**SYNGENTA POSITION ON DEVELOPMENTAL TOXICITY**

Syngenta agrees with the CRD proposal of category 2 for developmental toxicity. This conclusion is based on the observance of skeletal variations associated with delayed development, variations in the urinary tract and kidney, and foetal malformations (cleft palate in the rat) at the highest doses that were associated with severe maternal toxicity. As described in the CLH report, these variations occurred in a dose-responsive manner and are attributed to paclobutrazol administration. Therefore Syngenta agrees that classification as Category 2 (H361d) is appropriate.

The observation of cleft palate that are considered treatment related are ONLY made at dose levels that are significantly toxic to the dam (single incidences at low dose levels are within historical control range and considered spontaneous). It is therefore considered that a Category 2 (H361d) classification is the most appropriate for this substance.

The following detailed comments refer specifically to the observance of cleft palate in the database and its contribution to the overall weight of evidence.

**RAT**

**Regulatory studies**

Two regulatory rat developmental toxicity studies have been conducted (CTL/P/842 in 1983 & CTL/P/997 in 1984). The preliminary study to CTL/P/842 was reported as an embryotoxicity study in 1987 (CTL/P/1765).

**First rat study**

CTL/P/842 was reported in 1983. Rats were administered paclobutrazol in corn oil at doses of 0, 40, 100 and 250 mg/kg/d on days 6-15 of gestation. Five out of 25 dams died at 250 mg/kg/d (20% mortality). Surviving animals showed a slight decrease in body weight gain and food consumption during dosing, with recovery towards control values seen after the dosing period. Liver enlargement was also observed at this dose level. At 100 mg/kg/d, less marked decreases in body weight gain and food consumption were observed during dosing. No effects were observed at 40 mg/kg/d. There were no adverse treatment-related effects on litter parameters.

Cleft palate was observed at 40 mg/kg/d (n = 1) and 250 mg/kg/d (n = 3 foetuses from 2 litters). In one of the 3 foetuses with cleft palate at 250 mg/kg/d, exencephaly, bilateral anophthalmia and cleft lip were also observed.

The single incidence of cleft palate at 40mg/kg/d is considered to be spontaneous as it is within the historical control range (evidenced by the incidence in the control group of the preliminary rat study – see below). The only incidences of treatment related cleft palate are seen at a dose level that is severely toxic to the dams, resulting in 20% mortality.

**Second rat study**

CTL/P/997 was conducted in 1984. Rats were administered paclobutrazol at 0, 2.5, 10, 40 or 100 mg/kg/d in corn oil on days 7-16 of gestation. There was no evidence of maternal toxicity in this study nor were there any incidences of cleft palate.

**Preliminary rat study**

A preliminary study, conducted prior to the main studies, was reported in 1987 (CTL/P/1765). This study was not in the original data submission since it was preliminary and investigative in nature. Paclobutrazol was administered at doses of 0, 80, 160 or 240 mg/kg/d in corn oil on days 6-15 of gestation. Cleft palate was observed in 8 foetuses: 1/130 in controls, 1/110 at 80 mg/kg/d, 0/118 at 160 mg/kg/d and 6/85 (all from 1 litter) at 240 mg/kg/d. In addition to cleft palate, the foetus at 80 mg/kg/d had cleft lip and anophthalmia. There was evidence of maternal toxicity as two out of ten died at 240 mg/kg/d (20% mortality) with surviving animals showing a marginally reduced bodyweight gain and food consumption which persisted on completion of dosing. At 80 mg/kg/d, a small reduction in bodyweight gain was seen that reversed after the dosing period.

The single incidence of cleft palate at 80mg/kg/d is considered to be spontaneous as it is within the study control range. The only incidences of treatment related cleft palate are seen at a dose level that is severely toxic to the dams, resulting in 20% mortality.

**Published data**

Vergieva (1998) published a study that investigated the effects of paclobutrazol or benomyl on foetal development in the Wistar rat. The paper provides very limited methodology and reporting of data compared to a GLP study, and as such may be of limited reliability. Paclobutrazol was administered at dose levels of 50 and 200 mg/kg/d on days 6 through 15 of gestation (repeated dosing), and at 200 and 500 mg/kg on days 7, 9, 11 or 13 (single doses). A limited number of dams were included in experimental groups (n=5-12). Animals were sacrificed on day 21 and the following parameters were examined: number of *corpora lutea,* live foetuses, resorptions, and autolyses. Foetuses were weighed and examined for external abnormalities; two thirds underwent skeletal examinations and the remainder received soft tissue examinations. Not all experimental data were presented in the paper.

