AR 226-1341

Telomer Research Program

Daikin AGC Clariant DuPont

Report Title: 8-2 Telomer B Alcohol: Subchronic Toxicity 90-Day Oral Gavage Study in Rats

Author(s): Gregory S. Ladics, Ph.D

Contractor: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

Study Dates: Study Initiated: February 7, 2002
In-life Initiated: February 12, 2002
In-life Completed: August 22, 2002
Study Completed: April 1, 2003

Study Objective: The objective of this study was to evaluate the potential subchronic toxicity and the reversibility of the effects of 8-2 Telomer B Alcohol when administered by gavage to male and female rats. The oral route of administration was selected as the most efficient way to deliver an accurate dosage.

Materials and Methods: Five groups of young adult male and female Crl:CD®(SD)IGS BR rats were administered 8-2 Telomer B Alcohol daily by gavage at dosages of 0, 1, 5, 25, and 125 mg/kg/day for approximately 90 days. Body weights, food consumption, and clinical signs were evaluated weekly. Clinical pathology endpoints were evaluated on weeks 7, 13, and near the end of the three-month recovery period. Neurobehavioral assessments were also performed prior to dosing and during week 13. Biochemical evaluations (hepatic β-oxidation) were performed on animals after 10 and approximately 90 days of dosing and following the three-month recovery period. After approximately 90 days of dosing, 10 rats/sex/dose were sacrificed and given a gross and microscopic pathological examination. Approximately three months after the 90-day dosing period, 10 animals/sex in the control and high dose group were examined for recovery of toxic effects. A subgroup of 5 rats/sex/dose were designated as satellite bleed animals. An additional five animals/sex/dose were designated for hepatic biochemical analysis following a 10-day exposure to the test substance.

The range of mean daily dose volumes of 8-2 Telomer B Alcohol administered to male rats was 1.2 to 3.0 ml. The range administered to female rats was 0.9 to 1.6 ml.

Findings:
In-Life Toxicology Parameters: No test substance-related mortality occurred in the study. A test substance-related, toxicologically significant increase in the incidence of striated teeth was observed in the 125 mg/kg/day male and female dose groups. On test day 77, all male and female high dose animals were placed on ground chow due to test substance-induced alterations to the teeth. There were no test substance-related, toxicologically significant alterations in body

weight, body weight gain, food consumption, or food efficiency observed in any of the male or female dose groups.

Neurotoxicology Parameters: No adverse changes in neurobehavioral parameters were observed in male or female rats in any dose group.

Biochemical Toxicology: The rate of hepatic β-oxidation, a measure of peroxisome proliferation, was significantly increased in females administered 25 mg/kg/day 8-2 Telomer B Alcohol and in males and females administered 125 mg/kg/day 8-2 Telomer B Alcohol. Following a three-month recovery period, the rate of hepatic β-oxidation was lower but still significantly increased in males administered 125 mg/kg/day 8-2 Telomer B Alcohol, and had returned to control levels in female rats.

SUMMARY OF PEROXISOMAL BETA-OXIDATION ACTIVITY IN MALE RATS

		Hepatic Peroxisomal Beta-Oxidation Activity (nmol/min/mg protein)		
Group	Dosage (mg/kg/day)	10-Day Time point	90-Day Time point	3-Month Recovery Time point
I	0	14.8 ± 1.0[a]	12.4 ± 1.2	11.2 ± 1.8
III[b]	1	14.0 ± 1.7	13.6 ± 1.5	-----
V[b]	5	15.9 ± 2.1	13.6 ± 3.0	-----
VII[b]	25	14.7 ± 1.1	13.4 ± 2.1	-----
IX	125	24.1 ± 3.7#	20.6 ± 2.8#	14.9 ± 2.0*

[a] Mean ± standard deviation. The n = 5 for each group.
[b] Samples were not prepared for analysis from groups III, V and VII at the 90-day recovery time points.
Statistically significant difference from control ($p < 0.05$) by Jonckheere's Tend test.
* Statistically significant difference from control ($p < 0.05$) by Dunnett's test.

