

Telomer Research Program

Report Title: 8-2 Telomer B Alcohol: Developmental Toxicity Study in Rats

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Study Objective

The objective of this study was to evaluate the potential maternal and developmental toxicity of 8-2 Telomer B Alcohol when administered by oral gavage to pregnant rats daily from around the time of implantation to the end of gestation, days 6-20 of gestation (days 6-20G).

Materials and Methods

Groups of 22 time-mated Crl:CD[®](SD)IGS BR rats were administered formulations of the test substance in 0.5% aqueous methylcellulose over days 6 through 20 of gestation (G) at daily dose levels of 0, 50, 200, or 500 mg/kg. During the in-life portion of the study, body weight, food consumption, and clinical observation data were collected. On day 21G, all surviving dams were euthanized and dissected; the abdominal and thoracic viscera were examined grossly. The uterus of each pregnant rat was removed and dissected to permit examination of the uterine contents. The fetuses were removed, individually identified, weighed, sexed, and examined for external, visceral, head, and skeletal alterations.

Findings (Report Summary Tables Attached)

Under the conditions of this study, adverse maternal toxicity was produced at 500 mg/kg/day and consisted of maternal mortality, decreased body weights and body weight gains, and increased clinical observations of toxicity. One litter at 500 mg/kg/day consisted of one early resorption; persistent reductions in food consumption and body-weight gain and increased clinical observations indicative of toxicity were reported during the study for this female. Therefore, this litter consisting of a single early resorption is believed to be secondary to overt maternal toxicity in this dam. Developmental toxicity at 500 mg/kg/day consisted of increased skeletal variations (delayed pelvic bone ossification and wavy ribs). At 200 and 500 mg/kg/day, there were non-adverse, test substance-related, transient reductions in maternal food consumption. In addition, there were slight increases in the incidence of delayed skull bone ossification at 200 and 500 mg/kg/day that were not considered adverse.

Conclusion

The no-observed-effect level (NOEL) for both maternal and developmental toxicity was considered 200 mg/kg/day.

Publications / Presentations: None

Tables

Explanatory Notes

Notes

Day C21 = Corrected final maternal body weight = final maternal body weight minus the gravid uterine weight plus the empty uterine weight.

Abbreviations

--- = No Data
S.D. = Standard Deviation
S.E. = Standard Error
N = Number in Group

TABLE 1
SUMMARY OF DOSING ANALYSES

Nominal: Homogeneity Samples	Dosing Concentration of 8-2 Telomer B Alcohol (mg/mL)		
	<u>10</u>	<u>40</u>	<u>100</u>
Test Day 1			
Top	8.92 ^a (89.2) ^b	40.5 (101.3)	96.5 (96.5)
Middle	8.73 ^a (87.3)	40.0 (100.0)	98.3 ^a (98.3)
Bottom	10.0 (100.0)	39.8 (99.5)	94.8 (94.8)
Average Measured Conc. ^c	9.22	40.1	96.5
Average Percent Nominal	(92.2)	(100.3)	(96.5)
Standard Deviation ^c	± 0.69	± 0.37	± 1.8
Coefficient of Variation ^c	7%	1%	2%
Stability Samples			
5 Hour Room Temperature	9.56 (95.6)	41.1 (102.8)	104 (104.0)
Concentration Verification			
Test Day 11			
#1	7.28 ^a (72.8)	39.9 ^a (99.8)	100 ^a (100.0)
#2	7.23 ^a (72.3)	39.7 ^a (99.3)	101 ^a (101.0)
#3	8.71 ^d (87.1)	41.1 ^d (102.6)	98.2 ^d (98.2)
Average Measured Conc. ^e	7.74	40.2	99.7
Average Percent Nominal ^e	(77.4)	(100.5)	(99.7)
Standard Deviation ^e	± 0.84	± 0.76	± 1.4
Coefficient of Variation ^e	11%	2%	1%
Test Day 20			
#1	9.45 (94.5)	35.0 (87.5)	94.6 (94.6)
#2	9.57 (95.7)	37.8 (94.5)	91.9 (91.9)
Average Measured Conc. ^f	9.51	36.4	93.2
Average Percent Nominal ^f	(95.1)	(91.0)	(93.2)
Standard Deviation ^f	± 0.09	± 2.0	± 1.9
Coefficient of Variation ^f	1%	5%	2%

- a** Mean result of the original analysis and duplicate reanalysis of original sample.
b Numbers in parentheses are the respective percent of nominal values.
c Statistics based on the average measured concentration of the top, middle and bottom of each dosing level.
d Result of the analysis for the third sample collected from the dosing preparations.
e Statistics based on the average measured concentration of triplicate samples for the dosing level.
f Statistics based on the average measured concentration of duplicate samples for the dosing level.

