REGULATORY TOXICOLOGY

POSITION PAPER

Subject :

Diflufenican

CONTENTS :

Comments on the CLH dossier
proposing no classification
for reprotoxicity

[redacted]

We agree with the conclusion of the Dossier Submitter that Diflufenican does not require classification for reprotoxicity, based on the perinatal mortality observed in the two-generation study. However, there are some inaccuracies and inconsistencies which none the less require comment.

On pages 10 and 11 of the CLH report, the treatment of the P0 and F1 animals is implied to have ceased between the end of weaning of the first litter of pups and the recommencement of treatment prior to mating to produce the second litter of pups in the F1 and F2 generations, respectively. In fact, this is not correct, as dietary administration of X was continuous from the start of the pre-mating period to sacrifice of the respective adult animals after the weaning of the F2 pups.

On page 10, in the listing of perinatal mortality in the F0 generation, one female at 500 ppm is listed as having died on day 18. In fact, this animal died on day 18 of the mating period and was not pregnant; this animal should not be incuded in any listing of perinatal mortality. In the same paragraph, at 12500 ppm, the description of the number of mortalities is unclear. It would better be written as “12500 ppm, second mating: 1 female (humane sacrifice) at postnatal day 8 and one female at gestation day 22.” Similarly, in the description of the F1 mortalities, the description of mortalities at 12500 ppm would be better written as “12500 ppm, first mating: 1 female (humane sacrifice) at postnatal day 3 and 1 female at postnatal day 8; second mating, two females on postnatal day 1.”

The tables listing litter data for the F1 and F2 pups are largely correct. However, it must be noted that the litter size at birth for each of the F1 and F2 litters is in fact listed incorrectly; the figures entered into the table (i.e., 11.8, 12.0, 12.0, and 11.9 for 0, 500, 2500, and 12500 ppm in the F1A litters) for litter size at birth are actually the litter size on postnatal day 21. The correct litter sizes at birth are listed on page 94 and following of the study report, and this table should be corrected.

Of the females that died during the study, it is important to separate those which could be related to dystocia from those which cannot be considered dystocia.

Dystocia is commonly defined as prolonged or difficult parturition. Thus, animals which die or are humanely sacrificed during parturition, or which die or are humanely sacrificed with parturition incomplete, can be considered potentially related to dystocia. All other animals should be considered as mortalities which are not related to dystocia.

As stated above, at 500 ppm in the F0 generation, one female was humanely sacrificed on mating day 18. As this animal was not pregnant, this cannot be considered a dystocia-related death.

At 2500 ppm, one F0 female was found dead on day 22 of the second gestation period. None of this dam’s 15 fetuses had been delivered. Although the cause of death could not be determined, it is possible that this death could be linked to dystocia, and thus this animal cannot be excluded.

At 12500 ppm, one F0 female was humanely sacrificed on postnatal day 8 of the second littering. This dam had previously delivered all of her fetuses with no indication of a delay in parturition or a longer-than-normal gestation period, although all of the pups had died by postnatal day 7. This dam was in poor condition due to unexplained hind limb paralysis. There is at best an equivocal link between the physical condition of this animal and dystocia, especially as all of the fetuses had been delivered at the expected time.

Another F0 female was found dead on gestation day 22 of the second littering. This animal had not delivered any of her fourteen fetuses. Although the cause of death could not be determined, dystocia cannot be ruled out.

An F1 female at 12500 ppm was found dead on postnatal day 8 of the first littering. She had only delivered two fetuses, but at necropsy it was clear that she had delivered her entire litter. As the pups were delivered at the expected time and there were no undelivered fetuses, this mortality on postnatal day 8 cannot be described as dystocia.

Another F1 female at 12500 ppm was humanely sacrificed on postnatal day 3 of the first littering, with clinical and necropsy findings indicating prolonged parturition, and thus this animal must be considered a case of dystocia.

Finally, two F1 females at 12500 ppm were found dead on postnatal day 1 of the second littering. Both of these dams had incomplete delivery, and while it is not clear whether they were suffering difficult or prolonged parturition it cannot be excluded.

Thus, the animals which can be concluded to have possibly or clearly suffered from difficult or prolonged dystocia or incomplete parturition are:

- At 2500 ppm, one F0 female at the first littering
- At 12500 ppm, one F0 female at the second littering, one F1 female at the first littering, and two F1 females at the second littering.

As stated in the CLH dossier, historical control data complied from other reproduction studies conducted in the same laboratory showed cases of dystocia in two of the 10 studies available. Thus, the one animal observed at 2500 ppm can be considered to be within the historical control incidence and not related to treatment.

Dystocia may be a result of an alteration in endocrine control of gestation and parturition, or otherwise of uterine function. However, examination of the reproductive tracts both in the 2-generation study and in other repeat-dose studies conducted with diflifenican showed no macroscopic or microscopic abnormalities which would indicate an alteration in either endocrine function or in overall reproductive function.

