TERBUTHYLAZINE – POSITION ON REPRODUCTION IN SPRAGUE DAWLEY RATS

The notifiers agree with the UK HSE that the apparent effects on fertility in Sprague-Dawley rats at the top dose level (300ppm) are not attributable to a direct effect of terbuthylazine on mating or fertility. The findings reflect the high level of background variation in mating performance in the animals and/or are secondary to the general systemic toxicity observed, specifically a significantly lower bodyweight gain that is seen in concurrent controls.

In a 2-generation reproductive toxicology study with Sprague Dawley rats (Masters *et* all, 1992 – Study initiated in 1990) apparent effects on mating, pregnancy and *corpora lutea* were noted for F0 and F1 female rats administered terbuthylazine via the diet at 300 ppm. No similar effects were noted in females receiving 6 or 60 ppm. These data are summarised in Table 1, along with data for body weight gain and food consumption.

Table 1: Summary of mating and pregnancy parameters, *corpora lutea*, body weight gain and food consumption in female Sprague Dawley rats receiving terbuthylazine

	Dose (ppm)	No. Females Paired	No. Pregnant	Total No. Not Pregnant	Pregnancy Rate (%)	Reduced/ Absent *Corpora Lutea*	Pre-mate Body Weight Gain (% of Control)	Pre-mate Food Consumption (% of Control)
F0	0	32	31	1	96.9	0	100	100
	6	32	30	2	93.8	0	103	97
	60	32	30	2	93.8	0	94	94
	300	32	25	7	78.1	4	68	84
F1	Dose (ppm)	No. Females Paired	No. Pregnant	Total No. Not Pregnant	Pregnancy Rate (%)	Reduced/ Absent *Corpora Lutea*	Pre-mate Body Weight Gain (% of Control)	Pre-mate Food Consumption (% of Control)
	0	28	24	4	85.7	0	100	100
	6	28	24	4	85.7	0	100	99
	60	28	26	2	92.9	0	84	92
	300	28	22	6 / 4 (see note)	78.6 / 85.7 (see note)	7	75	84

Note: Initially 6 females were found not pregnant; however 2 of these animals became pregnant after mating with a proven male.

It is the notifiers' position that these apparent effects do not represent a direct effect of terbuthylazine because:

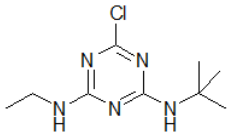
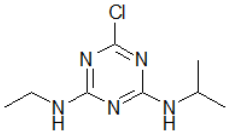
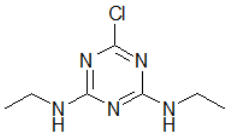
1. **The apparent effects are minor and not inconsistent with normal control variability.** Contemporary (date of study initiation ± 5 years) historical control data (HCD) from 2-generation reproductive toxicology studies using the same strain of rat have been provided by the performing laboratory (see Appendix 1). These data demonstrate a high level of normal variability for the parameters under consideration, particularly in the F1 generation, where control pregnancy rates vary between 36 and 100%.

2. **Reduced food consumption and body weight are known to affect pregnancy and numbers of *corpora lutea* in Sprague Dawley rats.** The effects noted on body weight gain of female rats administered 300 ppm are considerably in excess of what would be considered a suitable maximally tolerated dose (MTD) for this type of study. Terry *et al*. (2005) have demonstrated that reduced pre-mating body weight (achieved by feed restriction) resulted in a reduced pregnancy rate and reduced numbers of *corpora lutea* compared to controls.

3. **No evidence of effects on mating, pregnancy or *corpora lutea* in Han Wistar rats.** No effects on these parameters were noted in either 1-generation (Gainger, 1999) or 2-generation (Krishnappa, 1998) studies with Han Wistar rats administered terbuthylazine.

4. **No evidence of effects on mating, pregnancy or *corpora lutea* in Sprague Dawley rats administered closely structurally-related chlorotriazines.** 2-generation reproductive toxicology studies of near-identical design to that used for terbuthylazine were conducted using the same strain of rat in a contemporary time period, but in a different laboratory (hence they are not included in the HCD presented in Appendix 1) with two closely structurally-related chlorotriazines: atrazine and simazine. No effects on mating, pregnancy or *corpora lutea* were noted for these studies. Therefore, there is no evidence for a mode of action related effect on these parameters. The percentage pregnancy data are summarised in Table 2.

Table 2: Summary of percentage pregnancy in 2-generation reproductive toxicology studies with Sprague Dawley rats administered chlorotriazine herbicides.