TABLE 2
CLINICAL OBSERVATIONS

DAY OF GESTATION	OBSERVATION:	GROUP :	I	II	III	IV
		DOSE RATE (MG/KG/DAY):	0	50	200	500
		NO. EXAMINED :	22	22	22	22
6-21	NO. SACRIFICED IN EXTREMIS		0	0	0	4*
	NO. FOUND DEAD		0	0	0	1
	ALOPECIA		5	2	6	10*
	DIARRHEA		0	0	0	6*
	HUNCHED OVER		0	0	0	6*
	PALLOR		0	0	0	1
	SORE		0	0	0	1
	STAIN CAGEBOARD		0	0	1	1
	STAIN FACE		0	0	0	1
	STAIN PERINEUM		0	0	0	3*
	WEAK		0	0	0	5*
	WET PERINEUM		0	0	0	1

* Statistically significant trend (Cochran-Armitage test); $p < 0.05$.

TABLE 3
MEAN MATERNAL BODY WEIGHTS (grams)^a

		DAYS OF GESTATION						
		DAY 0	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9
GROUP I: 0 MG/KG/DAY								
GROUP MEAN		249.4	265.7	270.2	277.5	284.5	289.9	293.9
S.D.		10.26	11.68	11.37	12.33	11.77	11.88	11.65
S.E.		2.19	2.49	2.42	2.63	2.51	2.53	2.48
N		22	22	22	22	22	22	22
GROUP II: 50 MG/KG/DAY								
GROUP MEAN		249.6	264.6	271.0	276.3	281.5	287.9	291.6
S.D.		11.52	11.61	12.86	12.99	12.38	11.15	10.33
S.E.		2.51	2.53	2.81	2.84	2.70	2.43	2.26
N		21	21	21	21	21	21	21
GROUP III: 200 MG/KG/DAY								
GROUP MEAN		249.8	259.1	269.2	274.6	278.2	284.8	287.7
S.D.		11.38	16.20	13.81	13.09	12.21	13.59	11.90
S.E.		2.43	3.45	2.94	2.79	2.60	2.90	2.54
N		22	22	22	22	22	22	22
GROUP IV: 500 MG/KG/DAY								
GROUP MEAN		249.7	263.5	269.8	277.8	271.9*	278.3*	284.2*
S.D.		12.02	16.57	16.81	16.17	19.90	20.79	22.40
S.E.		3.00	4.14	4.20	4.04	4.97	5.20	5.60
N		16	16	16	16	16	16	16

TABLE 3 (Continued)

MEAN MATERNAL BODY WEIGHTS (grams)

	DAYS OF GESTATION						
	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	DAY 16
GROUP I: 0 MG/KG/DAY							
GROUP MEAN	299.6	306.8	314.3	322.4	326.0	332.8	344.3
S.D.	12.50	14.74	14.58	13.49	15.44	15.39	16.87
S.E.	2.67	3.14	3.11	2.88	3.29	3.28	3.60
N	22	22	22	22	22	22	22
GROUP II: 50 MG/KG/DAY							
GROUP MEAN	297.1	304.3	311.8	318.5	324.1	329.5	340.1
S.D.	11.84	11.02	11.99	10.86	12.77	10.78	11.37
S.E.	2.58	2.40	2.62	2.37	2.79	2.35	2.48
N	21	21	21	21	21	21	21
GROUP III: 200 MG/KG/DAY							
GROUP MEAN	292.5	299.3	308.1	316.7	320.7	330.2	341.5
S.D.	11.55	12.41	14.38	15.51	16.25	18.81	19.07
S.E.	2.46	2.65	3.07	3.31	3.46	4.01	4.07
N	22	22	22	22	22	22	22
GROUP IV: 500 MG/KG/DAY							
GROUP MEAN	287.8*	286.8*	296.0*	303.7*	313.2*	319.8*	331.2*
S.D.	17.89	21.52	25.14	23.68	20.46	23.03	23.55
S.E.	4.47	5.38	6.29	5.92	5.12	5.76	5.89
N	16	16	16	16	16	16	16

TABLE 3 (Continued)

MEAN MATERNAL BODY WEIGHTS (grams)

