current classification was agreed, this study is considered to add no significant new information. Syngenta considers that the combined data are insufficient to trigger a change to a H360D classification.

Chemicals having significant developmental toxicity can be recognized by certain well described and documented features seen in animal studies. These include a high degree of reproducibility and consistency within a database (especially within species) and a characteristic pattern of effects. At high exposures embryolethality predominates in combination with dead, growth retarded and malformed fetuses; at lower exposures developmental delay predominates (Neubert, 1980).

The database on cyproconazole developmental toxicity fails to provide a sufficient weight of the evidence for significant developmental toxicity. There is no clear consistency in the findings within and across studies and species and no discernable dose response relationship unifying embyrolethality, malformation and retardation. Moreover with cyproconazole many of the findings described are likely of a spontaneous etiology, are isolated and occur where an association to marked maternal toxicity cannot be discounted. Using established scientific criteria there is no convincing weight of the evidence that cyproconazole displays any significant developmental hazard that should lead to a classification in Category 1b; indeed the weakness of the evidence both in terms of a lack of consistency in the database and the failure to consistently and unequivocally ascribe major malformations to treatment in the study outcomes suggest that the current classification as category 2 H361 is appropriate.

This is based on:

- Some evidence of adverse findings on foetuses at maternally toxic dose levels
- In both species the incidence of hydrocephalus were present at levels reported in the HCD and did not follow a dose response
- Palatoschisis and malrotated hind limbs were observed in only 1 species (rat and rabbit respectively) and at maternally toxic doses.

These data are considered insufficient to provide **clear** evidence of an adverse effect on development, although they do provide **some** evidence of such effects, generally at dose levels producing significant maternal toxicity. In this case a Category 1b classification is not appropriate.

DEVELOPMENTAL TOXICITY ASSESSMENT

The developmental toxicity of cyproconazole has been evaluated in a number of studies, all of which are included in the EChA Annex VI Report (Proposal for Harmonised Classification & Labelling) submitted by Ireland 2014 and the EFSA evaluation and are listed in Table 1 below.

Table 1: Developmental toxicity studies conducted on cyproconazole.

Reference	Study type	Species/strain	Conducting laboratory
Becker, 1985a	Dose range finding teratogenicity study	Rat/KFM-Wistar	RCC Ltd, Switzerland
Becker, 1985b	Teratogenicity study	Rat/KFM-Wistar	RCC Ltd, Switzerland
Machera, 1995	Developmental toxicity	Rat/Wistar	Benaki phytopathological Institute, Greece
Becker, 1986	Teratogenicity study	Rabbit/Chinchilla (Kfm: Chin hybrid)	RCC Ltd, Switzerland
Muller, 1991	Teratogenicity study	Rabbit/NZW	Hazelton Deutschland GmbH, Germany

MATERNAL EFFECTS:

A summary of the effects of cyproconazole administration in dams of two species (rats and rabbits) in developmental toxicity studies have been evaluated and summarized in Table 2. Although treatment related maternal mortality was not observed in any of the studies with cyproconazole, marked effects on group mean bodyweight and bodyweight gain were observed, particularly at the onset of dosing, which occurs early in gestation. The effect on bodyweight and bodyweight gain is supported by a marked decrease in food consumption during the same period.

In the rat, administration of cyproconazole to dams at doses of 24 mg/kg/day or higher in two studies, resulted in markedly lower group mean bodyweight gain compared to controls at the onset of dosing (gestation days 6-11) (Becker, 1985a and b). It is acknowledged that maternal bodyweight, which has not been corrected for uterine contents can be influenced by the uterine contents if resorptions occur, however, effects on maternal bodyweight at the onset of dosing when fetal weights are insignificant do not influence the maternal bodyweight. Therefore in the rat, lower bodyweight gains observed in dams on gestation day 6-11 can be considered a marker of maternal toxicity.

In the rabbit, a consistent group mean bodyweight loss was noted in both studies at 50 mg/kg/day during gestation days 6 to 9, compared to a bodyweight gain in control animals (Becker, 1986 and Muller, 1991).

