

TABLE # 2 - Test 1 (With S9, 6 hr Treatment, 24 hr Harvest)

treatment	# cells eval.	relative cell count (vehicle)	slide observation	Structural Aberrations			Numerical Aberrations		
				aberration frequency lesions/cell	aberrant cell frequency (%)		% cells with		
					+ gaps	- gaps	AE	ER	PP
untreated control	100	1.48	"Nil toxicity" [no ppt]	0.00	0	0	0	0	0
	100	1.43		0.00	0	0	0	1	0
vehicle control (1%)	100	1.06	"Nil toxicity" [no ppt]	0.01	1	1	0	0	0
	100	0.94		0.00	0	0	0	0	0
TBZ 20 µg/ml	100	1.21	"Nil toxicity" [no ppt]	0.00	0	0	0	0	0
	100	1.22		0.00	0	0	0	0	1
TBZ 39 µg/ml	100	1.08	"Nil toxicity" [no ppt]	0.02	1	1	0	0	0
	100	0.92		0.00	0	0	0	1	0
TBZ 78 µg/ml	100	0.79	"Sparse metaphase cells" [no ppt]	0.00	0	0	0	0	0
	100	0.92		0.01	1	0	0	0	0
TBZ 156 µg/ml	100	0.15	"No metaphase cells, large amount of interphase cells." [no ppt]						
	100	0.05							
TBZ 313 µg/ml	-	0.00	"No metaphase cells, very few interphase cells." [slight cloudy]						
TBZ 625 µg/ml	-	0.05	"No metaphase cells, very few interphase cells" [fine ppt]						
	-	0.05							
TBZ 1250 µg/ml	-	0.08	"No metaphase cells, small dark interphase cells" [fine ppt]						
	-	0.20							
TBZ 2500 µg/ml	-	0.42	"Very sparse metaphase cells" [cloudy]						
	-	0.54							
TBZ 5000 µg/ml	-	0.89	"Sparse metaphase cells" [slight cloudy]						
	-	0.89							
CPH - 30	100	-	not reported	0.11	7	5	1	0	0
CPH - 40	100	-		0.27	17	17	1	0	0

AE = aneuploidy, ER = endoreduplication, PP = polyploidy

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TABLE # 3 - Test 1 (Without S9, 6 hr Treatment, 24 hr Harvest)

treatment	# cells eval.	relative cell count (vehicle)	slide observation	Structural Aberrations			Numerical Aberrations		
				aberration frequency lesions/cell	aberrant cell frequency (%)		% cells with		
					+ gaps	- gaps	AE	ER	PP
untreated control	100	1.60	"Nil toxicity" [no ppt]	0.00	0	0	0	0	1
	100	1.23		0.00	0	0	1	0	0
vehicle control (1%)	100	0.84	"Nil toxicity" [no ppt]	0.00	0	0	0	0	0
	100	1.16		0.00	0	0	0	0	1
TBZ 20 µg/ml	100	1.01	"Nil toxicity" [no ppt]						
	100	0.85							
TBZ 39 µg/ml	100	0.84	"Nil toxicity" [no ppt]	0.00	0	0	0	0	0
	100	0.95		0.01	1	0	0	1	0
TBZ 78 µg/ml	100	0.82	"Nil toxicity" [no ppt]	0.00	0	0	0	2	2
	100	0.79		0.00	0	0	0	0	0
TBZ 156 µg/ml	100	0.22	"Sparse metaphase cells" [no ppt]	0.02	2	2	0	0	0
	100	0.37		0.03	3	2	1	0	0
TBZ 313 µg/ml	-	0.00	"No metaphase cells" [slight ppt]						
	-	0.00							
TBZ 625 µg/ml	-	0.24	"No metaphase cells" [fine ppt]						
	-	0.08							
TBZ 1250 µg/ml	-	0.37	"Sparse metaphase cells" [fine ppt]						
	-	0.47							
TBZ 2500 µg/ml	-	0.38	"Sparse metaphase cells" [cloudy]						
	-	0.56							
TBZ 5000 µg/ml	-	0.72	"Nil toxicity" [fine ppt]						
	-	0.73							
MMS - 30	100	-	not reported	0.12	8	8	1	0	0
MMS - 40	100	-	not reported	0.29	16	16	1	0	0

AE = aneuploidy, ER = endoreduplication, PP = polyploidy

TABLE # 4 - Test 2 (With S9, 6 hr Treatment, 24 hour Harvest)

treatment	# cells eval.	relative cell count (vehicle)	slide observation	Structural Aberrations			Numerical Aberrations		
				aberration frequency lesions/cell	aberrant cell frequency (%)		% cells with		
					+ gaps	- gaps	AE	ER	PP
untreated control	100	0.88	"Nil toxicity"	0.00	0	0	0	0	1
	100	1.06		0.01	1	0	0	0	0
vehicle control (1%)	100	0.97	"Nil toxicity"	0.00	0	0	0	0	0
	100	1.03		0.03	3	0	1	1	3
TBZ 20 µg/ml	100	0.83	"Nil toxicity"						
	100	0.99							
TBZ 40 µg/ml	100	0.97	"Nil toxicity"						
	100	0.86							
TBZ 60 µg/ml	100	0.97	"Nil toxicity"	0.01	1	0	1	3	1
	100	0.94		0.02	1	1	0	6	0
TBZ 80 µg/ml	100	0.84	"Slightly sparse metaphase cells"	0.01	1	0	0	7	4
	100	0.81		0.01	1	0	0	3	2
TBZ 100 µg/ml	100	0.75	"Sparse metaphase cells"	0.27	13	13	0	1	1
	100	0.77		0.25	13	12	1	0	0
TBZ 150 µg/ml	100	0.15	"No metaphase cells"						
	100	0.11							
CPH - 20	100	-	not reported	0.20	11	9	1	0	1
CPH - 30	100	-	not reported	0.34	19	15	1	0	0

AE = aneuploidy, ER = endoreduplication, PP = polyploidy

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TABLE # 5 - Test 2 (Without S9, 22 hr Treatment, 24 hour Harvest)

treatment	# cells eval.	relative cell count (vehicle)	slide observation	Structural Aberrations			Numerical Aberrations		
				aberration frequency lesions/cell	aberrant cell frequency (%)		% cells with		
					+ gaps	- gaps	AE	ER	PP
untreated control	100	1.16	"Nil toxicity"	0.00	0	0	0	0	0
	100	1.13		0.00	0	0	0	0	0
vehicle control (1%)	100	1.04	"Nil toxicity"	0.00	0	0	0	0	2
	100	0.96		0.00	0	0	0	0	2
TBZ 10 µg/ml	100	1.02	"Nil toxicity"						
	100	0.96							
TBZ 20 µg/ml	100	0.81	"Nil toxicity"						
	100	0.92							
TBZ 40 µg/ml	100	0.78	"Nil toxicity"	0.00	0	0	0	0	0
	100	0.68		0.00	0	0	0	0	1
TBZ 80 µg/ml	100	0.62	"Nil toxicity"	0.00	0	0	0	0	0
	100	0.57		0.00	0	0	0	0	0
TBZ 100 µg/ml	100	0.44	"Nil toxicity"	0.00	0	0	0	1	1
	100	0.42		0.00	0	0	0	0	1
TBZ 150 µg/ml	-	0.00	"No metaphase cells"						
	-	0.00							
TBZ 200 µg/ml	-	0.00	"No metaphase cells"						
	-	0.00							
MMS - 20	100	-	-	0.19	13	12	1	0	0
MMS - 30	100	-	-	0.46	24	22	0	0	1

AE = aneuploidy, ER = endoreduplication, PP = polyploidy

TABLE # 6 - Test 3 (With S9, 6 hr Treatment, 24 hour Harvest)

treatment	# cells eval.	relative cell count (vehicle)	slide observation	Structural Aberrations			Numerical Aberrations		
				aberration frequency lesions/cell	aberrant cell frequency (%)		% cells with		
					+ gaps	- gaps	AE	ER	PP
untreated control	100	1.08	"Nil toxicity"	0.00	0	0	0	0	0
	100	1.04		0.00	0	0	0	0	0
vehicle control (1%)	100	1.03	"Nil toxicity"	0.00	0	0	0	0	0
	100	0.97		0.00	0	0	0	0	0
TBZ 80 µg/ml	100	0.82	"Sparse metaphase cells"						
	100	0.61							
TBZ 90 µg/ml	100	0.68	"Sparse metaphase cells"	0.26	16	13	0	0	1
	100	0.65		0.49	21	18	2	0	1
TBZ 100 µg/ml	54	0.56	"Very sparse metaphase cells, cell debris"	1.04	30	26	6	2	0
	22	0.52		0.95	27	23	0	5	0
TBZ 110 µg/ml	100	0.19	"No metaphase cells, cell debris"						
	100	0.21							
TBZ 120 µg/ml	-	0.21	"No metaphase cells, cell debris"						
	-	0.14							
CCP - 50	100	-	-	0.36	22	22	3	0	0

AE = aneuploidy, ER = endoreduplication, PP = polyploidy

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Study title: Tetrabenazine Micronucleus Test in Bone Marrow of Rats 0 h + 24 h Dosing and 48 h Sampling

Key findings: TBZ was negative for males and produced an equivocal response for females

Study no.: — Report Number 19434

Volume # 19, module 4

Conducting laboratory and location: _____

Date of study initiation: 11-Aug-00 (report dated 07-Aug-02)

GLP compliance: yes, OECD-GLP

QA reports: yes (x) no () The QA statement indicates that they relied on process inspections for this type of short term study; however, "The report is considered to describe accurately the methods and procedures used in the study. The reported results accurately reflect the original data generated during the study."

Drug, lot #, and % purity: Tetrabenazine, batch # 9950, chromatic purity by HPLC = 99.9%. TZB was dissolved in 0.5% carboxymethylcellulose. Solutions were prepared on the day of use, as close to the time of dosing as practicable.

Methods

Strains/species/cell line: CD rats from _____ . For all aspects of the study (DRF study, toxicity study and micronucleus test animals were acclimated for 6-10 days prior to dosing. All animals were 6-7 weeks old at dosing. The weight ranges for the different aspects of the study were as follows:

DRF study	males: 166-183 g	females: 142-160 g
toxicity study	males: 201-232 g	females: 151-179 g
micronucleus test	males: 140-169 g	females: 130-158 g

Doses used in definitive study: All doses were administered by oral gavage at a volume of 10 mg/kg. The doses used in the definitive study were 25, 50 and 100 mg/kg/day.

Basis of dose selection: preliminary DRF study and a toxicity study (see results section for details).

Vehicle controls: 0.5% carboxymethylcellulose

Positive controls: Cyclophosphamide (in distilled water)

Incubation and sampling times:

Micronucleus Test				
group	treatment/dose	dosing (hrs)	bone marrow sampling (hrs)	# rats
vehicle control	0.5% CMC (10 ml)	0 + 24	48	5/sex
low dose	TBZ – 25 mg/kg	0 + 24	48	5 males
mid dose	TBZ – 50 mg/kg	0 + 24	48	5 males
high dose	TBZ – 100 mg/kg	0 + 24	48	10/sex (*)
positive control	CPH – 50 mg/kg	0 + 24	48	5 males

(*) of these 5/sex were spare animals ("...contingency in case of unscheduled deaths or potential sex differences.")

Results

DRF Study				
dose (mg/kg/day)	# treated	dosing	parameters assessed	scheduled sacrifice
50	1/sex	0 hrs + 24 hrs	observed for mortality and clinical signs on day 1 (1 min, 0.25 hr, 0.5 hr, 0.75 hr, 1 hr, 2 h, 3.25 hr, and 4 hrs post dose) and day 2 (predose, 1 min, 0.5 hr, 1 hr, 2 hr, 3.25 hr, 4 hr, and 5 hr post dose), then twice daily until sacrifice	day 6
125	1/sex	0 hrs + 24 hrs		
350	1/sex	0 hrs + 24 hrs		
800	1/sex	0 hrs + 24 hrs		
2000	1/sex	0 hrs + 24 hrs		

Results of DRF study (taken directly from sponsor)

Dose Level (mg.kg ⁻¹ .day ⁻¹)	Animal Nos.	Clinical Signs Observed (Days 1 and 2 = Dosing Days)		No. of Deaths
50	1♂, 6♀	Day 1	Laboured breathing, subdued behaviour, unable to stand, hunched appearance, discharge (eyes), unwilling to move.	0
		Day 2	(predose: Subdued behaviour, rolling gait). Subdued behaviour, hunched appearance, unwilling to move, laboured breathing, rolling gait.	0
		Days 3-4	NAD.	0
125	2♂, 7♀	Day 1	Laboured breathing, subdued behaviour, unable to stand, hunched appearance, discharge (eyes), unwilling to move.	0
		Day 2	(predose: discharge (eyes), discharge (nose), subdued behaviour, tremors, rolling gait, hunched appearance, liquid faeces, eyes dull) Laboured breathing, subdued behaviour, hunched appearance, discharge (eyes), unwilling to move, faeces darker than normal, eyes half closed, tremors, rolling gait, liquid faeces, discharge (nose), piloerection, eyes dull/closed, KIE.	1♂, 1♀
350	3♂, 8♀	Day 1	Laboured breathing, subdued behaviour, unable to stand, hunched appearance, discharge (eyes), unwilling to move.	0
		Day 2 predose	Subdued behaviour, tremors, hunched appearance, liquid faeces, discharge (nose), staining around anus, eyes half closed, laboured breathing, rolling gait, hunched appearance, discharge (eyes), piloerection, unwilling to move, cold, eyes closed, no faeces evident in cage, KIE.	1♂, 1♀
800	4♂, 9♀	Day 1	Laboured breathing, subdued behaviour, unable to stand, hunched appearance, discharge (eyes), unwilling to move.	0
		Day 2 predose	Laboured breathing, subdued behaviour, tremors, rolling gait, hunched appearance, piloerection, unwilling to move, cold, eyes closed, FDC, KIE.	1♂, 1♀
2000	5♂, 10♀	Day 1	Laboured breathing, subdued behaviour, unable to stand, hunched appearance, discharge (eyes), unwilling to move, prostration, convulsions, FDC.	1♀
		Day 2	(predose: FDC.)	1♂

NAD = No abnormalities detected,

KIE = Killed in extremis

FDC = Found dead in cage

Main Toxicity Study (oral gavage)				
dose (mg/kg/day)	# treated	dosing	parameters assessed	scheduled sacrifice
50	3/sex	0 hrs + 24 hrs	observed for mortality and clinical signs after dosing (1 min, 0.5 hr, 1 hr, 2 h, 3 hr, and 4 hrs post dose) and then twice daily until sacrifice	day 7
75	3/sex	0 hrs + 24 hrs		
100	3/sex	0 hrs + 24 hrs		

Results of Main toxicity Study (taken directly from sponsor)

Dose Level (mg.kg ⁻¹ .day ⁻¹)	Animal Nos.	Clinical Signs Observed (Days 1 and 2 = Dosing Days)		No. of Deaths
		Day 1	Day 2	
50	11-13♂ 20-22♀	Day 1	Subdued behaviour.	0
		Day 2	Subdued behaviour, unwilling to move.	0
		Days 3-4	NAD.	0
75	14-16♂ 23-25♀	Day 1	Subdued behaviour, discharge (eyes), piloerection, staggering, unwilling to move, animal tense.	0
		Day 2	Laboured breathing, subdued behaviour, liquid faeces, piloerection, unwilling to move, vocalising when touched, eyes half/part closed, clear liquid from both eyes, hunched appearance.	0
		Days 3-4	NAD.	0
100	17-19♂ 26-28♀	Day 1	Subdued behaviour, hunched appearance, liquid faeces, piloerection, unwilling to move, animal tense, eyes half closed.	0
		Day 2	(predose: Subdued behaviour.) Laboured breathing, subdued behaviour, tremors, liquid faeces, piloerection, unwilling to move, eyes part closed, animal tense, hunched appearance, clear liquid from eyes.	0
		Days 3-4	Subdued behaviour.	0

NAD = No abnormalities detected

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Assessment: (taken directly from sponsor)

The better of the 2 prepared slides was selected for examination and the coded slides assessed blind by the same operator. Slides were scored in an ordered sequential fashion using the random number of each slide as guidance, *ie*, 201, 202, 203 etc. Two thousand (2000) polychromatic erythrocytes (PCE) per animal were scored for micronuclei and the frequency of micronucleated cells (MN-PCE) determined.

As a control against inclusion of artefacts, or action of a mutagen on the G₂ and/or mitotic phase of the cell cycle, the numbers of micronucleated normochromatic erythrocytes (MN-NCE) in mature red blood corpuscles were also recorded (Maier and Schmid, 1976; Hamoud *et al*, 1989). In addition, scored micronuclei were assigned on the basis of size into small or large categories, historically defined as micronuclei occupying less or more than 25% of the visible cellular area. This classification provided a non-specific measure of compound induced spindle dysfunction, as large micronuclei appear to derive from lagging chromosomes caused by damage to the mitotic apparatus during bone marrow erythropoiesis (Yamamoto and Kikuchi, 1980; Vanderkerken *et al*, 1989).

The PCE/NCE ratio, a measure of any induced systemic toxicity, was determined by counting a minimum total of 1000 erythrocytes (PCE + NCE) per marrow preparation.

Evaluation criteria: (taken directly from sponsor)

Negative Response

If no biologically relevant increases in the numbers of MN-PCE are observed, relative to the concurrent and established historical control frequencies for MN-PCE induction, the test is judged negative. No statistical analysis is performed if the levels of MN-PCE induction fall within the determined historical control frequencies. A similar biological approach to the data, which avoids the need for statistical evaluations, was recently described (Ashby and Tinwell, 1995). Variations in the MN-NCE frequencies and PCE/NCE ratios also are not analysed statistically, unless clearly different from concurrent control values.

Positive Response

If an increase in the numbers of micronucleated polychromatic erythrocytes (MN-PCE) (that is, an increase greater than 10% over the expected historical control range for a group of animals) is obtained for one or more of the test material treated dose groups, the test is judged positive. The increase observed should be biologically relevant and statistically significant relative to concurrent and historical control frequencies for MN-PCE and/or MN-NCE induction.

Inconclusive Response

If the levels of MN-PCE within any one dose group are increased above the established historical control frequencies for MN-PCE induction, but not high enough to meet the criteria for a positive response (that is an increase up to 10% over the maximum negative control frequency for a group of animals), the test is considered inconclusive.

Study outcome:

Clinical Observations: There were no unscheduled deaths and clinical signs were limited to subdued behavior, animals unwilling to move and eyes half closed. Recall that in the DRF study, 125 mg/kg/day resulted in unscheduled sacrifice (killed in extremis) for both animals.

Treatment	Animal No.	Clinical Signs Observed (Days 1 and 2 = Dosing Days)		No. of Deaths
10 ml 0.5% carboxymethyl cellulose.kg ⁻¹ .day ⁻¹	101-105♂ 106-110♀	Day 1	NAD.	0
		Day 2	NAD.	0
		Day 3	NAD.	0
25 mg Tetrabenzazine.kg ⁻¹ .day ⁻¹	111-115♂	Day 1	Subdued behaviour.	0
		Day 2	Subdued behaviour.	0
		Day 3	NAD.	0
50 mg Tetrabenzazine.kg ⁻¹ .day ⁻¹	116-120♂	Day 1	Subdued behaviour, unwilling to move, eyes half closed.	0
		Day 2	Subdued behaviour, unwilling to move, eyes half closed.	0
		Day 3	NAD.	0
100 mg Tetrabenzazine.kg ⁻¹ .day ⁻¹	121-130♂ 131-140♀	Day 1	Subdued behaviour, unwilling to move, eyes half closed.	0
		Day 2	Subdued behaviour, unwilling to move, eyes half closed	0
		Day 3	NAD.	0
50 mg Cyclophosphamide.kg ⁻¹ .day ⁻¹	141-145♂	Day 1	NAD.	0
		Day 2	NAD.	0
		Day 3	NAD.	0

NAD = No abnormalities detected

Micronucleus Assessment: The data are summarized in a sponsor-generated table that follows. Comparisons of the PCE/NCE ratio among groups did not indicate treatment-induced bone marrow toxicity for males or females. There was no evidence a treatment-related increase in MN-PCEs for males; however, the original five high dose treated females demonstrated an increase in the MN-PCEs compared to the vehicle control. The sponsor chose to assess the bone marrow of the five "spare" high dose treated females because the increase in MN-PCEs seen in the original five high dose treated females was "greater than 10% above the maximum range of the historical control data for a negative response." According to the sponsor two vehicle control slides and two positive control slides were chosen and assessed concurrently with the 'spare' high dose treated females in order to preserve the blinded evaluation. There was no evidence of a treatment-related increase in MN-PCE in the "spare" or contingency group females

when compared to the negative control or the historical control. It should be noted that according to report, the 'spare' animals were processed "in normal fashion" and kept "as a contingency in case of deaths or potential sex differences." When the results of the evaluation of the original and high dose females were combined, the combined frequency of MN-PCE was increased compared to the vehicle control and was outside of the historical control range (0.12 vs 0.09), although not statistically significant. The sponsor concluded that the TBZ did not induce a treatment-related increase in micronuclei in bone marrow at a maximally tolerated dose of 100 mg/kg/day.

Conclusion: TBZ did not result in the induction of micronuclei in male rats. The results in female are equivocal. In order to resolve the equivocal results in females the sponsor should conduct an additional study in female rats using multiple doses of TBZ, including the 100 mg/kg dose.

TK data were not collected as part of this assay. Relevant TK data are not available from other studies. The highest daily dose tested in the general toxicity studies conducted in rat was 15 mg/kg, administered bid (i.e., a total of 30 mg/kg/day). The highest dose tested in this micronucleus assay is 100 mg/kg/day.

Treatment	Dose (h)	Sex	No. of Rats Scored	Erythrocytes				PCE/NCE Mean ± S.D.
				Normochromatic Cells (NCE) No. of MN-NCE	Polychromatic Cells (PCE)			
					PCE Analysed	No. of MN-PCE	% MN-PCE	
10 ml 0.5% carboxymethyl cellulose kg ⁻¹ .day ⁻¹	0 + 24	♂	5	4	10013	9	0.09	0.95 ± 0.07
		♀	5	3	10048	7	0.07	0.92 ± 0.03
		♂♀	10	7	20061	16	0.08	0.94 ± 0.05
25 mg Tetrabenazine kg ⁻¹ .day ⁻¹	0 + 24	♂	5	4	10022	9	0.09	0.93 ± 0.06
50 mg Tetrabenazine kg ⁻¹ .day ⁻¹	0 + 24	♂	5	6	10032	3	0.03	0.92 ± 0.08
100 mg Tetrabenazine kg ⁻¹ .day ⁻¹	0 + 24	♂	5	11	10028	8	0.08	0.89 ± 0.09
		♀ a	5	4	10020	15	0.15 β	0.91 ± 0.05
		♀ b	5	9	10018	8	0.08	0.90 ± 0.07
		♀ a+b	10	13	20038	23	0.12 β	0.91 ± 0.06
50 mg Cyclophosphamide. kg ⁻¹ .day ⁻¹	0 + 24	♂	5	78 α	10017	352	3.51 φ	0.56 ± 0.10

- PCE = Polychromatic erythrocytes
 MN-PCE = Micronucleated PCE
 NCE = Normochromatic erythrocytes
 MN-NCE = Micronucleated NCE
 φ = Positive response in PCE
 α = Evident response in NCE
 β = Outside negative historical control range, but not statistically significant
 a = core group
 b = contingency group

Sponsor's historical control data (listed with audit date of 26-Sept-00)

Negative Control Data

Frequency of Micronucleated Polychromatic Erythrocytes (MN-PCE) in Vehicle and Untreated Male and Female Rats			
Mean		0.08%	
Standard Deviation		0.08%	
Ranges	Individual Rat	Group of 5-6 Rats	Group of 10-12 Rats
Min frequency	0.00%	0.01%	0.04%
Max frequency	0.20%	0.12%	0.09%
Number of Polychromatic Erythrocytes Assessed:		86000	
Number of Rats Assessed:		48	

Positive Control Data

Frequency of Micronucleated Polychromatic Erythrocytes (MN-PCE) in Positive Male and Female Rats	
Mean	3.04%
Standard Deviation	1.48%
Min - Max Frequencies	0.20-6.20%
Number of Polychromatic Erythrocytes Assessed:	32000
Number of Rats Assessed:	28

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ON ORIGINAL

Study title: *In Vivo* Mouse Micronucleus Assay

Key findings: negative (conducted in males only, both sexes should have been assessed)

Study no.: — Study # 7425-116

Volume #: NDA amendment #0007, volume 1 of 1

Conducting laboratory and location: _____

Date of study initiation: 13-July-05

GLP compliance: yes, FDA and OECD

QA reports: yes (x) no ()

Drug, lot #, and % purity: Tetrabenazine (TBZ) lot # 105481, with stated purity of 100.3%. Test formulations were made prior to dosing. Two sets of samples were obtained from each dosing solution from top, middle and bottom, for homogeneity and concentration analyses. "Stability analysis in the range of 2 to 30 mg/mL, under refrigerated conditions for 10 days, was performed under — Study No. 7425-102. Stability data was not available for the dose formulations at 1 mg/mL." Analysis of the samples revealed that the dosing formulations were homogeneous and for the 1, 2, and 4 mg/ml, concentrations were 96.7 – 102.0% of the nominal concentration. The highest concentration tested, 8 mg/ml was 87.7 – 92.1% of the nominal concentration. An additional concentration verification assay demonstrated that the concentrations were 98.4, 102.0, 98.8 and 92.1% the nominal concentration for 1.0, 2.0 4.0 and 8.0 mg/ml concentrations, respectively.

Methods

Strains/species/cell line: male CD-1@(ICR)BR mice (at treatment, ≈ 9 weeks old and 28.9 – 36.6 g).

"Systemic absorption of tetrabenazine in the mouse has been demonstrated and in addition, the two metabolites, α -dihydro-tetrabenazine and β -dihydro-tetrabenazine, are formed in the mouse."

Doses used in definitive study: 0, 10, 40, and 80 mg/kg TBZ, administered as a single oral gavage dose.

Basis of dose selection: "Based upon the results of — Study No. 745-105 with tetrabenazine, the Sponsor had relevant acute toxicity information in mice. Based upon the signs of systemic test article-induced toxicity, four test article doses were used in the definitive micronucleus assay (10, 20, 40 and 80 mg/kg)." "The high dose was expected to have produced some indication of toxicity, e.g., toxic signs, death or depression of the ratio of PCEs to NCEs."

Vehicle controls: 0.5% aqueous carboxymethylcellulose (CMC) and 0.1% Tween-80® in water.

Positive controls: cyclophosphamide (CP) dissolved in water.

Incubation and sampling times:

Design of main study						
treatment	dose (mg/kg)	concentration (mg/ml)	volume (ml/kg)	route	# animals for 24 hr timepoint	# animals for 48 hr timepoint
CP	80	8	10	oral gavage	5 males	-
vehicle	0	0	10	oral gavage	5 males	5 males
TBZ	10	1	10	oral gavage	5 males (*)	-
TBZ	20	2	10	oral gavage	5 males	-
TBZ	40	4	10	oral gavage	5 males	5 males (*)
TBZ	80	8	10	oral gavage	5 males	5 males

(*) - animals treated, but discarded without harvesting bone marrow.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

1. The study should have been conducted in both males and females. According to the OECD guidelines for this assay, "If at the time of the study there are data available from studies in the same species and using the same route of exposure that demonstrate that there are no substantial differences between sexes in toxicity, then testing in a single sex will be sufficient."

The choice of doses used in the main study was based on the clinical observations noted in a non-GLP 14-day oral gavage study with tetrabenazine in mice (— study # 7425-105) (module 4, volume 7). In this study mice —CD-1@(ICR)BR] (5/sex/gr) were administered daily oral (gavage) doses of vehicle (0.5% CMC) or TBZ at doses of 5, 10, 30 or 100 mg/kg. All 10 mice (5/sex) administered 100 mg/kg of TBZ were sacrificed on Day 1 due to the treatment-related clinical observations (time to sacrifice with reference to dosing was not reported). Clinical observations are summarized in the following reviewer-generated table. It appears that females are somewhat more sensitive to the CNS effects of TBZ and the sponsor did not note this. According to the sponsor, "The highest dose level in this study was selected based on the results of the 2-week study in which all animals treated at 100 mg/kg/day were sacrificed following a single dose because of signs of hypoactivity, recumbency, coldness, labored respiration, and squinting."

Dose (mg/kg/day)	Observations in Males	Observations in Females
0	-	-
5	eye partially closed (1/5, Days 1, 6, 13-14)	-
10	-	-
30	hypoactive (1/5, Days 8-9)	hypoactive (3/5, Days [1-3, 6, 8-10], [2, 8-10], [3, 5, 8-10])
100	-	sternal recumbency (3/5, Day 1)
	hypoactive (5/5, Day 1)	hypoactive (5/5, Day 1)
	labored respiration (5/5, Day 1)	labored respiration (5/5, Day 1)
	entire body cold to touch (5/5, Day 1)	entire body cold to touch (5/5, Day 1)
	squinting (periorbital) (2/5, Day1)	squinting (periorbital) (4/5, Day 1)
	sacrificed Day 1 (5/5)	sacrificed Day 1 (5/5)
(-) no significant findings		

This 14-day study was to serve as a DRF study for the 90-day oral gavage toxicity study conducted in mice. As part of the protocol for the 90-day study, the sponsor conducted a 5-day pilot study (non-GLP) in five female mice (administered daily oral doses TBZ at a dose of 60 mg/kg/day) "to provide additional data for selection of the high-dose level." Based on the results of this study the sponsor concluded, "These data confirmed a high dose level of 60 mg/kg/day would not exceed the maximum tolerated dose level in mice while still providing a high dose level at which tetrabenazine-related clinical effects could be expected." This suggests that the sponsor also considered female mice to be more sensitive to the treatment-induced toxicity of TBZ. (The in-life start date for this study was 15-Sept-03).

Limited toxicokinetic (TK) data are available in from the 90 day oral gavage toxicity study conducted in — CD-1@(ICR)BR mice (— study # 7425-102, module 4, volume 7). The revised TK study report was submitted as NDA amendment #0006). In this study, plasma tetrabenazine could not be determined due to technical difficulties. Plasma levels for the stereoisomeric metabolites of TBZ, α -dihydrotetrabenezine (α -HTBZ) and β -dihydrotetrabenezine (β -HTBZ) were evaluated for the LD, MD and HD (10, 30 and 60 mg/kg/day, QD). At 60 mg/kg/day, the highest doses evaluated, plasma exposure (based on AUC) to α -HTBZ and β -

HTBZ was much greater in females than in males. (see sponsor-provided figures and tables that follow).