Repeated dosing: Maternal body weight gain was depressed at both 50 and 200 mg/kg/d. At 200 mg/kg/d, 2/116 foetuses had open eyes and/or micrognathia, and 2/39 had cleft palate. Administration of 50 mg/kg/d was not associated with any treatment related anomalies.

Single dosing: After single treatment with 200 mg/kg on day 11, 14/76 foetuses were observed with open eyes and/or micrognathia, and 12/25 foetuses were observed with cleft palate. After single treatment with 500 mg/kg on day 11, 21/58 foetuses were observed with gross anomalies, and cleft palate was observed in all the foetuses. Shortened mandibula was also considered to be related to treatment at 500 mg/kg following treatment on days 9 and 13.

Table 1.Summary of cleft palate incidences reported in Vergieva\* (1998).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dose (mg/kg/d) | 200 | | 500 | | | |
| days | 6-15 | 11 | | 9 | 11 | 13 |
| No. Dams inseminated/pregnant | 13/11 | 7/6 | | 8/8 | 11/6 | 8/7 |
| Foetuses (litters) examined | 116(11) | 70(6) | | 90(8) | 58(6) | 84(7) |
| Cleft palate No./  % | 2/39(2/11)  5.1(18) | 12/25(4/6)  48(66.6) | | 4/32(4/8)  12.5(90) | 25/25(6/6)  100 | - |

\*Only groups with reported incidences of cleft palate included in table.

There are several confounding factors which reduce the reliability of the Vergieva (1998) study. First, in the repeated dose study at 200 mg/kg/day, it is impossible from the data presented to ascertain if the malformations in the few number of animals (2) are spontaneous or treatment related since there was no discussion of control animals or historical control data. Second, it does not seem logical that in the repeated dose study at 200 mg/kg/day, 2/39 foetuses were diagnosed with cleft palate, while a single dose of 200 mg/kg on day 11 produced 12/25 foetuses (noting that in the repeated dose study, treatment occurred on day 11 as well). Also, in the single dose study, cleft palate was reported to be observed when dosing occurred on days 9 or 11, but presumably not on day 13 of gestation. Palate closure in the rat typically occurs on about days 16-17, and most compounds that cause cleft palate are active from about days 12 to 17, so it is difficult to understand why a compound that caused 100% cleft palate on day 11 would be inactive on day 13. This inconsistency questions the biological plausibility of the reported findings. Minimal maternal toxicity was reported in dams administered 200 and 500 mg paclobutrazol/kg/d, in contrast to all other studies which demonstrate severe clinical toxicity and mortality at doses greater than 200 mg/kg/day. Additional findings which raise questions about the robustness of the study design include:

* Pregnancy of only 6/11 dams in the 500 mg/kg day 11 group
* Higher corpora lutea mean in the paclobutrazol-treated animals compared to the benomyl group; corporea lutea would not be altered by treatment as it occurs before dosing began
* Analysis of dose preparations and technical material not described
* Not all of the endpoints described in the Materials & Methods reported
* Clinical signs of toxicity not reported in dams.

Overall, the findings within the publication appear to be inconsistent which brings both their reliability and biological plausibility into question.

**RABBIT**

In the first rabbit developmental toxicity study (CTL/P/861),paclobutrazol was administered in corn oil during days 6-18 of gestation at0, 25, 75 and 125 mg/kg/d. Difficulties in mating were encountered in this study with the number of pregnant females falling below what would normally be required in this type of study (number pregnant was 9, 12, 13, and 8/18 animals respectively). Nevertheless, two animals died in each of the control, 75 mg/kg/d and 125 mg/kg/d dose groups, with the deaths not considered to be treatment-related. During the dosing period, animals in the 75 mg/kg/d and 125 mg/kg/d dose groups gained slightly less weight than controls with recovery to control levels seen in the post-dosing period. There were no incidences of cleft palate reported.

In the second rabbit developmental toxicity study (CTL/P/1460), paclobutrazol was administered in corn oil at doses of 0, 25, 75 or 125 mg/kg/d on days 7-19 of gestation. There was one non-treatment-related death at 75 mg/kg/d. Maternal toxicity was limited to initial body weight loss during dosing at 125 mg/kg/d with subsequent weight gain very close to control values. Cleft palate and encephalocoele (a neural tube defect) were found in a single foetus in the 75 mg/kg/d dose group. This single incidence of cleft palate in the mid dose group is considered to be spontaneous and unrelated to treatment. Cleft palate was not observed at a higher dose level in this study, nor was it observed in the first study which included the same and higher dose level.