	DAYS OF GESTATION					
	DAY 17	DAY 18	DAY 19	DAY 20	DAY 21	DAY C21
GROUP I: 0 MG/KG/DAY						
GROUP MEAN	358.2	374.5	391.1	409.0	433.8	330.3
S.D.	17.52	18.14	20.22	21.87	25.95	20.07
S.E.	3.74	3.87	4.31	4.66	5.53	4.28
N	22	22	22	22	22	22
GROUP II: 50 MG/KG/DAY						
GROUP MEAN	354.1	369.5	385.2	401.6	427.1	332.4
S.D.	12.58	13.83	14.86	15.38	18.14	14.87
S.E.	2.74	3.02	3.24	3.36	3.96	3.24
N	21	21	21	21	21	21
GROUP III: 200 MG/KG/DAY						
GROUP MEAN	356.8	373.2	389.2	405.3	426.5	325.7
S.D.	21.47	23.80	26.02	29.39	32.75	23.20
S.E.	4.58	5.07	5.55	6.27	6.98	4.95
N	22	22	22	22	22	22
GROUP IV: 500 MG/KG/DAY						
GROUP MEAN	346.1	360.5	374.1*	386.2*	395.1*	300.4*
S.D.	25.61	27.43	28.59	28.17	34.57	29.09
S.E.	6.40	6.86	7.15	7.04	8.64	7.27
N	16	16	16	16	16	16

a Data were excluded from females that were not pregnant, found dead, sacrificed *in extremis*, or had litters consisting entirely of resorptions.

* Statistically significant trend (linear contrast of means); $p < 0.05$.

TABLE 4

MEAN MATERNAL BODY WEIGHT CHANGES (grams)^a

	DAYS OF GESTATION						
	0-6	4-6	6-8	8-10	10-12	12-14	14-16
GROUP I: 0 MG/KG/DAY							
GROUP MEAN	28.1	11.8	12.4	9.7	14.7	11.8	18.2
S.D.	8.30	3.75	4.74	4.60	5.79	4.38	4.48
S.E.	1.77	0.80	1.01	0.98	1.23	0.93	0.96
N	22	22	22	22	22	22	22
GROUP II: 50 MG/KG/DAY							
GROUP MEAN	26.7	11.7	11.5	9.2	14.8	12.3	16.0
S.D.	6.12	3.67	5.27	4.23	6.61	5.72	7.95
S.E.	1.33	0.80	1.15	0.92	1.44	1.25	1.74
N	21	21	21	21	21	21	21
GROUP III: 200 MG/KG/DAY							
GROUP MEAN	24.8	15.4	10.2	7.7	15.6	12.6	20.8
S.D.	5.77	7.95	6.47	5.14	5.52	6.31	5.71
S.E.	1.23	1.70	1.38	1.10	1.18	1.35	1.22
N	22	22	22	22	22	22	22
GROUP IV: 500 MG/KG/DAY							
GROUP MEAN	28.1	14.2	0.5*	9.5	8.2*	17.2*	18.0
S.D.	7.99	5.09	9.68	9.26	14.95	10.08	8.65
S.E.	2.00	1.27	2.42	2.31	3.74	2.52	2.16
N	16	16	16	16	16	16	16

TABLE 4 (Continued)

MEAN MATERNAL BODY WEIGHT CHANGES (grams)

	DAYS OF GESTATION			
	16-18	18-21	6-21	6-C21
GROUP I: 0 MG/KG/DAY				
GROUP MEAN	30.3	59.3	156.4	52.9
S.D.	4.18	9.91	21.46	16.15
S.E.	0.89	2.11	4.58	3.44
N	22	22	22	22
GROUP II: 50 MG/KG/DAY				
GROUP MEAN	29.4	57.6	150.8	56.1
S.D.	6.63	7.96	19.57	13.63
S.E.	1.45	1.74	4.27	2.97
N	21	21	21	21
GROUP III: 200 MG/KG/DAY				
GROUP MEAN	31.8	53.3*	151.9	51.1
S.D.	7.02	11.41	28.17	19.49
S.E.	1.50	2.43	6.01	4.16
N	22	22	22	22
GROUP IV: 500 MG/KG/DAY				
GROUP MEAN	29.3	34.6*	117.4*	22.6*
S.D.	7.50	24.96	34.43	30.68
S.E.	1.88	6.24	8.61	7.67
N	16	16	16	16

a Data were excluded from females that were not pregnant, found dead, sacrificed *in extremis*, or had litters consisting entirely of resorptions.

* Statistically significant trend (linear contrast of means); $p < 0.05$.