Table 2: A summary of maternal toxicity findings from developmental toxicity studies with cyproconazole

Finding	Prelim study Rat (Han Wistar) (Becker, 1985a)	Rat (Han Wistar) (Becker, 1985b)	*Rat (Wistar)* *(Machera, 1995)$*	Rabbit (NZW) (Muller, 1991)	Rabbit (Chinchilla) (Becker, 1986)
Dose levels	0, 7.5, 30, 75, 120	0, 6, 12, 24, 48	*0, 20, 50, 75*	0, 2, 10, 50	0, 2, 10, 50
Maternal NOAEL	N/A	6 mg/kg/day	*Not assigned <20 mg/kg/day*	10 mg/kg/day	10 mg/kg/day
Developmental NOAEL	N/A	12 mg/kg/day	*<20 mg/kg/day*	10 mg/kg/day	50 mg/kg/day
Maternal mortality	None	None	*None*	Not considered treatment related 1at 10 mg/kg/day 1 at 50 mg/kg/day	None
Clinical signs	None	None	*Not reported*	Low water consumption at 50 mg/kg/day Others not considered treatment related	None
Bw gain D6-11 (as % (for uterine contents) controls)	↓29% (7.5mg/kg/day) ↓29% (30mg/kg/day) ↓41% (75mg/kg/day) ↓82% (120 mg/kg/d)	As per control (6mg/kg/day) ↓18% (12mg/kg/day) ↓29% (24mg/kg/day) ↓35% (48 mg/kg/day)	*↓37% (20mg/kg/day)* *↓37% (50mg/kg/day)* *↓63% (75mg/kg/day)*	Days 6-9 As per control (2mg/kg/day) ↓22.5% (10mg/kg/day) ↓152% (50mg/kg/day)*	Days 6-9 As per control (2mg/kg/day) As per control (10mg/kg/day) ↓254% (50mg/kg/day)^
Corrected bw gain (as % controls)	↓27% (7.5mg/kg/day) ↓11% (30mg/kg/day) ↓20% (75mg/kg/day) ↓25% (120 mg/kg/day)	No effects	*Not reported*	Data not available	No effect
Food consumption (as % controls)	Day 6-11 ↓11% (7.5mg/kg/day) ↓11% (30mg/kg/day) ↓22% (75mg/kg/day) ↓36% (120 mg/kg/day)	Day 6-11 ↓5% (6mg/kg/day) ↓6% (12mg/kg/day) ↓12% (24mg/kg/day) ↓18% (48 mg/kg/day)	*Not reported*	Day 6-9 As per control (2mg/kg/day) As per control (10mg/kg/day) ↓32% (50mg/kg/day)	Day 6-9 As per control (2mg/kg/day) As per control (10mg/kg/day) ↓73% (50mg/kg/day)

N/A – not applicable, NOAEL are not set based on dose range finding studies, however maternal toxicity evident by lower maternal bodyweight gain was present at 30 mg/kg/day

Corrected bw gain – takes into account the uterine content weight at termination, removing the confounding factor of lower fetal weight or foetal numbers on terminal bodyweight.

* - Rabbits at 50 mg/kg/day had a group mean weight loss of ~37g during days 6-9, compared to a group mean weight gain in the control group (~71g).

^ - Rabbits at 50 mg/kg/day had a group mean weight loss of ~57g during days 6-9, compared with a group mean weight gain in the control group (~37g).

$ - There are deficiencies in the Machera study which are highlighted within this document. An additional dose group of 100 mg/kg/day was used as a preliminary phase where marked maternal toxicity was evident as clinical signs, further information is not available. Bodyweight data were not included in the original paper but provided in personal communications with the Author.

FOETAL EFFECTS:
The foetal data from the teratogenicity studies listed in Table 1 have been collated and reviewed and included in a summary table (Table 3). This table excludes all findings which were:

- Present only in control animals.
- Present in treated groups at the same incidence as concurrent control.
- Present in treated groups at the same incidence in historical control data (HCD) (where this information was available and clear to interpret).
- Not following a dose response (i.e. identified at **low** frequency in low and mid dose groups, but not at high dose).