Figure 1: Plasma concentrations of α -HTBZ in composite mice on Day 1 after oral gavage administration of a single 10, 30, or 60 mg/kg dose of tetrabenzazine to male and female mice.

Table 1: Summary of toxicokinetic parameters for α -HTBZ after oral gavage administration of 10, 30, or 60 mg/kg/day doses of tetrabenzazine to male and female mice.

Parameter	10 mg/kg/day		30 mg/kg/day		60 mg/kg/day	
	Female	Male	Female	Male	Female	Male
Day 1						
C _{max} (ng/mL)	1,392	1,186	3,468	3,710	8,074	7,032
T _{max} (h)	0.25	0.25	0.25	0.25	1.00	0.50
AUC _{0-∞} (h•ng/mL)	398	397	2,832	1,487	22,749	8,939
Day 92						
C _{max} (ng/mL)	1,271	98	4,329	2,238	8,499	6,033
T _{max} (h)	0.25	0.25	0.25	0.25	0.50	0.50
AUC _{0-∞} (h•ng/mL)	384	75	4,806	838	12,713	4,662

Tetrabenzazine

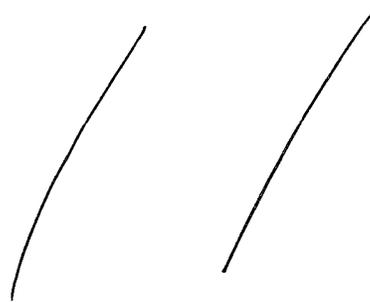
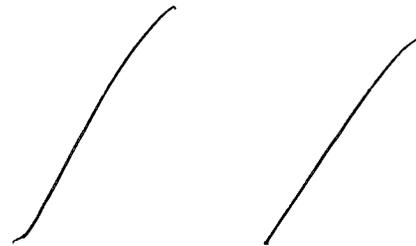


Figure 3: Plasma concentrations of β -HTBZ in composite mice on Day 1 after oral gavage administration of a single 10, 30, or 60 mg/kg dose of tetrabenzazine to male and female mice.

Table 2: Summary of toxicokinetic parameters for β -HTBZ after oral gavage administration of 10, 30, or 60 mg/kg/day doses of tetrabenzazine to male and female mice.

Parameter	10 mg/kg/day		30 mg/kg/day		60 mg/kg/day	
	Female	Male	Female	Male	Female	Male
Day 1						
C _{max} (ng/mL)	298	225	379	352	1,823	1,042
T _{max} (h)	0.25	0.25	0.25	0.25	0.25	0.50
AUC _{0-∞} (h•ng/mL)	127	126	997	599	4,676	1,791
Day 92						
C _{max} (ng/mL)	330	53	1,190	482	2,041	1,073
T _{max} (h)	0.25	0.50	0.25	0.25	0.50	0.50
AUC _{0-∞} (h•ng/mL)	121	34	1,207	280	3,441	1,467



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- According to the OECD guidelines for this assay, "The highest dose is defined as the dose producing signs of toxicity such that higher dose levels, based on the dosing regimen, would be expected to produce lethality." The selected high dose for this study (80 mg/kg/day) did demonstrate treatment-related toxicity after the single dose. The only data available for a higher dose (100 mg/kg/day) resulted in moribund sacrifice on Day 1 of dosing (see table above for details). Based on the limited information about the clinical observations in that study, it would appear that the choice of high dose (80 mg/kg/day) was adequate; however, concentration verification of the dosing formulation revealed that the formulation was approximately 90% nominal and therefore the actual dose administered was approximately 72 mg/kg.
- According to the study report, the slides were evaluated in a blinded fashion. Micronucleus frequency was determined by analyzing at least 2000 PCE/animal and the PCE:NCE ratio was determined in at least 500 erythrocytes per animal. The study had appropriate responses in the vehicle and positive control groups and thus, within the limitation of its single sex design, the study appears to be a valid.
- Sponsor's evaluation criteria: "The criteria for a positive response is the detection of a statistically significant increase in micronucleated PCEs for at least one dose level, and a

statistically significant dose-related response. A test article that does not induce both of these responses is considered negative. Statistical significance is not the only determinant of a positive response; the Study Director also considers the biological relevance of the results in the final evaluation.”

Study outcome: The animals were observed for signs of toxicity, immediately after dosing, 1 hr post dose and “at least daily for the duration of the assay.” All of the animals survived until scheduled sacrifice and the clinical observations are summarized in the following sponsor-supplied summary table that follows. With regard to hypoactivity the sponsor states that there was a “moderate decrease in movement but all animals responded well to stimulus.” The duration of the clinical observations in the 40 and 80 mg/kg dose groups was not reported, except that all animals appeared normal the next day.

The group mean ratio of PCE:NCE was decreased for the 40 and 80 mg/kg treated males when compared to the vehicle control males at 24 hrs, and for the 80 mg/kg treated males compared with the vehicle treated controls at 48 hrs; however the decreases were not statistically significant. There was no evidence of a treatment-related increase in the frequency of micronuclei at the 24 or 48 hr time points. (See the sponsor-provided summary table that follows).

Conclusion: The assay was negative; however, it was conducted in males only and should have been conducted in both males and females

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Target Dose Level (mg/kg)	Harvest Timepoint	Animal ID	Time After Dosing			
			IPD	1 hour PD	1 day	2 days
Vehicle Control 0	24	All	0	0	0	NA
	48	All	0	0	0	0
Positive Control 80	24	All	0	0	0	NA
10	24	7900	0	0	0	NH
		7914	0	0	0	NH
		7917	0	0	0	NH
		7918	0	0	0	NH
		7937	0	0	0	NH
20	24	7907	0	0	0	NA
		7909	0	0	0	NA
		7919	0	0	0	NA
		7938	0	0	0	NA
		7941	0	0	0	NA
40	24	7912	0	1,2,3	0	NA
		7913	0	1,2,3	0	NA
		7922	0	1,2,3,4	0	NA
		7930	0	1,2,3	0	NA
		7939	0	1,2,3	0	NA
	48	7920	0	0	0	NH
		7923	0	1,2,3	0	NH
		7928	0	1,2,3	0	NH
		7935	0	1,2,3	0	NH
		7936	0	1,2,3	0	NH
80	24	7905	0	1,2,5	0	NA
		7916	0	1,2,5	0	NA
		7927	0	1,2,5	0	NA
		7931	0	1,2,5	0	NA
		7940	0	1,2,5	0	NA
	48	7921	0	1,2,5	0	0
		7926	0	1,2,3	0	0
		7929	0	1,2,5	0	0
		7933	0	1,2,3	0	0
		7943	0	1,2,5	0	0

Key: 0 = Normal, 1 = Squinted eyes, 2 = Hunched posture, 3 = Slightly hypoactive, 4 = Irregular breathing, 5 = Hypoactive
 NA = not applicable, animal sacrificed at the 24-hour harvest timepoint.
 NH = not applicable, animal sacrificed at the 24-hour timepoint, bone marrow was not harvested, and the animal was disposed.
 IPD = Immediately post dosing
 PD = Post dosing

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Table 3: Micronucleus Assay – Summary Table

Assay No.: 27327-0-455OECD
 Test Article: Tetrabenazine
 Initiation of Dosing: 19 July 2005

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle	VC 10 ml/kg	24 hr	0.04 ± 0.02	0.55 ± 0.04
		48 hr	0.04 ± 0.02	0.59 ± 0.04
Positive	CP 80 mg/kg	24 hr	1.34 ± 0.29 *	0.41 ± 0.03 **
Test Article	20 mg/kg	24 hr	0.01 ± 0.01	0.56 ± 0.06
		48 hr	0.03 ± 0.02	0.49 ± 0.06
	40 mg/kg	24 hr	0.04 ± 0.01	0.48 ± 0.05
		48 hr	0.03 ± 0.01	0.47 ± 0.04

* Significantly greater than the corresponding vehicle control, p ≤ 0.01.

** Significantly less than the corresponding vehicle control, p ≤ 0.01.

VC = 0.5% CMC and 0.1% Tween-80

CP = Cyclophosphamide

PCE = Polychromatic erythrocyte

NCE = Normochromatic erythrocyte

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Study title: α -Dihydropyridazinone – Bacterial Reverse Mutation Test

Key findings: negative

Study no.: BCR 001/042467

Volume #19, module 4

Conducting laboratory and location.

(formulation chemistry was conducted at

Date of study initiation: 06-Apr-04

GLP compliance: Yes, UK-GLP, OECD-GLP and the EC Commission Directive

QA reports: yes (x) no (), the protocol and report had individual based audits; however, the rest were process based inspections conducted "at or about the time this study was in progress."

Drug, lot #, and % purity: α -Dihydropyridazinone (α -HTBZ) Batch # RUS 0406, stated purity of >98%. α -HTBZ was dissolved in DMSO. The stability of α -HTBZ in DMSO was evaluated as part of the study (0.39 and 50 mg/ml for 4 hrs or 2 days at room temperature and 2 days refrigerated. The greatest change in concentration was 2.2% seen at 50 mg/ml stored for 2 days at room temperature. (In the analytical section it states, that these test periods represent "the maximum time from preparation to completion of use"). The concentration evaluations (of the second assay) revealed that the actual concentrations of the test formulations was increased by 14.6 – 18.6% the nominal concentration; therefore, according to the sponsor, they were diluted by 10% prior to use.

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* strain, WP2 *uvrA* (pKM101)

Doses used in definitive study: see the reviewer generated summary tables for details.

Basis of dose selection: The highest concentration tested, 5000 μ g/plate, is the maximum suggested concentration for non cytotoxic, freely soluble agents based on the current OECD guidelines for this assay.

Negative controls: DMSO served as the vehicle and vehicle control

Positive controls: as stated in the following reviewer-generated table:

agent	solvent	concentration	S9	strains
sodium azide	DMSO	0.5 μ g/plate	- S9	TA1535, TA100
9-aminoacridine	DMSO	50 μ g/plate	- S9	TA1537
2-nitrofluorene	DMSO	1 μ g/plate	- S9	TA98
2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.05 μ g/plate	- S9	WP2 <i>uvrA</i> (pKM101)
2-aminoanthracene	DMSO	2 μ g/plate & 10 μ g/plate	+ S9	TA1535 & WP2 <i>uvrA</i> (pKM101)
benzo[a]pyrene	DMSO	5 μ g/plate	+ S9	TA1537, TA98, and TA100

Metabolic activation system: S9 from male SD-derived rats treated with Aroclor 1254

Incubation and sampling times: The first test employed the plate incorporation technique and the second test employed the pre-incubation technique (30 minute preincubation period). For both tests it appears that plates were run in triplicates and incubated for approximately 72 hrs, background lawn examined and revertant colonies counted (automated colony counter).

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): For both tests it appears that plates were run in triplicates and incubated for approximately 72 hrs, background lawn examined and revertant colonies counted (automated colony counter).

The protocol specified that the following criteria were to be used to assess mutagenic potential:

If exposure to a test substance produces a reproducible increase in revertant colony numbers of at least twice (three times in the case of strains TA1535 and TA1537) the concurrent vehicle controls, with some evidence of a positive dose-response relationship, the test substance is considered to exhibit mutagenic activity in this test system. No statistical analysis was performed.

If exposure to a test substance does not produce a reproducible increase in revertant colony numbers, the test substance is considered to show no evidence of mutagenic activity in this test system. No statistical analysis was performed.

If the results obtained failed to satisfy the criteria for a clear "positive" or "negative" response, even after additional testing, the test data were to be subjected to possible analysis to determine the statistical significance of any increases in revertant colony numbers. The statistical procedures planned were those described by Mahon *et al* (1989) and were to use Dunnett's test followed, if appropriate, by trend analysis. Biological importance was to be considered along with statistical significance. In general, treatment-associated increases in revertant colony numbers below two or three times the vehicle controls (as described above) were not to be considered biologically important. It should be noted that it was acceptable to conclude an equivocal response if no clear results could be obtained.

Occasionally, these criteria may not be appropriate to the test data and, in such cases, the Study Director was to use his/her scientific judgement.

Study outcome: See the reviewer-generated summary tables that follow for details. In the first assay (plate incorporation technique), the sponsor covered test article concentrations from 5 – 5000 µg/plate in seven steps (5, 15, 50, 150, 500, 1500, and 5000 µg/plate). For the second assay (preincubation) the sponsor eliminated the two lowest concentrations (5 and 15 µg/plate) and covered the range from 50 – 500 µg/plate with the same concentrations as used in the first assay. In certain strains the highest concentration tested, 5000 µg/plate was cytotoxic, as demonstrated by a decrease in mutation frequency. Ideally, the sponsor should have tested a concentration between 1500 and 5000 µg/plate; however, that was not done. In the second assay (pre-incubation technique) for *Escherichia coli* strain, WP2 *uvrA* (pKM101), in the presence of metabolic activation, the positive control produced a weak, but positive response (mean number of revertant was 2.3x the vehicle control). Under the conditions of the assays, the test article, α-HTBZ, did not produce a mutagenic response in the tester strains.

1 st Assay Plate Incorporation (mean revertant colony count per plate ± SD)						
test substance	concentration (µg/plate)	TA 98	TA100	TA1535	TA1537	WP2 <i>uvrA</i> (pKM101)
absence of metabolic activation						
vehicle	0	31 ± 1	134 ± 12	21 ± 3	12 ± 3	109 ± 12
α-HTBZ	5	32 ± 5	141 ± 22	22 ± 2	11 ± 2	111 ± 14
α-HTBZ	15	40 ± 3	142 ± 12	23 ± 2	9 ± 1	117 ± 9
α-HTBZ	50	34 ± 4	146 ± 7	20 ± 4	10 ± 2	105 ± 13
α-HTBZ	150	33 ± 5	150 ± 19	20 ± 1	13 ± 3	131 ± 15
α-HTBZ	500	30 ± 2	153 ± 16	22 ± 6	11 ± 3	120 ± 16
α-HTBZ	1500	35 ± 4	159 ± 16	21 ± 1	11 ± 3	111 ± 14
α-HTBZ	5000	33 ± 2	160 ± 10	17 ± 3	7 ± 2	118 ± 10
positive control	variable	372 ± 69	495 ± 22	709 ± 90	662 ± 157	570 ± 9
presence of metabolic activation						
vehicle	0	41 ± 5	167 ± 16	20 ± 4	28 ± 1	147 ± 6
α-HTBZ	5	48 ± 6	161 ± 12	22 ± 3	26 ± 5	151 ± 13
α-HTBZ	15	37 ± 10	187 ± 18	20 ± 3	23 ± 3	153 ± 21
α-HTBZ	50	47 ± 4	173 ± 13	23 ± 1	27 ± 4	166 ± 15
α-HTBZ	150	39 ± 6	156 ± 14	19 ± 3	22 ± 6	147 ± 9
α-HTBZ	500	39 ± 2	161 ± 15	21 ± 6	21 ± 4	158 ± 12
α-HTBZ	1500	41 ± 3	178 ± 14	21 ± 2	21 ± 3	145 ± 20
α-HTBZ	5000	37 ± 2	156 ± 11	20 ± 3	22 ± 8	142 ± 10
positive control	variable	953 ± 65	1074 ± 135	498 ± 73	160 ± 24	527 ± 42

2 nd Assay Pre-incubation Technique (mean revertant colony count per plate ± SD)						
test substance	concentration	TA 98	TA100	TA1535	TA1537	WP2 <i>uvrA</i> (pKM101)
absence of metabolic activation						
vehicle	0	26 ± 4	110 ± 13	14 ± 2	9 ± 1	145 ± 18
α-HTBZ	50	22 ± 2	116 ± 8	18 ± 4	10 ± 0	135 ± 14
α-HTBZ	150	23 ± 5	92 ± 10	17 ± 3	9 ± 1	117 ± 26
α-HTBZ	500	30 ± 6	92 ± 31	16 ± 4	8 ± 1	138 ± 18
α-HTBZ	1500	24 ± 4	107 ± 6	14 ± 1	10 ± 3	130 ± 3
α-HTBZ	5000	16 ± 3	81 ± 6	8 ± 2	4 ± 1	94 ± 13
positive control	variable	304 ± 27	499 ± 55	396 ± 6	422 ± 68	772 ± 273
presence of metabolic activation						
vehicle	0	36 ± 4	102 ± 7	22 ± 5	27 ± 6	152 ± 10
α-HTBZ	50	35 ± 7	93 ± 1	13 ± 2	26 ± 4	130 ± 23
α-HTBZ	150	33 ± 4	97 ± 4	18 ± 5	19 ± 5	132 ± 32
α-HTBZ	500	32 ± 1	117 ± 27	17 ± 3	21 ± 6	123 ± 25
α-HTBZ	1500	25 ± 6	118 ± 14	16 ± 8	30 ± 5	148 ± 12
α-HTBZ	5000	22 ± 6	96 ± 7	13 ± 3	24 ± 4	108 ± 10
positive control	variable	303 ± 121	481 ± 81	94 ± 1	200 ± 40	353 ± 72

Study title: α -Dihydrotrabenazine – *In Vitro* Mammalian Chromosome Aberration Test in CHL Cells

Key findings: positive in the presence and absence of metabolic activation

Study no.: BCR 002/043071

Volume # 19, module 4

Conducting laboratory and location: _____

Date of study initiation: 19-Apr-04

GLP compliance: yes – UK-GLP, OECD-GLP, and the European Community Commission Directive with the following exception, “The sampling and determination of homogeneity of the test substance in the vehicle was not determined as part of the study.”

QA reports: yes (x) no () There were protocol and report audits; however, the rest of the inspections were “process based inspections” conducted “at or about the time this study was in progress.”

Drug, lot #, and % purity: α -Dihydrotrabenazine, (α -HTBZ), (2-hydroxy-3-isobutyl-1,2,3,4,6,7-hexahydro-9,10-dimethoxy-11b-*H*-benzo[a]quinolizine), batch # RUS 0406, with >98% purity.

According to the report. “The stability of α -dihydrotrabenazine in the vehicle was determined as part of _____ Study No. BCR/001. [report not included] The homogeneity of α -dihydrotrabenazine in the vehicle was not determined as part of the study.” Concentration determinations were conducted on the dosing formulations from Test #2. The mean concentrations varied from 95-109.6% nominal except at the highest concentration (43.38 mg/ml) which was 115.5% nominal. The dose volume of for this dose was adjusted based on the concentration.

Methods

Strains/species/cell line: Chinese Hamster Lung (CHL) cells (strain IU). According to the sponsor, they have a doubling time of approximately 10 hrs and a modal chromosome number of 25.

Doses used in definitive study: see the following reviewer generated tables for details.

Basis of dose selection: Test article-induced cytotoxicity (see results for further discussion).

Solvent controls: DMSO. According to the sponsor, the test article was soluble in DMSO at 48.6645 mg/ml. “On dosing at 2% v/v into aqueous tissue culture medium, giving a final concentration of 973.29 μ g/ml, no precipitation was evident. A color change in the culture medium was observed, however the pH change was less than 1.0 unit. ...In this case, the highest final concentration used for subsequent testing was 1000 μ g/ml.”

Positive controls: Mitomycin C (MMC) (-S9), and Cyclophosphamide (CPH) (+S9)

Metabolic Activation System: S9 from Aroclor 1254 treated male “Sprague Dawley derived” rats

Incubation and sampling times:

TABLE # 1 - List of Tests and Incubation Conditions					
S9 mix	Test	Treatment period	Recovery period	Colcemid	Harvest
+ S9	test 1	0-3 hrs	3-15 hrs	13-15 hrs	15 hrs
- S9	test 1	0-3 hrs	3-15hrs	13-15 hrs	15 hrs
+ S9	repeat test 1	0-3 hrs	3-15 hrs	13-15 hrs	15 hrs
- S9	repeat test 1	0-3 hrs	3-15 hrs	13-15 hrs	15 hrs
+ S9	test 2	0-3 hrs	3-15 hrs	13-15 hrs	15 hrs
- S9	test 2	0-3 hrs	3-15 hrs	13-15 hrs	15 hrs

Cultures established approximately 24 hrs prior to exposure period
Vehicle controls, positive controls, and test article treated cultures were run in duplicates.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Vehicle controls, positive controls and test article treated cultures were run in duplicates.

- “The proportion of mitotic cells per 1000 cells in all cultures was recorded.”
- “From the cell count data, the dose level causing a reduction of at least 50% (approximately) of the solvent control value or, if there is no decrease, the maximum achievable concentration was used as the highest dose level for metaphase analysis. Mitotic index data were used to assist the selection of the highest dose level. The intermediate and low dose levels were also selected.”
- “The concentration of each positive control compound selected for analysis was the lowest concentration dosed unless a preliminary scan of metaphase figures indicated an insufficient level of aberrant cells.”
- “The selected slides were then coded. ...One hundred metaphase figures were examined, where possible, from each culture. Chromosome aberrations were scored according to the classification of the ISCN (1985). Only cells with 23 – 27 chromosomes were analysed. Polyploid and endoreduplicated cells were noted when seen.”
- “The incidence of polyploidy metaphase cells, out of 500 metaphase cells, was determined quantitatively for all cultures used in the analysis for chromosomal aberrations.”
- “The number of aberrant polyploid metaphase cells in each treatment group was compared with the solvent control value using a one-tailed Fisher’s exact test...”

Sponsor’s criteria for clastogenicity:

The test substance was to be considered to have caused a positive response if the following conditions were met:

Statistically significant increases ($P < 0.01$) in the frequency of metaphases with aberrant chromosomes (excluding gaps) are observed at one or more test concentration.

The increases exceed the negative control range of this laboratory, taken at the 99% confidence limit.

The increases are reproducible between replicate cultures.

The increases are not associated with large changes in pH, osmolality of the treatment medium or extreme toxicity.

Evidence of a dose-relationship is considered to support the conclusion.

A negative response was to be claimed if no statistically significant increases in the number of aberrant cells above concurrent control frequencies were observed, at any dose level.

A possible further evaluation was to be carried out if the above criteria for a positive or a negative response were not met.

Study outcome: See the reviewer-generated summary tables that follow for details. Test 1 was conducted in the presence and absence of metabolic activation at test article concentrations of 7.81 – 1000 µg/ml. Cytotoxicity analysis revealed demonstrated a “steep toxic response in both the absence and presence of S9 mix at concentrations above 500 µg/ml” (see Table #2). The sponsor did not analyze these cultures for evidence of clastogenicity. Instead, the sponsor conducted a repeat of Test 1, using the same procedure,

with adjusted concentrations of the test article (see Tables #3 and #4). In the presence and absence of metabolic activation α -HTBZ caused an increase in the mean percentage of cells with structural aberrations and the total number of structural aberrations. There was no treatment related increase in polyploidy in the presence or absence of metabolic activation. It appears that higher concentrations of α -HTBZ could have been evaluated in both the presence and absence of metabolic activation.

Test 2 was conducted in the presence and absence of metabolic activation (see Tables 5 and 6 for details). In the absence of metabolic activation α -HTBZ demonstrated cytotoxicity at lower concentrations than in Test #1, and the sponsor did not conduct a metaphase analysis. In the presence of metabolic activation (Table # 6) α -HTBZ caused an increase in the mean percentage of cells with structural aberrations, the total number of structural aberrations and a possible increase in the frequency of polyploidy. It should be noted that the frequency of polyploidy was within the historical control range. The sponsor made the following comments about the α -HTBZ induced increase in aberration frequency:

“The quantitative increase in aberration frequency observed at 800 μ g/ml was inconsistent with accompanying results, as only at 800 μ g/ml was a significant reduction in mitotic index (62%) observed. This reduction in cytotoxicity was also associated with increased aberration frequency.

Additional slides from 800 μ g/ml formulations [reviewer note – from cultures exposed in the absence of metabolic activation] with no associated cytotoxicity (mitotic index 95% were therefore analysed to clarify the inconsistency. The results showed no increased aberration frequency and would therefore suggest the previous results may be an irregularity.”

The relevance of data generated in the absence of metabolic activation to clarify findings generated in the presence of metabolic activation (as proposed by the sponsor) is not clear.

Conclusion: α -Dihydropyridazinone, (α -HTBZ) was clastogenic in the *in vitro* CHL assay in the presence (two independent assays) and absence (in the only assay conducted) of metabolic activation.

Potential problems: The importance of the technical problem listed below is diminished; in the face of repeatably positive results.

1. Stability/homogeneity was not reported here, nor was the time elapsed between test article preparation and use.

TABLE # 2 - Results of First Test – not evaluated further

treatment – concentration (μ g/ml)	- S9, 3 hrs treatment, 12 hr recovery			+ S9, 3 hrs treatment, 12 hr recovery		
	relative cell count	mitotic index (%)	relative mitotic index (%)	relative cell count	mitotic index (%)	relative mitotic index (%)
solvent control	100	13.1	100	100	15.0	100
α -HTBZ – 7.81	95	12.8	98	102	16.2	108
α -HTBZ 15.63	99	12.5	95	95	16.2	108
α -HTBZ – 31.25	99	12.5	95	89	16.8	112
α -HTBZ – 62.5	89	12.1	92	102	17.3	115
α -HTBZ – 125	85	12.4	95	81	16.3	109
α -HTBZ – 250	88	11.3	86	78	15.9	106
α -HTBZ – 500	74	11.1	85	79	13.6	91
α -HTBZ – 1000	5	(b)	(b)	59	6.8	45
MMC – 0.1	73	12.6	96	-	-	-
MMC – 0.2	80	7.7	59	-	-	-
CPH – 5	-	-	-	79	8.9	59
CPH – 10	-	-	-	75	5.7	38

(b) - “No cells, no metaphases present on slide.”

TABLE # 3 - Repeat Test 1 (-S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	14.0	100	200	14	4	5	1.5	0.5
α-HTBZ - 125	86	16.0	114						
α-HTBZ - 250	76	13.8	99						
α-HTBZ - 500	79	15.3	109						
α-HTBZ - 550	68	13.2	94						
α-HTBZ - 600	66	12.9	92	200	17	8	6.5	3.0	0.5
α-HTBZ - 650	69	11.8	84	200	19	14	7.0	5.5	0.3
α-HTBZ - 700	62	8.0	57						
α-HTBZ - 750	50	7.7	55	200	38	31	11.0	9.0***	0.3
α-HTBZ - 800	23	3.3	24						
α-HTBZ - 850	23	^	-						
α-HTBZ - 900	9	^	-						
α-HTBZ - 950	12	^	-						
MMC - 0.1	75	14.6	104	200	42	39	17.5***	16.5***	0.1
MMC - 0.2	70	10.1	72						

^ - "Very few viable cells present on the slide."
 (§) not evaluated statistically
 shaded area not evaluated further
 *** p<0.001

TABLE #4 - Repeat Test 1: +S9, 3 hrs treatment, 12 hr recovery

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	12.2	100	200	16	4	5.0	2.0	0.3
α-HTBZ - 250	87	12.3	101						
α-HTBZ - 500	84	11.6	95	200	29	20	9.5	6.0	0.1
α-HTBZ - 600	90	9.2	75						
α-HTBZ - 700	83	8.7	71	200	26	19	10.5	8.0**	0.3
α-HTBZ - 800	80	6.4	52	200	36	22	11.5**	7.5**	0.2
α-HTBZ - 900	67	4.9	40						
α-HTBZ - 1000	48	1.4	11						
CPH - 5	97	6.8	56	200	143	110	37.0***	33.0***	0.1
CPH - 10	68	3.7	30						

(§) not evaluated statistically
 shaded area not evaluated further
 *** p<0.001, ** p<0.01

TABLE # 5 - Test 2 (-S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % of cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	6.5	100						
α-HTBZ - 400	59.1	9.3	143						
α-HTBZ - 500	69.8	8.9	137						
α-HTBZ - 600	38.6	4.3	66						
α-HTBZ - 625	19.1	1.7	26						
α-HTBZ - 650	7.0	^	-						
α-HTBZ - 675	2.8	^	-						
α-HTBZ - 700	2.8	^	-						
α-HTBZ - 725	3.3	^	-						
α-HTBZ - 750	2.8	^	-						
α-HTBZ - 775	11.2 §	^	-						
α-HTBZ - 800	2.8	^	-						
MMC - 0.1	55.3	12.9	198						
MMC - 0.2	64.7	10.1	155						

§ - individual values are 0.04 and 0.43
 ^ - "Very few viable cells, no metaphases present on slide."

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TABLE # 6 - Test 2: (+ S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations		mean % of cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	17.1	100	200	8	4	3.5	2.0	0.2
α-HTBZ - 250	94.4	17.4	102						
α-HTBZ - 500	76.8	17.5	102						
α-HTBZ - 550	84.3	18.6	109						
α-HTBZ - 600	78.3	17.1	100						
α-HTBZ - 650	76.3	16.9	99						
α-HTBZ - 700	84.3	18.7	109						
α-HTBZ - 750	77.3	15.8	92	200	26	14	10.0**	6.0	0.4
α-HTBZ - 800	60.6	10.6	62	200	45	40	14.5***	13.5***	0.1
α-HTBZ - 867.6	75.8	14.0	82	200	22	16	8.5	6.5	0.4
α-HTBZ - 400 [§]	93.4	15.7	92						
α-HTBZ - 800 [§]	75.3	16.3	95						
CPH - 5	80.8	11.3	66	200	70	67	19.5***	19.5***	0.1
CPH - 10	66.2	8.0	47						

§ - individual values are 0.04 and 0.43
 ^ - "Very few viable cells, no metaphases present on slide."
 & - "Additional cultures dosed from - S9 formulations."
 *** p<0.001, ** p<0.01

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APPEARS THIS WAY ON ORIGINAL

Study title: β -Dihydropyridazinone – Bacterial Reverse Mutation Test

Key findings: negative

Study no.: BCR 003/042468

Volume # 19 module 4

Conducting laboratory and location:

formulation chemistry was conducted at

Date of study initiation: 06-Apr-04

GLP compliance: yes, UK-GLP, OECD-GLP, and EC Commission Directive

QA reports: yes (x) no () the protocol and report had individual based audits; however, the rest were process based inspections conducted "at or about the time this study was in progress."