TABLE 5

MEAN MATERNAL FOOD CONSUMPTION (grams/day) ^a

	DAYS OF GESTATION						
	4-6	6-8	8-10	10-12	12-14	14-16	16-18
GROUP I: 0 MG/KG/DAY							
GROUP MEAN	22.8	23.3	24.1	25.5	26.1	27.2	29.3
S.D.	2.78	3.47	2.35	2.94	2.68	2.65	3.83
S.E.	0.59	0.74	0.50	0.63	0.57	0.56	0.82
N	22	22	22	22	22	22	22
GROUP II: 50 MG/KG/DAY							
GROUP MEAN	23.8	21.9	23.4	25.7	26.4	26.8	30.2
S.D.	2.56	2.42	2.49	2.71	2.29	3.68	3.42
S.E.	0.56	0.53	0.54	0.59	0.50	0.80	0.75
N	21	21	21	21	21	21	21
GROUP III: 200 MG/KG/DAY							
GROUP MEAN	23.5	21.3*	22.1*	24.6	26.1	28.4	31.9*
S.D.	2.39	2.40	2.22	3.65	3.57	3.28	2.96
S.E.	0.51	0.51	0.47	0.78	0.76	0.70	0.63
N	22	22	22	22	22	22	22
GROUP IV: 500 MG/KG/DAY							
GROUP MEAN	23.7	19.9*	21.4*	20.6*	25.8	27.9	31.8*
S.D.	3.03	5.91	3.25	6.76	4.28	4.44	5.31
S.E.	0.76	1.48	0.81	1.69	1.07	1.11	1.33
N	16	16	16	16	16	16	16

TABLE 5 (Continued)

MEAN MATERNAL FOOD CONSUMPTION (grams/day)

	DAYS OF GESTATION	
	18-21	6-21

GROUP I: 0 MG/KG/DAY

GROUP MEAN	28.1	26.4
S.D.	3.84	2.64
S.E.	0.82	0.56
N	22	22

GROUP II: 50 MG/KG/DAY

GROUP MEAN	28.2	26.2
S.D.	2.47	2.00
S.E.	0.54	0.44
N	21	21

GROUP III: 200 MG/KG/DAY

GROUP MEAN	28.4	26.3
S.D.	3.88	2.46
S.E.	0.83	0.52
N	22	22

GROUP IV: 500 MG/KG/DAY

GROUP MEAN	25.4	24.7
S.D.	5.55	2.88
S.E.	1.39	0.72
N	16	16

a Data were excluded from females that were not pregnant, found dead, sacrificed *in extremis*, or had litters consisting entirely of resorptions.

* Statistically significant trend (linear contrast of means); $p < 0.05$.

TABLE 6

REPRODUCTIVE OUTCOME

Group:		I	II	III	IV
Dose (mg/kg/day):		0	50	200	500
No. Mated		22	22	22	22
No. Pregnant		22	21	22	22
No. Delivered Early		0	0	0	0
No. Deaths		0	0	0	5*
No. With Total Resorptions		0	0	0	1
No. Litters		22	21	22	16
Mean Corpora Lutea ^a		15.7 (1.61) ^b	16.0 (3.04)	16.0 (3.40)	16.1 (2.51)
Implantations (Nidations)		14.7 (1.62)	13.9 (1.56)	13.9 (1.64)	13.4 (3.87)
Resorptions:	Total	0.0 (0.21)	0.7 (1.06)	0.4 (0.79)	0.4 (0.81)
	Early	0.0 (0.21)	0.7 (1.06)	0.4 (0.79)	0.4 (0.81)
	Late	0.0	0.0	0.0	0.1 (0.25)
Dead Fetuses		0.0	0.0	0.0	0.0
Live Fetuses ^c :	Total	14.6 (1.56)	13.2 (1.81)	13.5 (1.77)	13.8 (1.95)
	Males	7.2 (1.79)	6.0 (1.58)	6.7 (2.16)	7.1 (1.84)
	Females	7.5 (1.74)	7.2 (1.69)	6.8 (1.97)	6.7 (1.89)
Mean Fetal Weight:	Total	5.55 (0.31)	5.66 (0.29)	5.77 (0.39)	5.40 (0.44)
	Males	5.68 (0.33)	5.83 (0.34)	5.94 (0.40)	5.57 (0.47)
	Females	5.43 (0.32)	5.51 (0.26)	5.59 (0.42)	5.20 (0.39)
Sex Ratio ^d		0.49 (0.11)	0.45 (0.11)	0.50 (0.14)	0.51 (0.12)

a Statistical analyses are not conducted on mean corpora lutea data; these data are presented for information only.

b Standard deviation is reported in parentheses.

c Statistical analyses are only conducted on the mean total number of live fetuses per litter and total mean fetal weight. The means for males and females are presented for information only.

d Number male fetuses/total number fetuses per litter.