Table 3: An updated summary of implantation losses and key foetal findings from developmental toxicity studies with cyproconazole

Finding	Prelim study Rat (Han Wistar) (Becker, 1985a)	Rat (Han Wistar) (Becker, 1985b)	*Rat (Wistar)* *(Machera, 1995)$*	Rabbit (NZW) (Muller, 1991)	Rabbit (Chinchilla) (Becker, 1986)
Dose levels	0, 7.5, 30, 75, 120	0, 6, 12, 24, 48	*0, 20, 50, 75*	0, 2, 10, 50	0, 2, 10, 50
Maternal NOAEL	N/A	6 mg/kg/day	*Not assigned <20 mg/kg/day*	10 mg/kg/day	10 mg/kg/day
Developmental NOAEL	N/A	12 mg/kg/day	*<20 mg/kg/day*	10 mg/kg/day	50 mg/kg/day
Dams with total resorptions	3 (120 mg/kg/day) 2 (75 mg/kg/day)	1 (48 mg/kg/day)	*None*	Not considered treatment related	None
Post implantation loss	54.2% (120 mg/kg/day) 55.6% (75 mg/kg/day) 24.2% (30 mg/kg/day) 2.1% (7.5 mg/kg/day) 7.8% (control)	34.2% (48 mg/kg/day) 22.2% (24 mg/kg/day) 2.9% (12 mg/kg/day) 4.2% (6 mg/kg/day) 5.0% (control)	↑ *Post-implantation loss not calculated*	No treatment related effects	16.4% (50 mg/kg/day) 7.0% (10 mg/kg/day) 0.8% (2 mg/kg/day) 2.4% (control)
Tail reduced/rudimentary				3 (50 mg/kg/day)	
Malrotated hind limbs				4 (50 mg/kg/day) 1 (10 mg/kg/day)	
Lumbar vertebrae absent				3 (50 mg/kg/day)	
Hydrocephalus internus		1 (48 mg/kg/day) 1 (24 mg/kg/day)	*16 (75 mg/kg/day)* *10 (50 mg/kg/day)* *5 (20 mg/kg/day)*		1 (50 mg/kg/day) 1 (10 mg/kg/day) 1 (2 mg/kg/day)
Palatoschisis	10 (120 mg/kg/day) 1 (75 mg/kg/day) 1 (30 mg/kg/day)	1 (48 mg/kg/day)	*9 (75 mg/kg/day)* *11 (50 mg/kg/day)* *2 (20 mg/kg/day)*		
Runt, including palatoschisis, hydrocephalus		1 (48 mg/kg/day)			
Supernumerary ribs		% increased incidence 24 & 48 mg/kg/day			

Actual foetal numbers presented, not litter incidence

N/A – not applicable, NOAEL are not set based on dose range finding studies, however maternal toxicity evident by lower maternal bodyweight gain was present at 30 mg/kg/day

$ - There are deficiencies in the Machera study which are highlighted within this document. An additional dose group of 100 mg/kg/day was used as a preliminary phase where marked maternal toxicity was evident as clinical signs, further information is not available at this dose 7/10 litters were totally resorbed.

From the data in Table 3 the findings of concern are evaluated below:

Total resorptions - there are no effects of cyproconazole administration on the number of dams with total litter resorptions in rabbits. In rats, total resorptions were associated with high doses of cyproconazole in the preliminary range finding study only (≥75 mg/kg/day), a single incidence was also observed at 48 mg/kg/day in the main study (Becker, 1985b), the association with treatment in an isolated incidence is uncertain.

Post-implantation loss – in rabbits there are no consistent treatment related effects of cyproconazole administration on post-implantation loss. Increases were observed at 50 mg/kg/day in the first study (Becker, 1986), but there was no increase at the same dose level in the later study by Muller (1991). In rats, a dose related increase in post-implantation loss was observed in both studies. The majority of losses were documented as early embryonic and could be a consequence of maternal toxicity manifested as marked effects on bodyweight gain in the dam during the early dosing period (gestation days 6-11).