Chlorotriazine	Terbuthylazine	Atrazine	Simazine
Structure			
Laboratory	Huntingdon, UK	Ciba-Geigy, USA	Ciba-Geigy, USA
Strain	Sprague Dawley (CrL:CD)	Sprague Dawley (CrL:CD)	Sprague Dawley (CrL:CD)
Dose Levels (ppm)	0, 6, 60, 300	0, 10, 100, 500	0, 10, 100, 500
% Pregnancy (F0)	97, 94, 94, 78	97, 93, 87, 87	80, 83, 90, 93
% Pregnancy (F1)	86, 86, 93, 79 / 86	80, 60, 98, 87	67, 73, 66, 98

In conclusion, there is no evidence for a direct effect of terbuthylazine on reproduction, with any apparent effects noted in females treated with 300 ppm being a reflection of normal biological variability, excessive systemic toxicity (as evidenced by reduced body weight gain and food consumption) or a combination of both factors.

Reference

Terry *et al*., 2005. *Birth Defects Res. (Part B)* **74**: 431-441.

MATING PERFORMANCE AND FERTILITY

F0 GENERATION

Study No.	Study Start date	Fertility rate (%) [A]		Copulation rate [B]		Females in prolonged oestrus after pairing	Number pregnant		Number littered		Incidence of absent/ reduced corpora lutea
		Mate 1	Mate 2	Mate 1	Mate 2		Mate 1	Mate 2	Mate 1	Mate 2	
1	Jul 1985	100	92	36/36 (100%)	33/36 (92%)	NA	36/36	33/36	36/36 (100%)	33/36 (92%)	0/36
2	Dec 1985	93	NA	27/28 (96%)	NA	0/28	26/28	NA	26/28 (93%)	NA	0/28
3	Jan 1986	93	100	27/28 (96%)	27/27 (100%)	0/28	26/28	27/27	25/28 (89%)	25/27 (93%)	0/25
4	May 1986	100	NA	28/28 (100%)	NA	0/28	28/28	NA	28/28 (100%)	NA	0/28
5	Jul 1986	89	NA	26/28 (93%)	NA	NA	25/28	NA	25/28 (89%)	NA	0/28
6	Jul 1986	100	NA	27/28 (96%)	NA	0/28	28/28	NA	28/28 (100%)	NA	0/28
7	Aug 1986	96	100	28/28 (100%)	25/28 (89%)	0/28	27/28	28/28	27/28 (96%)	28/28 (100%)	0/28
8	Sep 1986	96	NA	27/28 (96%)	NA	0/28	27/28	NA	27/28 (96%)	NA	0/28
9	Dec 1986	100	NA	27/28 (96%)	NA	0/28	28/28	NA	28/28 (100%)	NA	0/28
10	Mar 1987	96	NA	27/28 (96%)	NA	0/28	27/28	NA	27/28 (96%)	NA	0/28
11	Dec 1987	97	100	30/31 (97%)	29/31 (94%)	0/31	31/32	31/31	30/31 (97%)	31/31 (100%)	0/31
12	Oct 1988	97	NA	31/32 (97%)	NA	0/32	31/32	NA	30/32 (94%)	NA	0/31
13	Dec 1989	97	NA	31/32 (97%)	NA	NA	31/32	NA	31/32 (97%)	NA	0/32
14	Apr 1990	97	NA	30/32 (94%)	NA	0/32	31/32	NA	31/32 (97%)	NA	0/32
15	Oct 1991	84	NA	30/32 (94%)	NA	0/32	27/32	NA	26/32 (81%)	NA	1/32
16	Apr 1992	100	NA	31/32 (97%)	NA	0/32	32/32	NA	32/32 (100%)	NA	3/32
17	May 1992	86	NA	27/28 (96%)	NA	0/28	24/28	NA	24/28 (86%)	NA	NA
18	Sep 1992	91	NA	30/32 (94%)	NA	1/32 5 days of cornified cells	29/32	NA	29/32 (91%)	NA	2/20
19	Nov 1992	88	NA	28/32 (88%)	NA	1/32 5 days of cornified cells	28/32	NA	28/32 (88%)	NA	2/31
20	Mar 1993	91	NA	32/32 (100%)	NA	0/32	29/32	NA	29/32 (91%)	NA	5/32
21	Jul 1993	94	NA	31/32 (97%)	NA	0/32	30/32	NA	30/32 (94%)	NA	2/32
22	Aug 1993	91	NA	31/32 (97%)	NA	0/32	29/32	NA	29/32 (91%)	NA	0/32
23	May 1994	96	NA	28/28 (100%)	NA	0/28	27/28	NA	27/28 (96%)	NA	0/28
24	Nov 1995	94	NA	32/32 (100%)	NA	0/32	30/32	NA	30/32 (94%)	NA	2/32

