TERBUTHYLAZINE – POSITION ON LEYDIG CELL TUMOURS IN SPRAGUE DAWLEY-DERIVED RATS

The notifiers agree with UK HSE that the apparent increase in Leydig (interstitial) cell tumours in male Sprague-Dawley rats is an artefact of increased survival in the high dose group. Details of the notifiers' position are given in Annex II of the CLH report and are summarised below:

Leydig cell tumours are a common spontaneous age-related tumour. It is, therefore, not surprising that the incidence of these tumours is highest in dose groups where survival is increased.

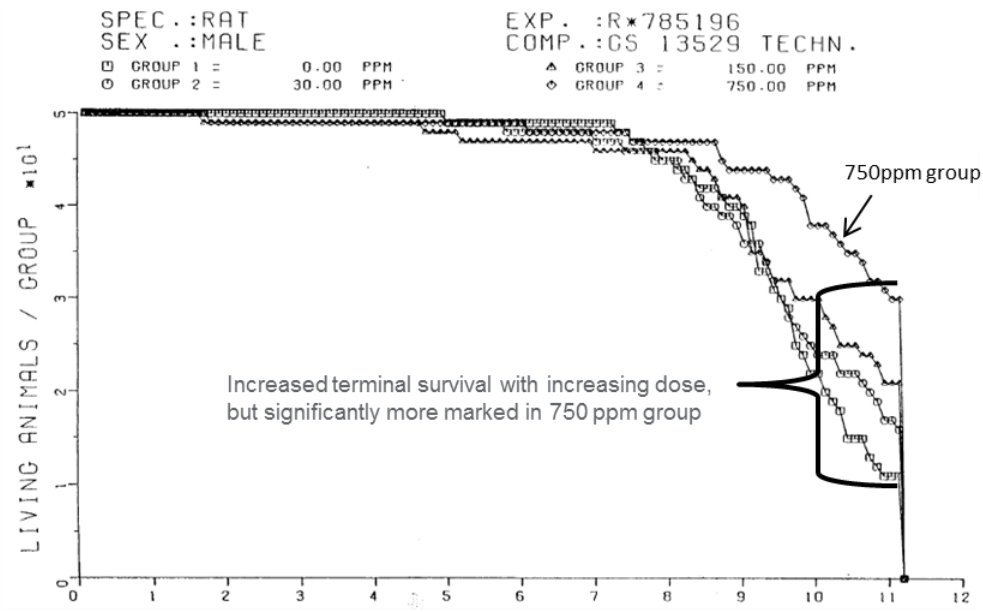
- **Significantly lower bodyweight/weight gain and reduced food consumption in the top dose group resulted in improved survival**. The increase in survival in the top dose group represented a step change compared to the survival of control and low and mid dose animals (see Table 1 and Fig 1).

Table 1: Body weight gain, body weight, survival and Leydig cell tumour incidence

Dose (ppm)	Body Weight Gain (% of Control) Week 1-105*	Body Weight (% of Control) Week 105*	Survival Time – 75th Percentile (days)	Leydig Cell Tumour Incidence
0	100	100	642	3/79 (3.8%)
30	91	93	647	4/79 (5.1%)
150	78	81	660	2/80 (2.5%)
750	54	59	740	10/80 (12.5%)

*: Animals received test item for 24 months, after which all groups were placed on control diet until survival in one group dropped to ~20% (in this case, the control group). Therefore, the measurements taken at week 105 are the best for evaluating effects of the test item on body weight gain and body weight.

Fig 1: Male survival in Carcinogenicity group (n=50) terminated at day 779



- **A non-standard study design resulted in an extended observation time (to 779 days) in males.** A high proportion of Leydig cell tumours developed between the scheduled sacrifice (2 years) and the study termination (2, 1, 1, 7 in control, 30, 150 and 750 ppm from a total of 28, 27, 32, 46 animals)

The increase in Leydig cell tumours is not statistically significant when appropriate survival-adjusting statistical techniques are used. In the original study report (Gfeller, 1983a) a Peto trend test was used to test for a statistically significant increase in tumours adjusted for survival. A Peto trend test requires a fatal/non-fatal designation of each tumour by the study pathologist, which was not conducted in this study. Revised statistical analyses using the Poly-k trend test, a statistical technique that adjusts for survival without the requirement for a fatal/non-fatal designation are presented in Annex II, Appendix 1 of the CLH report. The apparent increase in Leydig cell tumours was not statistically significant when using the Poly-k trend test, nor in subsequent pair-wise tests using Fishers exact test.

Higher incidences have been noted in control rats in other studies. Although slightly higher than the upper limit of the laboratory historical control data, similar/higher incidences have been reported in control Sprague Dawley rats in other laboratories and in the Registry of Industrial Toxicology Animal (RITA) database (see Table 2).

Table 2: Ranges of Leydig cell tumour incidences in control Sprague Dawley rats

Data Source	Range of Leydig Cell Tumour Incidence (%)
750 ppm terbuthylazine	12.5
Laboratory historical control data	0 – 7.5
RITA database	0 – 12
McMartin *et al*., 1992	1.4 – 13.3
Nakazawa *et al*., 2001	22.5 – 27.5

No increase in tumours was noted at 150 ppm, a dose which would have represented a robust MTD for the evaluation of carcinogenicity. Based on the significant reductions in body weight gain (78% of control over weeks 1 -105) and body weight (81% of control at week 105). The reduction in bodyweight gain at the top dose (up to 50% of control) was of sufficient magnitude to suggest that the MTD had been exceeded.

No increase in Leydig cell tumours was noted in a chronic toxicity and carcinogenicity study with Han Wistar rats (Ramesh, 2001).