Note: The pregnancy rate data, adult mortality data, and the total resorption data were statistically analyzed using the Cochran-Armitage test. All litter mean data (except for fetal weight and sex ratio) were analyzed using Jonckheere's test. Fetal weight and sex ratio were analyzed using a linear contrast of least square means.

* Statistically significant trend; $p < 0.05$.

TABLE 7

INCIDENCE OF FETAL MALFORMATIONS

	Group:	I	II	III	IV
	Dose (mg/kg/day):	0	50	200	500
<u>EXTERNAL</u>					
No. Examined ^a		322[22]	277[21]	297[22]	220[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
<u>VISCERAL</u>					
No. Examined		167[22]	142[21]	156[22]	113[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
<u>HEAD</u>					
No. Examined		167[22]	142[21]	156[22]	113[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
<u>SKELETAL</u>					
No. Examined		322[22]	277[21]	297[22]	220[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
TOTAL NUMBER AFFECTED		0(0)	0(0)	0(0)	0(0)

a Number examined and affected, including the number affected with the listed malformations are expressed as Fetuses[Litters] or Fetuses (Litters).

b For ease of reading, zeros have been replaced with dashes for the listed malformations.

No significant trends were detected (Jonckheere-Terpstra Trend test); $p < 0.05$.

Note: Statistical analyses are only conducted on the individual endpoints. The overall total and totals by exam type are presented for information only.

TABLE 8
INCIDENCE OF FETAL VARIATIONS

	Group:	I	II	III	IV
	Dose (mg/kg/day):	0	50	200	500
DEVELOPMENTAL VARIATIONS					
<u>EXTERNAL</u>					
No. Examined ^a		322[22]	277[21]	297[22]	220[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
<u>VISCERAL</u>					
No. Examined		167[22]	142[21]	156[22]	113[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
<u>HEAD</u>					
No. Examined		167[22]	142[21]	156[22]	113[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
<u>SKELETAL</u>					
No. Examined		322[22]	277[21]	297[22]	220[16]
Rib					
- Rudimentary Cervical		---	---	2(1)	1(1)
- Supernumerary		25(11)	20(13)	32(15)	34(10)
Skull - Fused		---	---	---	1(1)
No. Affected		25(11)	20(13)	34(16)	35(11)
TOTAL WITH DEVELOPMENTAL VARIATIONS		25(11)	20(13)	34(16)	35(11)
VARIATIONS DUE TO RETARDED DEVELOPMENT					
<u>EXTERNAL</u>					
No. Examined ^a		322[22]	277[21]	297[22]	220[16]
No. Affected		0[0]	0[0]	0[0]	0[0]
<u>VISCERAL</u>					
No. Examined		167[22]	142[21]	156[22]	113[16]
Kidney ^b					
Small Papilla - Size 1		2(2)	---	1(1)	2(2)
Small Papilla - Size 2		4(3)	2(2)	1(1)	1(1)
Papilla - Size 3		6(6)	5(5)	3(3)	2(1)
No. Affected		12[9]	7[7]	5[5]	5[3]

TABLE 8 (Continued)

INCIDENCE OF FETAL VARIATIONS

VARIATIONS DUE TO RETARDED DEVELOPMENT (Continued)HEAD

No. Examined	167[22]	142[21]	156[22]	113[16]
No. Affected	0[0]	0[0]	0[0]	0[0]

SKELETAL

No. Examined	322[22]	277[21]	297[22]	220[16]
Pelvis - Retarded Ossification	1(1)	---	---	9(5)*
Rib - Wavy	---	---	---	5(2)*
Skull - Retarded Ossification	22(8)	31(12)	45(16)*	55(12)*
Sternebra - Retarded Ossification	5(3)	2(2)	3(2)	8(5)
Vertebra - Retarded Ossification	37(12)	30(8)	34(13)	12(6)
No. Affected	56([15]	56[13]	72[19]	73[14]

TOTAL WITH VARIATIONS

DUE TO RETARDED DEVELOPMENT	66(17)	63(15)	76(19)	78(15)
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TOTAL NUMBER FETUSES WITH VARIATIONS	89(21)	80(19)	99(20)	106(15)
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- a Number examined and affected, including the number affected with the listed variations, are expressed as Fetuses[Litters] or Fetuses (Litters).
- b Statistical analyses are conducted on the combined incidences of small renal papillae; the details for each size are presented for information only.

* Significant trend (Jonckheere's test); $p < 0.05$.

Note: Statistical analyses are only conducted on the individual endpoints. The overall total and totals by exam are presented for information only.