SUMMARY OF PEROXISOMAL BETA-OXIDATION ACTIVITY IN FEMALE RATS

Group	Dosage (mg/kg/day)	Hepatic Peroxisomal Beta-Oxidation Activity (nmol/min/mg protein)		
		10-Day Time point	90-Day Time point	3-Month Recovery Time point
II	0	22.5 ± 2.2[a]	25.6 ± 3.3	23.8 ± 1.3
IV[b]	1	22.2 ± 1.5	25.4 ± 1.4	-----
VI[b]	5	23.8 ± 4.5	27.3 ± 2.1	-----
VIII[b]	25	23.2 ± 2.2	29.7 ± 3.3#	-----
X	125	29.5 ± 4.4#	39.1 ± 4.7#	27.0 ± 5.6

[a] Mean ± standard deviation. The n = 5 for each group.
[b] Samples were not prepared for analysis from groups IV, VI and VIII at the 90-day recovery time point.
Statistically significant difference from control ($p < 0.05$) by Jonckheere's Tend test.

Clinical Pathology: Samples were collected at mid-study, at the end of treatment, and after three months of recovery for hematology, clinical chemistry, urinalysis, coagulation (end of study only), and plasma and urine fluoride (end of study and three-month recovery period).

There were no toxicologically significant changes in clinical pathology parameters observed in rats dosed with up to 125 mg/kg/day 8-2 Telomer B Alcohol. Other changes during treatment or after a three-month recovery were considered treatment-related but non-adverse because the magnitude of change was small, transient, and/or in a direction not associated with toxicity. Treatment-related changes observed in rats administered ≥ 25 mg/kg/day included decreases in some red cell mass parameters along with reticulocytosis and increased serum cholesterol (females only). Test substance-related changes in clinical pathology of rats dosed with 125 mg/kg/day included transiently increased alkaline phosphatase (males only), increased phosphorus (males only), decreased triglycerides (males only), increased total protein and albumin, increased calcium (females only), increased urine volume (males only), and decreased urine ketone concentrations (males only).

Plasma fluoride levels were significantly increased in males and females dosed with 125 mg/kg/day, but returned to control levels following three months of recovery. Urine fluoride excretion was increased in males and females dosed with ≥ 5 mg/kg/day. After three months of recovery, urine fluoride levels in females returned to control levels, while levels in males were significantly reduced compared to those at the end of the exposure period.

Red cell mass parameters and reticulocytes (expressed as a percent of control group mean)*

		Males				Females			
Dosage (mg/kg/day):		1	5	25	125	1	5	25	125
	Test Day								
RBC	48/49	99%	98%	***95%***	***93%***	98%	98%	97%	***91%***
	93/94	100%	97%	***95%***	***91%***	100%	102%	98%	***93%***
	183	--	--	--	96%	--	--	--	100%
HGB	48/49	101%	100%	98%	96%	99%	99%	96%	***91%***
	93/94	100%	98%	98%	***94%***	102%	102%	98%	***94%***
	183	--	--	--	98%	--	--	--	103%
HCT	48/49	100%	100%	97%	96%	98%	99%	***96%***	***91%***
	93/94	101%	99%	98%	***95%***	101%	102%	97%	***94%***
	183	--	--	--	97%	--	--	--	103%
ARET	48/49	97%	111%	113%	***123%***	88%	97%	***85%***	94%
	93/94	92%	108%	112%	***126%***	98%	110%	99%	***125%***
	183	--	--	--	105%	--	--	--	107%

* Statistical significance indicated by bold italicized type

ABBREVIATIONS:

RBC - red blood cell count
HGB - hemoglobin
HCT - hematocrit
ARET - absolute reticulocyte count