Drug, lot #, and % purity: β -Dihydropyridazinone (β -HTBZ), batch #RUS 0407, stated purity of >98%. The stability of β -HTBZ in DMSO was evaluated as part of the study (05 and 100 mg/ml for 4 hrs or 2 days at room temperature and 2 days refrigerated. The greatest change in concentration was +2.7% seen at 0.5 mg/ml stored for 2 days at room temperature. (In the analytical section it states, that these test periods represent "the maximum time from preparation to completion of use"). The concentration evaluations of the 0.5, 5 and 50 mg/ml solutions revealed that the actual concentrations of the test formulations were slightly decreased (0.7-3.2%) compared to the nominal concentration.

Methods

Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* strain, WP2 *uvrA* (pKM101)

Doses used in definitive study: see the reviewer generated summary tables for details.

Basis of dose selection: The highest concentration tested, 5000 μ g/plate, is the maximum suggested concentration for non-cytotoxic, freely soluble agents based on the current OECD guidelines for this assay.

Negative controls: DMSO served as the vehicle and vehicle control

Positive controls: as stated in the following reviewer-generated table:

agent	solvent	concentration	S9	strains
sodium azide	DMSO	0.5 μ g/plate	- S9	TA1535, TA100
9-aminoacridine	DMSO	50 μ g/plate	- S9	TA1537
2-nitrofluorene	DMSO	1 μ g/plate	- S9	TA98
2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.05 μ g/plate	- S9	WP2 <i>uvrA</i> (pKM101)
2-aminoanthracene	DMSO	2 μ g/plate & 10 μ g/plate	+ S9	TA1535 & WP2 <i>uvrA</i> (pKM101)
benzo[a]pyrene	DMSO	5 μ g/plate	+ S9	TA1537, TA98, and TA100

Metabolic activation system: S9 from male SD-derived rats treated with Aroclor 1254

Incubation and sampling times: The first test employed the plate incorporation technique and the second test employed the pre-incubation technique (30 minute preincubation period). For both tests it appears that plates were run in triplicates and incubated for approximately 72 hrs, background lawn examined and revertant colonies counted (automated colony counter).

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): For both tests it appears that plates were run in triplicates and incubated for approximately 72 hrs, background lawn examined and revertant colonies counted — automated colony counter).

The protocol specified that the following criteria were to be used to assess mutagenic potential:

If exposure to a test substance produces a reproducible increase in revertant colony numbers of at least twice (three times in the case of strains TA1535 and TA1537) the concurrent vehicle controls, with some evidence of a positive dose-response relationship, the test substance is considered to exhibit mutagenic activity in this test system. No statistical analysis was performed.

If exposure to a test substance does not produce a reproducible increase in revertant colony numbers, the test substance is considered to show no evidence of mutagenic activity in this test system. No statistical analysis was performed.

If the results obtained failed to satisfy the criteria for a clear "positive" or "negative" response, even after additional testing, the test data were to be subjected to possible analysis to determine the statistical significance of any increases in revertant colony numbers. The statistical procedures planned were those described by Mahon *et al* (1989) and were to use Dunnett's test followed, if appropriate, by trend analysis. Biological importance was to be considered along with statistical significance. In general, treatment-associated increases in revertant colony numbers below two or three times the vehicle controls (as described above) were not to be considered biologically important. It should be noted that it was acceptable to conclude an equivocal response if no clear results could be obtained.

Occasionally, these criteria may not be appropriate to the test data and, in such cases, the Study Director was to use his/her scientific judgement.

Study outcome: See the reviewer-generated summary tables that follow for details. In the first assay (plate incorporation technique), the sponsor tested test article concentrations from 5 – 5000 µg/plate in seven steps (5, 15, 50, 150, 500, 1500, and 5000 µg/plate). For the second assay (preincubation) the sponsor eliminated the two lowest concentrations (5 and 15 µg/plate) and covered the range from 50 – 500 µg/plate with the same concentrations as used in the first assay. In certain strains the highest concentration tested, 5000 µg/plate was cytotoxic, as demonstrated by a decrease in mutation frequency. Ideally, the sponsor should have tested a concentration between 1500 and 5000 µg/plate; however, that was not done. Under the conditions of the assays, the test article, β-HTBZ, did not produce a mutagenic response in the tester strains.

1 st Assay Plate Incorporation Technique (mean revertant colony count per plate \pm SD)						
test substance	concentration (μ g/plate)	TA 98	TA100	TA1535	TA1537	WP2 <i>uvrA</i> (pKM101)
absence of metabolic activation						
vehicle	0	33 \pm 3	133 \pm 12	17 \pm 1	11 \pm 3	135 \pm 10
β -HTBZ	5	24 \pm 4	115 \pm 13	16 \pm 6	10 \pm 2	126 \pm 7
β -HTBZ	15	31 \pm 3	106 \pm 12	17 \pm 4	14 \pm 2	127 \pm 3
β -HTBZ	50	33 \pm 4	119 \pm 6	21 \pm 2	11 \pm 2	118 \pm 8
β -HTBZ	150	31 \pm 3	108 \pm 10	20 \pm 2	11 \pm 3	125 \pm 23
β -HTBZ	500	35 \pm 6	103 \pm 11	19 \pm 2	12 \pm 3	122 \pm 3
β -HTBZ	1500	35 \pm 6	113 \pm 8	17 \pm 3	11 \pm 1	117 \pm 8
β -HTBZ	5000	30 \pm 4	97 \pm 7	15 \pm 4	9 \pm 1	106 \pm 3
positive control	variable	321 \pm 3	706 \pm 104	621 \pm 79	1580 \pm 228	623 \pm 37
presence of metabolic activation						
vehicle	0	49 \pm 4	144 \pm 3	24 \pm 2	28 \pm 4	167 \pm 17
β -HTBZ	5	44 \pm 10	158 \pm 24	18 \pm 2	27 \pm 4	152 \pm 9
β -HTBZ	15	43 \pm 9	153 \pm 26	16 \pm 3	27 \pm 7	159 \pm 13
β -HTBZ	50	41 \pm 6	147 \pm 20	15 \pm 4	27 \pm 5	151 \pm 8
β -HTBZ	150	45 \pm 5	149 \pm 11	18 \pm 4	27 \pm 6	152 \pm 7
β -HTBZ	500	39 \pm 8	151 \pm 11	14 \pm 4	30 \pm 7	157 \pm 10
β -HTBZ	1500	40 \pm 5	132 \pm 16	14 \pm 5	25 \pm 7	157 \pm 15
β -HTBZ	5000	40 \pm 4	124 \pm 9	14 \pm 1	30 \pm 6	159 \pm 19
positive control	variable	958 \pm 60	928 \pm 102	209 \pm 32	381 \pm 57	599 \pm 127

2 nd Assay Preincubation Technique (mean revertant colony count per plate \pm SD)						
test substance	concentration (μ g/plate)	TA 98	TA100	TA1535	TA1537	WP2 <i>uvrA</i> (pKM101)
absence of metabolic activation						
vehicle	0	32 \pm 2	101 \pm 15	13 \pm 3	14 \pm 2	104 \pm 9
β -HTBZ	50	27 \pm 2	111 \pm 7	21 \pm 1	10 \pm 2	104 \pm 17
β -HTBZ	150	23 \pm 3	109 \pm 11	17 \pm 4	13 \pm 3	114 \pm 12
β -HTBZ	500	23 \pm 2	121 \pm 12	13 \pm 1	10 \pm 5	108 \pm 19
β -HTBZ	1500	21 \pm 5	136 \pm 14	12 \pm 2	13 \pm 1	122 \pm 15
β -HTBZ	5000	16 \pm 1	120 \pm 16	5 \pm 1	6 \pm 2	86 \pm 15
positive control	variable	257 \pm 55	575 \pm 137	373 \pm 77	1676 \pm 80	593 \pm 105
presence of metabolic activation						
vehicle	0	34 \pm 7	131 \pm 6	15 \pm 2	23 \pm 6	125 \pm 6
β -HTBZ	50	30 \pm 1	124 \pm 23	13 \pm 4	24 \pm 3	137 \pm 19
β -HTBZ	150	38 \pm 5	122 \pm 25	13 \pm 3	21 \pm 4	123 \pm 10
β -HTBZ	500	33 \pm 4	126 \pm 16	13 \pm 3	21 \pm 1	137 \pm 14
β -HTBZ	1500	33 \pm 4	116 \pm 23	11 \pm 3	21 \pm 2	118 \pm 6
β -HTBZ	5000	25 \pm 2	118 \pm 12	10 \pm 2	15 \pm 6	108 \pm 18
positive control	variable	341 \pm 39	473 \pm 44	81 \pm 7	266 \pm 13	534 \pm 67

Study title: β -Dihydropyridazinone – *In Vitro* Mammalian Chromosome Aberration Test in CHL Cells

Key findings: positive in the presence and absence of metabolic activation

Study no.: BCR 004/042969

Volume # 19, module 4

Conducting laboratory and location: _____

Date of study initiation: 19-Apr-04

GLP compliance: yes – UK-GLP, OECD-GLP, and the European Community Commission Directive with the following exception, “The sampling and determination of homogeneity of the test substance in the vehicle was not determined as part of the study.”

QA reports: yes (x) no () There were protocol and report audits; however, the rest of the inspections were “process based inspections” conducted “at or about the time this study was in progress.”

Drug, lot #, and % purity: β -Dihydropyridazinone, (β -HTBZ) (2-hydroxy-3-isobutyl-1,2,3,4,6,7-hexahydro-9,10-dimethoxy-11b-*H*-benzo[*a*]quinolizine), batch # RUS 0407, with > 98% purity.

According to the report, “The stability of β -dihydropyridazinone in the vehicle was determined as part of

_____ Study No. BCR/003. [report not included] The homogeneity of β -dihydropyridazinone in the vehicle was not determined as part of the study.” Concentration determinations were conducted on the dosing formulations from Test #2. The mean concentrations varied from 98-99% nominal for the concentrations of 30, 45 and 60 mg/ml. The lower concentrations were notably lower than the nominal concentrations (88.7% nominal for 1 mg/ml, 51.9% nominal for 1.875 mg/ml, and 82.8% nominal for 2.5 mg/ml).

Methods

Strains/species/cell line: Chinese Hamster Lung (CHL) cells (strain IU). According to the sponsor, they have a doubling time of approximately 10 hrs and a modal chromosome number of 25.

Doses used in definitive study: see the following reviewer-generated tables for details

Basis of dose selection: Test article induced cytotoxicity (see results for further discussion).

Vehicle control: DMSO. “Prior to commencing testing, the solubility of the test substance in solvents compatible with the test system was assessed. After vortexing, sonication and incubation at 37°C β -Dihydropyridazinone was found to be soluble in dimethyl sulphoxide (DMSO) at a concentration of 97.12 mg/ml. On dosing at 2% v/v into aqueous tissue culture medium, giving a final concentration of 1942.43 μ g/ml, no precipitate was evident. A color change in the culture medium was observed, however the pH change was less than 1.0 unit... In this case, the highest final concentration used for subsequent testing was 2000 μ g/ml.”

Positive controls: Mitomycin C (MMC) (-S9), and Cyclophosphamide (CPH) (+S9)

Metabolic Activation System: S9 from Aroclor 1254 treated male “Sprague Dawley derived” rats.

Incubation and sampling times:

TABLE # 1 - List of Tests and Incubation Conditions					
metabolic status	Test	Treatment period	Recovery period	Colcemid	Harvest
+ S9	first test	0-3 hrs	3-15 hrs	13-15 hrs	15 hrs
- S9	first test	0-3 hrs	3-15 hrs	13-15 hrs	15 hrs
+ S9	1 st repeat first test	0-3 hrs	3-12 hrs	13-15 hrs	15 hrs
- S9	1 st repeat first test	0-3 hrs	3-12 hrs	13-15 hrs	15 hrs
+ S9	2 nd repeat first test	0-3 hrs	3-12 hrs	13-15 hrs	15 hrs
+ S9	second test	0-3 hrs	3-12 hrs	13-15 hrs	15 hrs
- S9	second test	0-3 hrs	3-12 hrs	13-15 hrs	15 hrs

Cultures established approximately 24 hrs prior to exposure period
 Vehicle controls, positive controls and test article treated cultures were run in duplicates.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Vehicle controls, positive controls and test article treated cultures were run in duplicates.

- “The proportion of mitotic cells per 1000 cells in all cultures was recorded.”
- “From the cell count data, the dose level causing a reduction of at least 50% (approximately) of the solvent control value or, if there is no decrease, the maximum achievable concentration was used as the highest dose level for metaphase analysis. Mitotic index data were used to assist the selection of the highest dose level. The intermediate and low dose levels were also selected.”
- “The concentration of each positive control compound selected for analysis was the lowest concentration dosed unless a preliminary scan of metaphase figures indicated an insufficient level of aberrant cells.”
- “The selected slides were then coded. ...One hundred metaphase figures were examined, where possible, from each culture. Chromosome aberrations were scored according to the classification of the ISCN (1985). Only cells with 23 – 27 chromosomes were analysed. Polyploid and endoreduplicated cells were noted when seen.”

Sponsor’s criteria for clastogenicity:

The test substance was to be considered to have caused a positive response if the following conditions were met:

Statistically significant increases ($P < 0.01$) in the frequency of metaphases with aberrant chromosomes (excluding gaps) are observed at one or more test concentration.

The increases exceed the negative control range of this laboratory, taken at the 99% confidence limit.

The increases are reproducible between replicate cultures.

The increases are not associated with large changes in pH, osmolality of the treatment medium or extreme toxicity.

Evidence of a dose-relationship is considered to support the conclusion.

A negative response was to be claimed if no statistically significant increases in the number of aberrant cells above concurrent control frequencies were observed, at any dose level.

A possible further evaluation was to be carried out if the above criteria for a positive or a negative response were not met.

Study outcome: See the reviewer-generated summary tables that follow for details. Test 1 (Table #2) was conducted in the presence and absence of metabolic activation at test article concentrations of 15.63 – 2000 µg/ml. According to the sponsor, “Due to a steep toxic response at concentrations above 1000 µg/ml in the absence of S9 mix and 62.5 µg/ml in the presence of S9 mix, a repeat test was performed...”. The sponsor did not analyze these cultures for evidence of clastogenicity. Instead, the sponsor conducted a repeat of Test 1, using the same procedure, with adjusted concentrations of the test article (see Tables #3 and #4). In the absence of metabolic activation β-HTBZ caused an increase in the mean percentage of cells with structural aberrations and the total number of structural aberrations. There was no treatment related increase in polyploidy. In the presence of metabolic activation sufficient cytotoxicity was not achieved (the sponsor referred to it as an “inappropriate toxicity profile”) (see Table #4) and the sponsor did not analyze these cultures for evidence of clastogenicity. The sponsor conducted an additional test in the presence of metabolic activation (referred to as first test, second repeat) (Table # 5), in which β-HTBZ caused an increase in the mean percentage of cells with structural aberrations and the total number of structural aberrations. There was no treatment related increase in polyploidy.

A second test was carried out in the presence and absence of metabolic activation (Tables #6 and #7). In the presence and absence of metabolic activation β-HTBZ caused an increase in the mean percentage of cells with structural aberrations and the total number of structural aberrations. There was no treatment related increase in polyploidy compared to solvent control.

Conclusion: In two independent assays β-Dihydrotrabenzazine (β-HTBZ) is clastogenic in the *in vitro* CHL assay in the presence and absence of metabolic activation.

Potential problems: The assays had some technical problems that are listed below; however, since the results are repeatably positive, the importance of these issues is diminished.

1. Stability/homogeneity was not reported here, nor was the time elapsed between test article preparation and use.
2. Concentration evaluations were notably low for the lower concentration range in test 2 (only test evaluated for concentration).
3. The QA statement did not state that the report accurately reflected the data.
4. The aberration frequency for some of the solvent controls and positive controls were outside the range of historical control values
Table 3 – aberration frequency for solvent control (+ gaps) (7.0 vs 6.0 upper limit for h.c.)
Table 6 – aberration frequency for solvent control (- gaps) (0.5 vs 1.0 lower limit for h.c.)
Table 7 – aberration frequency for positive control (+ gaps) (17.0 vs 18 lower limit for h.c.)
5. frequency of polyploidy for some solvent controls were outside limit of historical control
Table 5 – solvent control – 0.7 vs upper limit of 0.6 in historical control
Table 6 – solvent control – 0.8 vs upper limit of 0.7 in historical control
Table 7 – solvent control – 0.9 vs upper limit of 0.6 in historical control

TABLE # 2 - Results of First Test - not evaluated further

treatment (µg/ml)	- S9, 3 hrs treatment, 12 hr recovery			+ S9, 3 hrs treatment, 12 hr recovery		
	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)
vehicle control	100	11.4	100	1.72	100	100
β-HTBZ - 15.63	115	11.0	96	1.44	84	110
β-HTBZ - 31.25	111	10.8	95	0.99	58	40
β-HTBZ - 62.5	118	9.9 (b)	-	0.68	40	8
β-HTBZ - 125	120	10.6	93	0.49	28	(d)
β-HTBZ - 250	110	10.1	89	0.50	29	(d)
β-HTBZ - 500	91	8.7	76	0.49	28	(e)
β-HTBZ - 1000	30	4.6	40	0.37	22	(e)
β-HTBZ - 2000	11	c	-	0.06	3	(f)
MMC - 0.1	101	11.5	101	-	-	-
MMC - 0.2	92	8.7	76	-	-	-
CPH - 5	-	-	-	1.30	76	(g)
CPH - 10	-	-	-	1.25	73	(g)

(b) - based on single culture - duplicate culture was "lost during treatment, no data obtainable"
(c) - "Few viable cells, no metaphases present on slides."
(d) - "Cells, no metaphases present on slides"
(e) - "No viable cells or metaphases present on slides"
(f) - "No cells or metaphases present on slides"
(g) - "Due to chromosome scattering accurate results are unobtainable"

TABLE # 3 - First Test Repeat (- S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % of cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	10.2	100	200	17	7	7.0	3.0	0.6%
β-HTBZ - 125	70	10.1	99						
β-HTBZ - 250	85	10.2	100						
β-HTBZ - 375	93	10.3	101						
β-HTBZ - 500	62	9.5	93						
β-HTBZ - 625	69	10.9	107						
β-HTBZ - 750	57	9.9	97	200	4	1	2.0	0.5	0.5%
β-HTBZ - 875	49	8.9	87						
β-HTBZ - 1000	51	7.3	72	200	47	36	13.5	10.5 ***	0.5%
β-HTBZ - 1125	34	5.3	52	200	90	84	24.0 ***	23.0 ***	0.5%
MMC - 0.1	77	14.5	142	200	58	43	20.0 ***	17.0 ***	0.1%
MMC - 0.2	67	10.4	102						

(§) not evaluated statistically
*** p < 0.001
shaded area not evaluated further

TABLE # 4 - First Test Repeat (+ S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % of cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	11.9	100						
β-HTBZ - 15.0	80	13.7	115						
β-HTBZ - 17.5	51	12.1	102						
β-HTBZ - 20.0	60	11.5	97						
β-HTBZ - 22.5	63	11.4	96						
β-HTBZ - 25.0	54	14.3	120						
β-HTBZ - 27.5	54	12.8	108						
β-HTBZ - 30.0	53	11.1	93						
β-HTBZ - 32.5	42	10.6	89						
β-HTBZ - 35.0	49	10.0	84						
CPH - 5	53	6.8	57						
CPH - 10	43	3.9	33						

shaded area not evaluated further

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TABLE # 5 - First Test 2nd Repeat (+ S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % of cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	13.9	100	200	11	9	5.0	4.0	0.7
β-HTBZ - 15.0	99	16.3	117						
β-HTBZ - 20.0	87	13.9	100	200	14	11	5.0	3.5	0.3
β-HTBZ - 25.0	75	13.2	95						
β-HTBZ - 30.0	66	9.4	68	200	94	89	31.5 ***	30.0 ***	0.3
β-HTBZ - 35.0	66	8.5	61						
β-HTBZ - 40.0	57	5.4	39						
β-HTBZ - 45.0	57	6.0	43	200	146	131	40.0 ***	38.0 ***	0.4
β-HTBZ - 50.0	51	4.0	29						
β-HTBZ - 55.0	55	4.9	35						
β-HTBZ - 60.0	52	4.0 (b)	-						
β-HTBZ - 65.0	48	1.8	13						
CPH - 5	75	9.9	71	200	149	139	40.5 ***	39.0 ***	0.3
CPH - 10	83	7.0	50						

(b) - derived from single culture, duplicate was unusable ("Very few viable cells, 1000 cells unobtainable from culture.")
 (§) - not evaluated statistically
 *** p < 0.001

TABLE # 6 - Second Test (- S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	Relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % of cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	15.2	100	200	7	1	2.5	0.5	0.8
β-HTBZ - 600	79	12.0	79						
β-HTBZ - 700	78	15.0	99						
β-HTBZ - 750	67	13.6	89	200	17	13	8.0 **	6.0 ***	0.4
β-HTBZ - 800	55	12.1	80						
β-HTBZ - 850	54	10.0	66	200	19	12	8.0 **	5.0 **	0.3
β-HTBZ - 900	54	7.9	52						
β-HTBZ - 950	54	9.4	62						
β-HTBZ - 1000	60	9.2	61						
β-HTBZ - 1050	55	7.6	50						
β-HTBZ - 1100	53	8.9	59	200	31	19	12.5 ***	7.5 ***	0.4
β-HTBZ - 1150	51	6.1	40						
β-HTBZ - 1200	20	2.0	13						
MMC - 0.1	71	16.3	107	200	56	47	19.0 ***	16.5 ***	0.3
MMC - 0.2	75	11.3	74						

(§) - not evaluated statistically
 shaded area not evaluated further
 *** p < 0.001, ** p < 0.01

TABLE # 7 - Second Test (+ S9, 3 hrs treatment, 12 hr recovery)

treatment - concentration (µg/ml)	relative cell count (%)	mitotic index (%)	relative mitotic index (%)	# cells evaluated	total # structural aberrations (§)		mean % of cells with structural aberrations		incidence of polyploidy (mean %)
					+ gaps	- gaps	+ gaps	- gaps	
vehicle control	100	18.6	100	200	16	13	4.5	3.0	0.9
β-HTBZ - 20	92	18.3	98						
β-HTBZ - 25	75	17.3	93						
β-HTBZ - 27.5	87	18.8	101						
β-HTBZ - 30	74	16.7	90	200	23	22	8.0	7.5	0.9
β-HTBZ - 32.5	70	16.7	90						
β-HTBZ - 35	60	12.2	66	200	67	58	20.5 ***	18.0 ***	0.1
β-HTBZ - 37.5	68	12.2	66						
β-HTBZ - 40	63	9.5	51						
β-HTBZ - 42.5	61	9.8	53						
β-HTBZ - 45	58	7.4	40						
β-HTBZ - 47.5	48	8.2	44						
β-HTBZ - 50	52	8.3	45	200	105	102	31.5 ***	30.5 ***	0.2
CCP - 5	81	10.1	54	200	42	40	17.0 ***	16.0 ***	0.0
CCP - 10	74	5.4	29						

(§) - not evaluated statistically
 shaded area not further evaluated
 *** p < 0.001

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food consumption were observed at doses up to 15 mg/kg/day, and no clinical observations or change in food consumption were observed after a single dose at 30 mg/kg/day. At 60 mg/kg/day, clinical observations were constricted pupils, slight droop of the eyelids, and mild stupor that resolved within 3 hours of dosing. Decreased food consumption (assessed qualitatively) was also observed in rabbits that received 60 mg/kg. Following a single oral administration of 120 mg/kg, the rabbits had constricted pupils and were recumbent at least an hour following administration. There was little or no food consumption for either of these rabbits for at least 24 hours following dose administration. Following 5 consecutive days of dosing at 45 mg/kg/day, two rabbits displayed constricted pupils, and decreased food consumption in one of the rabbits was observed.

A high-dose of 60 mg/kg/day was used for the definitive study. It was anticipated that this dose level would produce maternal toxicity, including remarkable clinical observations and decreased food consumption. Other dose levels were selected at intervals that were predicted to be narrow enough to reveal any dose-related trends. Though priority was given to detecting a dose-related trend, it was expected that the low-dose level would be a “no-observed-adverse-effect level.”

Results

Mortality (dams): Dams were checked twice daily for mortality, signs of pain, or distress. There were three unscheduled deaths as depicted in the following reviewer-generated table.

dose (mg/kg/day)	animal	date of sac.	results
C: 0 mg/kg/day	-	-	-
LD: 10 mg/kg/day	F61172	GD23	sacrificed following abortion, BW and FC comparable to group, <u>no clinical observations</u> – according to the sponsor, abortion not attributed to treatment – no remarkable necropsy findings
MD: 30 mg/kg/day	F61248	GD19	sacrificed following abortion, BW and FC comparable to group – <u>constricted pupils on GD 11</u> - no remarkable necropsy findings – according to the sponsor, abortion not attributed to treatment
HD: 60 mg/kg/day	F61214	GD27	sacrificed following abortion, attributed to TBZ – based on clinical obs, (thin appearance-GD-27, recumbent-GD-19, constricted pupils-GD8-12, 15-17, 19-20, squinted eyes-GD-14, rapid respiration-GD-17, 19-20, and few or no feces-GD-11, 27) necropsy finding (dark material found in the stomach, one placenta found in stomach) decreased FC and decreased BW gain

Clinical signs (dams): Cageside observations were conducted daily (≈1 hr post dose). Detailed observations were conducted on GDs: 0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27 and 29 (at the time of weighing). GD0 observations were taken from the animal supplier. The following reviewer-generated table summarizes the treatment-related clinical observations (see observation marked with (*). The report did not define the abbreviation “CSO” that appeared in the summaries (possibly, cage side observation). According to the sponsor, the clinical observations present in the HD group combined with the decrease in food consumption were dose-limiting toxicities.

Clinical Observations	
group/dose	Observations
C – 0 mg/kg/day	20/22 had no clinical observations * few or no feces in 3/22 (GD: 13, 24, 29) (1 on given day)
LD – 10 mg/kg/day	19/22 had no clinical observations * thin appearance in 1/22 (GD: 11, 13, 15) (#61173) * few or no feces in 3/33 (GD: 9, 13, 15, 21, 24) (1-2 on given day)
MD – 30 mg/kg/day	3/22 had no clinical observations * CSO-constricted pupils in 18/22 (GD: 7-20) (1-7 on given day) * squinted eye in 1/22 (GD: 11) * CSO- squinted eye in 2/22 (GD: 15, 17) (1-2 on given day) * CSO – closed eye in 3/22 (GD: 14, 18, 19) (1-2 on given day) * CSO – rapid respiration in 9/22 (GD 14-20) (1-3 on given day) * few or no feces in 2/22 (GD: 4, 15, 18) (1 on given day) * red fluid in pan in 1/22 (GD: 27) (#61249)
HD – 60 mg/kg/day	0/22 had no clinical observations * thin appearance in 1/22 (GD: 27) (#61214) * CSO-recumbent in 2/22 (GD: 16, 17, 19, 20) (1-2 on given day) (#61256, #61214) * CSO – constricted pupil in 22/22 (GD: 7-20) (9-18 on given day) * squinted eyes in 5/22 (GD: 12, 16, 17, 18) (1-2 on given day) * CSO – squinted eye in 15/22 (GD: 7, 9-20) (1-4 on given day) * CSO – closed eye in 15/22 (GD: 8-20) (2-7 on given day) * rapid respiration in 1/22 (GD: 12) * CSO – rapid respiration in 21/22 (GD: 7-20) (1-14 on given day) * few or no feces in 8/22 (GD: 11, 15, 21, 27, 29) (1-3 on given day)

Body weight (dams): Dams were weighed on GDs: 0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27 and 29. GD0 body weights were taken from the animal supplier. There was a very slight treatment related decrease in group mean body weight in the HD group (maximum of 5% when compared to control). There was a significant decrease in body weight gain GD 7-21 for HD animals; however this was predominantly noted GD7-13. There was an increase in group mean body weight change in the LD and HD from GD21-27. A copy of the sponsor-supplied summary table follows.

Mean Maternal Body Weight Changes During Gestation (g)

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY	60 MG/KG/DAY
DAYS 0 TO 4	MEAN	139.9	138.5	59.9	124.4
	S.D.	88.4	122.7	172.6	74.8
	N	22	22	21	20
DAYS 4 TO 7	MEAN	70.5	30.1	34.5	38.0
	S.D.	64.8	63.5	101.0	59.2
	N	22	22	21	20
DAYS 7 TO 9	MEAN	3.8	-16.8	31.5	-61.8**
	S.D.	43.4	48.7	116.8	41.2
	N	22	22	21	20
DAYS 9 TO 11	MEAN	17.8	28.0	31.3	-10.8
	S.D.	48.7	38.9	46.7	61.5
	N	22	22	21	20
DAYS 11 TO 13	MEAN	37.5	31.0	21.0	3.9
	S.D.	36.6	42.5	61.9	61.3
	N	22	22	21	20
DAYS 13 TO 15	MEAN	36.0	50.7	40.5	34.9
	S.D.	32.0	34.7	70.8	61.7
	N	22	22	21	20
DAYS 15 TO 18	MEAN	37.0	62.4	34.6	49.0
	S.D.	83.1	103.6	60.1	72.2
	N	22	22	21	20
DAYS 18 TO 21	MEAN	61.3	23.6	42.5	46.7
	S.D.	73.8	84.7	59.1	77.0
	N	22	22	20	20
DAYS 21 TO 24	MEAN	70.0	86.6	58.9	89.0
	S.D.	52.9	72.6	51.3	96.4
	N	22	21	20	20
DAYS 24 TO 27	MEAN	15.4	30.7	35.8	34.4
	S.D.	59.6	46.1	41.5	83.9
	N	22	21	20	20

DAYS 27 TO 29	MEAN	45.5	46.0	44.6	47.7
	S.D.	37.0	44.9	31.0	82.8
	N	22	21	20	19
DAYS 0 TO 7	MEAN	210.4	159.7	94.4	162.4
	S.D.	99.1	153.6	197.0	98.5
	N	22	22	21	20
DAYS 7 TO 29	MEAN	324.3	340.8	321.3	259.8
	S.D.	113.0	129.7	212.8	168.3
	N	22	21	20	19
DAYS 0 TO 29	MEAN	534.6	501.8	437.5	425.0
	S.D.	160.5	158.7	193.5	195.0
	N	22	21	20	19
DAYS 7 TO 21	MEAN	193.5	178.8	182.0	61.9*
	S.D.	94.3	120.2	201.2	139.2
	N	22	22	20	20
DAYS 21 TO 29	MEAN	130.9	163.4	139.3	192.8
	S.D.	99.9	87.0	77.0	120.2
	N	22	21	20	19

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.