[A]: Total pregnant/total paired; [B]: Number with positive evidence of copulation (sperm or plug)

F1 GENERATION

Study No.	Study Start date	Fertility rate (%) [A]		Copulation rate [B]		Females in prolonged oestrus after pairing	Number pregnant		Number littered		Incidence of absent/ reduced corpora lutea
		Mate 1	Mate 2	Mate 1	Mate 2		Mate 1	Mate 2	Mate 1	Mate 2	
1	Jul 1985	78	NA	25/32 (78%)	NA	NA	25/32	NA	25/32 (78%)	NA	0/32
2	Dec 1985	100	NA	24/24 (100%)	NA	0/24	24/24	NA	24/24 (100%)	NA	0/24
3	Jan 1986	96	92	24/24 (100%)	24/24 (100%)	0/24	23/24	22/24	23/24 (96%)	22/24 (92%)	0/24
4	May 1986	96	NA	23/24 (96%)	NA	0/24	23/24	NA	22/24 (92%)	NA	0/24
5	Jul 1986	100	71	21/24 (88%)	20/24 (83%)	NA	24/24	17/24	24/24 (100%)	17/24 (71%)	0/24
6	Jul 1986	92	NA	23/24 (96%)	NA	1/24 5 days of cornified cells	22/24	NA	22/24 (92%)	NA	0/24
7	Aug 1986	89	89	27/28 (93%)	25/28 (89%)	0/28	25/28	25/28	25/28 (89%)	25/28 (89%)	0/28
8	Sep 1986	67	71	20/24 (83%)	19/24 (79%)	0/24 first mate; 1/24 second mate 5 days of cornified cells	16/24	17/24	16/24 (67%)	17/24 (71%)	0/24
9	Dec 1986	92	NA	20/24 (83%)	NA	0/24	22/24	NA	22/24 (92%)	NA	0/24
10	Mar 1987	88	NA	24/24 (100%)	NA	0/24	21/24	NA	21/24 (88%)	NA	0/24
11	Dec 1987	82	82	26/28 (93%)	27/27 (100%)	1/28 11 days of cornified cells	23/28	22/27	23/28 (82%)	22/28 (79%)	0/27
12	Oct 1988	61	36	16/28 (57%)	5/11 (45%)	0/28 1st mate; 3/11 second mate 5 days of cornified cells	17/28	4/11	17/28 (61%)	4/11 (36%)	0/28
13	Dec 1989	96	NA	26/28 (93%)	NA	NA	27/28	NA	27/28 (96%)	NA	0/28
14	Apr 1990	86	NA	20/28 (71%)	NA	0/28	24/28	NA	24/28 (86%)	NA	0/28
15	Oct 1991	56	56	19/27 (70%)	20/27 (74%)	0/27	15/27	15/27	15/27 (56%)	15/27 (56%)	2/27
16	Apr 1992	86	NA	27/28 (96%)	NA	0/28	24/28	NA	24/28 (86%)	NA	0/28
17	May 1992	83	NA	24/24 (100%)	NA	0/24	20/24	NA	20/24 (83%)	NA	NA
18	Sep 1992	86	NA	25/28 (89%)	NA	0/28	24/28	NA	24/28 (86%)	NA	NA
19	Nov 1992	75	NA	27/28 (96%)	NA	0/28	21/28	NA	21/28 (75%)	NA	2/28
20	Mar 1993	82	NA	27/28 (96%)	NA	0/28	23/28	NA	23/28 (82%)	NA	0/28
21	Jul 1993	96	NA	28/28 (100%)	NA	0/28	27/28	NA	27/28 (96%)	NA	0/28
22	Aug 1993	57	37	24/28 (86%)	19/27 (70%)	0/28	16/28	10/27	15/28 (54%)	10/27 (37%)	0/28
23	May 1994	83	NA	22/24 (92%)	NA	1/28 5 days of cornified cells	20/24	NA	20/24 (83%)	NA	0/23
24	Nov 1995	96	NA	28/28 (100%)	NA	0/28	27/28	NA	26/28 (93%)	NA	5/27

[A]: Total pregnant/total paired; [B]: Number with positive evidence of copulation (sperm or plug)