Fluoride (expressed as mean and as percent of control group mean)*

Parameter	**Sex**	**Test Day**	**I/II**	**III/IV**	**V/VI**	**VII/VIII**	**IX/X**
			0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	25 mg/kg/day	125 mg/kg/day
PFLU (ug/mL)	Males	93	0.1	0.1	0.1	0.1	***0.4***
				100%	100%	100%	***400%***
		183	0.1	--	--	--	0.1
			--	--	--	--	***100%***
PFLU (ug/mL)	Females	94	0.1	0.1	0.1	0.2	***0.5***
			--	100%	100%	200%	***500%***
		183	0.1	--	--	--	0.1
			--	--	--	--	100%
UFLU (ug)	Males	93	10.7	13	***38.8***	***202.4***	***997.2***
			--	121%	***363%***	***1892%***	***9320%***
		183	9.9	--	--	--	***27.6***
			--	--	--	--	***279%***
UFLU (ug)	Females	94	6	5.7	14.1	***88***	***702.6***
			--	95%	235%	***1467%***	***11710%***
		183	11.9	--	--	--	15.7
			--	--	--	--	132%

* Statistical significance indicated by bold italicized type.

ABBREVIATIONS:

PFLU - plasma fluoride
UFLU - urine fluoride

Anatomical Pathology: Test substance-related, toxicologically significant degeneration / disorganization of enamel organ ameloblast cells occurred in male rats administered 125 mg/kg/day 8-2 Telomer B Alcohol.

A test substance-related, adverse increase in the incidence and severity of focal hepatic necrosis was observed in males administered 25 and 125 mg/kg/day. An increased incidence and severity of chronic progressive nephropathy occurred in the 125 mg/kg/day female group and was considered adverse.

Test-substance related and statistically significant increases in liver weight parameters were present in males and females administered 25 or 125 mg/kg/day for approximately 90 days. The increased liver weights correlated with microscopic hepatocellular hypertrophy in the 125 mg/kg/day male dose group. Liver weight changes and microscopic hypertrophy were considered to be a physiological adaptive response to metabolism of the test substance and not adverse. Test-substance related and statistically significant increases in kidney weight parameters occurred in males and females administered 25 or 125 mg/kg/day. The increased kidney weights correlated with microscopic renal tubular hypertrophy in the 25 and 125 mg/kg/day male groups only. However, no correlative microscopic or clinical pathological evidence of renal toxicity were present. Therefore, kidney weight changes and microscopic tubular hypertrophy were not considered adverse findings. Altered colloid, a common spontaneous finding in rats, was increased in incidence and/or severity in treated male groups. This change was not associated with other findings in the thyroid gland and was also not considered an adverse finding.

After three months of recovery, rats dosed with 125 mg/kg/day showed reversibility of some effects. The ameloblastic degeneration/disorganization persisted with decreased incidence and severity in the 125 mg/kg/day male group. Alterations in thyroid colloid persisted in male rats. Increased liver weight parameters and hepatocellular hypertrophy (males only) were no longer observed in the 125 mg/kg/day male and female groups. However, the incidence of hepatocellular necrosis in the 125 mg/kg/day male group increased after three months of recovery. Increased kidney weight parameters and renal tubular hypertrophy (males only) were not observed in rats sacrificed after three months of recovery. In contrast, the incidence and severity of chronic progressive nephropathy were increased in the 125 mg/kg/day female group following three months of recovery.

MEAN FINAL BODY AND ORGAN WEIGHTS FOR MALE RATS

RATS DESIGNATED FOR SUBCHRONIC TOXICITY

MEAN FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams)

Group:	I	III	V	VII	IX
Dosage(mg/kg/day)	0	1	5	25	125
LIVER	14.876	14.109	15.575	17.219#	21.976#
	1.770(10)	1.254(10)	1.744(10)	1.693(10)	1.928(10)
KIDNEYS	3.924	3.804	4.087	4.351#	4.636#
	0.463(10)	0.263(10)	0.501(10)	0.417(10)	0.346(10)
FINAL BODY WEIGHT	552.940	540.790	582.650	564.140	536.980
	49.145(10)	37.400(10)	46.186(10)	43.119(10)	31.734(10)

52

MEAN FINAL BODY AND ORGAN WEIGHTS FOR MALE RATS

RATS DESIGNATED FOR SUBCHRONIC TOXICITY

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

Group:	I	III	V	VII	IX
Dosage(mg/kg/day)	0	1	5	25	125
LIVER/					
FINAL BODY * 100	2.690	2.609	2.669	3.050#	4.102#
	0.225(10)	0.140(10)	0.148(10)	0.124(10)	0.410(10)
KIDNEYS/					
FINAL BODY * 100	0.712	0.707	0.702	0.771	0.867#
	0.091(10)	0.076(10)	0.069(10)	0.035(10)	0.096(10)