Food consumption (dams): FC was recorded for the body weight intervals GDs: 0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27 and 29 (with additional recordings as needed). There were significant decreases in food consumption GD 7-21 in HD animals. The food consumption in the HD animals was not significantly different from control on GD21-29. A copy of the sponsor provided table follows.

DOSE LEVEL	MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION (G/ANIMAL/DAY)				
	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY	
DAYS 13 TO 15	MEAN S.D. N SPILLED	152.2 28.8 21 1	149.7 50.3 22 0	148.8 45.6 21 0	105.5** 49.7 20 0
DAYS 15 TO 17	MEAN S.D. N SPILLED	159.6 24.9 21 1	168.8 39.2 22 0	162.0 52.1 21 0	122.4* 49.4 20 0
DAYS 17 TO 18	MEAN S.D. N SPILLED	163.3 43.5 21 1	175.0 38.5 22 0	163.4 51.8 21 0	130.9 42.8 20 0
DAYS 18 TO 20	MEAN S.D. N SPILLED	160.1 29.2 22 0	145.6 49.3 22 0	149.2 49.4 20 0	139.9 37.8 20 0
DAYS 20 TO 21	MEAN S.D. N SPILLED	155.4 32.2 22 0	131.7 56.9 22 0	144.4 43.9 20 0	132.5 38.2 20 0
DAYS 21 TO 23	MEAN S.D. N SPILLED	158.5 28.1 22 0	146.5 43.2 21 0	154.7 35.9 20 0	160.7 29.0 20 0
DAYS 23 TO 24	MEAN S.D. N SPILLED	130.7 35.4 22 0	129.1 50.3 21 0	131.2 38.7 19 1	144.2 45.6 20 0
DAYS 24 TO 26	MEAN S.D. N SPILLED	115.7 32.8 22 0	114.0 39.8 21 0	120.8 30.4 20 0	117.6 43.1 20 0
DAYS 26 TO 27	MEAN S.D. N SPILLED	98.5 36.7 22 0	110.2 29.7 22 0	116.6 35.3 20 0	103.0 45.7 20 0
DAYS 27 TO 29	MEAN S.D. N SPILLED	109.6 36.4 22 0	128.3 21.8 21 0	117.9 30.2 19 1	109.6 42.7 19 0
DAYS 7 TO 29	MEAN S.D. N SPILLED	145.0 33.1 20 2	143.1 26.6 21 0	140.5 27.0 19 2	125.2 27.5 18 1
DAYS 4 TO 29	MEAN S.D. N SPILLED	146.9 22.5 19 2	143.8 27.1 20 0	141.8 25.9 19 2	130.0 26.2 18 1
DAYS 7 TO 21	MEAN S.D. N SPILLED	159.1 24.7 20 2	151.6 34.6 22 0	150.3 34.8 20 1	119.3** 36.9 19 1
DAYS 21 TO 29	MEAN S.D. N SPILLED	124.6 27.0 22 0	127.0 27.3 21 0	127.3 23.7 19 1	130.5 30.2 19 0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.

Toxicokinetics: not evaluated

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Animals showing signs of abortion were sacrificed and necropsied. Scheduled sacrifices were performed on GD29 (no mention of necropsy). Uterine contents were examined and uterine weight was recorded. "Abnormal tissues were retained in 10% neutral buffered formalin." Techniques used were not discussed in the report.

Maternal necropsy	
dose	Finding
C: 0 mg/kg/day	<ul style="list-style-type: none"> 22/22 – pregnant, no remarkable observations
LD: 10 mg/kg/day	<ul style="list-style-type: none"> 21/22 – pregnant, no remarkable observation 1/22 – aborted, no other remarkable observations
MD: 30 mg/kg/day	<ul style="list-style-type: none"> 19/22 – pregnant, no remarkable observations 1/22 – not pregnant, no remarkable observations 1/22 – pregnant, no viable fetuses, no other remarkable observations 1/22- aborted – no other remarkable observations
HD: 60 mg/kg/day	<ul style="list-style-type: none"> 19/22 – pregnant, no remarkable observations 2/22 – not pregnant, no remarkable observations 1/22 – aborted stomach – dark material, "one placenta found in stomach"

According to the sponsor, "Mean gravid uterine weights and corrected terminal body weights were similar across groups." However, examination of the data reveals that mean weight of the gravid uterus was decreased at the MD and that the mean corrected body weight was decreased for the MD and HD. The mean net change in body weight (minus uterine weight) decreased in a dose related fashion in LD, MD and HD. See the sponsor-generated table for details.

Summary of Uterine and Net Body Weights (g)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY
GRAVID UTERUS	MEAN	502.50	495.96	449.32	503.68
	S.D.	81.53	118.57	108.86	93.50
	N	22	21	20	19
CORRECTED WEIGHT	MEAN	3137.05	3127.76	3069.43	3056.64
	S.D.	336.77	330.16	336.35	350.95
	N	22	21	20	19
NET CHANGE FROM DAY 0	MEAN	32.14	5.81	-11.82	-78.68
	S.D.	154.86	161.89	177.92	189.96
	N	22	21	20	19

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.
 CORRECTED WEIGHT = TERMINAL BODY WEIGHT MINUS GRAVID UTERINE WEIGHT
 NET WEIGHT CHANGE FROM DAY 0 = CORRECTED WEIGHT MINUS DAY 0 BODY WEIGHT

The cesarean data are presented in the reviewer-generated tables that follow (one including all dams that were pregnant at C-section, and the other containing data only from those dams with viable fetuses at c-section). As noted in the mortality section, one dam from each the LD, MD and HD groups aborted. According to the sponsor, only the abortion in the HDF was associated with treatment (secondary to decreased food consumption). The lack of increase in incidence of abortion with increasing dose does support the occurrences in the LD and MD as spontaneous and not related to treatment. According to the sponsor, mean post-implantation loss was slightly increased in the MD group due to animal #F61252 (noted with no viable fetuses, 3 corpora lutea, 2 implantation sites and 2 early resorptions). Examination of the data reveals that this dam did contribute to the finding; however, this dam was not the major contributing factor. If the data from this dam were eliminated the total number of early resorptions would still be increased compared to control (5 resorptions from 5 litters compared to 2 resorptions from 2 litters). Relationship to treatment is questionable because a similar effect was not seen in the HD.

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Cesarean Data for All Dams					
		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
# mated		22	22	22	22
# pregnant/# mated (%)		22/22 (100 %)	22/22 (100 %)	21/21 (95 %)	20/22 (91%)
# dams that aborted (%)		0/22 (0%)	1/22 (4.5 %)	1/21 (4.5 %)	1/20 (5 %)
# dams found dead		0	0	0	0
# dams with early deliveries		0	0	0	0
# pregnant at C section		22/22 (100%)	21/22 (95%)	20/21 (95%)	19/20 (95%)
# dams with viable fetuses		22/22 (100 %)	21/22 (100 %)	19/20 (95%)	19/19 (100%)
# dams with no viable fetuses		0/22	0/21	1/20 (5%)	0/19

Cesarean Data for All Dams Pregnant at C-Section					
		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
corpora lutea	total (# litters)	215 (22)	209 (21)	178 (20)	184 (19)
	mean ± SD	9.8 ± 2.2	10.0 ± 2.0	8.9 ± 2.0	9.7 ± 1.5
implantation sites	total (# litters)	187 (22)	176 (21)	152 (20)	168 (19)
	mean ± SD	8.5 ± 1.7	8.4 ± 2.1	7.6 ± 1.9	8.8 ± 1.6
preimplantation loss	total	28	33	26	16
	mean % ± SD	11.4 ± 12.9	15.6 ± 13.7	14.6 ± 11.7	8.9 ± 8.0
resorptions (total)	total (# affected litters)	4 (3/22)	3 (3/21)	8 (7/20)	3 (2/19)
	mean ± SD	0.2 ± 0.5	0.1 ± 0.4	0.4 ± 0.6	0.2 ± 0.5
	mean % ± SD	1.6 ± 4.4	2.1 ± 5.7	8.6 ± 22.2	2.4 ± 8.0
resorptions (early)	total (# affected litters)	2 (2/22)	1 (1/21)	7 (6/20)	2 (2/19)
	mean ± SD	0.1 ± 0.3	0.0 ± 0.2	0.4 ± 0.6	0.1 ± 0.3
	mean % ± SD	0.9 ± 2.8	1.0 ± 4.4	8.0 ± 22.3	1.5 ± 4.7
resorptions (late)	total (# affected litters)	2 (1/22)	2 (2/21)	1 (1/20)	1 (1/19)
	mean ± SD	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.2
	mean % ± SD	0.8 ± 3.6	1.2 ± 3.9	0.6 ± 2.5	0.9 ± 3.8
dead fetuses	Total	0	0	0	0
postimplantation loss	mean ± SD	1.6 ± 4.4	2.1 ± 5.7	8.6 ± 22.2	2.4 ± 8.0
live fetuses	total (# litters)	183 (22)	173 (21)	144 (20)	165 (19)
	mean ± SD	8.3 ± 1.4	8.2 ± 2.1	7.2 ± 2.1	8.7 ± 1.9
	mean % ± SD	98.4 ± 4.4	97.9 ± 5.7	91.4 ± 22.2	97.6 ± 8.0
females	total (# litters)	91 (22)	76 (21)	68 (19)	85 (19)
	mean ± SD	4.1 ± 1.7	3.6 ± 1.4	3.6 ± 1.4	4.5 ± 1.6
	mean % ± SD	49.6 ± 18.3	45.8 ± 20.2	47.7 ± 18.9	51.9 ± 15.5
males	total (# litters)	92 (22)	97 (21)	76 (19)	80 (19)
	mean ± SD	4.2 ± 1.5	4.6 ± 2.2	4.0 ± 1.7	4.2 ± 1.8
	mean % ± SD	50.4 ± 18.3	54.2 ± 20.2	52.3 ± 18.9	48.1 ± 15.5
ratio of M:F	-	50:50	56:44	53:47	48:52

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Cesarean Data for All Dams with Viable Fetuses at C-Section					
		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
corpora lutea	total (# litters)	215 (22)	209 (21)	175 (19)	184 (19)
	mean ± SD	9.8 ± 2.2	10.0 ± 2.0	9.2 ± 1.5	9.7 ± 1.5
implantation sites	total (# litters)	187 (22)	176 (21)	150 (19)	168 (19)
	mean ± SD	8.5 ± 1.7	8.4 ± 2.1	7.9 ± 1.4	8.8 ± 1.6
preimplantation loss	total	28	33	25	16
	mean ± SD	11.4 ± 12.9	15.6 ± 13.7	7 ± ?	8.9 ± 8.0
resorptions (total)	total (# affected litters)	4 (3/22)	3 (3/21)	6 (6/19)	3 (3/19)
	mean ± SD	0.2 ± 0.5	0.1 ± 0.4	0.3 ± 0.5	0.2 ± 0.5
	mean % ± SD	1.6 ± 4.4	2.1 ± 5.7	3.8 ± 5.7	2.4 ± 8.0
resorptions (early)	total (# affected litters)	2 (2/22)	1 (1/21)	5 (5/19)	2 (2/19)
	mean ± SD	0.1 ± 0.3	0.0 ± 0.2	0.3 ± 0.5	0.1 ± 0.3
	mean % ± SD	0.9 ± 2.8	1.0 ± 4.4	3.2 ± 5.5	1.5 ± 4.7
resorptions (late)	total (# affected litters)	2 (1/22)	2 (2/21)	1 (1/19)	1 (1/19)
	mean ± SD	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.2
	mean % ± SD	0.8 ± 3.6	1.2 ± 1.9	0.6 ± 2.5	0.9 ± 3.8
dead fetuses		0	0	0	0
postimplantation loss	mean ± SD	1.6 ± 4.4	2.1 ± 5.7	3.8 ± 5.7	2.4 ± 8.0
live fetuses	total (# litters)	183 (22)	173 (21)	144 (19)	165 (19)
	mean ± SD	8.3 ± 1.4	8.2 ± 2.1	7.6 ± 1.3	8.7 ± 1.9
	mean % ± SD	98.4 ± 4.4	97.9 ± 5.7	96.2 ± 5.7	97.6 ± 8.0
females	total (# litters)	91 (22)	76 (21)	68 (19)	85 (19)
	mean ± SD	4.1 ± 1.7	3.6 ± 1.4	3.6 ± 1.4	4.5 ± 1.6
	mean % ± SD	49.6 ± 18.3	45.8 ± 20.2	47.7 ± 18.9	51.9 ± 15.5
males	total (# litters)	92 (22)	97 (21)	76 (19)	80 (19)
	mean ± SD	4.2 ± 1.5	4.6 ± 2.2	4.0 ± 1.7	4.2 ± 1.8
	mean % ± SD	50.4 ± 18.3	54.2 ± 20.2	52.3 ± 18.9	48.1 ± 15.5
(viable only) sex ratio M:F		50:50	56:44	53:47	48:52

There were no notable effects of treatment on group mean fetal weights even when covariate adjustment (see the sponsor-provided summary table below for details).

Summary of Mean Fetal Weights

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY	
FETAL WEIGHTS	UNITS: GRAMS					
	of all Viable Fetuses	MEAN	42.56	42.62	44.06	41.04
		S.D.	4.27	4.11	5.15	5.90
		N	22	21	19	19
	Covariate Adjusted MEAN	42.72	42.67	43.09	41.77	
of Male Fetuses	MEAN	42.52	43.33	44.42	41.74	
	S.D.	4.60	4.87	5.63	6.30	
	N	22	20	19	19	
	Covariate Adjusted MEAN	42.65	43.52	43.32	41.98	
of Female Fetuses	MEAN	42.27	41.84	42.87	40.58	
	S.D.	4.43	4.38	5.30	5.68	
	N	22	21	19	19	
	Covariate Adjusted MEAN	42.42	41.98	41.97	41.26	

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.

Offspring (malformations, variations, etc.): The fetal examination was described in the report as follows:

Each fetus was weighed and examined for external abnormalities. Live fetuses were sacrificed by _____ via intraperitoneal injection followed by exsanguination and/or thoracic penetration. A mid-coronal slice was made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses were examined in the fresh state using Staples' technique; and the sex of each fetus (live or dead) was determined. Viscera were then removed and discarded. Carcasses were processed for skeletal examination using the Alizarin Red S staining method and evaluated. All fetuses were retained in glycerin..."

External findings: There were no external findings in any fetus based on examination of 183, 173, 144 and 165 fetuses from the C, LD, MD and HD, respectively.

Soft tissue findings: There were no treatment-related soft tissue malformations. There was an apparent treatment-related decrease in the total fetal soft tissue variations. This was based on the decreased fetal incidence and litter incidence of variations of the major vessels in LD, MD and HD groups when compared to the concurrent control, and decreased fetal incidence (LD, MD and HD) and litter incidence (HD) of small or missing intermediate lobe of the lung. Increased renal pelvic cavitation, the only other finding noted, was seen in a single HD pup, and this finding should be considered incidental. Copies of the sponsor-provided summary tables follow.

Summary of Fetal Soft Tissue Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY
Litters Evaluated	N	22	21	19	19
Fetuses Evaluated	N	183	173	144	165
Live	N	183	173	144	165
Dead	N	0	0	0	0
HEART AND/OR GREAT VESSEL MALFORMATIONS					
Fetal Incidence	N %	1 0.5	0 0.0	0 0.0	0 0.0
Litter Incidence	N %	1 4.5	0 0.0	0 0.0	0 0.0
GALL BLADDER AGENESIS					
Fetal Incidence	N %	0 0.0	1 0.6	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	1 4.8	0 0.0	0 0.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N %	1 0.5	1 0.6	0 0.0	0 0.0
Litter Incidence	N %	1 4.5	1 4.8	0 0.0	0 0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.
N = NUMBER

Summary of Fetal Soft Tissue Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY
Litters Evaluated	N	22	21	19	19
Fetuses Evaluated	N	183	173	144	165
Live	N	183	173	144	165
Dead	N	0	0	0	0
VARIATIONS OF THE MAJOR VESSELS					
Fetal Incidence (-)	N %	13 7.1	8 4.6	3 2.1*	3 1.8*
Litter Incidence (-)	N %	9 41	7 33	3 16	3 16
INTERMEDIATE LOBE OF LUNG SMALL/MISSING					
Fetal Incidence (-)	N %	12 6.6	7 4.0	7 4.9	1 0.6**
Litter Incidence (-)	N %	6 27	7 33	7 37	1 5.3
INCREASED RENAL PELVIC CAVITATION					
Fetal Incidence	N %	0 0.0	0 0.0	0 0.0	1 0.6
Litter Incidence	N %	0 0.0	0 0.0	0 0.0	1 5.3
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence (-)	N %	23 13	15 8.7	10 6.9	5 3.0**
Litter Incidence (-)	N %	11 50	11 52	10 53	4 21

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.
(-) = NEGATIVE TREND
N = NUMBER

Skeletal findings: There were no skeletal malformations in the HD group and no clear evidence of a treatment-related effect on skeletal malformations. One fetus from the MD (#9 from dam F61188) accounted for 4 of the 5 malformations in the MD group. The sponsor notes that the incidence of fused bone in the skull (incomplete suture between the nasals) in the MD animal was outside of the historic control (value for this finding not listed). A copy of the sponsor-supplied table follows for details.

Best Possible Copy

Summary of Fetal Skeletal Malformations

	DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY
Litters Evaluated	N	22	21	19	19
Fetuses Evaluated	N	183	173	144	165
Live	N	183	173	144	165
Dead	N	0	0	0	0
BONE(S) FUSED IN SKULL					
Fetal Incidence	N %	0 0.0	0 0.0	1 0.7	0 0.0
Litter Incidence	N %	0 0.0	0 0.0	1 5.3	0 0.0
VERTEBRAL ANOMALY WITH/WITHOUT ASSOCIATED RIB ANOMALY					
Fetal Incidence	N %	1 0.5	3 1.7	2 1.4	0 0.0
Litter Incidence	N %	1 4.5	2 9.5	2 11	0 0.0
MISALIGNED, FUSED AND/OR ABSENT CAUDAL VERTEBRA(E)					
Fetal Incidence	N %	0 0.0	0 0.0	1 0.7	0 0.0
Litter Incidence	N %	0 0.0	0 0.0	1 5.3	0 0.0
MAJDA FUSION OF STERNEBRAE					
Fetal Incidence	N %	1 0.5	0 0.0	1 0.7	0 0.0
Litter Incidence	N %	1 4.5	0 0.0	1 5.3	0 0.0
FORKED/FUSED RIB(S)					
Fetal Incidence	N %	0 0.0	1 0.6	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	1 4.8	0 0.0	0 0.0
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N %	2 1.1	4 2.3	2 1.4	0 0.0
Litter Incidence	N %	2 9.1	3 14	2 11	0 0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.
N = NUMBER

The fetal incidence of total fetal skeletal variations was slightly decreased in the LD and HD groups, and according to the sponsor, the skeletal variations were "the type and frequency commonly seen in this strain of rabbits." The sponsor noted that the total fetal incidence for skeletal variations decreased in the LD and HD despite the fact that the fetal incidence of unossified 6th sternebrae was significantly increased in these groups (the sponsor noted that the incidence of unossified 6th sternebrae was within historical control range. The sponsor noted no other effects of treatment.

Examination of the data indicate an apparent treatment-related increases in variations of skull (angulated hyoid wings, incomplete/unossified hyoid body, and incomplete ossification of skull), spinal column (less than 16 caudal vertebra(e) ossified), sternebrae (6th sternebra unossified, other sternebra(e) bipartite, and sternebra(e) asymmetrically ossified), ribs (13th full rib(s)), and the appendicular skeleton (talus(i) unossified). Examination of the data also reveals an apparent treatment related decrease in the following two variations, 5th sternebra(e) unossified and 13th unilateral full rib. In total, these skeletal variations do not suggest a significant treatment-related toxicity to the fetus due to low fetal and/or litter incidence, no increase in litter incidence with increasing dose, or combination of apparent treatment-related increases and decreases for similar structures. In addition, the relevance of an apparent treatment-related decrease in a skeletal variation is unknown.

Based on examination of the data, the NOEL for maternal toxicity was 10 mg/kg/day (approximately 1.9 times the MRHD on a mg/m² basis), based on treatment-related clinical observations (constricted pupils, squinted/closed eyes, rapid respiration, few or no feces, and recumbency) in MD and HD and changes in body weigh and food consumption seen at the HD. The NOEL for embryo fetal development was 60 mg/kg/day (the highest dose tested) (approximately 11.6 times the MRHD on a mg/m² basis).

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Summary of Fetal Skeletal Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY
Litters Evaluated	N	22	21	19	19
Fetuses Evaluated	N	183	173	144	165
Live	N	182	173	144	165
Dead	N	0	0	0	0
ANGULATED HYOID WING(S)					
Fetal Incidence (+)	N	4	5	6	10
	%	2.2	2.9	4.2	6.1
Litter Incidence	N	3	3	3	2
	%	14	14	16	11
ACCESSORY BONE(S) IN SKULL					
Fetal Incidence	N	3	0	2	1
	%	1.6	0.0	1.4	0.6
Litter Incidence	N	2	0	2	1
	%	9.1	0.0	11	5.3
INCOMPLETE/UNOSSIFIED HYOID BODY					
Fetal Incidence (+)	N	2	0	1	4
	%	1.1	0.0	0.7	2.4
Litter Incidence	N	2	0	1	2
	%	9.1	0.0	5.3	11
INCOMPLETE/UNOSSIFIED HYOID WING(S)					
Fetal Incidence	N	2	0	1	1
	%	1.1	0.0	0.7	0.6
Litter Incidence	N	2	0	1	1
	%	9.1	0.0	5.3	5.3
INCOMPLETE OSSIFICATION OF SKULL					
Fetal Incidence (+)	N	0	0	0	3
	%	0.0	0.0	0.0	1.8
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	5.3
26 PRESACRAL VERTEBRAE					
Fetal Incidence	N	35	44	39	31
	%	19	25	27	19
Litter Incidence	N	12	13	14	11
	%	55	62	74	58
LESS THAN 16 CAUDAL VERTEBRAE OSSIFIED					
Fetal Incidence	N	6	5	2	9
	%	3.3	2.9	1.4	5.5
Litter Incidence	N	5	4	2	5
	%	23	19	11	26
MISALIGNED OR BIPARTITE DISTAL CAUDAL VERTEBRA(E)					
Fetal Incidence	N	1	0	1	0
	%	0.5	0.0	0.7	0.0
Litter Incidence	N	1	0	1	0
	%	4.5	0.0	5.3	0.0
24 PRESACRAL VERTEBRAE					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.5	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.8	0.0	0.0
5TH/6TH STERNESRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	50	53	32	49
	%	27	31	22	30
Litter Incidence	N	18	17	14	16
	%	82	81	74	84
6TH STERNESRA UNOSSIFIED					
Fetal Incidence	N	4	16	5	14
	%	2.2	9.2**	3.5	8.5**
Litter Incidence	N	4	7	4	7
	%	18	33	21	37
5TH/6TH STERNESRA(B) BIPARTITE					
Fetal Incidence	N	4	1	1	3
	%	2.2	0.5	0.7	1.8
Litter Incidence	N	3	1	1	2
	%	14	4.8	5.3	11
5TH STERNESRA UNOSSIFIED					
Fetal Incidence	N	22	5	9	10
	%	12	2.9**	6.2	6.1*
Litter Incidence	N	10	5	4	6
	%	45	24	21	32
MINOR FUSION OF STERNESRAE					
Fetal Incidence	N	5	4	0	5
	%	2.7	2.3	0.0	3.0
Litter Incidence	N	4	3	0	2
	%	18	14	0.0	11
OTHER STERNESRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N	2	1	0	0
	%	1.1	0.6	0.0	0.0
Litter Incidence	N	2	1	0	0
	%	9.1	4.8	0.0	0.0

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DOSE LEVEL	UNIPOLAR AND BIPOLAR UNILATERAL OSSIFICATIONS			
	GROUP 1 0 MG/KG/DAY	GROUP 2 10 MG/KG/DAY	GROUP 3 30 MG/KG/DAY	GROUP 4 60 MG/KG/DAY
Litters Evaluated	N 22	21	19	19
Fetuses Evaluated	N 183	173	144	159
Live	N 183	173	144	159
Dead	N 0	0	0	0
OTHER STERNBERG(E) BIPARTITE				
Fetal Incidence	N %	0 0.0	1 0.6	0 0.0
Litter Incidence	N %	0 0.0	1 4.8	0 0.0
STERNBERG(E) ASYMMETRICALLY OSSIFIED				
Fetal Incidence	N %	1 0.5	1 0.6	0 0.0
Litter Incidence	N %	1 4.5	1 4.8	0 0.0
STERNBERG(E) ASYMMETRICALLY OSSIFIED WITH MINOR FUSION				
Fetal Incidence	N %	0 0.0	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	0 0.0	1 5.3
13TH FULL RIB(S)				
Fetal Incidence	N %	83 45	70 40	78 54
Litter Incidence	N %	20 91	16 76	18 95
13TH REDUCED RIB(S)				
Fetal Incidence	N %	24 13	29 17	25 17
Litter Incidence	N %	12 55	14 67	13 68
13TH UNILATERAL FULL RIB				
Fetal Incidence (-)	N %	13 7.1	5 2.9	1 0.7**
Litter Incidence (-)	N %	9 41	5 24	1 5.3**
11TH FULL RIB(S)				
Fetal Incidence	N %	0 0.0	1 0.6	0 0.0
Litter Incidence	N %	0 0.0	1 4.8	0 0.0
VALVUS (I) UNOSSIFIED				
Fetal Incidence	N %	0 0.0	1 0.6	1 0.7
Litter Incidence	N %	0 0.0	1 4.8	1 5.3
TOTAL FETAL SKELETAL VARIATIONS				
Fetal Incidence	N %	159 87	134 77*	121 84
Litter Incidence	N %	22 100	21 100	19 100

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * - P<0.05 ** - P<0.01.
 [-] = NEGATIVE TREND
 N = NUMBER

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Study title: Oral Developmental Toxicity Study and Pre-and Postnatal Development with Tetrabenazine, in the Rat (Segment II Study)**Key study findings:** embryoletality (an increase in early and total resorptions in the HD group)**Study no.:** _____ Study # 7425-106**Volume # 22, module 4****Conducting laboratory and location:** _____**Date of study initiation:** 13-Oct-03**GLP compliance:** yes – US-FDA, Japanese and OECD with the following exception:

“The Gestation Day 0 clinical observations and body weights received from the supplier and used for randomization of animals prior to study initiation were collected in a non-GLP facility; however, these data did not affect the integrity of the study.”

QA reports: yes (X) no ()

Drug, lot #, and % purity: Tetrabenazine (TBZ), lot # 105481, with stated purity of 100%. TBZ was suspended in carboxymethylcellulose (CMC) and Tween® 80 in water at TBZ concentrations of 0, 2.0, 6.0 and 12.0 mg/ml. Dosing formulations were prepared at least weekly (and stored refrigerated). Homogeneity was assessed week 1 and the data indicated that the formulations were homogeneous (99.9 – 101.3% of nominal). The formulation analyses carried out weeks 1, 2 and 3 demonstrated that the formulations were within 6% of the nominal concentrations (94.7 – 102.8%). “Stability was determined in a previous study conducted at _____ and reported in 7425-102; concentrations ranged from 2.0 to 30.0 mg/mL and were stored refrigerated for 10 days.”

Methods**Doses:** 0, 5, 15 and 30 mg/kg/day (QD dosing) GD6-17.**Species/strain:** _____ CD®(SD)IGS BR mated female rats from _____ The animals were approximately 10-11 weeks of age at randomization, and on GD0 weighed 201 – 250 g. Animals were individually housed.**Number/sex/group:** 25/group**Route, formulation, volume, and infusion rate:** oral gavage (2.5 ml/kg), QD dosing, GD 6-17**Satellite groups used for toxicokinetics:** none**Study design and parameters and endpoints evaluated are described in the text.**

Justification for Dose: based on the results of the 4- and 26-wk toxicity studies in rat / _____ studies #455298 and #455738). “The data from these studies demonstrated that rats treated with tetrabenazine for up to 26 weeks resulted in dose-dependent sedation that was marked at the 30 mg/kg/day dose level. Although females treated for longer durations (13 or 26 weeks) experienced increased food consumption and body weight gain compared to control, the females treated for only 4 weeks experienced reduced body weight gains after receiving 30 mg/kg/day. It was therefore anticipated that the high-dose level would show drug-specific effects, producing decreased body weight gains in the females but not jeopardizing embryo/fetal viability. Other dose levels were selected at intervals that were predicted to be narrow enough to reveal any dose-related trends. Though priority was given to detecting a dose-related trend, it was expected that the low-dose level would be a ‘no-observed-adverse-effect level.’” It should be noted that 4- and 26-week toxicity studies in rat utilized BID dosing; whereas, this reproductive toxicology study utilizes QD dosing.

Results

Mortality (dams): Animals were checked twice daily for signs of mortality, morbidity and distress. There were no unscheduled deaths.

Clinical signs (dams): During the dosing period daily cageside observations were conducted at approximately 1 hr post dose. Detailed observations were conducted at the time of weighing (GD0, 4, 6,

8, 10, 14, 17, and 20). GD0 observations were provided by the supplier. According to the sponsor, the only treatment related clinical observations (approximately 1hr post dose) were hypoactivity and squinted or closed eyes in MD and HD. The following reviewer-generated table summarizes the findings. The observation abbreviations "CSO" and "PDO" were not defined.

Clinical Observations	
group/dose	Observations
C - 0 mg/kg/day	-
LD - 5 mg/kg/day	missing digits in 1/25 (GD20)
MD - 15 mg/kg/day	CSO - hypoactivity in 1/25 (GD 8) PDO - hypoactivity in 25/25 (GD6-17) (7-23 on a given day) PDO - closed eyes in 13/25 (GD6-7, 9-13, 16-17) (1-3 on a given day) PDO- squinted eyes in 25/25 (GD6-17) (6-14 on a given day)
HD - 30 mg/kg/day	CSO - hypoactivity in 1/25 (GD 10) PDO - hypoactivity in 25/25 (GD6-17, 14-25 on a given day) PDO - closed eyes in 20/25 (GD6-17) (2-9 on a given day) CSO - squinted eyes in 1/25 (GD6) PDO-squinted eyes in 25/25 (GD 6-17) (7-21 on a given day) PDO - excessive salivation in 1/25 (GD17) Red crust, nose - 2/25 (GD17, 20) (1 per day) bent tail (distal portion) in 1/25 (GD8, 10, 17, 20)

on GD 14 - observations for animal B71796 were not recorded.