Palatoschisis - was observed in all rat studies, with a generally increased frequency at high dose levels in the dose range finding study only. For the OECD guideline study in the rat (Becker, 1985b) a single incidence of palatoschisis was observed at the high dose (48 mg/kg/day) and another incidence was recorded at the same dose level in a runt. Single incidences of palatoschisis are reported in the cumulative historical control data presented in the study report (n=3), suggesting that this may be a background finding (Becker, 1985b). Palatoschisis was only seen at maternally toxic dose levels. Palatoschisis was not identified in either of the two studies conducted in rabbits.

Malrotated hind limbs - was identified only in one of the two rabbit teratogenicity studies at dose levels of 10 and 50 mg/kg/day (Muller, 1991). At a dose of 10 mg/kg/day the incidence in the study was within the range seen in the HCD (n=2). The detail surrounding the malrotated limbs in the study report is limited, it is not reported if the finding is uni or bilateral or the severity of the malrotation. Malrotation of the limbs is generally seen as a subtle inward rotation of the limb and in many cases is a result of reduced amnion levels and/or positioning of the foetus in the uterus, it is unlikely to be caused by skeletal malformations, but could be a consequence of maternal toxicity.

Hydrocephalus internus - was observed in the OECD guideline study conducted in rats (Becker, 1985b) at a single incidence at 24 and 48 mg/kg/day, in addition 1 foetus at 48 mg/kg/day described as a runt also had hydrocephalus. Given this finding was not seen in the dose range finding study conducted at a much higher dose (120 mg/kg/day), and was also observed in historical control data (n=2), the relationship to treatment is unclear. In rabbits, a single incidence of hydrocephalus was reported at each dose level (2, 10 and 50 mg/kg/day) (Becker, 1986). The incidence did not demonstrate a dose response despite a 25 fold increase in dose level between the low and high doses, is consistent with incidences observed in historical control animals of the same strain (n=9) and was not seen in the second rabbit study conducted using identical dose levels. Thus this finding in the rabbit is considered to be incidental to treatment.

STUDY ACCEPTABLILITY:

The acceptability of the developmental toxicity studies is summarized in Table 4:

Table 4: A summary of the acceptability of the developmental toxicity studies of cyproconazole.

Study	GLP	Guidelines	EFSA Acceptablity	Klimisch Reliability Score	Comments
Dose range finding teratogenicity study – rat (Becker, 1985a)	No	Range finding study	Yes, supplementary	2	Dose range finding study
Teratogenicity study – rat (Becker, 1985b)	**Yes**	**OECD 414**	**Yes**	**1**	
Developmental toxicity – rat (Machera, 1995)	No	No	Yes, supplementary	2	Maternal parameters and individual findings not reported
Teratogenicity study – rabbit (Becker, 1986)	Yes	OECD 414	[Yes]	1	Inconsistencies in determined concentrations of dose preparations→no confidence in dose received
Teratogenicity study (Muller, 1991)	**Yes**	**OECD 414**	**Yes**	**1**	

The study reported by Machera (1995) (Dose levels 0, 20, 50, 75mg/kg bw/day) is considered not to be reliable for the assessment of the developmental toxicity potential of cyproconazole for the following reasons:

- It was not conducted according to Internationally recognized test guidelines
- It was not conducted to Good Laboratory Practice (GLP)
- The purity of the test item was not stated
- Dose preparations were not analysed
- The animals were grouped housed throughout the duration of the study
- Humidity controls were not available
- In the pilot study using a single dose level (100 mg/kg/day) and no control, clinical signs were not reported but the general condition of the dams was described as “not very good” and 7/10 litters were totally resorbed, indicating the maximum tolerated dose was exceeded.
- A NOEL for maternal toxicity or developmental endpoints was not established
- Level of significance of statistical calculations were not stated

- Animals from the top dose gained less weight (-17%) before initiation of dosing (days 0-6) than animals from the other groups.
- Historical Control Data for the strain and laboratory were not available
- Scientific interpretation of the data is not supported by actual data
- The conclusions drawn from the study are not justified since fundamentally important factors such as maternal toxicity during treatment period were not adequately considered