MEAN FINAL BODY AND ORGAN WEIGHTS FOR FEMALE RATS

RATS DESIGNATED FOR SUBCHRONIC TOXICITY

MEAN FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams)

Group:	II	IV	VI	VIII	X
Dosage(mg/kg/day)	0	1	5	25	125
LIVER	7.904	8.224	8.185	9.230#	11.513#
	0.849(10)	0.982(10)	0.845(10)	1.076(10)	0.972(10)
KIDNEYS	2.239	2.147	2.155	2.453	2.462#
	0.188(10)	0.239(10)	0.173(10)	0.267(10)	0.265(10)
FINAL BODY WEIGHT	286.830	294.600	294.090	297.520	288.960
	25.304(10)	25.107(10)	25.820(10)	26.045(10)	17.911(10)

53

MEAN FINAL BODY AND ORGAN WEIGHTS FOR FEMALE RATS

RATS DESIGNATED FOR SUBCHRONIC TOXICITY

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

Group:	II	IV	VI	VIII	X
Dosage(mg/kg/day)	0	1	5	25	125
LIVER/					
FINAL BODY * 100	2.755	2.790	2.784	3.098#	3.988#
	0.158(10)	0.212(10)	0.161(10)	0.171(10)	0.287(10)
KIDNEYS/					
FINAL BODY * 100	0.782	0.732	0.734	0.825	0.852#
	0.043(10)	0.087(10)	0.043(10)	0.056(10)	0.064(10)

Incidences and Average Lesion Grades of Test substance-Related Microscopic Findings In Male and Female Rats

Dose (mg/kg/day):	0	1	5	25	125
	Males: 90-Day Exposure				
Liver:					
Hypertrophy, hepatocellular	0/10[a] (0.0)[b]	0/10 (0.0)	0/10 (0.0)	0/10 (0.0)	10/10 (1.6)
Necrosis, focal	0/10 (0.0)	0/10 (0.0)	1/10 (1.0)	5/10 (1.0)	3/10 (1.0)
Kidneys:					
Hypertrophy, tubular	0/10 (0.0)	0/10 (0.0)	0/10 (0.0)	9/10 (1.0)	10/10 (1.0)
Thyroid gland:					
Alteration, colloid	3/10 (1.0)	8/10 (1.1)	9/10 (1.0)	10/10 (1.0)	8/10 (2.3)
Teeth:					
Degeneration, ameloblasts (nose)	0/10 (0.0)	0/10 (0.0)	0/10 (0.0)	0/10 (0.0)	5/10 (1.6)
	Males: Three-Month Recovery				
Liver:					
Necrosis, focal	3/10 (1.3)	NA	NA	NA	7/10 (1.1)
Thyroid gland:					
Alteration, colloid	7/10 (1.4)	NA	NA	NA	10/10 (1.6)
Teeth:					
Degeneration, ameloblasts (nose)	0/10 (0.0)	NA	NA	NA	2/10 (1.0)
	Females: 90-Day Exposure				
Kidneys:					
Chronic progressive nephropathy	2/11 (1.0)	2/10 (1.0)	3/10 (1.0)	3/10 (1.0)	7/10 (1.0)
	Females: Three-Month Recovery				
Kidneys:					
Chronic progressive nephropathy	5/9 (1.0)	NA	NA	NA	8/10 (1.4)

a. Numerator indicates incidence of rats with microscopic lesion. Denominator indicates number of rats in group.
b. Number in parentheses indicates average severity of lesion.

NA Tissue not available because there were no intermediate recovery groups.

Conclusion: The no-observed-effect level (NOEL) for males was 5 mg/kg/day 8-2 Telomer B Alcohol based on the increased incidence of hepatic necrosis observed in males administered ≥ 25 mg/kg/day. The NOEL for females was 25 mg/kg/day 8-2 Telomer B Alcohol based on the increased incidence and severity of chronic progressive nephropathy observed in females administered 125 mg/kg/day.

Publications / Presentations: None