Body weight (dams): Body weights were obtained on GD0, 4, 6, 8, 10, 14, 17, and 20. GD0 weights were provided by the supplier. There were no significant differences in the group mean body weights at any of the designated weighing dates. The maximum decrease seen in the HD group was 3%. At day 17, the last day of treatment, the group mean body weights for the control group was 329.8 ± 15.8 compared with 319.2 ± 17.6 in the HD group (on day 20, the value for control was 379.9 ± 18.2 compared with 369.9 ± 25.7 in the HD group). There were statistically significant decreases in mean group body weight changes as depicted in the following sponsor provided table; however the difference is small.

TABLE 4
MEAN MATERNAL BODY WEIGHT CHANGES DURING GESTATION (G)

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	5 MG/KG/DAY	15 MG/KG/DAY	30 MG/KG/DAY
DAYS 6 TO 20	MEAN	127.7	126.5	122.0	113.4*
	S.D.	12.3	10.8	13.3	17.0
	N	24	24	25	24
DAYS 0 TO 20	MEAN	155.1	154.8	149.8	143.7
	S.D.	17.4	14.4	19.3	19.6
	N	24	24	25	24

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

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Mean Maternal Body Weight Changes During Gestation (g)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
DAYS 0 TO 4	MEAN	14.6	15.6	15.9	17.9
	S.D.	6.5	5.3	6.0	6.0
	N	24	24	25	24
DAYS 4 TO 6	MEAN	12.8	12.8	11.9	12.3
	S.D.	5.7	6.4	6.0	4.8
	N	24	24	25	24
DAYS 6 TO 8	MEAN	7.9	8.4	5.4	0.8**
	S.D.	6.2	5.2	4.2	5.3
	N	24	24	25	24
DAYS 8 TO 10	MEAN	15.0	11.7	10.6*	9.2**
	S.D.	7.6	4.9	5.8	5.5
	N	24	24	25	24
DAYS 10 TO 14	MEAN	22.8	22.9	21.9	25.8
	S.D.	7.6	5.7	5.9	5.9
	N	24	24	25	24
DAYS 14 TO 17	MEAN	31.9	33.1	27.3*	26.8*
	S.D.	4.4	6.3	5.8	6.8
	N	24	24	25	24
DAYS 17 TO 20	MEAN	50.1	50.5	54.8	50.7
	S.D.	4.8	8.1	6.1	11.4
	N	24	24	25	24
DAYS 0 TO 6	MEAN	27.5	28.4	27.8	30.2
	S.D.	9.1	6.8	10.5	7.9
	N	24	24	25	24
DAYS 6 TO 17	MEAN	77.6	76.0	67.2**	62.7**
	S.D.	10.2	9.8	9.7	10.2
	N	24	24	25	24

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

Food consumption (dams): Food consumption was assessed on GD4, 6, 8, 10, 14, 17, and 20. Mean food consumption was decreased in the MD and HD groups as depicted in the following sponsor-provided summary table.

Mean Maternal Food Consumption During Gestation (g/animal/day)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
DAYS 4 TO 6	MEAN	25.0	25.5	25.6	25.7
	S.D.	3.2	3.3	3.4	2.6
	N	24	24	25	24
	SPILLED	0	0	0	0
DAYS 6 TO 8	MEAN	26.8	27.4	25.2	23.3**
	S.D.	3.0	2.9	3.4	2.7
	N	24	23	25	24
	SPILLED	0	1	0	0
DAYS 8 TO 10	MEAN	28.2	27.6	26.4	25.8*
	S.D.	3.0	3.0	2.8	3.0
	N	24	24	25	24
	SPILLED	0	0	0	0
DAYS 10 TO 14	MEAN	29.9	29.4	27.3**	28.0
	S.D.	2.9	2.9	3.1	2.5
	N	24	24	25	24
	SPILLED	0	0	0	0
DAYS 14 TO 17	MEAN	30.9	31.0	28.4**	27.3**
	S.D.	2.9	2.3	2.5	3.0
	N	24	24	25	24
	SPILLED	0	0	0	0
DAYS 17 TO 20	MEAN	31.4	31.0	29.6	28.3**
	S.D.	2.9	2.4	3.4	3.7
	N	24	24	25	24
	SPILLED	0	0	0	0
DAYS 6 TO 17	MEAN	29.3	29.2	27.1**	26.6**
	S.D.	2.7	2.5	2.8	2.2
	N	24	23	25	24
	SPILLED	0	1	0	0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

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TABLE 5
MEAN MATERNAL FOOD CONSUMPTION DURING GESTATION (G/ANIMAL/DAY)

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	5 MG/KG/DAY	15 MG/KG/DAY	30 MG/KG/DAY
DAYS 6 TO 20	MEAN	29.8	29.6	27.6*	26.9**
	S.D.	2.6	2.4	2.8	2.4
	N	24	23	25	24
	SPIILLED	0	1	0	0
DAYS 4 TO 20	MEAN	29.2	29.1	27.3*	26.8**
	S.D.	2.5	2.4	2.8	2.2
	N	24	23	25	24
	SPIILLED	0	1	0	0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

Toxicokinetics: not assessed

Terminal and necropsy evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): Animals were sacrificed (CO₂ inhalation and exsanguination) on GD20. Gross abnormalities of the cervical, thoracic and abdominal viscera were noted. The number of corpora lutea and implantation sites was determined for each dam. Each gravid uterus was weighed and the contents were examined.

There were no remarkable findings at necropsy for any dam in any group. There was no effect of treatment on group mean gravid uterine weight. The mean corrected body weight (terminal body weight minus the gravid uterine weight) was slightly (4%), but not significantly, decreased in the HD group. There was a dose-related non-significant decrease in the mean change in body weight (corrected by subtracting the gravid uterine weight) from Day 0 until sacrifice for the LD (3%), MD (9%) and HD (16%) groups. This data is summarized in the following sponsor-supplied summary table.

Summary of Uterine and Net Body Weights (g)

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	5 MG/KG/DAY	15 MG/KG/DAY	30 MG/KG/DAY
GRAVID UTERUS	MEAN	78.97	80.93	80.23	79.58
	S.D.	7.91	8.75	10.07	13.27
	N	24	24	25	24
CORRECTED WEIGHT	MEAN	300.95	300.28	296.25	290.30
	S.D.	17.75	17.82	19.06	18.26
	N	24	24	25	24
NET CHANGE FROM DAY 0	MEAN	76.16	73.90	69.53	64.09
	S.D.	17.52	15.05	18.03	15.97
	N	24	24	25	24

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.
CORRECTED WEIGHT = TERMINAL BODY WEIGHT MINUS GRAVID UTERINE WEIGHT
NET WEIGHT CHANGE FROM DAY 0 = CORRECTED WEIGHT MINUS DAY 0 BODY WEIGHT

The cesarean data are summarized in the sponsor-provided table that follows. Pregnancy rate was similar across groups. No dams aborted or had early deliveries, and all dams had litters with viable fetuses. There were no differences among groups for the group mean corpora lutea, implantation sites or preimplantation loss. Although not acknowledged by the sponsor, there appears to be a treatment-related increase in total resorptions and early resorptions in the HD group, and an increase in post-implantation loss in the HD group. This is summarized in the reviewer-generated table that follows. The mean number of live fetuses per litter was similar across groups.

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Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	24	24	25	24
	%	96	96	100	96
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	24	24	25	24
Dams with Viable Fetuses	N	24	24	25	24
	%	100	100	100	100
Dams with no Viable Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Corpora Lutea	MEAN	15.8	16.1	15.6	16.7
	S.D.	2.4	2.6	2.8	4.0
	N	24	24	25	24
	TOTAL	378	386	391	400
Implantation Sites	MEAN	13.7	13.9	14.1	14.0
	S.D.	1.2	1.2	2.0	1.9
	N	24	24	25	24
	TOTAL	328	333	352	336
Preimplantation Loss	MEAN%	11.7	12.4	8.9	13.6
	S.D.	12.7	9.8	9.3	12.4

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

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Cesarean Data					
		0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	30 mg/kg/day
total resorptions	total	9	6	7	13
	affected litters	8/24 litters	3/24 litters	6/25 litters	6/24 litters
	# affected/litter	(0-2 per litter)	(0-3 per litter)	(0-2 per litter)	(0-6 per litter)
	mean ± SD	0.4 ± 0.6	0.2 ± 0.7	0.3 ± 0.5	0.5 ± 1.4
	mean % ± SD	2.7 ± 4.1	1.8 ± 5.3	2.0 ± 3.8	3.9 ± 9.3
early resorptions	total	7	6	7	13
	affected litters	6/24 litters	3/24 litters	6/25 litters	6/24 litters
	# affected/litter	(0-2 per litter)	(0-3 per litter)	(0-2 per litter)	(0-6 per litter)
	mean ± SD	0.3 ± 0.6	0.2 ± 0.7	0.3 ± 0.5	0.5 ± 1.4
	mean % ± SD	2.1 ± 3.9	1.8 ± 5.3	2.0 ± 3.8	3.9 ± 9.3
late resorptions	total	2	0	0	0
	affected litters	2/24 litters	0/24 litters	0/25 litters	0/24 litters
	# affected/litter	(0-1 per litter)	0 per litter	(0 per litter)	0 per litter
	mean ± SD	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	mean % ± SD	0.6 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
dead fetuses	total	0	0	0	0
	mean ± SD	2.7 ± 4.1	1.8 ± 5.3	2.0 ± 3.8	3.9 ± 9.3
post-implantation loss		0% for 16 litters	0% for 21 litters	0% for 19 litters	0% for 18 litters
		7% for 5 litters	8% for 1 litter	7% for 2 litters	7% for 3 litters
		8% for 2 litters	17% for 1 litter	8% for 3 litters	8% for 1 litter
		14% for 1 litter	20% for 1 litter	13% for 1 litter	30% for 1 litter
					35% for 1 litter

SUMMARY OF CESAREAN SECTION DATA

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
Pregnant at C-section	N	24	24	25	24
Live Fetuses	MEAN	13.3	13.6	13.8	13.5
	S.D.	1.3	1.5	2.1	2.2
	N	24	24	25	24
	TOTAL	319	327	345	323
	MEAN%	97.3	98.2	98.0	96.1
	S.D.	4.1	5.3	3.8	9.3
Females	MEAN	7.0	6.7	6.7	6.3
	S.D.	2.0	2.4	2.6	1.9
	N	24	24	25	24
	TOTAL	167	161	168	151
	MEAN%	52.3	48.6	48.0	46.7
	S.D.	14.1	14.9	15.0	12.6
Males	MEAN	6.3	6.9	7.1	7.2
	S.D.	1.9	1.8	1.9	2.0
	N	24	24	25	24
	TOTAL	152	166	177	172
	MEAN%	47.7	51.4	52.0	53.3
	S.D.	14.1	14.9	15.0	12.6
Sex Ratio M:F		48:52	51:49	51:49	53:47

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

There is also a slight (nonsignificant) dose-related increase in mean fetal weight, and covariate adjusted (for number of fetuses per litter) fetal weight for total fetuses, male fetuses and females fetuses, as demonstrated in the following sponsor-provided summary table.

Summary of Mean Fetal Weights

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
FETAL WEIGHTS UNITS: GRAMS					
of all Viable Fetuses	MEAN	3.75	3.78	3.86	3.90
	S.D.	0.26	0.31	0.32	0.29
	N	24	24	25	24
	Covariate Adjusted MEAN	3.74	3.78	3.87	3.90
of Male Fetuses	MEAN	3.84	3.88	3.97	3.98
	S.D.	0.29	0.32	0.34	0.28
	N	24	24	25	24
	Covariate Adjusted MEAN	3.84	3.88	3.98	3.98
of Female Fetuses	MEAN	3.66	3.67	3.72	3.80
	S.D.	0.25	0.31	0.33	0.34
	N	24	24	25	24
	Covariate Adjusted MEAN	3.65	3.67	3.72	3.79

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

Offspring (malformations, variations, etc.):

"Each fetus (live or dead) was sexed, weighed, and examined for external abnormalities. Live fetuses were sacrificed by _____ via intraperitoneal injection followed by exsanguination and/or thoracic penetration. Approximately one-half of all fetuses from each litter were processed for visceral examination. The heads were removed, prepared/stained in Bouin's fixative, and evaluated by Wilson's technique. The internal organs of the thoracic and abdominal cavities of the fetuses were examined in the fresh state using Staples' technique. The fetuses were processed (but not evaluated) for skeletal examination using Alizarin Red S staining method. The remaining fetuses were eviscerated, processed for skeletal examination using the Alizarin Red S staining method, and evaluated. Findings were judged to be variations or malformations. All fetuses were retained in Bouin's fixative or glycerin..."

It should be noted that historical control data were not submitted for fetal variations and malformations. Summary tables of the fetal findings follow. Fetal external findings: there were no fetal external findings in any of the 319, 327, 345 and 323 fetuses from the control, LD, MD and HD groups, respectively. There were no head malformations in any fetus and no evidence of a treatment-related effect on variations of the

head. Soft tissue malformations were confined to single fetuses in the LD and HD groups. These fetuses were each noted with a malformed kidney with rough surface area. These same two fetuses were the only fetuses noted with the variation of a pale (white) kidney. A single MD fetus was noted with the variation of mottled kidney (white and light pink). Although these findings occurred only in TBZ treated groups, the incidence is low (1 fetus per group) and shows no increase with increasing dose; therefore, this should be considered an incidental finding. Other soft tissue variations did not appear to be related to treatment.

No skeletal malformations were noted in the study. This seems unusual, and the sponsor has not provided historical control data from the contract laboratory for interpretation. According to the sponsor, the skeletal variations noted were "the type and frequency commonly seen in this strain of rat." Examination of the data revealed no clear evidence of a treatment-related effect on skeletal variations. There were apparent treatment-related increased fetal and litter incidences of bipartite vertebral centrum(a) (MD and HD) and in 14th rudimentary rib(s) (LD, MD, and HD); however, the overall relevance of this finding is diminished when considered in conjunction the apparent treatment-related decrease in overall skeletal variations that are discussed below.

It should be noted that the fetal incidence of total skeletal variations decreased in the LD, MD and HD when compared with the concurrent control. For the MD and HD groups, the sponsor attributed this decrease to a treatment related decrease in the incidence of sternovertebral variations. However, apparent treatment-related decreases in the following skeletal variations occurred as follows: (1) incomplete ossification of the skull (MD, HD), (2) less than 4 caudal vertebrae ossified (MD, HD), (3) unossified vertebral arch(es) (HD), (4) 5th/6th sternebra(e) incomplete ossification (MD), (5) 5th sternebra unossified (MD, HD), (6) 6th sternebra unossified (HD), (7) 5th/6th sternebra(e) bipartite (MD, HD), (8) wavy bent rib(s) (LD, MD, HD). The relevance of these findings (if any) is not clear.

The sponsor concludes, "the NOEL for maternal toxicity is 5 mg/kg/day based on clinical signs, and reductions in maternal food consumption and body weight gain in the two higher dose groups. The NOEL for embryo/fetal viability and growth (based on fetal weight), and fetal development (teratogenicity) is 30 mg/kg/day, the highest dose tested in this study."

Based on examination of the data the following conclusions can be made: (1) the NOEL for maternal toxicity is 5 mg/kg/day (approximately 0.48 x the MRHD on a mg/m² basis), (2) the NOEL for embryo-fetal viability is 15 mg/kg/day (approximately 1.5 x the MRHD on a mg/m² basis), and (3) the NOEL for fetal development is 30 mg/kg/day (the highest dose tested) (approximately 3 x the MRHD on a mg/m² basis).

Total External Findings					
# fetuses examined		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
Total External Findings	fetal incidence	0/319 (0%)	0/327 (0%)	0/345 (0%)	0/323 (0%)
	litter incidence	0/24 (0%)	0/24 (0%)	0/25 (0%)	0/24 (0%)

Head Examination					
# fetuses examined		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
Head Malformations	fetal incidence	0/160 (0%)	0/162 (0%)	0/170 (0%)	0/164 (0%)
	litter incidence	0/24 (0%)	0/24 (0%)	0/25 (0%)	0/24 (0%)
Head Variations	fetal incidence	1/160 (0.6%)	0/162 (0%)	1/170 (0.6%)	1/164 (0.6%)
	litter incidence	1/24 (4.2%)	0/24 (0%)	1/25 (4%)	1/24 (4.2%)
• dilated lateral ventricles	fetal incidence	1/160 (0.6%)	0/162 (0%)	1/170 (0.6%)	1/164 (0.6%)
	litter incidence	1/24 (4.2%)	0/24 (0%)	1/25 (4%)	1/24 (4.2%)

Soft Tissue Exam					
# fetuses examined		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
Total Malformations	fetal incidence	0/159 (0%)	1/163 (0.6%)	0/167 (0.0%)	1/164 (0.6%)
	litter incidence	0/24 (0%)	1/24 (4.2%)	0/25 (0%)	1/24 (4.2%)
• malformed kidney(s) with surface area rough	fetal incidence	0/159 (0%)	1/163 (0.6%)	0/167 (0.0%)	1/164 (0.6%)
	litter incidence	0/24 (0%)	1/24 (4.2%)	0/25 (0%)	1/24 (4.2%)
Total Variations	fetal incidence	18/159 (11%)	12/163 (7.4%)	11/167 (6.6%)	18/164 (11%)
	litter incidence	13/24 (54%)	9/24 (38%)	7/25 (28%)	12/24 (50%)
• kidney(s) pale white – same fetuses as malformed kidney	fetal incidence	0/159 (0%)	1/163 (0.6%)	0/167 (0%)	1/164 (0.6%)
	litter incidence	0/24 (0%)	1/24 (4.2%)	0/25 (0%)	1/24 (4.2%)
• kidney(s) mottled	fetal incidence	0/159 (0%)	0/163 (0%)	1/167 (0.6%)	0/164 (0%)
	litter incidence	0/24 (0%)	0/24 (0%)	1/25 (4%)	0/24 (0%)
• dilated ureter	fetal incidence	17/159 (11%)	9/163 (5.5%)	10/167 (6.0%)	18/164 (11%)
	litter incidence	12/24 (50%)	6/24 (25%)	7/25 (28%)	12/24 (50%)
• enlargement of atrium of the heart	fetal incidence	0/159 (0%)	2/163 (1.2%)	0/167 (0%)	0/164 (0%)
	litter incidence	0/24 (0%)	2/24 (8.3%)	0/25 (0%)	0/24 (0%)
• variations of the major vessels	fetal incidence	1/159 (0.6%)	0/163 (0%)	0/167 (0%)	0/164 (0%)
	litter incidence	1/24 (4.2%)	0/24 (0%)	0/25 (0%)	0/24 (0%)

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Skeletal Exam					
# fetuses examined		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
Total Malformations	fetal incidence	0/159 (0%)	0/165 (0%)	0/175 (0%)	0/159 (0%)
	litter incidence	0/24 (0%)	0/24 (0%)	0/25 (0%)	0/24 (0%)
Total Variations	fetal incidence	122/159 (77%)	112/165 (68%)*	108/175 (62%)*	106/159 (67%)*
	litter incidence	24/24 (100%)	23/24 (96%)	25/25 (100%)	24/24 (100%)
• unossified hyoid body	fetal incidence	47/159 (30%)	46/165 (28%)	66/175 (38%)	57/159 (36%)
	litter incidence	19/24 (79%)	19/24 (79%)	23/25 (92%)	19/24 (79%)
• incomplete ossification of skull	fetal incidence	18/159 (11%)	17/165 (10%)	11/175 (6.3%)	11/159 (6.9%)
	litter incidence	8/24 (33%)	10/24 (42%)	7/25 (28%)	8/24 (33%)
• < 4 caudal vertebrae ossified	fetal incidence	19/159 (12%)	27/165 (16%)	18/175 (10%)	14/159 (8.8%)
	litter incidence	14/24 (58%)	12/24 (50%)	10/25 (40%)	6/24 (25%)*
• bipartite vertebral centrum(a)	fetal incidence	1/159 (0.6%)	2/165 (1.2%)	7/175 (4.0%)*	5/159 (3.1%)
	litter incidence	1/24 (4.2%)	2/24 (8.3%)	6/25 (24%)	5/24 (21%)
• Incomplete ossific. of vertebral arch(es)	fetal incidence	16/159 (10%)	30/165 (18%)*	15/175 (8.6%)	15/159 (9.4%)
	litter incidence	10/24 (42%)	14/24 (58%)	7/25 (28%)	8/24 (33%)
• unossified vertebral arch(es)	fetal incidence	3/159 (1.9%)	5/165 (3.0%)	3/175 (1.7%)	1/159 (0.6%)
	litter incidence	2/24 (8.3%)	3/24 (13%)	2/25 (8%)	1/24 (4.2%)
• unossified vertebral centrum(a)	fetal incidence	0/159 (0%)	1/165 (0.6%)	0/175 (0%)	0/159 (0%)
	litter incidence	0/24 (0%)	1/24 (4.2%)	0/25 (0%)	0/24 (0%)
• 5 th /6 th sternebra(e) incomplete ossific.	fetal incidence	69/159 (43%)	63/165 (38%)	39/175 (22%)**	63/159 (40%)
	litter incidence	20/24 (83%)	20/24 (83%)	19/25 (76%)	21/24 (88%)
• 5 th sternebra unossified	fetal incidence	40/159 (25%)	33/165 (20%)	29/175 (17%)*	18/159 (11%)**
	litter incidence	17/24 (71%)	16/24 (67%)	12/25 (48%)	11/24 (46%)
• 6 th sternebra unossified	fetal incidence	7/159 (4.4%)	10/165 (6.1%)	8/175 (4.6%)	4/159 (2.5%)
	litter incidence	5/24 (21%)	6/24 (25%)	3/25 (12%)	4/24 (17%)
• 5 th /6 th sternebra(e) bipartite	fetal incidence	3/159 (1.9%)	2/165 (1.2%)	0/175 (0%)	0/159 (0%)
	litter incidence	2/24 (8.3%)	2/24 (8.3%)	0/25 (0%)	0/24 (0%)
• other sternebra(e) incomplete ossific.	fetal incidence	3/159 (1.9%)	2/165 (1.2%)	3/175 (1.7%)	5/159 (3.1%)
	litter incidence	2/24 (8.3%)	2/24 (8.3%)	3/25 (12%)	4/24 (17%)
• other sternebra(e) bipartite	fetal incidence	0/159 (0%)	0/165 (0%)	0/175 (0%)	1/159 (0.6%)
	litter incidence	0/24 (0%)	0/24 (0%)	0/25 (0%)	1/24 (4.2%)
• other sternebra(e) unossified	fetal incidence	0/159 (0%)	1/165 (0.6%)	1/175 (0.6%)	0/159 (0%)
	litter incidence	0/24 (0%)	1/24 (4.2%)	1/25 (4.0%)	0/24 (0%)
• 14 th rudimentary rib(s)	fetal incidence	3/159 (1.9%)	6/165 (3.6%)	8/175 (4.6%)	10/159 (6.3%)*
	litter incidence	3/24 (13%)	5/24 (21%)	4/25 (16%)	7/24 (29%)
wavy/bent rib(s)	fetal incidence	7/159 (4.4%)	3/165 (1.8%)	1/175 (0.6%)*	0/159 (0%)**
	litter incidence	4/24 (17%)	2/24 (8.3%)	1/25 (4.0%)	0/24 (0%)
• incomplete ossification of rib(s)	fetal incidence	1/159 (0.6%)	0/165 (0%)	2/175 (1.1%)	1/159 (0.6%)
	litter incidence	1/24 (4.2%)	0/24 (0%)	1/25 (4.0%)	1/24 (4.2%)

Skeletal Exam					
# fetuses examined		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
• 13 th rudimentary rib(s)	fetal incidence	2/159 (1.3%)	0/165 (0%)	0/175 (0%)	1/159 (0.6%)
	litter incidence	2/24 (8.3%)	0/24 (0%)	0/25 (0%)	1/24 (4.2%)
7 th cervical rib(s)	fetal incidence	0/159 (0%)	0/165 (0%)	0/175 (0%)	1/159 (0.6%)
	litter incidence	0/24 (0%)	0/24 (0%)	0/25 (0%)	1/24 (4.2%)
thickened rib(s)	fetal incidence	0/159 (0%)	0/165 (0%)	2/175 (1.1%)	0/159 (0%)
	litter incidence	0/24 (0%)	0/24 (0%)	1/25 (4.0%)	0/24 (0%)
• incomplete ossific. of ischium(a)	fetal incidence	2/159 (1.3%)	2/165 (1.2%)	2/175 (1.1%)	3/159 (1.9%)
	litter incidence	2/24 (8.3%)	1/24 (4.2%)	2/25 (8.0%)	2/24 (8.3%)
unossified pubis(es)	fetal incidence	0/159 (0%)	1/165 (0.6%)	3/175 (1.7%)	1/159 (0.6%)
	litter incidence	0/24 (0%)	1/24 (4.2%)	3/25 (12%)	1/24 (4.2%)
unossified ischium(a)	fetal incidence	0/159 (0%)	1/165 (0.6%)	1/175 (0.6%)	0/159 (0%)
	litter incidence	0/24 (0%)	1/24 (4.2%)	1/25 (4.0%)	0/24 (0%)

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Prenatal and postnatal development

Study title: Oral Developmental Toxicity Study and Pre- and Postnatal Development Study with Tetrabenazine, in the Rat (Segment III Study)

Key study findings: Tetrabenazine produced treatment-induced perinatal pup lethality and developmental delays.

Study no.: — Study # 7425-106

Volume # 22, module 4

Conducting laboratory and location: _____

Date of study initiation: 13-Oct-03

GLP compliance: yes – US-FDA, Japanese and OECD with the following exception:

“The Gestation Day 0 clinical observations and body weights received from the supplier and used for randomization of animals prior to study initiation were collected in a non-GLP facility; however, these data did not affect the integrity of the study.”

QA reports: yes (X) no (.)

Drug, lot #, and % purity: Tetrabenazine (TBZ), lot # 105481, with stated purity of 99.3-100%. TBZ was suspended in carboxymethylcellulose (CMC) and Tween® 80 in water at TBZ concentrations of 0, 2.0, 6.0 and 12.0 mg/ml. Dosing formulations were prepared at least weekly (and stored refrigerated). Homogeneity was assessed weeks 1 and 5 and the data indicated that the formulations were homogeneous (\pm 5% nominal). The formulation analyses carried out weeks 1, 2, 3, 4 and 5 demonstrated that the formulations were within 6% of the nominal concentrations (94.7 – 102.8%). “Stability was determined in a previous study conducted at _____ and reported in 7425-102; concentrations ranged from 2.0 to 30.0 mg/mL and were stored refrigerated for 10 days.”

Methods

Doses: 0, 5, 15 and 30 mg/kg/day (QD dosing) GD6-LD20.

Species/strain: ~~SD~~SD(SD)IGS BR mated female rats from _____ The animals were approximately 10-11 weeks of age at randomization, and on GD0 weighed 200 – 250 g. Animals were individually housed.

Number/sex/group: 25/gr

Route, formulation, volume, and infusion rate: oral gavage (2.5 ml/kg), QD dosing, from GD6-LD20. If a dam was in the process of giving birth at the scheduled dosing period, the dose was skipped.

Satellite groups used for toxicokinetics: none

Study design: standard segment III

Parameters and endpoints evaluated: described with the results, below.

Justification for Dose: “The choice of the high-dose level was based on the outcome of previously conducted 4- and 26-week rat toxicity studies provided by the sponsor. It was anticipated that the high-dose level would show drug-specific effects. Other dose levels were selected at intervals that were predicted to be narrow enough to reveal any dose-related trends. Though priority was given to detecting a dose-related trend, it was expected that the low-dose level would be a ‘no-observed-adverse-effect level’.” It should be noted that 4- and 26-week toxicity studies in rat utilized BID dosing; whereas, this reproductive toxicology study utilizes QD dosing.

Results

Mortality F₀ dams: Animals were checked twice daily for signs of mortality, morbidity and distress. There were no unscheduled deaths during the gestation period; however, a control female that had not delivered a litter by GD30 was sacrificed and found not to be pregnant.

Clinical signs F₀ dams: During the dosing period daily cageside observations were conducted at approximately 1 hr post dose, during the first two weeks of dosing. From 03-Nov-03 (corresponds roughly to late gestation/early lactation for all dams) through LD14, the cageside observations were made twice daily (at 1 and 3 hrs post dose), noting any change in maternal care of pups, especially pup retrieval. From LD15 onward, cageside observations were conducted once daily (at ≈ 1hr post dose). Detailed observations were conducted at the time of weighing (GD0, 4, 6, 8, 10, 14, 17, 20, and LD0, 4, 7, 10, 14, 17 and 21). GD0 clinical observations were provided by the supplier. Clinical observations were not recorded in 1-LD dam (LD14), 1-MD dam (LD20) and in 2-HD dams (LD4, LD7, LD10 in one, and LD21 in the other).

During the gestation and lactation periods, the observations in the LD group were isolated and similar to the background findings noted in the control group. The MD and HD groups were noted with treatment-related hypoactivity, closed eyes, and squinted eyes throughout the gestation and lactation periods. Since observations were made at approximately 1 hr post dose or 1 and 3 hrs post dose, it is not clear how long the clinical signs persisted on a day-to-day basis. MD and HD dams were subjectively rated as not tending to their litters. In the MD group this was noted for 10-11 dams on LD 0-17, with 0-5 dams affected per day. In the HD group this was noted for 15-18 dams on LD 0-16, with 0-6 dams affected per day. There were entire litter losses in 1-MD dam (LD1) and in 3-HD dams (LD2 for 2 dams, and LD7 for 1 dam).