Bodyweight and bodyweight gain data were not reported in the paper, however a summary of the maternal weight gain was presented in a communication from the author, although individual animal data were not provided. These data indicate a significant effect on maternal weight gain during days 6-11 (up to -63%) at 75 mg/kg bw/day. This initial reduction, coupled with a significantly lower number of foetuses per litter and significantly lower foetal bodyweight (-22% at 75 mg/kg/day), are indicative of significant maternal toxicity at this dose level.
A comparison of this study with the Syngenta study of Becker (1985b) has been made previously (Muller 1996). This comparison also concluded that there were uncertainties with respect to the reproductive performance of the rat strain (for example, a decrease in the number of implants was reported for all treated groups, despite implantation occurring before dose initiation), and that reproductive and developmental effects were only seen at doses where maternal toxicity was evident.

During the EFSA evaluation of Cyproconazole, the DAR considered this study as “supplementary information only”. This study is considered unreliable for assessment of the teratogenic potential of cyproconazole.

SUMMARY AND CONCLUSION

Chemicals having significant developmental toxicity can be recognized by certain well described and documented features seen in animal studies. These include a high degree of reproducibility and consistency within a database (especially within species) and a characteristic pattern of effects. At high exposures embryolethality predominates in combination with dead, growth retarded and malformed fetuses; at lower exposures developmental delay predominates (Neubert, 1980).

The database on cyproconazole developmental toxicity fails to provide a sufficient weight of the evidence for significant developmental toxicity. There is no clear consistency in the findings within and across studies and species and no discernable dose response relationship unifying embyrolethality, malformation and retardation. Moreover with cyproconazole many of the findings described are likely of a spontaneous etiology, are isolated and occur where an association to marked maternal toxicity cannot be discounted (see summary Table 5).

Table 5: A summary of the developmental toxicity studies of cyproconazole

Finding	**Study**				
	Prelim study Rat (Becker, 1985a)	**Rat (Becker, 1985b)**	***Rat (Machera, 1995)***	**Rabbit (Muller, 1991)**	**Rabbit (Becker, 1986)**
Maternal toxicity	≥7.5 mg/kg/day	≥6 mg/kg/day	*<20 mg/kg/day*	≥10 mg/kg/day	≥10 mg/kg/day
Post-implantation loss	Yes (early) in conjunction with maternal bodyweight effects	Yes (early) in conjunction with maternal bodyweight effects	*Yes at maternally toxic dose levels*	None	Yes (early) in conjunction with maternal bodyweight effects
Palatoschisis	Yes at maternally toxic dose levels (120 mg/kg/day; single incidence only at 75 & 30 mg/kg/day)	Single incidence at 48 mg/kg/day, (likely spontaneous)	*Yes at maternally toxic dose levels (75, 50 & 20 mg/kg/day)*	No	No
Malrotation of limbs	No	No	*No*	Yes at maternally toxic dose levels (50 mg/kg/day); single incidence at 10 mg/kg/day	No
Hydrocephalus	No	Single incidences at 48 and 24 mg/kg/day (likely spontaneous), not see at higher doses in prelim study	*Yes at maternally toxic dose levels (75, 50 & 20 mg/kg/day)*	No	Single incidences, no dose response (likely spontaneous)

Using established scientific criteria there is no convincing weight of the evidence that cyproconazole displays any significant developmental hazard that should lead to a classification in Category 1b; indeed the weakness of the evidence both in terms of a lack of consistency in the database and the failure to consistently and unequivocally ascribe major malformations to treatment in the study outcomes suggest that the current classification as category 2 H361 is appropriate.

This is based on:

- Some evidence of adverse findings on foetuses at maternally toxic dose levels
- In both species the incidence of hydrocephalus were present at levels reported in the HCD and did not follow a dose response
- Palatoschisis and malrotated hind limbs were observed in only 1 species (rat and rabbit respectively) and at maternally toxic doses.

These data are considered insufficient to provide **clear** evidence of an adverse effect on development, although they do provide **some** evidence of such effects, generally at dose levels producing significant maternal toxicity. In this case a Category 1b classification is not appropriate.

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