Subchronic Toxicity 90-Day Oral Gavage Study of of 8-2 Telomer B Alcohol in Rats

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Abstract

8-2 Telomer B Alcohol (8-2 TBA) is a fluorinated chemical intermediate used to manufacture specialty polymers and surfactants. The potential subchronic toxicity and the reversibility of the effects of this chemical were evaluated following approximately 90 days of oral gavage dosing to Crl:CD®(SD)IGS BR rats. A complete toxicology profile including neurobehavioral assessments and hepatic β-oxidation were conducted at selected intervals and a group of rats was included for a 90-day post-dosing recovery period. Dose levels tested were 0 (control), 1, 5, 25, and 125 mg/kg/day. No test-substance-related mortality occurred. Rats at 125 mg/kg/day developed striated teeth such that these animals were switched to ground chow at 77 days. No treatment-related alterations in body weight, food consumption, neurobehavioral parameters, or hematology/clinical chemistry were found. Hepatic β-oxidation was increased in males at 125 mg/kg/day and in females at 25 mg/kg/day. In both males and females, plasma fluorine levels were significantly increased at 125 mg/kg/day and urinary fluorine was elevated at ≥ 5 mg/kg/day. Degeneration/disorganization of enamel organ ameloblast cells was seen in males, but not females at 125 mg/kg/day. Liver weight increases accompanied by focal hepatic necrosis were seen at both 25 and 125 mg/kg/day in males and chronic progressive nephrotoxicity occurred in females administered 125 mg/kg/day. With the exception of hepatocellular necrosis observed in males administered 125 mg/kg/day and the incidence and severity of chronic progressive nephropathy in the 125 mg/kg/day female group, which increased after 3 months, all other changes showed evidence of reversibility. The no-observed-adverse-effect level for the 90-day study was 5 mg/kg/day.

Introduction

The test substance, 8-2 Telomer B Alcohol, is an organofluorine compound. The 1, 5, 25, and 125 mg/kg/day dose levels for this study were selected based on the toxicity observed in an 84-day range finding study with 5, 25, and 125 mg/kg/day 8-2 Telomer B Alcohol (2004 SOT Abstract #778) and subchronic studies with similar fluorotelomer-based alcohols and perfluorooctanoic acid (PFOA).[1,2,3,4]

Objective

The objective of this study is to evaluate the potential subchronic toxicity and reversibility 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.

Experimental Design

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 samples were collected on weeks 7, 13, and near the end of the 3-month recovery period for hematology, clinical chemistry, urinalysis, coagulation (end of study only), and plasma and urine fluoride (end of study and 3-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 3-month recovery period. After approximately 90 days of dosing, 10 rats/sex/dose were sacrificed and given a gross and microscopic pathology examination. Approximately 3 months after the 90-day dosing period, 10 animals/sex in the control and high dose group were examined for recovery of toxic effects.

Results

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 3-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.

Clinical Pathology

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. Plasma fluoride levels were significantly increased in males and females dosed with 125 mg/kg/day, but returned to control levels following 3 months of recovery. Urine fluoride excretion was significantly increased in males and females dosed with ≥ 5 mg/kg/day and ≥ 25 mg/kg/day, respectively. After 3 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. Increased plasma and urine fluoride indicates exposure to and metabolism of a fluorine-containing compound. The presence of fluoride after 90 days of recovery indicates continued metabolism of the test substance.

Anatomic 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. In addition, alterations in thyroid colloid were observed in male and female rats at all dose levels. Because altered colloid occurs spontaneously in healthy Sprague-Dawley rats,[5] and since there were no other microscopic alterations in the thyroid gland, altered colloid was interpreted as not biologically meaningful and non-adverse.

A test substance-related, biologically 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 that 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. Hepatic enzyme levels, however, were not elevated. Test-substance related and statistically significant increases in kidney weight parameters occurred in males administered 25 mg/kg/day and males and females administered 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, there was no histomorphologic evidence of renal damage, and renal clinical pathology parameters were not indicative of adverse effects.

After 3 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. 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 3 months of recovery. Increased kidney weight parameters and renal tubular hypertrophy (males only) were not observed in rats sacrificed after 3 months of recovery. In contrast, the incidence and severity of chronic progressive nephropathy were increased in the 125 mg/kg/day female group following 3 months of recovery.

Summary of Peroxisomal β-Oxidation Activity in Male Rats

	Hepatic Peroxisomal β-Oxidation Activity (nmol/min/mg protein)		
	10-Day	90-Day	3-Month Recovery
0 mg/kg/day	14.8 ± 1.0[a]	12.4 ± 1.2	11.3 ± 1.8
1 mg/kg/day[b]	14.0 ± 1.7	13.6 ± 1.5	-----
5 mg/kg/day[b]	15.9 ± 2.1	13.6 ± 3.0	-----
25 mg/kg/day[b]	14.7 ± 1.1	13.4 ± 2.1	-----
125 mg/kg/day	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 1, 5 or 25 mg/kg/day groups at the 3-month recovery.
Statistically significant difference from control at p < 0.05 by Jonckheere's Trend test.
* Statistically significant difference from control at p < 0.05 by Dunnett's test.