**WEIGHT OF EVIDENCE**

In the rat, administration of paclobutrazol at significantly maternally toxic doses induces cleft palate. In 2 out of the 3 developmental toxicity studies conducted with paclobutrazol in the rat, severe maternal toxicity including mortality was observed in dams at doses of 240-250 mg/kg/d. In these same studies, cleft palate was observed in 6/86 foetuses and 3/234 foetuses at 240 and 250 mg/kg/d, respectively. A published study reported findings of cleft palate at similar and higher levels, but was considered unreliable due to lack of consistency and biological implausibility. No treatment related incidences of cleft palate were seen in the rabbit studies. At doses below maternally toxic levels, cleft palate was isolated to single incidences, which were considered to reflect the background incidence and hence to be spontaneous and not treatment related.

Hence the overall picture is one of observation of treatment related cleft palate only in the rat, and only at dose levels that are severely maternally toxic, and clearly exceeding a maximally tolerated dose. The EChA definition for classification in Category 1b is “Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on *development in the absence of other toxic effects*, or if occurring together with other toxic effects the adverse effect on reproduction *is considered not to be a secondary non-specific consequence* of other toxic effects” (EChA 2015). Treatment related cleft palate was only observed at the highest doses in conjunction with severe maternal toxicity, such that the dams were significantly compromised in their ability to support the developing foetus. Hence the adverse effects may be considered to be a secondary non-specific consequence of the severe maternal toxicity, and a Category 1b classification is inappropriate.

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**Table 2: Summary of findings of cleft palate in developmental toxicity studies with Paclobutrazol**

|  |  |  |  |
| --- | --- | --- | --- |
| Reference | Study type/dose levels | Observations | Comments |
| Rat | | | |
| CTL/P/1765 Preliminary study (1987) | Rat (Wistar)  0, 80, 160 & 240 | 240 mg/kg/d: Maternal toxicity (20% mortality)  Cleft palate 6/85 (all from a single litter)  80 mg/kg/d: Small reduction in maternal bodyweight gain  Cleft palate (1/110)  0 mg/kg/d: Cleft palate (1/130) | Incidences at 0 and 80mg/kg/d considered spontaneous and not treatment related. |
| CTL/P/842  Main Study (1983) | Rat (Wistar)  0, 40, 100 & 250 | 250 mg/kg/d: Maternal toxicity (20% mortality)  Cleft palate - 3/234 at 250 (1 of the fetuses had multiple malformations exencephaly, anophthalmia, cleft lip)  40 mg/kg/d: Cleft palate 1/297 | Incidence at 40mg/kg/d considered spontaneous and not treatment related. |
| CTL/P/997 Main study (1984) | Rat (Wistar)  0, 2.5, 10, 40 & 100 | No maternal toxicity  No cleft palate |  |
| Vergeiva 1998 | Rat Wistar  Repeat dose: 50 or 200mg/kg/d GD 6-15  Single dose: 200 or 500 mg/kg on day 7 or 9 or 11 or 13 | n=5-12 dams/grp  Maternal effects reduced bw gain≥50mg/kg repeat dose  Repeat dose (200 mg/kg) – Cleft palate 2/39  Single dose: 200 mg/kg d11 Cleft palate 12/25  500 mg/kg d9 Cleft palate 4/32  500 mg/kg d11 Cleft palate 25/25 | Several inconsistencies were noted in study making interpretation of results unreliable |
| Rabbit | | | |
| CTL/P/1861 (1983) | Rabbit NZW  0, 25, 75 & 125 | 125 mg/kg/d: slightly decreased maternal body weight gain  Single incidence malformations including clubbed hindpaws, exencephaly, vertebral defects  75 mg/kg/d: slightly decreased maternal body weight gain | Inadequate - no pregnant dams for full evaluation |
| CTL/P/1460 | Rabbit  NZW  0, 25, 75, 125 | 125 mg/kg/d: decreased maternal body weight gain initially with recovery to levels comparable to controls  75 mg/kg – Cleft palate and exencephaly 1/103. | Cleft palate in mid dose not considered treatment related. |