Body weight F₀ dams: Body weights were obtained on GD0, 4, 6, 8, 10, 14, 17, 20, and LD0, 4, 7, 10, 14, 17 and 21. GD0 body weights were provided by the supplier. There was a treatment-related slight decrease in group mean maternal body weight during gestation in the MD and HD groups (in the HD group the maximum decrease was 6%) and during lactation (in the HD group the maximum decrease was 11% on LD4, returning to a 7% decrease by LD14). According to the sponsor, the decreases in group mean body weight noted during lactation are the result of the decreases that occurred during the gestation period. Examination of the data indicates that the group mean maternal body weight changes noted during intervals of LD0-4, LD4-7, LD7-10, LD10-14, LD14-17, LD17-21 and LD0-21 do not demonstrate treatment-related decreases. Copies of the sponsor's summary tables follow.

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Mean Maternal Body Weights During Gestation (g) – F₀ Generation

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	5 MG/KG/DAY	15 MG/KG/DAY	30 MG/KG/DAY
DAY 0	MEAN	226.2	225.9	226.6	225.2
	S.D.	13.9	13.2	13.2	13.9
	N	24	25	25	25
DAY 4	MEAN	242.8	241.9	243.4	241.4
	S.D.	12.2	13.4	15.4	14.2
	N	24	25	25	25
DAY 6	MEAN	253.9	254.3	254.9	252.5
	S.D.	13.3	13.7	14.6	13.4
	N	24	25	25	25
DAY 8	MEAN	263.2	262.0	259.6	255.5
	S.D.	13.1	15.2	15.3	13.1
	N	24	25	25	25
DAY 10	MEAN	276.2	273.9	269.8	265.0
	S.D.	13.6	15.7	16.8	13.3
	N	24	25	25	25
DAY 14	MEAN	300.3	298.9	293.4	287.5*
	S.D.	13.6	17.1	17.3	15.0
	N	24	25	25	25
DAY 17	MEAN	334.3	332.0	321.2*	314.1**
	S.D.	18.2	15.4	15.6	14.7
	N	24	25	25	25
DAY 20	MEAN	379.9	380.3	366.4	355.3**
	S.D.	25.3	23.1	26.4	18.4
	N	24	25	25	25

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

Mean Maternal Body Weights During Lactation (g) – F₀ Generation

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	5 MG/KG/DAY	15 MG/KG/DAY	30 MG/KG/DAY
DAY 0	MEAN	290.2	287.2	272.8**	260.6**
	S.D.	18.3	20.1	20.5	17.5
	N	24	25	25	25
DAY 4	MEAN	308.6	308.9	290.2	273.7**
	S.D.	24.9	21.9	28.8	13.1
	N	24	25	24	23
DAY 7	MEAN	315.1	317.3	303.2	283.1**
	S.D.	24.0	23.0	22.5	16.3
	N	24	25	24	23
DAY 10	MEAN	325.7	323.4	317.0	297.1**
	S.D.	22.4	21.4	26.8	18.5
	N	24	25	24	22
DAY 14	MEAN	335.5	331.0	324.5	311.8**
	S.D.	21.8	22.2	24.2	17.0
	N	24	25	24	22
DAY 17	MEAN	337.1	335.4	330.6	313.0**
	S.D.	22.7	19.2	26.3	26.8
	N	24	25	24	22
DAY 21	MEAN	321.1	307.9	309.8	297.1**
	S.D.	23.4	29.2	21.9	26.0
	N	24	25	24	22

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

Food consumption F₀ dams: Food consumption was assessed on GD4, 6, 8, 10, 14, 17, 20, and LD0, 4, 7, 10, and 14 (not on LD17 and 21). Due to technical problems, food consumption data for LD14 were invalid for 13-C, 12-LD, 11-MD, 6-HD dams. There was a treatment related decrease in food consumption during the gestation period in the MD and HD for the intervals GD 6-8, GD8-10, GD10-14, GD14-17, GD17-20 and GD6-20 and during lactation period at intervals LD0-4, LD4-7, LD7-14 and LD0-14 (all that were recorded). Copies of the sponsor-provided summary tables follow.

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Mean Maternal Food Consumption During Gestation (g/animal/day) – F₀ Generation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
DAYS 4 TO 6	MEAN	25.2	26.2	26.5	26.9
	S.D.	3.7	3.2	3.5	3.9
	N	24	25	25	25
	SPILLED	0	0	0	0
DAYS 6 TO 8	MEAN	27.4	27.2	26.2	24.4**
	S.D.	2.9	3.2	3.6	2.9
	N	24	25	25	25
	SPILLED	0	0	0	0
DAYS 8 TO 10	MEAN	28.1	28.1	27.8	27.0
	S.D.	2.5	2.9	3.9	2.6
	N	24	25	25	25
	SPILLED	0	0	0	0
DAYS 10 TO 14	MEAN	30.1	30.3	29.5	28.1*
	S.D.	2.5	3.1	3.1	2.3
	N	24	25	25	25
	SPILLED	0	0	0	0
DAYS 14 TO 17	MEAN	32.2	31.0	29.6**	27.6**
	S.D.	2.3	3.0	2.9	2.6
	N	24	25	25	25
	SPILLED	0	0	0	0
DAYS 17 TO 20	MEAN	34.1	32.8	30.8**	27.7**
	S.D.	4.3	3.3	4.6	2.7
	N	24	25	25	25
	SPILLED	0	0	0	0
DAYS 6 TO 20	MEAN	30.7	30.2	29.1	27.2**
	S.D.	2.6	2.6	3.1	2.0
	N	24	25	25	25
	SPILLED	0	0	0	0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

Mean Maternal Food Consumption During Lactation (g/animal/day) – F₀ Generation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
DAYS 0 TO 4	MEAN	40.1	39.9	35.1	31.6**
	S.D.	7.3	5.5	8.8	7.3
	N	24	25	24	23
	SPILLED	0	0	0	0
DAYS 4 TO 7	MEAN	50.7	51.3	48.7	42.4**
	S.D.	9.8	6.0	7.5	8.9
	N	24	25	24	23
	SPILLED	0	0	0	0
DAYS 7 TO 14	MEAN	64.7	64.3	61.1	54.3**
	S.D.	6.5	4.9	7.7	11.6
	N	11	13	13	16
	SPILLED	0	0	0	0
DAYS 0 TO 14	MEAN	54.6	54.8	50.9	45.2**
	S.D.	4.6	4.4	7.6	8.8
	N	11	13	13	16
	SPILLED	0	0	0	0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

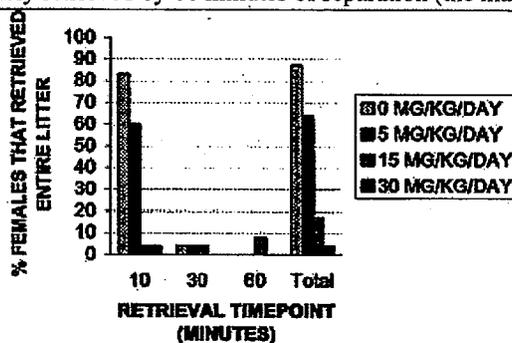
F₀ delivery and litter data: The data are summarized in the following reviewer-generated table. One control female was not pregnant. All pregnant dams delivered litters with some live fetuses. Although not acknowledged by the sponsor there was a slight increase in the duration of the gestation period in the MD and HD groups. The mean number of pups delivered was similar across groups; however, the number of still born pups was increased in the MD and HD (as well as the number of affected litters). It should be noted that the individual animal line listings for each dam states that there were no still born pups delivered to any litter, so there is no way to independently verify this finding. It appears that the sponsor looked at any pup death on postpartum day 0 (LD0) and determined whether the dead pup met one of their two criteria for designation as a stillborn pup, i.e., (1) pup has no milk in its stomach (indicating that the pup has never nursed), or (2) lungs do not float (indicating that the pup has never breathed). There was also a treatment-related increase in the number of dead/missing/cannibalized pups in the MD and HD groups between birth and LD4. There were entire litter losses in 1-MD dam (LD1) and in 3-HD dams (LD2 for 2 dams) (for 1 dam, 11/12 pups died between LD0-LD4 and the remaining pup died LD7). Thus, the live-birth index and the viability index were decreased in the MD and HD groups. There was no effect of treatment on weaning index.

It is not clear that lack of milk in a pup's stomach should be used as a criterion establishing a stillbirth. The liveborn pups could be affected by their in-utero exposure to TBZ or its metabolites (a new born pup may not be able to metabolize and/or excrete the TBZ and/or its metabolites) resulting in their inability to nurse and thus their death. Since the pup status is not assessed immediately after birth, the definitive designation of some perinatal deaths as stillbirths can be problematic.

The sponsor provided the following discussion regarding the increase in stillborn pups and increase in pup death.

- “There are several possible factors that could result in stillborn pups and include difficult or prolonged delivery (although not noted in this study, these factors could potentially result in suffocation of pups during the delivery process), lack of maternal care at birth (pups who are not quickly and adequately cleaned of mucus and membranes could also suffocate), or pup defects that are incompatible with viability (there were no data to support this in either this study or the Segment II phase [Oral Developmental Toxicity Study and Pre- and Postnatal Development Study with tetrabenazine, in the Rat; Segment II Study]). Therefore, it is possible that lack of maternal care could contribute to an increase in stillborn pups.”
- “During lactation, increased incidences of remarkable pup clinical observations were observed at doses ≥ 15 mg/kg/day including cold to touch, weakness, and pale and thin appearance. It is possible that lack of maternal care is responsible for many of these pup observations as supported by the observation “dam not attending litter” on many occasions in the 15 and 30 mg/kg/day dams at the one-hour and three-hour postdose timepoints (... NOTE: This is a subjective observation used by the technicians when they notice that the dam is not in proximity to the litter, the pups are scattered in the box, or the dam takes no interest in the pups). Other pup clinical observations were sporadic and not attributed to tetrabenazine.”

F₀ maternal pup retrieval was assessed on LD3 (≈ 1 hr post dose) by distributing the pups around the cage, with the dam in the center. The number of pups not retrieved by the dam and placed in the nest was recorded at 10, 30 and 60 minutes post distribution. Pups not returned to the nest after 60 min were returned to the nest. The purpose of this assessment was to help determine whether pup death was secondary to the lack of maternal care (particularly, lack of maternal or litter group body heat). Data for this parameter are presented in the reviewer-generated summary table that follows and in the sponsor-provided figure below. At approximately 1 hr post dose on LD3 (the only day and time tested) there is a treatment related decrease in pup retrieval in the LD, MD and HD treated groups. Note that 3/24 control litters have not been completely retrieved by 60 minutes of separation (the maximum time tested).



The sponsor notes that from 04-Nov-03 (on or prior to LD3), each time the dam was returned to the cage she was placed near the nest, or the majority of pups (except after the 1 hr post dose period on LD3 during the retrieval assessment). According to the sponsor, this amendment to the protocol was instituted in hopes of ameliorating any adverse effects on the pups that were caused by the pharmacological effects of treatment on the dam.

Summary of F ₀ Delivery and Litter Data					
		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
females pregnant / females mated		24/25 (96%)	25/25 (100%)	25/25 (100%)	25/25 (100%)
females delivering/females pregnant		24/24 (100%)	25/25 (100%)	25/25 (100%)	25/25 (100%)
duration of gestation	mean ± SD	21.9 ± 0.4	21.9 ± 0.3	22.0 ± 0.0	22.1 ± 0.3
	# @ 21 days	4	3	0	0
	# @ 22 days	19	22	25	22
	# @ 23 days	1	0	0	3
females with no live-born pups		0/24	0/25	0/25	0/25
implantation sites	total (# litters)	333 (24)	367 (25)	355 (25)	359 (25)
	mean ± SD	13.88 ± 2.89	14.68 ± 1.55	14.20 ± 2.04	14.36 ± 1.55
post-implantation loss	total #	16	19	17	22
	affected litters	11	11	11	13
	range per litter	(0-3)	(0-5)	(0-3)	(0-4)
pups delivered	total (# litters)	317 (24)	348 (25)	338 (25)	337 (25)
	mean ± SD	13.21 ± 2.87	13.92 ± 1.50	13.52 ± 1.85	13.48 ± 1.66
	range per litter	(3 - 18)	(12 - 17)	(9 - 16)	(11 - 17)
Live-born pups	total # pup	316	347	322	305
	mean	13.17	13.88	12.88	12.20
	# litters (%)	24/24 (100%)	25/25 (100%)	25/25 (100%)	25/25 (100%)
still-born pups (defined in note A)	# pups	1	1	15	32
	# litters affected (%)	1 (4.2%)	1 (4.0%)	4 (16%)	11 (44%)
uncertain live-born/still-born	# pups	0	0	1	0
pups dying, killed, missing and/or cannibalized	days 0-4	6	8	41	82
	days 5-21	2	0	0	1
entire litter died, killed, missing and/or cannibalized	days 0-4	0	0	1	2
	days 5-21	0	0	0	1
Pup disposition	culled LD4	123	139	95	67
	killed	0	0	0	0
	died	5	7	36	75
	cannibalized	1	0	3	1
	missing	2	1	2	7
pups surviving until LD21		185	200	186	155
gestation index (defined in note B)		24 (100%)	25 (100%)	25 (100%)	25 (100%)
Live-birth index (defined in note C)		100	100	96	90
viability index (defined in note D)		98	98	86 **	72 **
weaning index (defined in note E)		99	100	100	96
total # males, and mean %/litter	day 0	148 (49%)	183 (52%)	160 (48%)	157 (52%)
	day 4 precull	146 (49%)	181 (53%)	137 (49%)	114 (56%)
	day 21	93 (51%)	99 (50%)	93 (51%)	82 (55%)
Live pups/litter with live pups	day 0	13.17 ± 2.87 (24)	13.88 ± 1.54 (25)	12.88 ± 2.35 (25)	12.20 ± 2.63 (25)
	day 4 precull	12.92 ± 2.93 (24)	13.56 ± 1.66 (25)	11.71 ± 3.04 (24)	9.70 ± 4.16 (23) **
	day 4 post cull	7.79 ± 1.02 (24)	8.00 ± 0.00 (25)	7.75 ± 1.03 (24)	6.78 ± 2.15 (23)
	day 7	7.75 ± 1.03 (24)	8.00 ± 0.00 (25)	7.75 ± 1.03 (24)	7.05 ± 1.79 (22)
	day 14	7.71 ± 1.04 (24)	8.00 ± 0.00 (25)	7.75 ± 1.03 (24)	7.05 ± 1.79 (22)
	day 21	7.71 ± 1.04 (24)	8.00 ± 0.00 (25)	7.75 ± 1.03 (24)	7.05 ± 1.79 (22)
maternal retrieval on LD3	dams with 100% retrieval				
	at 10 min	20/24 (83%)	15/25 (60%)	1/25 (4%)	1/25 (4%)
	at 30 min	21/24 (88%)	16/25 (64%)	2/25 (8%)	1/25 (4%)
	at 60 min	21/24 (88%)	16/25 (64%)	4/25 (16%)	1/25 (4%)

- Note A – Stillborn pups – “defined as dead pups found on LD0 without milk in their stomachs (indicating they never nursed) or whose lungs do not float (indicating that these pups never took a breath).”
- Note B – gestation index litters with live born pups / # pregnant females
- Note C – live birth index - (# born alive) / (# born)
- Note D – viability index – (# alive LD4_{precull}) / (# born alive)
- Note E – weaning index – (# alive LD21) / (number alive LD4_{postcull})

F₀ necropsy: Any dams that had not delivered by GD 26 were sacrificed. Dams that delivered were sacrificed as soon as possible after weaning the F1 litters (Post partum day 21) or as soon as possible after the death of an entire litter. Gross necropsy included the cervical, thoracic and abdominal viscera. Gross visceral lesions as well as the ovaries and uterus were preserved in 10% neutral buffered formalin (NBF). There were no treatment related effects on necropsy parameters. The only notations consisted of the following: (1) a single control female was not pregnant (noted as unremarkable), (2) a single control female was noted as having delivered 14 pups but having only 13 implantation sites. There were entire

litter losses in 1-MD dam (LD1) and in 3-HD dams (LD2 for 2 dams, and LD7 for 1 dam). The necropsies of these dams (1-MD, 3-HD) were considered unremarkable.

F₁ litter observations: General observations were conducted at birth, LD4, 7, 14 and 21. At each time point litter size was recorded and the sex, weight and general observations for each individual pup. On LD4, larger litters were culled to 8 (4 males and 4 females, or as close as possible) using a random card draw. Culled pups and any pup found dead were examined for visceral abnormalities (thoracic, cervical and abdominal). Pups found dead were preserved in alcohol.

The clinical observations of the F₁ pups during lactation are summarized in the following reviewer-generated table. When compared to the control, there were treatment related increases in pups noted as cold, weak or thin in the MD groups, and pups noted as cold, weak, thin or pale or lacking milk in the stomach in HD group.

Clinical Observations of Pups During Lactation	
group/dose	observations
C – 0 mg/kg/day	pup(s) s cold to touch in 1/24 litters (LD4) pup (s) pale in 1/24 litters (LD1) pup(s) with no visible milk in stomach in 2/24 litters (LD0, 1) (1-2 on a given day)
LD – 5 mg/kg/day	pup(s) s cold to touch in 1/25 litters (LD0) pup (s) pale in 1/25 litters (LD2) pup(s) with no visible milk in stomach in 2/25 litters (LD0, 2) (1 on a given day) pup(s) with eye(s) white spot in 1/25 litters (LD14-17, 19, 21)
MD – 15 mg/kg/day	pup(s) s cold to touch in 5/25 litters (LD0, 1, 4) (1-3 on a given day) pup(s) weak in 2/25 litters (LD 4, 5) (1 on a given day) pup (s) pale in 2/25 litters (LD1, 4) (1 on a given day) pup(s) with no visible milk in stomach in 2/25 litters (LD 0, 4) (1 on a given day) pup (s) thin in 1/25 litters (LD 5,6,7) pup(s) with cloudy eyes in 1/25 litters (LD21) pup(s) with protruding eye(s) in 1/25 litters (LD21)
HD – 30 mg/kg/day	pup(s) s cold to touch in 9/25 litters (LD0, 2, 4-10) (1-6 on a given day) pup(s) weak in 7/25 litters (LD0, 2, 4-10) (1 – 4 on a given day) pup (s) pale in 4/25 litters (LD0, 2, 4, 5, 6) (1-2 on a given day) pup(s) with no visible milk in stomach in 4/25 litters (LD2-10) (1-2 on a given day) pup (s) thin in 4/25 litters (LD2-5, 8-11) (1-2 on a given day) pup(s) with bent tail in 1/25 litters (LD21)
<ul style="list-style-type: none"> LD0 observations were not recorded for F₁ females as follows: 2-C (#B73483, #B73494), 0-LD, 2-MD (#B73528, #B73539), and 2-HD (#B73558, #B73559) LD21 observations were not recorded for F₁ males #B73426 and #B73427 	

F₁ pup weights (preweaning): Fetal weights were decreased in the MD and HD group at all time points. The data are summarized in the following sponsor-provided tables. Group mean pup weights covariate adjusted (for litter size) were generally slightly decreased in the MD and HD groups compared to the control (data expressed as male/females) on LD0 (MD-3%/3%, HD-7%/6%), LD4_{preculling} (MD-5%/1%, HD-12%/8%), LD4_{postculling} (MD-5%/1%, HD-12%/8%), LD7 (MD-6%/0%, HD-10%/6%), LD14 (MD-5%/4%, HD-9%/9%), and LD21 (MD-7%/6%, HD-8%/9%).

TABLE 10
NATURAL DELIVERY DATA AND LITTER DATA SUMMARY - F0 GENERATION

DOSE LEVEL		GROUP 1 0 MG/KG	GROUP 2 5 MG/KG	GROUP 3 15 MG/KG	GROUP 4 30 MG/KG
Pup Weight/Litter (g)					
day 0 MALES	MEAN	6.82	6.65	6.54	6.28
	S.D.	0.85	0.56	0.49	0.38
	N	24	25	25	25
	Covariate Adjusted MEAN	6.77	6.71	6.54	6.27**
day 0 FEMALES	MEAN	6.33	6.29	6.16	5.94
	S.D.	0.50	0.59	0.59	0.47
	N	23	25	25	25
	Covariate Adjusted MEAN	6.33	6.32	6.15	5.93*
day 4 MALES - Precull	MEAN	10.92	10.84	10.36	9.67
	S.D.	1.63	1.30	1.45	1.76
	N	24	25	23	23
	Covariate Adjusted MEAN	10.94	10.88	10.35	9.61*
day 4 FEMALES - Precull	MEAN	10.20	10.39	10.21	9.67
	S.D.	1.13	1.21	1.47	1.38
	N	23	25	23	21
	Covariate Adjusted MEAN	10.28	10.49	10.18	9.50
day 4 MALES - Postcull	MEAN	10.95	10.94	10.39	9.72
	S.D.	1.59	1.33	1.46	1.80
	N	24	25	23	23
	Covariate Adjusted MEAN	10.96	10.95	10.39	9.63*
day 4 FEMALES - Postcull	MEAN	10.23	10.42	10.24	9.66
	S.D.	1.13	1.26	1.43	1.39
	N	23	25	23	21
	Covariate Adjusted MEAN	10.30	10.50	10.22	9.51
day 7 MALES	MEAN	17.70	17.91	16.59	15.62
	S.D.	2.25	1.92	2.42	2.68
	N	24	25	23	22
	Covariate Adjusted MEAN	17.65	17.73	16.53	15.54*
day 7 FEMALES	MEAN	16.69	17.26	16.70	15.37
	S.D.	2.02	1.91	2.55	2.00
	N	23	25	23	21
	Covariate Adjusted MEAN	16.63	17.18	16.63	15.61
day 14 MALES	MEAN	34.94	35.55	33.16	31.01
	S.D.	3.30	2.77	3.05	5.29
	N	24	25	23	22
	Covariate Adjusted MEAN	34.87	35.12	33.08	31.75**
day 14 FEMALES	MEAN	34.00	34.35	32.75	30.57
	S.D.	3.38	2.38	3.71	3.00
	N	23	25	23	21
	Covariate Adjusted MEAN	33.96	34.26	32.69	30.79**
day 21 MALES	MEAN	57.04	57.65	53.42	51.15
	S.D.	6.76	5.79	4.89	9.00
	N	24	25	23	22
	Covariate Adjusted MEAN	56.90	56.84	53.12	52.54
day 21 FEMALES	MEAN	55.36	54.97	52.05	50.32
	S.D.	5.83	5.51	5.83	5.06
	N	23	25	23	21
	Covariate Adjusted MEAN	55.34	54.92	52.02	50.44*

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01. N = NUMBER OF LITTERS.

Necropsy observations for F₁ pups (culled, stillborn or found dead): The findings are presented in the sponsor-supplied summary table below. The analysis of the data and resulting conclusions are somewhat limited by the increased incidence of autolysis (abdominal region, or entire fetus) in the HD group (2/129-control, 0/146-low dose, 5/147-mid dose and 28/173-HD). There were pups in the MD and HD groups noted with no milk in the stomach. The sponsor did not mention that there were discrepancies in number of pups that were supposed to be examined in each group and the number that were actually examined in each group as presented in the reviewer-generated table that follows. Based on the data provided, there were no other findings clearly indicative of a treatment related effect. However, given the incidence of abdominal autolysis and autolysis of the entire fetus, it would be possible to miss a treatment related effect.

Summary of Pup Necropsy Observations – F₁ Generation

	DOSE LEVEL	GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	5 MG/KG/DAY	15 MG/KG/DAY	30 MG/KG/DAY
Litters Evaluated	N	23	25	29	25
Pups Evaluated	N	129	146	147	173
Liveborn	N	128	145	132	141
Stillborn	N	1	1	15	32
AUTOLYSIS-ABDOMINAL REGION					
Pup Incidence	N	2	0	5	20
%		1.6	0.0	3.4	12
Litter Incidence	N	2	0	5	8
%		8.7	0.0	20	32
AUTOLYSIS-ENTIRE FETUS					
Pup Incidence	N	0	0	0	8
%		0.0	0.0	0.0	4.6
Litter Incidence	N	0	0	0	3
%		0.0	0.0	0.0	12
V VARIATION OF THE MAJOR BLOOD VESSELS					
Pup Incidence	N	0	0	1	0
%		0.0	0.0	0.7	0.0
Litter Incidence	N	0	0	1	0
%		0.0	0.0	4.0	0.0
STOMACH-NO MILK PRESENT					
Pup Incidence	N	0	0	8	19
%		0.0	0.0	5.4	11
Litter Incidence	N	0	0	2	8
%		0.0	0.0	8.0	32
KIDNEY(S)-DILATED PELVIS(ES)					
Pup Incidence	N	0	1	1	1
%		0.0	0.7	0.7	0.6
Litter Incidence	N	0	1	1	1
%		0.0	4.0	4.0	4.0
URINARY BLADDER DISTENDED					
Pup Incidence	N	0	0	0	1
%		0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
%		0.0	0.0	0.0	4.0
URETER(S)-DISTENDED					
Pup Incidence	N	6	14	8	5
%		4.7	9.6	5.4	2.9
Litter Incidence	N	3	8	7	3
%		13	32	26	12
TOTAL PUP NECROPSY OBSERVATIONS					
Pup Incidence	N	8	15	23	46
%		6.2	10	16	27
Litter Incidence	N	4	8	13	13
%		17	32	52	52

N = NUMBER

		F ₁ necropsy designation			
		C: 0 mg/kg/day	LD: 5 mg/kg/day	MD: 15 mg/kg/day	HD: 30 mg/kg/day
values from the necropsy summary	total # pups examined	129	146	147	173
	• # liveborn	• 128	• 145	• 132	• 141
	• # stillborn	• 1	• 1	• 15	• 32
values from the litter summary	total # pups eligible for necropsy (*)	129	147	146	174
	• culled	• 123	• 139	• 95	• 67
	• dead	• 5	• 7	• 36	• 75
	• stillborn	• 1	• 1	• 15	• 32

(*) excludes those missing and presumed cannibalized or those partially cannibalized

F₁ physical development: beginning on the stated day the following parameters were assessed for each pup using — SOPs (SOPs not provided: pinna unfolding (LD1), surface righting reflex (LD4), hair growth (LD7), incisor eruption (LD7), eye opening (LD11), auditory startle (LD21). "Pups were weaned on LD 21, and 20 rats/sex/group (from 1 pup/sex/litter, supplemented randomly from appropriate groups, if needed, to achieve 20 rats/sex/group) were randomly selected for the F₁ maturation phase (7 weeks duration)."

Although the sponsor states that there was no effect of treatment on pinna unfolding, examination of the individual pup data suggest a treatment-related very slight delay in the HD pups (see the reviewer-generated table that follows for details).

Pinna Unfolding (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 1	2/311 - 1%	0/339 - 0%	5/283 - 2%	1/244 - 0%
day 2	35/310 - 11%	17/339 - 5%	11/281 - 4%	19/231 - 8%
day 3	83/310 - 27%	112/339 - 33%	88/281 - 31%	91/231 - 39%
day 4	183/310 - 59%	214/339 - 63%	158/281 - 56%	144/230 - 63%
day 5	170/199 - 85%	199/213 - 93%	164/190 - 86%	152/171 - 89%
day 6	197/199 - 99%	213/213 - 100%	190/190 - 100%	162/171 - 95%
day 7	199/199 - 100%			170/171 - 99%
day 8				171/171 - 100%
mean ± SD	5.29 ± 0.95	5.28 ± 0.61	5.29 ± 0.62	5.18 ± 1.26
covariate adj. mean	5.32	5.33	5.28	5.11

Are all these left in bloc of safety?
Just curious!

There was no effect of treatment on incisor eruption (see the reviewer-generated table that follo

Incisor Eruption (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 7	0/186 - 0%	0/200 - 0%	0/186 - 0%	0/155 - 0%
day 8	1/186 - 1%	0/200 - 0%	0/186 - 0%	3/155 - 2%
day 9	10/186 - 5%	8/200 - 4%	5/186 - 3%	10/155 - 6%
day 10	25/186 - 13%	24/200 - 12%	28/186 - 15%	29/155 - 19%
day 11	79/186 - 42%	147/200 - 74%	94/186 - 51%	103/155 - 66%
day 12	174/185 - 94%	194/200 - 97%	165/186 - 89%	137/155 - 88%
day 13	184/185 - 99%	200/200 - 100%	184/186 - 99%	150/155 - 97%
day 14	184/185 - 99%		186/186 - 100%	154/155 - 99%
day 15	185/185 - 100%			155/155 - 100%
mean ± SD	12.13 ± 1.15	11.76 ± 0.66	12.29 ± 0.69	12.14 ± 1.13
covariate adj. mean	12.12	11.74	12.28	12.17

The sponsor notes a significant delay in hair growth in the HD pups, which they attribute to decreased pup weight. Examination of the individual pup data suggest treatment-related delays in hair growth in LD (slight) and MD also (see the reviewer-generated table that follows for details, note that the HD never reaches 100%).

APPEARS THIS WAY
ON ORIGINAL

Hair Growth (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 7	0/186 – 0%	0/200 – 0%	0/186 – 0%	0/155 – 0%
day 8	0/186 – 0%	0/200 – 0%	0/186 – 0%	0/155 – 0%
day 9	0/186 – 0%	0/200 – 0%	0/186 – 0%	0/155 – 0%
day 10	0/186 – 0%	0/200 – 0%	0/186 – 0%	0/155 – 0%
day 11	5/186 – 3%	0/200 – 0%	0/186 – 0%	0/155 – 0%
day 12	35/185 – 19%	56/200 – 28%	37/186 – 20%	20/155 – 13%
day 13	136/185 – 74%	151/200 – 76%	127/186 – 68%	73/155 – 47%
day 14	183/185 – 99%	180/200 – 90%	155/186 – 83%	117/155 – 75%
day 15	185/185 – 100%	198/200 – 99%	174/186 – 94%	147/155 – 95%
day 16		200/200 – 100%	182/186 – 98%	151/155 – 97%
day 17			183/186 – 98%	153/155 – 99%
day 18			186/186 – 100%	153/155 – 99%
day 19				153/155 – 99%
day 20				153/155 – 99%
day 21				154/155 – 99%
mean ± SD	13.33 ± 0.76	13.72 ± 1.02	14.13 ± 1.48	14.43 ± 1.40
covariate adj. mean	13.33	13.70	14.12	14.45**

Although the sponsor states that there was no effect of treatment on eye opening, examination of the individual pup data suggest a treatment-related slight delay in eye opening in the HD pups (see the reviewer-generated table that follows for details).