Summary of Peroxisomal β-Oxidation Activity in Female Rats

	Hepatic Peroxisomal β-Oxidation Activity (nmol/min/mg protein)		
	10-Day	90-Day	3-Month Recovery
0 mg/kg/day	22.5 ± 2.2[a]	25.6 ± 3.3	23.8 ± 1.3
1 mg/kg/day[b]	22.2 ± 1.5	25.4 ± 1.4	-----
5 mg/kg/day[b]	23.6 ± 4.5	27.3 ± 2.1	-----
25 mg/kg/day[b]	23.2 ± 2.2	29.7 ± 3.3#	-----
125 mg/kg/day	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 1, 5 or 25 mg/kg/day groups at the 3-month recovery.
Statistically significant difference from control at p < 0.05 by Jonckheere's Trend test.

Plasma and Urine Fluoride (expressed as mean and as percent of control group mean)

Parameter	Sex	Day	Test 0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	25 mg/kg/day	125 mg/kg/day
PFLU (ug/mL)	Male	93	0.1	0.1	0.1	0.1	0.4@
				100%	100%	100%	400%
		183	0.1	--	--	--	0.1
			--	--	--	--	100%
	Female	94	0.1	0.1	0.1	0.2	0.5@
			--	100%	100%	200%	500%
		183	0.1	--	--	--	0.1
			--	--	--	--	100%
UFLU (ug)	Male	93	10.7	13	38.8@	202.4@	997.2@
			--	121%	363%	1892%	9320%
		183	9.9	--	--	--	27.6*
			--	--	--	--	279%
	Female	94	6	5.7	14.1	88@	702.6@
			--	95%	235%	1467%	11710%
		183	11.9	--	--	--	15.7
			--	--	--	--	132%

@ Statistically significant difference from control at p < 0.05 by Dunn's test.
* Statistically significant difference from control at p < 0.05 by Dunnett/Fershaw-Dunnett test.

Mean Final Body, Liver, and Kidney Weights for Male Rats after 90-Day Test Substance Exposure

	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	25 mg/kg/day	125 mg/kg/day
MEAN FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams)					
LIVER	14.876	14.109	15.575	17.259#	21.976#
	1.770(10)	1.254(10)	1.744(10)	1.693(10)	1.928(10)
KIDNEYS	3.034	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 (grams)					
	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)
MEAN RELATIVE ORGAN WEIGHTS (% of body weight)					
LIVER/ FINAL BODY * 100	2.690	2.609	2.669	3.050#	4.103#
	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)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)
Statistically significant difference from control at p < 0.05 by Jonckheere-Terpstra trend test.

Mean Final Body, Liver, and Kidney Weights for Female Rats after 90-Day Test Substance Exposure

	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	25 mg/kg/day	125 mg/kg/day
MEAN FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams)					
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 (grams)					
	285.830	294.600	294.090	297.520	288.960
	25.304(10)	25.107(10)	25.820(10)	26.045(10)	17.911(10)
MEAN RELATIVE ORGAN WEIGHTS (% of body weight)					
LIVER/ FINAL BODY * 100	2.755	2.790	2.784	3.098#	3.988#
	0.158(10)	0.213(10)	0.161(10)	0.171(10)	0.287(10)
KIDNEYS/ FINAL BODY * 100	0.783	0.732	0.734	0.825	0.852#
	0.043(10)	0.087(10)	0.043(10)	0.056(10)	0.064(10)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)
Statistically significant difference from control at p < 0.05 by Jonckheere-Terpstra trend test.

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

		0 mg/kg/day	1 mg/kg/day	5 mg/kg/day	25 mg/kg/day	125 mg/kg/day
		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: 3-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: 3-Month Recovery				
Kidneys	Chronic progressive nephropathy	3/9 (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.

Summary

- Test substance-related, toxicologically significant increases in the incidence of striated teeth occurred in the 125 mg/kg/day male and female dose groups. Test substance-related, toxicologically significant degeneration/disorganization of enamel organ ameloblasts cells occurred in male rats dosed with 125 mg/kg/day 8-2 Telomer B Alcohol. This tooth lesion, along with the increased incidence of striated teeth, is consistent with fluoride toxicosis.[6] After 3 months, the degeneration/disorganization of ameloblasts persisted, at a lower incidence and severity.
- The observed alterations in liver and kidney weights, as well as the incidence of hepatocellular and renal tubular hypertrophy, were considered to be a pharmacologically adaptive response by these organs to the test substance and, therefore, not considered adverse.[7,8,9]
- A statistically significant increase in the rate of hepatic β-oxidation occurred in the 25 mg/kg/day female and 125 mg/kg/day male and female groups. After the recovery period, the increased rate of β-oxidation persisted, albeit at a reduced level compared to the 90-day time point, in the 125 mg/kg/day male group.
- A test substance-related, toxicologically significant 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 that was considered adverse. After 3 months of recovery, both the incidence of hepatocellular necrosis in the 125 mg/kg/day male group and the incidence and severity of chronic progressive nephropathy in the 125 mg/kg/day female group were increased compared to control.

Conclusions

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 5 mg/kg/day 8-2 Telomer B Alcohol based on the increased rate of hepatic β-oxidation observed in females administered ≥ 25 mg/kg/day.

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