Overall health status of an animal may also affect parturition. As noted in the CLH dossier, body weight and body weight gain were sharply impacted by diflufenican administration during the 2-generation study. In fact, the body weight data from the F0 and F1A adults shown in the following two figures (reproduced from pages 44 and 45 of the original study report) show that at both 2500 ppm and 12500 ppm, there was a marked effect on body weight in females beginning immediately after the start of compound administration. Although the animals showed a similar pattern in body weight development over time as did animals in the control and 500 ppm groups, their overall body weight was biologically significantly reduced compared to control and 500 ppm animals.

FIGURE 4:0

Group mean weekly bodyweights of males and females - F0 generation

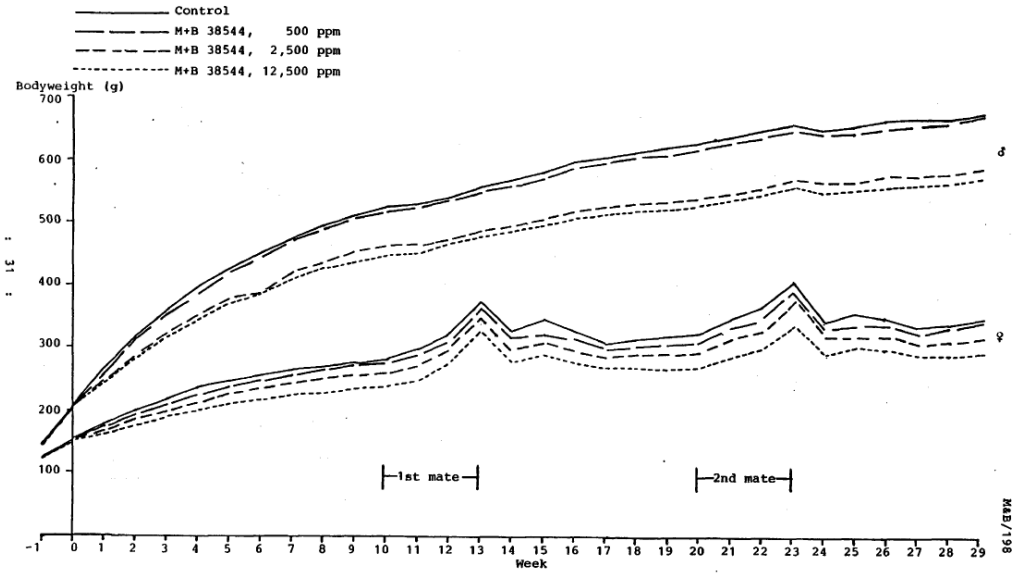
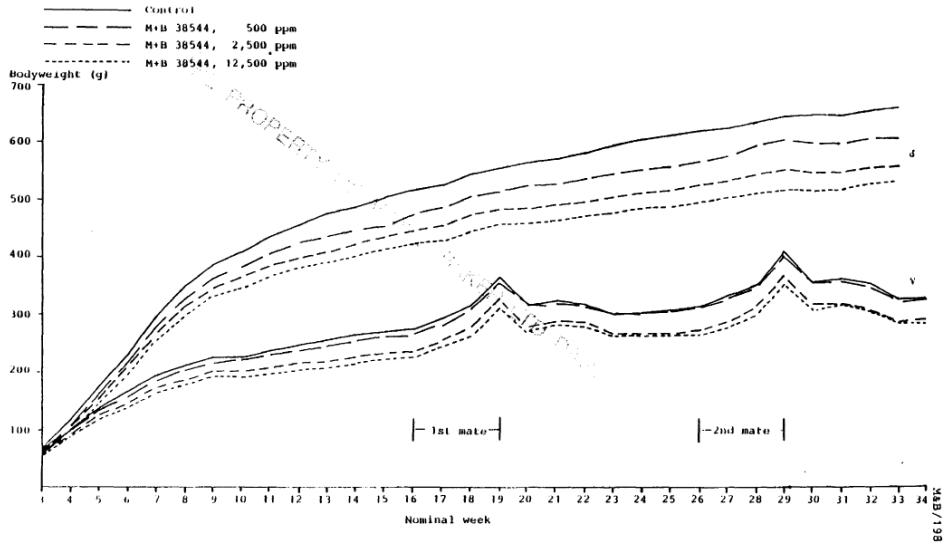


FIGURE 4:1

Group mean weekly bodyweights of males and females - F1A generation



It is therefore the position of Bayer that the four animals which were either sacrificed humanely or found dead at 12500 ppm and showed signs of prolonged or difficult parturition were not indicative of a direct effect of diflufenican on parturition. Rather, these animals were in a general state of ill health due to administratin of difufenican well above the maximum tolerated dose, and the effects of parturition were secondary to that excessive general toxicity. We therefore agree with the CLH dossier that diflufenican does not warrant classification for reprotoxicity.