Eye Opening (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 11	0/186 – 0%	0/200 – 0%	0/186 – 0%	0/155 – 0%
day 12	0/186 – 0%	4/200 – 2%	0/186 – 0%	1/155 – 1%
day 13	14/185 – 8%	20/200 – 10%	6/186 – 3%	14/155 – 9%
day 14	115/185 – 62%	125/200 – 63%	94/186 – 51%	70/155 – 45%
day 15	179/185 – 97%	194/200 – 97%	175/186 – 94%	147/155 – 95%
day 16	185/185 – 100%	200/200 – 100%	186/186 – 100%	153/155 – 99%
day 17				153/155 – 99%
day 18				155/155 – 100%
mean ± SD	14.96 ± 0.46	14.80 ± 0.65	14.96 ± 0.69	15.14 ± 0.83
covariate adj. mean	14.97	14.85	14.97	15.05

There was no effect of treatment on auditory startle eruption (see the reviewer-generated table that follows for details). Since the test was conducted on only one day, when all offspring had achieved the endpoint, an effect of treatment could be missed.

Auditory Reflex (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 21	185/185 – 100%	200/200 – 100%	186/186 – 100%	155/155 – 100%

There is no clear effect of treatment on surface righting reflex; however, there appear to be some discrepancies in the data for the control and LD groups.

Surface Righting Reflex (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 4	76/215 – 35%	69/237 – 29%	50/204 – 25%	37/177 – 21%
day 5	84/186 – 45%	84/200 – 42%	87/186 – 47%	64/156 – 41%
day 6	111/186 – 60%	107/200 – 54%	119/186 – 64%	72/156 – 46%
day 7	154/186 – 83%	129/200 – 65%	132/186 – 71%	92/156 – 59%
day 8	171/186 – 92%	174/200 – 87%	160/186 – 86%	124/156 – 79%
day 9	176/186 – 95%	191/200 – 96%	170/186 – 91%	142/156 – 91%
day 10	173/186 – 93%	194/200 – 97%	170/186 – 91%	151/156 – 97%
day 11	184/186 – 99%	198/200 – 99%	181/186 – 97%	153/156 – 98%
day 12	185/186 – 99%	197/200 – 99%	181/186 – 97%	155/156 – 99%
day 13	180/186 – 97%	198/200 – 99%	183/186 – 98%	155/156 – 99%
day 14	182/186 – 98%	198/200 – 99%	184/186 – 99%	155/156 – 99%
day 15	186/186 – 100%	199/200 – 100%	186/186 – 100%	156/156 – 100%
day 16		199/200 – 100%		
day 17		156/156 – 100%		
mean ± SD	8.17 ± 2.08	8.76 ± 2.01	8.83 ± 2.63	8.86 ± 2.27
covariate adj. mean	8.18	8.82	8.85	8.76

F₁ maturation parameters and behavioral evaluation (post weaning): Body weights and abnormal/normal responses were recorded weekly during the 7-week maturation phase. The following maturation parameters were assessed (according to SOPs): vaginal opening (PPD30), cleavage of the balanopreputial gland (PPD35), locomotor activity using a photocell chamber (20 min) (PPD22 ± 1 day and ‘during week 4 of maturation’), papillary reflex (PPD 22 ± 1 day and ‘during week 4 of maturation’), water maze learning and memory assessment (“beginning at Week 2 of maturation”).

There is no clear effect of treatment on pupil reflex as conducted by the sponsor; however, since the all animals had a positive response of the first day of testing, it is not clear that appropriate dates were chosen which would permit testing for an effect of treatment.

Pupil Reflex (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 21	12/12 – 100%	12/12 – 100%	12/12 – 100%	18/18 – 100%
day 22	26/26 – 100%	22/22 – 100%	25/25 – 100%	20/20 – 100%
day 23	26/26 – 100%	21/22 – 95%	25/25 – 100%	20/20 – 100%

Although the sponsor states that there was no effect of treatment on vaginal opening, examination of the individual pup data suggest a treatment-related slight delay in the LD, MD and HD group (see the reviewer-generated table that follows for details). In addition, there appear to be some discrepancies in the data.

Vaginal Opening (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 30	1/28 – 4%	0/30 – 0%	0/29 – 14%	0/27 – 0%
day 31	0/28 – 0%	1/30 – 3%	4/29 – 14%	1/27 – 4%
day 32	3/28 – 11%	5/30 – 17%	8/29 – 28%	7/27 – 26%
day 33	9/28 – 32%	12/30 – 40%	11/29 – 38%	7/27 – 26%
day 34	14/28 – 50%	20/30 – 67%	21/29 – 72%	9/27 – 33%
day 35	23/28 – 82%	21/30 – 70%	25/29 – 86%	16/27 – 59%
day 36	23/28 – 82%	24/30 – 80%	26/29 – 90%	18/27 – 67%
day 37	27/28 – 96%	25/30 – 83%	27/29 – 93%	19/27 – 70%
day 38	27/28 – 96%	25/30 – 83%	27/29 – 93%	21/27 – 78%
day 39	27/28 – 96%	28/30 – 93%	28/29 – 97%	22/27 – 81%
day 40	28/28 – 100%	28/30 – 93%	28/29 – 97%	24/27 – 89%
day 41		30/30 – 100%	28/29 – 97%	25/27 – 93%
day 42			28/29 – 97%	27/27 – 100%
day 43			26/27 – 96%	
day 44			27/27 – 100%	
mean ± SD	34.35 ± 2.17	34.60 ± 2.31	34.13 ± 2.75	35.71 ± 3.32
covariate adj. mean	34.35	34.65	34.09	35.70
<ul style="list-style-type: none"> • C #B71912 – observation not documented on day 30, negative on day 31, positive on day 32. • MD # B71775 – observation not documented on day 43, negative on day 42, and positive on day 44 • The body weight of #B73519 was not recorded on the day the landmark was achieved These deviations do not significantly change the interpretation of the study.				

The sponsor notes a significant delay in preputial separation in the HD pups, which they attribute to decreased pup weight. Examination of the individual pup data suggest treatment-related delays in the MD and the HD (see the reviewer-generated table that follows for details). In addition, there appear to be some discrepancies in the data.

Preputial Separation (Cumulative)				
day of observation	Control 0 mg/kg/day	Low dose 5 mg/kg/day	Mid dose 15 mg/kg/day	High dose 30 mg/kg/day
day 35	0/28 – 0%	0/30 – 0%	0/29 – 0%	0/27 – 0%
day 36	0/28 – 0%	0/30 – 0%	0/29 – 0%	0/27 – 0%
day 37	1/29 – 3%	2/30 – 7%	0/29 – 0%	0/27 – 0%
day 38	2/29 – 7%	4/30 – 13%	0/29 – 0%	1/27 – 4%
day 39	7/29 – 24%	11/30 – 37%	5/29 – 17%	2/27 – 7%
day 40	7/29 – 24%	23/30 – 77%	10/29 – 34%	7/27 – 26%
day 41	12/29 – 41%	26/30 – 87%	14/29 – 48%	10/27 – 37%
day 42	22/29 – 76%	29/30 – 97%	19/29 – 66%	14/27 – 52%
day 43	29/29 – 100%	30/30 – 100%	23/29 – 79%	19/27 – 70%
day 44			24/29 – 83%	21/27 – 78%
day 45			24/29 – 83%	24/27 – 89%
day 46			26/29 – 90%	27/27 – 100%
day 47			28/29 – 97%	
day 48			27/27 – 100%	
mean ± SD	8.17 ± 2.08	8.76 ± 2.01	8.83 ± 2.63	8.86 ± 2.27
covariate adj. mean	8.18	8.82	8.85	8.76
<ul style="list-style-type: none"> • C # B71864 – observation not documented on days 35, 36 and 39, negative on day 40, positive on day 42. • MD - #B71927 – negative on day 46, observation not documented on day 47, positive on day 48 • HD - #B71783 – negative on day 40, observation not documented on day 41, positive on day 42 The deviation in control animal had no impact on the study. The deviations in the MD and HD group would shift the data slightly; however, not significantly affect the interpretation of the results.				

There was no obvious effect of treatment on open field testing (mean activity counts using a photocell chamber) conducted on postpartum day 22 and post weaning week 4 in the test system as conducted. The sponsor-provided summary tables follow.

Open-Field Testing – Mean Activity Counts – F₁ Generation

DAY 22 POSTPARTUM										
MINUTES:	1-5	6-10	11-15	16-20	TOTAL	1-5	6-10	11-15	16-20	TOTAL
GROUP: 1 Male (0 MG/KG/DAY)						GROUP: 1 Female (0 MG/KG/DAY)				
N	24	24	24	24	24	24	24	24	24	24
MEAN	344	216	128	95	784	304	188	98	48	638
S.D.	97.6	107.7	120.9	113.3	367.5	91.7	113.4	103.5	81.3	261.0
GROUP: 2 Male (5 MG/KG/DAY)						GROUP: 2 Female (5 MG/KG/DAY)				
N	25	25	25	25	25	25	25	25	25	25
MEAN	382	224	130	88	823	354	190	130	79	753
S.D.	105.8	121.0	125.7	101.9	364.2	127.4	103.7	95.7	88.2	327.8
GROUP: 3 Male (15 MG/KG/DAY)						GROUP: 3 Female (15 MG/KG/DAY)				
N	24	24	24	24	24	24	24	24	24	24
MEAN	325	180	109	77	698	290	137	78	70	574
S.D.	96.2	81.3	86.3	75.8	230.2	100.5	84.2	90.0	88.0	248.2
GROUP: 4 Male (30 MG/KG/DAY)						GROUP: 4 Female (30 MG/KG/DAY)				
N	22	22	22	22	22	22	22	22	22	22
MEAN	330	122	102	60	673	359	177	135	79	749
S.D.	109.9	106.7	80.9	63.1	257.6	99.7	103.7	83.4	80.4	277.9

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

WEEK 4 POSTWEANING										
MINUTES:	1-5	6-10	11-15	16-20	TOTAL	1-5	6-10	11-15	16-20	TOTAL
GROUP: 1 Male (0 MG/KG/DAY)						GROUP: 1 Female (0 MG/KG/DAY)				
N	24	24	24	24	24	24	24	24	24	24
MEAN	439	324	238	213	1214	512	364	310	242	1427
S.D.	105.3	67.6	70.7	57.6	230.6	74.1	62.3	96.2	82.5	243.9
GROUP: 2 Male (5 MG/KG/DAY)						GROUP: 2 Female (5 MG/KG/DAY)				
N	25	25	25	25	25	25	25	25	25	25
MEAN	458	344	265	227	1294	514	399	300	240	1453
S.D.	107.9	100.6	82.3	82.0	331.4	102.6	84.3	96.5	109.3	313.5
GROUP: 3 Male (15 MG/KG/DAY)						GROUP: 3 Female (15 MG/KG/DAY)				
N	24	24	24	24	24	24	24	24	24	24
MEAN	465	331	260	218	1275	498	390	309	244	1442
S.D.	64.1	62.2	82.7	68.4	206.5	75.1	84.5	72.2	98.6	258.1
GROUP: 4 Male (30 MG/KG/DAY)						GROUP: 4 Female (30 MG/KG/DAY)				
N	22	22	22	22	22	22	22	22	22	22
MEAN	448	351	258	241	1298	520	395	321	252	1489
S.D.	115.7	91.1	64.2	75.6	277.3	100.8	80.3	65.4	72.7	235.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

There was no obvious effect of treatment in the water maze assessment of learning and memory. Data were presented as dichotomized positive or negative response; however, no SOP was supplied and no description of the conduct of the study. This test was conducted during week 2 of the maturation phase (maturation phase begins on post partum day 28 ± 3).

Water M-Maze Test 1: Learning – F₁ Generation

Trial #:	Males						Females					
	1	2	3	4	5	6	1	2	3	4	5	6
Group 1 – 0 mg/kg/day												
Number of animals tested	24	24	24	24	24	24	24	24	24	24	24	24
Number of animals with a positive response	6	11	21	22	24	23	5	14	20	23	18	22
Percent of animals with a positive response	25	45	88	92	100	96	21	58	83	96	75	92
Group 2 – 5 mg/kg/day												
Number of animals tested	25	25	25	25	25	25	25	25	25	25	25	25
Number of animals with a positive response	1	12	19	22	22	25	7	10	19	23	24	23
Percent of animals with a positive response	4	48	76	88	88	100	28	40	76	92	96	92
Group 3 – 15 mg/kg/day												
Number of animals tested	24	24	24	24	24	24	24	24	24	24	24	24
Number of animals with a positive response	10	11	16	22	21	22	4	9	23	22	21	19
Percent of animals with a positive response	42	46	67	92	88	92	17	38	96	92	88	79
Group 4 – 30 mg/kg/day												
Number of animals tested	22	22	22	22	22	22	22	22	22	22	22	22
Number of animals with a positive response	2	13	14	19	21	21	5	14	20	17	19	22
Percent of animals with a positive response	9	59	64	86	95	95	23	64	91	77	86	100

Note: All trials were conducted with the escape ramp positioned in the right-hand compartment.

Water M-Maze Test 2: Memory and Reversal Learning – F₁ Generation

Trial #:	Males							Females						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
	Group 1 – 0 mg/kg/day													
Number of animals tested	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Number of animals with a positive response	21	3	2	10	14	17	20	24	4	3	9	7	8	17
Percent of animals with a positive response	88	13	8	42	58	71	83	100	17	13	38	29	33	71
	Group 2 – 5 mg/kg/day													
Number of animals tested	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Number of animals with a positive response	21	2	1	4	14	14	19	18	1	1	6	15	18	21
Percent of animals with a positive response	84	8	4	16	56	56	76	72	4	4	24	60	72	84
	Group 3 – 15 mg/kg/day													
Number of animals tested	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Number of animals with a positive response	23	5	1	6	13	14	17	22	4	5	8	7	17	18
Percent of animals with a positive response	96	21	4	25	54	58	71	92	17	21	33	29	71	75
	Group 4 – 30 mg/kg/day													
Number of animals tested	22	22	22	22	22	22	22	22	22	22	22	22	22	22
Number of animals with a positive response	17	3	3	14	16	19	19	21	2	3	11	14	16	16
Percent of animals with a positive response	77	14	14	64	73	86	86	95	9	14	50	64	73	73

Note: Trial 1 was conducted with the escape ramp positioned in the right-hand compartment (memory)
 Trial 2 was conducted immediately after trial 1
 Trials 2-7 were conducted with the escape ramp in the left-hand compartment (reversal learning)

F₁ post weaning mortality and clinical observations: According to the sponsor, “There were no unscheduled deaths or treatment-related clinical observations in males or females during the maturation and subsequent mating phase or unscheduled deaths in females during gestation or lactation phase. Clinical observations during gestation and lactation (for both F₁ females and F₂ offspring) were few and not attributed to treatment.” This summary is inaccurate. MD F₁ female #B73526 was found dead on day 89. In a different section of the text the sponsor states that this female was found dead during the rest phase (Day 89) and that prior to death this animal had no remarkable clinical observations. The sponsor considered its death and the gross observations as incidental). The individual parental necropsy observation table (pg 477) states that the animal was found dead and was noted with a pale (light red spleen) and dark area(s) on the stomach (entire glandular mucosa dark brown). According to the protocol, the earliest day of mating would have been 74 (after the end of the 7-week maturation period that began on post partum day 28 ± 3). The individual animal mating listing for this animal (pg 426) was as an unconfirmed pregnancy. Body weights for this animal during its gestation period are missing from the individual animal data and it would appear to be missing from the group mean summary table. In addition, the length of the gestation period for this animal is not provided. The delivery results from this dam (17 pups delivered [16-live, 1-stillbirth] and all 16 pups still alive on post partum day 1, with pup weights ranging from 5.5 – 7.0 gr), suggest a full term pregnancy and these data are incorporated into the group mean.

The clinical observations during maturation and resting phases were mostly unremarkable and isolated, with the exception of rough haircoat in 3-HDM on days 119 and/or 120. Clinical observations during the gestation period for F₁ females were unremarkable. Noteworthy clinical observations during lactation (days 0-1) consisted of red vaginal discharge in 1-C and 1-MD dam, and black vaginal discharge in 1-C and 1-MD dam.

F₁ post weaning body weight: Weekly group mean body weights were slightly decreased (although not significantly) in a dose-related way in males from the MD and HD groups from maturation day 0 (corresponds to approximately post partum day 28) through the end of the study (maturation day 120). The decreases in the HD varied from 3% to 9%. The group mean body weight changes duration the 120 day post-weaning maturation and resting phases were 527.0 ± 49.0 for the C, 529.7 ± 50.9 for the LD, 526.4 ± 57.1 for the MD, and 513.0 ± 55.0 for the HD.

For females, the weekly mean body weights were slightly decreased in MD and HD groups from maturation day 0 through maturation day 28. The decreases for the HD female offspring varied from 2% to 9%. Group mean weekly body weights in females from day 35 through day 56 were not notably

different from control. The group mean body weight changes duration the first 56 days post-weaning maturation phase (just prior to mating) were 193.6 ± 25.3 for the C, 201.9 ± 24.2 for the LD, 194.8 ± 25.9 for the MD, and 207.1 ± 19.9 for the HD. The group mean body weights for pregnant females was not notably different from control throughout gestation or on lactation day 0 (last point examined).

F₁ reproduction: The breeding period began after a 7-week postweaning period. At this time each female was cohabitated with a male from the same treatment group (with sibling pairs avoided) and each pair had a maximum of 21 days to mate. Animals were observed twice daily for mortality and morbidity. Detailed clinical observations were conducted at weighing (GD0, 7, 14, 20 and LD0). The data are summarized in the following reviewer-generated table. The reproductive effects on the F₁ generation were not adequately described by the sponsor. There were discrepancies between the summary table and individual line listings. Pregnancy rate did not appear to be affected by treatment (96% for the control and 100% for the LD, MD and HD). According to the sponsor, there were no effects of treatment of reproductive parameters in the F₁ generation. It should be noted that corpora lutea counts and an evaluation of preimplantation loss do not appear to have been submitted for the F₁ females. The discrepancies in the reporting of the data preclude any further definitive conclusions.

- According to the report MD F₁ female #B73526 (corresponds to Note B in the summary table) was found dead on day 89 during the rest phase (the period that follows LD1). The individual animal mating listing for this animal (pg 426) was as an unconfirmed pregnancy. According to the protocol, the earliest day of mating would have been 74 (after the end of the 7-week maturation period that began on post partum day 28 ± 3). This would indicate that the animal should be no further along in pregnancy than GD12 when it died. The delivery results from this dam, suggest a full term pregnancy (17 pups delivered [16-live, 1-stillbirth] and all 16 pups still alive on post partum day 1, with pup weights ranging from 5.5 – 7.0 gr), and these data are incorporated into the group mean. Body weights for this animal during its gestation period are missing from the individual animal data and it would appear to be missing from the group mean summary table. In addition, the length of the gestation period for this animal is not provided. The sponsor needs to clarify the circumstances of this animal's pregnancy (e.g., age at mating, age at parturition, duration of gestation, date of death relative to date of parturition).
- LD F₁ female #B73509 (corresponds to Note A in the summary table) was noted as pregnant in the individual animal mating listing (pg 425); however, data from this animal was eliminated from the individual litter and delivery table (pg 429) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 25g from gestation days 0 through 20). According to the protocol, any F₁ female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites. The individual parental necropsy observation table (pg 475) notes "No remarkable observations" at necropsy; however, no further information is provided about its reproductive status. All relevant data (including date of necropsy relative to date of mating and assessment of reproductive status) should be provided for this animal.
- HD F₁ female #B73557 (corresponds to Note C in the summary table) was noted as pregnant on in the individual animal mating listings (pg 427); however, data from this animal was eliminated from the individual litter and delivery table (pg 431) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 27g from gestation days 0 through 20). According to the protocol, any F₁ female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites. The individual parental necropsy observation table (pg 479) notes "No remarkable observations" at necropsy; however, no further information is provided about its reproductive status. All relevant data (including date of necropsy relative to date of mating, and assessment of reproductive status) should be provided for this animal.

- There appears to be a discrepancy between the summary table and the individual line listings in the numbers of pups surviving on postpartum days 0 and 1. There also appears to be a discrepancy between the summary table and the individual line listings in the number of pups dying, killed, missing and/or cannibalized between postpartum days 0 and 1 for the LD and MD groups. These discrepancies should be resolved.

Summary of F ₁ Delivery and Litter Data					
		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
females mated		24	25	24	22
females pregnant	sponsor's summary	23	25	24	22
	review of data	23	25	24	22
females delivering	sponsor's summary	23	24	24	21
	review of data	23	24 (see Note A)	23 (see Note B)	21 (see Note C)
duration of gestation	mean ± SD (litters)	21.7 ± 0.5 (23)	21.9 ± 0.3 (24)	21.7 ± 0.4 (23)	21.7 ± 0.5 (21)
	# @ 21 days	7	2	6	6
	# @ 22 days	16	22	17	15
	# @ 23 days	0	0	0	0
females with live-born pups	sponsor's summary	23/23	24/24	24/24	21/21
females with no live-born pups	sponsor's summary	0	0	0	0
implantation sites	total (# litters)	363 (23)	409 (24)	407 (24)	317 (21)
	mean ± SD	15.78 ± 2.13	17.04 ± 2.69	16.96 ± 2.51	15.10 ± 2.49
post-implantation loss	total # (%)	9 (2.5%)	17 (4.2%)	39 (9.6%)	17 (5.4%)
	affected litters	9	13	16	13
	range per litter	(0 - 1)	(0 - 2)	(0 - 6)	(0 - 3)
pups delivered	total (# litters)	354 (23)	392 (24)	368 (24)	300 (21)
	mean ± SD	15.39 ± 2.13	16.33 ± 2.57	15.33 ± 3.21	14.29 ± 2.45
	range per litter	(10 - 19)	(9 - 21)	(4 - 21)	(10 - 19)
live-born pups	total # pup	352	390	360	300
	mean	15.30 ± 2.05	16.25 ± 2.54	15.00 ± 3.20	14.29 ± 2.45
	# litters (%)	23 (100%)	24 (?)	24 (?)	21 (?)
still-born pups	# pups	2	2	8	0
	# litters affected (%)	2 (8.7%)	2 (?)	6 (?)	0 (?)
uncertain live-born/still-born	# pups	0	0	0	0
pups surviving at day 0 → day 1	sponsor's summary	351 → 350	389 → 386	358 → 351	299 → 296
	review of data	352 → 351	390 → 386	360 → 352	300 → 297
pups dying, killed, missing and/or cannibalized days 0-1	sponsor's summary	1	3	7	3
	review of data	1 litter	4 from 4 litters	8 from 8 litters	3 from 3 litters
entire litter died, killed, missing and/or cannibalized	sponsor's summary	0	0	0	0
total # and mean males %/litter	day 0	186 (53%)	206 (53%)	164 (46%)	151 (51%)
live pups and litters with pups	day 0	15.30 ± 2.05 (23)	16.25 ± 2.54 (24)	15.00 ± 3.20 (24)	14.29 ± 2.45 (21)
	day 1	15.26 ± 1.98 (23)	16.08 ± 2.57 (24)	14.67 ± 3.07 (24)	14.14 ± 2.48 (21)

- Note A – Group 2 female #B73509 was noted as pregnant in the individual animal mating listing (pg 425); however, data from this animal was eliminated from the individual litter and delivery table (pg 429) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 25g from gestation days 0 through 20). According to the protocol, any F₁ female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites.
- Note B - Group 3 female #B73526 was found dead on day 89. The individual animal mating listing for this animal (pg 426) was as an unconfirmed pregnancy. According to the protocol, the earliest day of mating would have been 74 (after the end of the 7-week maturation period that began on post partum day 28 ± 3). This would indicate that the animal should be no further along in pregnancy than GD12 when it died. The delivery results from this dam, suggest a full term pregnancy (17 pups delivered [16-live, 1-stillbirth] and all 16 pups still alive on post partum day 1, with pup weights ranging from 5.5 – 7.0 gr), and these data are incorporated into the group mean. Body weights for this animal during its gestation period are missing from the individual animal data and it would appear to be missing from the group mean summary table. In addition, the length of the gestation period for this animal is not provided.
- Note C – Group 4 female #B73557 was noted as pregnant in the individual animal mating listings (pg 427); however, data from this animal was eliminated from the individual litter and delivery table (pg 431) with no explanation. Body weight data for this animal were available during the gestation period (indicating a weight gain of only 27g from gestation days 0 through 20). According to the protocol, any F₁ female that did not delivery its litter by gestation day 26 was to be sacrificed, and the uterus examined for implantation sites.

F₁ female necropsy: Any dams that had not delivered by GD 26 were sacrificed. Dams that delivered were given a rest period starting on LD1, until the sponsor made the decision not to rebreed. At that point the F₁ dams were sacrificed. Gross necropsy included the cervical, thoracic and abdominal viscera. Gross visceral lesions as well as the ovaries and uterus were preserved in 10% NBF. Examination of the data did not reveal any clearly treatment related effect; however, one MD F₁ female was found dead on D89, and the only abnormalities noted at necropsy were a pale (light red) spleen and dark brown coloration of the entire glandular mucosa of the stomach.

F₁ male necropsy: According to the sponsor, after the birth of all F₂ litters, the decision was made not to rebreed, so the males were sacrificed. Gross necropsy included the cervical, thoracic and abdominal viscera. Gross visceral lesions as well as the reproductive organs were preserved in 10% NBF. Examination of the data did not real any effect of treatment.

F₂ findings: For each F₁ dam the litter size was recorded (at birth) and for each offspring, the body weight and sex were noted. F₂ pups were observed then killed on LD1 (preserved in 10% neutral-buffered formalin). Any pup found dead was examined for visceral abnormalities (cervical, thoracic and abdominal) and preserved in alcohol.

There was not effect of treatment on F₂ covariate adjusted mean body weights for males or females (see the sponsor supplied summary table below for details).

		DOSE LEVEL	GROUP 1 0 MG/KG/DAY	GROUP 2 5 MG/KG/DAY	GROUP 3 15 MG/KG/DAY	GROUP 4 30 MG/KG/DAY
day 0	MALES -	MEAN	6.47	6.42	6.32	6.65
		S.D.	0.59	0.46	0.56	0.49
		N	23	24	24	21
	Covariate Adjusted	MEAN	6.47	6.49	6.31	6.57
day 0	FEMALES -	MEAN	6.16	6.18	6.01	6.33
		S.D.	0.52	0.41	0.55	0.43
		N	23	24	24	21
	Covariate Adjusted	MEAN	6.17	6.25	6.01	6.26

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01. N = NUMBER OF LITTERS.

F₂ Pup observations were noted only as part of the clinical observations of the dams. The results are summarized in the following reviewer-generated table. Of note is one MD litter that had at least one pup missing the proximal tail and one HD litter that has at least one pup with a filamentous tail. One LD litter was noted with at least one pup cold to touch on day 0 and two HD litters were noted with at least a single pup cold to touch. The sponsor notes no effect of treatment.

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F ₂ Pup observations	
group/dose	observations (noted as part of the clinical observation of dams, no further details supplied)
C – 0 mg/kg/day	pup(s) bruised in 1/23 litters (days 0 and 1) pup(s) with teeth marks/cuts in 2/23 litters (one on day 0, one on day 1) pup(s) partially cannibalized, alive in 1/23 litters (days 0 and 1) pup(s) weak in 1/23 litters (day 0) pup(s) no visible milk in stomach in 1/23 litters (day 0) (same litter was noted with weak pup(s))
LD – 5 mg/kg/day	pup(s) cold to touch in 1/24 litters day 0
MD – 15 mg/kg/day	pup(s) missing proximal tail in 1/24 litters (day 0) pup(s) with teeth marks/cuts in 1 /24 litters (day 0) pup(s) pale in 1/24 litters (day 0) pup(s) partially cannibalized, dead in 1/24 litters (day 0) pup(s) weak in 1/24 litters (day 0) pup(s) no visible milk in stomach in 1/24 litters (day 0) (same litter was noted with weak pup(s))
HD – 30 mg/kg/day	pup(s) cold to touch in 2/21 litters (one on day 0, one on day 1) pup(s) bruised in 1/21 litters (day 1) pup(s) bruised in axillary region in 1/21 litters (day 1) pup(s) with teeth marks/cuts in 1/21 litters (day 0) pup(s) with a filamentous tail in 1/21 litters (day 0) pup(s) no visible milk in stomach in 2/21 litters (day 0) (one litter was also noted with weak pups) pup(s) weak in 1/21 litters (day 0)

F₂ necropsies were confined to stillborn pups and dead (originally liveborn) pups (see reviewer generated table that follows for details). In the MD group one stillborn pup was not evaluated (the reason was not mentioned) and 2 could not be definitively evaluated due to the degree of autolysis of the entire fetus. There was no evidence of a treatment-related effect; however, the sample size is too small to make definitive statements.

F ₂ necropsy observations of stillborn and dead (originally liveborn) pups					
		Control 0 mg/kg/day	Low Dose 5 mg/kg/day	Mid Dose 15 mg/kg/day	High Dose 30 mg/kg/day
pups evaluated	total pups (# litters)	3 (3 litters)	5 (5 litters)	10 (8 litters)	2 (2 litters)
	stillborn	2 (2 litters)	2 (2 litters)	7 (6 litters)	0
	dead (originally liveborn)	1 (1 litter)	3 (3 litters)	3 (3 litters)	2 (2 litters)
stillborn pups not evaluated	-	0	0	1	0
no remarkable observations	pup incidence	3/3 (100%)	5/5 (100%)	7/10 (70%)	2/2 (100%)
	litter incidence	3/3 (100%)	5/5 (100%)	6/8 (75%)	2/2 (100%)
post mortem autolysis of abdominal cavity	pup incidence	-	-	1/10 (10%)	-
	litter incidence	-	-	1/8 (12.5%)	-
autolysis of entire fetus	fetal/pup incidence	-	-	2/10 (20%)	-
	litter incidence	-	-	2/8 (25%)	-

Sponsor's Conclusion: "...the Noel is 5 mg/kg/day for F₀ maternal effects (based on clinical observations, significantly decreased gestation body weight gain and food consumption, and pup retrieval data) and F₁ embryofetal survival (primarily attributed to a pharmacological effect on the dam affecting maternal behavior rather than a specific effect on the pups and based on the increased number of still born pups reflected in the lower liveborn index, as well as neonatal deaths), 15 mg/kg/day for F₁ offspring growth and development through maturation (based on decreased pup weight and delayed hair growth in both male and female pups, and delayed preputial separation in the males), and 30 mg/kg/day for F₁ offspring behavioral and reproductive parameters."

Based on examination of the data, the following conclusions can be made:

1. This study report has several discrepancies in the data that should be resolved before definitive conclusions can be made about the acceptability of the study, and the effects of treatment of the F₀ generation on the subsequent (F₁) generation and the resulting offspring of that generation (F₂).
2. Without further information, it can be stated that a NOEL for findings in the F₀ dams may not have been achieved, based on an effect on pup retrieval data at the lowest dose tested (despite lack of clinical observation in the dams). It should be noted that it is not possible to ascribe the effect to dam or pup, since it is very difficult to distinguish between dam and pup effects.
3. It appears that 5 mg/kg/day (approximately 0.48 times the MRHD on a mg/m² basis) was a NOEL for F₁ perinatal pup survival (The sponsor attributes the treatment related increase in still births and early pup deaths primarily to "a pharmacological effect on the dam affecting maternal behavior rather than a specific effect on the pups." While the pharmacological effects of TBZ on the dams may be contributory to the increase in perinatal pup deaths, a direct effect of treatment on the pup cannot be excluded.
4. There were treatment-related delays in the pinna unfolding (HD), hair growth (all doses), eye opening (HD), vaginal opening (all doses) and preputial separation (MD and HD). Therefore, a NOEL for development was not established.
5. Conclusions about an effect of treatment (of the F₀ dams) on the reproductive function in the F₁ generation cannot be made until the sponsor resolves the discrepancies in the data and provides, if available, corpora lutea counts and an evaluation of preimplantation loss for the F₁ females.

2.6.6.7 Local tolerance

No studies were conducted by the sponsor.

2.6.6.8 Special toxicology studies

No studies were conducted by the sponsor.

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2.6.6.9 Discussion and Conclusions

Summary for General Toxicology Study in Rat:

The chronic toxicity of TBZ in rat was determined in a 26-week study with a 13-week interim kill (Report # 20730). The doses chosen for the chronic rat toxicity study were selected based on the results of a 4-week toxicity study in rats (Report # 19371, module 4, volume 11). The 4-week GLP study was a complete toxicity study conducted in 10/sex/gr. It was not reviewed in detail because it was conducted with the same doses and dosing regimen as employed in the chronic toxicity study and in the same strain of rats. With regard to the justification of doses for this 4-week study, the sponsor refers to a study that does not appear to have been submitted to the NDA. The information provided about this study seems to be confined to the following statement from the sponsor:

- “Dose levels were agreed with the Sponsor after evaluation of preliminary study data (Project No. 455282). This study showed very low body weight gain and decreased food consumption in males receiving 20 mg.kg⁻¹.bid, hence 15 mg.kg⁻¹.bid was considered a more suitable level for 4 weeks of administration. Dose levels took into account the maximum tolerated dose in the test model and other factors such as anticipated therapeutic doses.”

Thus, studies in rat evaluating the toxicity of TBZ at doses greater than 15 mg/kg bid do not appear to have been submitted for review.

In the 4-wk study there were no unscheduled deaths. Histopathology was carried out on the control and HD groups. There were no treatment-related gross findings at necropsy and no clear evidence of treatment-related histopathology. With regard to the brain, sections of the forebrain, midbrain and cerebellum were evaluated by histopathologic exam, and no abnormalities detected in any control or high dose animal. As in the chronic toxicity study in rat, the summary table for clinical observations was based on “selected” observations. Satellite animals were used to assess the TK of TBZ and the metabolite dihydrotetabenazine (HTBZ) on Days 1 and 29. HTBZ was measured in a non-chiral assay that did not differentiate between α -HTBZ and β -HTBZ.

In the 13- wk/26-wk chronic study, Sprague-Dawley CD@ (SD) IGS BR were treated twice daily, via oral gavage, with TBZ at doses of: 0, 2.5, 7.5 and 15 mg/kg, bid (or 0, 5, 15, and 30 mg/kg/day). The study was designed such that at week 14, the first 10 rats/sex/group were sacrificed and the sample size for the 26 week sacrifice was, therefore, ≤ 20 /sex/group. Unscheduled deaths (moribund sacrifices or animals found dead) occurred in all treatment groups. However, within each treatment group the number of animals surviving until the week 26 terminal sacrifice was adequate (15-20 /sex/group).

Prior to the initial NDA filing, it was noted that the original study report did not include a delineation of unscheduled deaths and moribund sacrifices. A request for this information was communicated to the sponsor in the NDA withdrawal letter that issued on 12-Aug-05. The requested information was submitted on 15-Dec-05. Among the listing was the sacrifice of a female animal that became pregnant during the course of the study, suggesting a lack of vigilance in the conduct of the study.

The moribund sacrifices of four MDM (due to wounds/lesions on the neck, thorax and abdomen) were possibly indirectly related to treatment; the animals were group housed, and TBZ administration was noted to induce hyperactivity and aggressive behavior in a dose-related fashion. The moribund sacrifices of the three HDM and the deaths of the single HDM and single HDF found dead are assumed to be treatment-related.

Upon review of information submitted to describe the circumstances of the unscheduled sacrifices and deaths, the adequacy of the report to fully describe the results of the study came into question. For example, one of the high animals was sacrificed in moribund condition during week 23 of the study, and the sponsor listed the cause of moribund condition as chronic dermatitis (the dermatitis was listed as

moderate in severity and appeared to be confined to the muzzle). In the description of this animal's condition there was no mention of convulsions; however, the line listings for this animal noted convulsions on days 133, 142 and 154 (and the animal was sacrificed during week 23). An additional high dose animal that survived until terminal sacrifice was noted to have had convulsions on days 172 and 176. Convulsions were not mentioned in the general discussion of clinical observations and did not appear in the summary table. Furthermore, the sponsor's summary table presents only "selected" post dose clinical signs and is labeled as such and was focused on lethargy, hyperactive behavior, and aggressive behavior. Examination of the individual animal data suggested that the clinical observation data sets for each animal may not be complete. A request for further information regarding the conduct and reporting of the clinical observations, clarification of discrepancies between the summary table and the individual animal observations, and the day of sacrifice or death for each animal was sent to the sponsor on 24-Jan-06. A response was not received in time for incorporation into the review. Until an adequate response has been submitted and reviewed, there is no assurance that the sponsor has submitted a full an accurate data set for clinical observation of each animal.

Further interpretation of a treatment-related effect on behavior and potential signs leading to mortality or morbidity should be deferred until the submission and review of the requested data. However, even without further information it is possible to state that a no-effect dose for treatment related lethargy and aggressiveness was not established in this study and that treatment-related convulsions and deaths occurred at the HD (approximately 2.9 times the maximum recommended daily dose in humans on a mg/m² basis).

There were treatment related-decreases in mean body weight gain in MDM and HDM throughout the study and a slight decrease in mean body weight gain for the LDM from wk 21 until the end of the study. At wk 26, the decreases in mean body weight (from control) was 4%, 15% and 19% for LDM, MDM and HDM respectively. For HDF mean body weight gain was transiently decreased (through wk 6), after which there was a slight increase. LDF and MDF demonstrated increases in mean body weight gain throughout the study. At wk 26, the increases in group mean body weight were 1%, 7% and 4% for LDF, MDF, and HDF, respectively. Interpretation of food consumption data is somewhat limited since animals were group housed (5/cage). MDM and HDM demonstrated a transient decrease (wks 1-3) in group mean food consumption followed by an increase in LDM, MDM and HDM. From wk 3 onward, group mean food consumption was generally increased in MDF and HDF. There were no treatment-related effects on ophthalmoscopy.

There were several potentially treatment-related changes in hematology. Theses changes include: decreased (3-4%) RBCs in HDM and HDF (wks 14 and 26), increased (3-6%) MCH in MDM (wk 26) and HDM (wks 14 and 26), increased (3-5%) MCV in MDM, HDM, and HDF (wk 26), decreased (7-29%) WBCs in MDM (wk 26) and HDM (wk 14 and 26) due to a decrease in lymphocytes (9-34%), monocytes (5-44%), eosinophils (28-45%) and basophils (20-50%). Monocytes were also decreased (18-32%) in LDF, MDF and HDF (wk 26). There were decreases (20-53%) in LUC (large unclassified cells) at wk 26 in MDM, HDM and HDF. Platelets were decreased (3-15%) in LDM, MDM and HDM (wks 14 and 26) and in MDF and HDF (wk 26). Peripheral smears were obtained only from unscheduled sacrifices; however, it is not clear that they were evaluated and bone marrow smears were obtained in all animals, but not evaluated.

Apparent treatment-related changes in clinical chemistry parameters were seen; however, they were not correlated to treatment-induced histopathology and, thus their relevance would be questionable. Examination of the urinalysis data did not reveal an obvious signal for renal toxicity; however, there were increases in urinary volume at 26 wks in LDM, MDM, HDM, MDF, and HDF.

It should be noted that there was no separate pathology report, and the integrated summary report did not contain the signature the study pathologist. There was no evidence of treatment-related findings at the

14-wk interim sacrifice; however, examination of the 26-wk terminal sacrifice (plus unscheduled sacrifice) data revealed the following potentially treatment-related findings (1) pale focus in the lungs (all lobes, many) in 2/20 HD, (2) damaged/swollen or abnormally colored ears, open sores and hair loss, predominantly in MDM and HDM (this cluster of findings may be due, in part, to treatment-related aggressive behavior and group housing of the animals), (3) low incidence finding of flaccid testes in MDM (1/20) and HDM (2/20) (additional low incidence findings in male reproductive tract include reddened testes, small testes, and small epididymis).

There were dose-related decreases in absolute organ weights for many organs for the MDM and HDM groups, based on the decreases in mean body weight (compared to control) in those groups (and the decreases in thymus weight in these groups were not associated with thymic atrophy). A finding that appears to be related to treatment is the decreases in absolute uterine weight in the LD, MD and HD females after 13 wks of treatment and at terminal sacrifice (+ unscheduled deaths), possibly related to treatment-related changes in endocrine status. The sponsor noted that the increase in adrenal weight in males was possibly related to treatment (however, it was not associated with abnormal histopathology).

An adequate battery of tissues was examined microscopically; however, the sponsor did not conduct histopathology on gross lesions unless they occurred in the control or high dose, or in low and mid dose animals that were unscheduled sacrifices. It is standard practice to examine gross lesions in all animals, and the rationale for this was not discussed. The histopathology summary table did not incorporate any findings from the single LD unscheduled sacrifice animal or seven MD unscheduled sacrifice animals (except for those in the mammary gland and vagina), nor was there a separate table summarizing this information.

In the discussion of the pathology findings there was no mention that 24 of 60 control animals and 17 of 60 high dose animals were infected with pinworm parasites. Only five animals (of 40) were noted as infected at the 13-week interim sacrifice; suggesting that the infestation occurred primarily between weeks 13 and 26. Since the intestines from low and mid dose animals were not microscopically examined, the number infected in those groups is unknown. Pinworms are generally a local problem confined to the distal GI tract, and there were no background lesions in the intestines, other than parasites, and no treatment-related lesions in the GI tract. Toxicology studies are supposed to be conducted in normal healthy animals, and clearly this was not the case for this study. The occurrence of the parasite infestation and implications (if any) on the validity of the study were not discussed, and the sponsor should provide this (presumably it would be part of the histopathology report).

There was no relevant treatment-related histopathology in the 13-week interim sacrifice animals. With regard to the C and HD animals sacrificed after 26-weeks of treatment and C and HD unscheduled deaths, plus specified tissues (mammary and vaginal tissues only) from the MD animals, the following treatment-related findings were identified:

1. Minimal to mild multifocal accumulations of alveolar macrophages. This was assessed only in the C and HD; tissues from the MD and possibly LD should have been assessed to establish a NOEL). According to the sponsor, "These accumulations tended to be at the bronchoalveolar junctions (centroacinar regions) and were not associated with an inflammatory response."
2. An increased incidence of "physiological" hyperplasia of the mammary gland was noted in MD and HD males and females; however, tissues from the LD should have been assessed to establish a NOEL. It should be noted that the mammary glands from the C and HD animals were unremarkable after 13 weeks of treatment; however, HDF #228 found dead wk-14 was noted with mild physiologic hyperplasia of the mammary gland.

The sponsor attributes the “physiological” hyperplasia to “an increase in circulatory prolactin levels or a change in the pattern of release of prolactin due to administration of the test item.” The sponsor states that TBZ “directly blocks dopaminergic inhibition of prolactin secretion” (Login *et al.* 1982). In the referenced study, female SD rats administered a single intraperitoneal injection of tetrabenazine (30 mg/kg) had significantly higher serum prolactin levels than a concurrent control at 1 hr (20-fold increased) and 16 hrs (4-fold increased) post dose and were indistinguishable from control by 24 hrs post dose. The sponsor has not provided data to support an increase of serum prolactin after oral administration of TBZ in rats (serum prolactin levels were not assessed in the 4-wk or the 13/26-wk study). Prolactin levels were supposed to be determined as part of study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats). The results of the prolactin analysis were to be submitted separately from the study report and do not appear to have been submitted to the NDA.

3. Vaginal cycle of proestrus in 100% of HDF and minimal vaginal epithelial degeneration in 2/20 HDF. According to the sponsor, “All Group 4 [HD] females showed vaginal mucification, with epithelial thinning and occasional epithelial degeneration, and appeared to be in proestrus.” It should be noted that the vaginal tissues from the C and HD interim sacrifice animals were unremarkable after 13 weeks of treatment; however, HDF # 228, found dead wk 14 was noted with the vaginal cycle designation of proestrus (and with “vaginal mucification and epithelial degeneration”). The histopathology finding of the vaginal tissue listed in the summary table and individual line listings were confined to the determination of stage of the estrus cycle and a finding of minimal vaginal epithelial degeneration in 2/20 HDF. No incidences of “vaginal mucification, with epithelial thinning” were reported. Without further information about the occurrence of vaginal mucification and epithelial thinning in all groups examined, it would not be possible to verify that a NOEL was achieved in the MD. The sponsor states that TBZ “directly blocks dopaminergic inhibition of prolactin secretion” (Login *et al.* 1982). The sponsor references Batten and Ingleton (1987), stating that “There is an increase in prolactin levels during pro-oestrus in the normal rat oestrus cycle. This increase in circulating prolactin causes vaginal mucification in rats.” The sponsor did not supply this reference and it was unavailable through the FDA at the time of the review.

Vaginal mucification and epithelial thinning are not consistent with the description of rat vaginal histopathology during proestrus discussed in Greaves (Greaves P, 2000), “During proestrus the number of cell layers increases and the granular layer (stratum granulosum) develop. As oestrus approaches, a horny stratum corneum also becomes prominent. During the latter part of oestrus and metoestrus, the upper layers of the squamous epithelium become desquamated and there is a return of increasing numbers of leukocytes before the cycle is repeated. In pseudopregnancy and pregnancy the superficial cells of the rodent vaginal mucosa become cuboidal or cylindrical with vacuolation of immediate cell layers. In late pregnancy the superficial cells become mucus secreting.”

Furthermore, the uterine histopathology (estrus dilatation in 4/20-HD versus 8/19-C) and the treatment-related decrease in uterine weight (27-45%) are not consistent with treatment-induced proestrus state in 100% of the HD animals. Greaves (Greaves P, 2000), describes the uterus during proestrus as follows: “In prooestrus the uterine horns enlarge and fill with fluid. Epithelial cells lining the uterine cavity become more cuboidal. These cells meet a maximum during prooestrus, before the establishment of oestrus.”

4. There was an increase in the incidence of cysts in the intermediate lobe of the pituitary gland in the HD (1/20 HDM and 2/19 HDF versus 0/40 controls). This is a low incidence finding may be associated with age, or with changes in prolactin secretion.

5. There was an increase in calculus in the urinary bladder in HDM (3/20 versus 1/20 in CM; only C and HD examined). There were no other abnormal histopathology of the urinary bladder in the HDM, and none of the 10-CM or 10-HDM sacrificed after 13 weeks of treatment had any abnormalities of the urinary bladder. In the recently conducted mass balance study in humans, the majority of the drug-related compounds are excreted renally, and there is evidence of urinary excretion of metabolites in rat. The relevance of this low incidence finding is unknown.
6. There was an increase in incidence of minimal to moderate chronic dermatitis in HDM (3/20-HDM versus 0/20-CM). This would appear to be a treatment related finding and a no-effect level was not established; however, an argument could be made for not requiring additional histopathology examination of the lower doses to establish a no-effect dose level.

The sponsor elected to examine additional sections of the brain (i.e., the pons) based on the demonstration of tetrabenazine-induced neurotoxicity in the literature (Satou *et al.* 2001. Repetitive administration of tetrabenazine induces irreversible changes in locomotion and morphology of the substantia nigra in rats, *Exp Toxic Pathol.* 53: 303-308), and no treatment-related changes in the brain were noted in the chronic rodent study. In the Satou *et al.* study, male Wistar rats were administered tetrabenazine by intraperitoneal injection (1 mg/kg) either as a single injection or as daily injections for seven consecutive days. Animals were sacrificed 1, 8 or 15 days after the last injection (the 15 day recovery period was studied only in the animals treated for seven day). Animals were assessed for spontaneous locomotion and a morphometric analysis of the substantia nigra pars compacta (SNpc). Brains were perfusion fixed, sectioned, and morphometric analysis was conducted on a single H&E stained section per animal (5.3 mm from bregma) chosen as a representative sample of the SNpc. In addition, the next serial section was stained for the presence of GFAP. The results of the multiple dose portion of the study demonstrated statistically significant treatment-related neuronal cell loss in the SNpc and a decrease in SNpc neuron area and cell size that progressed with increasing survival time (up to approximately 50% neuronal cell loss and approximately 30% decrease in area). An increase in staining for GFAP indicating glial proliferation was also noted in the SNpc. The single dose portion of the study did not demonstrate treatment-related neuropathological changes. In addition, animals treated with a single dose of tetrabenazine demonstrated a reversible decrease in spontaneous locomotion, whereas the animals treated daily for 7-days demonstrated a treatment-related decrease in locomotion that was not completely reversible, even after a 15 day recovery period.

Direct correlations between the findings in Satou *et al.* and the 26 wk toxicity study in rats cannot be made. The studies were conducted in different strains of rat (Wistar [Satou] versus Sprague Dawley [sponsor]). TBZ is highly metabolized, and the routes of administration were different (intraperitoneal injection [Satou] versus oral gavage [sponsor]) and the daily doses were different (1 mg/kg, ip [Satou] versus a maximum of 15 mg/kg, bid, po [sponsor]) and PK evaluation was not provided for the Satou study (however, Mehvar *et al.*, (1987b) has determined that the oral bioavailability of TBZ (1 mg/kg) in male SD rats was 17%). The timing of neuropathological evaluation with regard to first drug exposure (maximum of 22 days [Satou] versus 6-months [sponsor]) was different. There were differences in the tissue fixation technique (perfusion fixation [Satou] versus standard immersion fixation [sponsor]). Further comparisons of the neuropathologic assessment cannot be made because the sponsor did not describe the neuropathologic assessment conducted beyond stating that in addition to the standard histopathologic examination of the forebrain, midbrain and cerebellum, the pons was examined in selected animals (C and HD animals sacrificed after 26 weeks of treatment and C and HD unscheduled deaths). Without further information it is assumed that the examination was standard light microscopic examination of H&E stained sections (with no additional special staining such as GFAP). It also appears that the sponsor did not conduct a detailed morphometric analysis of the SNpc. The Satou article did state "Hematoxylin and eosin showed clear neuronal loss and atrophy in SNpc ... associated with glial cell

proliferation. GFAP immunopositive cells were clearly revealed in SNpc." The demonstration of a treatment-related effect was based on morphometric analysis (neuronal count, area and neuronal size).

The toxicokinetics of TBZ and the metabolite, HTBZ (the unresolved mixture of α -HTBZ and β -HTBZ), were determined as part of the chronic study. Examination of the data for TBZ indicates highly variable TK. In addition, for LDM, TBZ was not found in the plasma at any time point. Exposure to HTBZ was much greater than exposure to TBZ in each group, suggesting extensive metabolism. In each dose group, the C_{max} (obs) and the AUC for HTBZ were greater in males than in females. Exposure to TBZ and HTBZ increased with increasing dose (except for TBZ in males at Wk 25).

Additional TK data are available from a draft report of — study # 7425-114, a 14-Day Oral Gavage with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats (module 4, volume 11 and revised TK report as submitted in NDA amendment #0005). In this study — CD@ (SD) IGS BR rats were administered TBZ at a daily dose of 15 mg/kg, bid (approximately 12 hrs apart, except on Days 1 and 14, when only the morning dose was administered). According to the revised TK report, "Interfering peaks were found to be present in the tetrabenazine chromatograms ... and tetrabenazine plasma concentrations were reported for information only. Consequently, the tetrabenazine concentrations ... should be considered as information not relevant to the primary objective of the assessment of the toxicokinetics of α -HTBZ and β -HTBZ." At this dose (15 mg/kg), exposure to α -HTBZ was greater in males and exposure to β -HTBZ was greater in females. The ratio of α -HTBZ to β -HTBZ was approximately 4 times greater in males than females. Exposure data from this study are presented in the PK section of the NDA review.

In conclusion, additional information is necessary to determine the acceptability of this study to characterize the toxicity of TBZ given chronically to rats. This information includes: (1) a response to the request for information sent to the sponsor on 24-Jan-06, (2) a copy of the pathologist's report, which should include a discussion of the parasite infection and implications (if any) on the validity of the study, (3) a detailed description of the histopathologic examination of the brain, with an emphasis on the techniques used, and the extent of the examination, especially with regard to the substantia nigra, and (4) the results of the serum prolactin assessment conducted in — Study # 7425-114 (14-Day Oral Gavage Study with Tetrabenazine to Assess Toxicokinetics and Prolactin Levels in Rats).

It should be noted that without further information, it can be stated that no-effect doses for treatment related lethargy and aggressiveness were not established, and that potentially treatment-related convulsions and deaths occurred at the HD (approximately 2.9 times the maximum recommended human dose (MRHD) of 100 mg on a mg/m^2 basis). Furthermore, no-effect levels were not established for treatment-related physiological hyperplasia of the mammary gland, multifocal accumulations of alveolar macrophages and chronic dermatitis.

Finally, there appears to be a notable lack of vigilance in the conduct and reporting of this study that should be further investigated.

Summary for General Toxicology Study in Dog:

The chronic toxicity of TBZ in nonrodent was evaluated in a 9-month toxicity study in dog — Study #7425-101). The doses for this study were selected based on a non-GLP (and apparently non-Q/Aed) exploratory study (15-Day Capsule Dosing Toxicity Study in Dogs, Study # 7425-100), conducted at — This dose-range finding study was not reviewed in detail. Beagle dogs (2/sex) (at least 6 months old and weighing 7.8 – 13.3 kg) were administered TBZ at doses of 0, 2.5, 5, 10 or 20 mg/kg, bid (with at least 6 hrs between daily doses). Treatment of the low dose (2.5 mg/kg/dose) group was started 2 days later than the rest of the study. TBZ (batch #105481) was administered by gelatin capsule and the control group received empty gelatin

capsules. A dosing error on Day 1 resulted in the full day's dose being administered as a single dose for all groups except the lowest dose group. Animals were observed twice daily for signs of morbidity and/or mortality. Cage-side observations were conducted approximately 60-90 minutes after each of the daily doses and detailed observations were conducted prior to the initiation of treatment, weekly during treatment and prior to sacrifice. Additional parameters assessed included: body temperature, blood pressure, body weights, food consumption, hematology, coagulation, clinical chemistry, TK analysis (on Days 1 and 14 of treatment for TBZ, and HTBZ [the unresolved mixture of the metabolites α - and β -HTBZ]), necropsy and organ weights (adrenals, brain, heart, kidneys, liver, lung, ovaries, pituitary, prostate, spleen and testes). Although the following tissues were preserved (10% neutral buffered formalin), they were not examined microscopically: adrenals, brain, eyes, gallbladder, heart, kidneys, gross lesions, liver, lung, ovary, pancreas, pituitary, prostate, spleen, substantia nigra, and testis.

Unscheduled deaths were confined to the 20 mg/kg/dose (40 mg/kg/day) group. One of the four animals was sacrificed in moribund condition on Day 7 (dosing for this animal was discontinued after the first dose on Day 4), and the remaining three animals in this group were sacrificed in moribund condition on Day 11 (they were dosed up through Day 11).

The sponsor described the clinical observations for male #40300 (sacrificed on Day 7) as follows, with reviewer annotations contained in square brackets and italics, [].

- “This dog was laterally recumbent, chewing on its metal cage, and walked into corner of room and did not leave during a veterinary examination. Signs noted after Day 4 included laceration on its face on Day 5, the wound was cleaned and sutured; significantly deteriorated condition on Day 6; cool to touch, vomiting, drooling, swollen face, and was judged to be in pain on Day 7. At this point, clinical pathology samples were collected, and the dog was euthanized for humane reasons and extremely poor prognosis.

Clinical chemistry evaluation revealed pronounced elevations in the alanine aminotransferase (ALT) [*≈ 4x from baseline*], aspartate aminotransferase (AST) [*≈ 23x from baseline*], and cholesterol (T CHOL) [*≈ 1.4 fold from baseline*] values and slight decreases in calcium [13%] and inorganic phosphorus (IN PHOS) [51%] values; these findings are suggestive of an effect on hepatobiliary tissue, whereas the lower calcium and inorganic phosphorus values are consistent with anorexia. The increased transaminase activities in this male may represent an effect of the test article.” [*Necropsy remarks were confined to abnormal behavior, broken skin and scab on muzzle swollen lip (corresponding to broken skin on gums) and brown oral discharge. The liver and gallbladder appeared unremarkable and histopathologic examination was not conducted*].

In general, the 20 mg/kg/dose (40 mg/kg/day) group were noted with the following treatment-related clinical signs: hunched posture, tremors, ataxia, hypoactivity, recumbency, repetitive behavior (head pressing, excessive chewing, digging and non-directed growling and barking), excessive salivation, constricted pupils, and reddened conjunctiva, gums and ears. The sponsor noted that these signs were present by 60-90 minutes post-dose, and were still present 5-7 hours post-dose, but had resolved before the next dose was administered. The sponsor noted that lymphocyte counts were slightly decreased in one male and one female in this group. The only gross necropsy finding of note was dark pancreas in one female (histopathology was not conducted).

Treatment-related clinical signs similar to those described above occurred in all doses, with some decrease in frequency with decreasing dose, and with the addition of pale gums, aggressive behavior and occasional dilated pupils present in the lower groups. According to the sponsor, by Day 9 the daily planned “commingling” was eliminated for all groups due to treatment-related aggression. Examination of the clinical pathology data from the 2.5, 5 or 10 mg/kg/dose groups did not reveal any notable signals for treatment related toxicity.

According to the sponsor, plasma exposure ($C_{\text{max-observed}}$ and AUC) for TBZ and HTBZ increased in a dose-related manner, and the $t_{1/2}$'s were independent of dose and duration of treatment. Examination of the data indicated extremely variable data that would preclude definitive conclusions (TK data are presented in the Pharmacokinetics section of the review).

In the definitive 9-month toxicity study, beagle dogs (4/sex/group) were treated once daily, via oral gelatin capsule, with TBZ at doses of 0, 1, 3, or 10 mg/kg/day. A protocol deviation resulted in the administration of a lower than nominal dose to all groups during wk 25 and 1st day of wk 26 [estimates of decreases: 24-26%-LD, 16-21%-MD, and 18-21%-HD]. This deviation should not affect the integrity of the study.

Unscheduled deaths were confined to a single HDF that was sacrificed in moribund condition on Day 244 (Wk 32). Although not clearly stated by the sponsor, this animal appears to have been sacrificed due to a combination of a treatment-related clinical observations (including: reddened and swollen muzzle, tremors, hypoactivity, recumbency, repetitive behavior [e.g., head pressing and chewing], excessive salivation, and panting, as well as reddened gums and inner ears), and recurrent damage to the oral cavity, presumably due to treatment-induced stereotypic head pressing and chewing. TK data for this animal were only available for Day 1 and Wk 13 and were unremarkable.

In general, treatment-related clinical signs were confined to the MD and HD groups and consisted of: frequent hypoactivity and tremors (limb and/or whole body) throughout the duration of the study and less frequent, or isolated incidences of repetitive behavior (e.g., excessive head pressing, chewing and digging, as well as non-directed barking, growling, and biting), hunched posture (HD), recumbency, rigidity of limbs, sensitivity to touch (HD), and ataxia (HD). Also noted were red gums, skin (particularly the ears) and conjunctiva, abnormal respiration (panting/labored breathing), excessive salivation, squinting eyes (HD), and clear discharge from eyes (HD), infrequent notations of thin appearance, and swollen muzzle. In general, these signs occurred in a dose-related fashion and were confined to the scheduled observation periods ranging from 60-90 minutes post dose and 5-7 hrs post dose, according to the notations.

Mean body weights were slightly decreased in MD and HD males and notably decreased in HDF throughout the study (At wk 40, the mean decrease in body weight compared to the control was 9%-MDM, 7%-HDM, 6%-LDF, 10%-MDF and 20%-HDF). The mean weekly food consumption was generally decreased in HDF. Ophthalmoscopy and ECGs were not assessed as part of the study. Blood pressure was assessed and according to the sponsor, there were no adverse effects of treatment; however, examination of the data suggests that the data are unreliable due to technical problems. There were no significant effects of treatment on body temperature.

The clinical pathology assessment consisted of evaluations of hematology, coagulation parameters and clinical chemistry. The sponsor did not conduct urinalyses. The sponsor stated that there were no toxicologically relevant effects of treatment on hematology and coagulation parameters; however, examination of the data suggests a slight treatment-related decrease in WBCs in HD males and females at wk 40 (due to lymphocytes, neutrophils, and eosinophils in both males and females, and also monocytes and basophils in females). With regard to clinical chemistry, there were a few noteworthy increases in AST (transiently increased [approximately 10-fold baseline] in a single HDF) and ALT (increased at termination in a single LDM [approximately 5-fold baseline] and a single HDM [approximately 4-fold baseline]); however, these changes were not associated with abnormal histopathology, therefore, a relationship to treatment is questionable.

Thickening of the mammary gland, uterine wall and vaginal wall occurred only in tetrabenazine treated animals (however, with no relationship to dose). The thickening of the mammary gland and uterine wall