AR226 - 1340

8-2 Telomer B Alcohol Repeated-Dose Oral Toxicity

Range-Finding Study in Rats

Work Request Number 13690

Service Code 1583

Data Summary

Summary

The data in this document are from a range-finder study that was performed in order to set dose levels for a 90-day subchronic toxicity study with 8-2 Telomer B Alcohol. The data from the 90-day subchronic toxicity study are located in Haskell Laboratory Report 9478. The protocol and protocol amendments for the range-finder study are located in appendix 1 of this document. In the 8-2 Telomer B Alcohol range finder study, male (10/group) and female (5/group) rats were dosed until the attainment of steady state blood fluorine levels, and allowed to recover for 75 days post-dosing (5/group, males and females). Serial bleeding for analysis of total fluorine and parent/metabolites was conducted during the dosing and recovery phases of the study. Blood fluorine levels reached steady state between test days 60-84. At steady state blood fluorine levels, the liver and kidneys of 5 males/group were weighed; females were not evaluated. At necropsy, liver kidney, and fat were saved from males and females for analysis of parent compound, PFOA, and PFNA. In males (on test day 81), mean body weights were significantly decreased and relative liver weights were significantly increased in rats administered 125 mg/kg/day 8-2 Telomer B Alcohol; kidney weights were not affected. After 75 days of recovery (test day 159), relative kidney weights were significantly increased in male rats administered 125 mg/kg/day 8-2 Telomer B Alcohol; final body, liver, and testes weights were not affected. In female rats (test day 159), kidney (absolute and relative) and relative liver weights were significantly increased in rats administered 125 mg/kg/day 8-2 Telomer B Alcohol; final body weights were not affected.

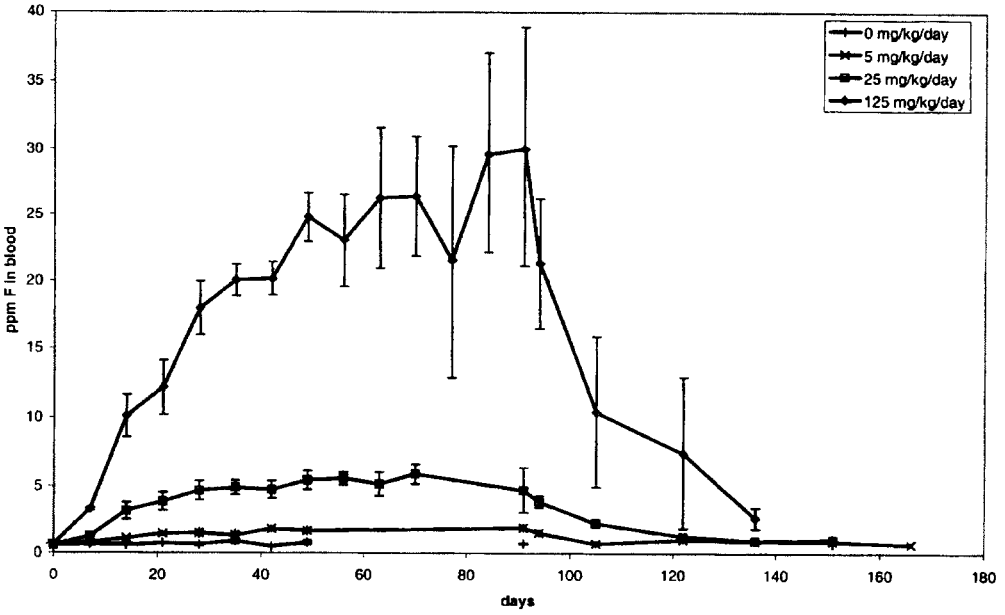
In addition, pooled plasma samples obtained from males and females over the duration of the in-life phase of the study were analyzed for parent compound, the 8-2 acid, PFOA, and PFNA. At steady state (test day 84) pooled plasma were analyzed for PFOA, parent compound, and total fluorine levels. At steady state, the level of parent compound detected did not exceed 0.074 ppm in either males or females. In males, PFOA represented approximately 85% of the total plasma fluorine detected at test day 84. In females, PFOA represented approximately 5% of the total plasma fluorine detected at test day 84. The 8-2 acid of 8-2 Telomer B alcohol and PFNA were also found in both males and females over the duration of the study.

Study Parameters

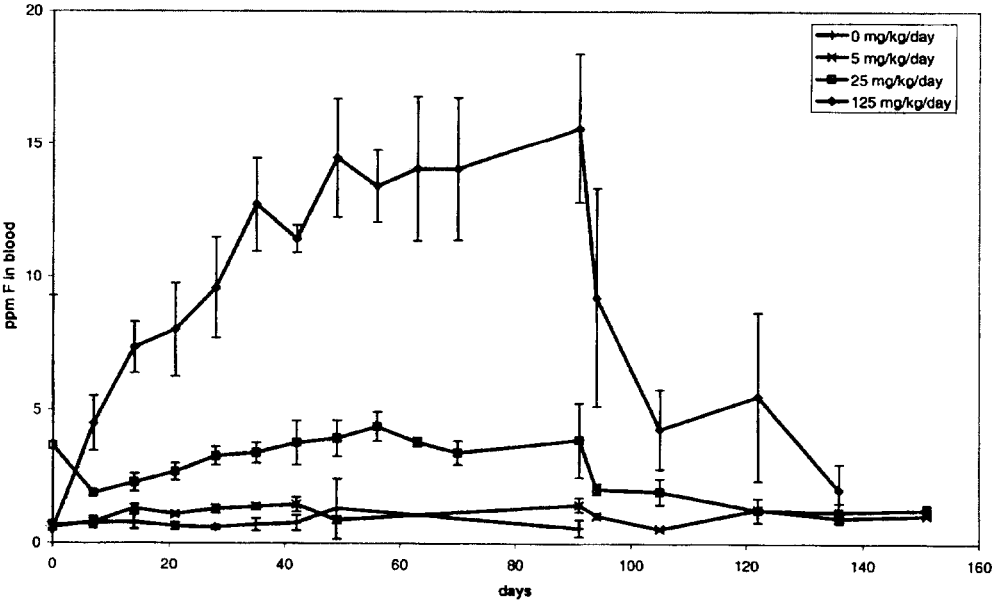
Test Species	Male & female Crl:CD®(SD)IGS BR rats
Dose Administration	Test Day 0 – to attainment of steady state (test day 84). All animals were dosed until the last dose group attained steady state for total fluorine in the blood.
Body Weight	Twice per week at 3-4 day intervals during dosing; once per week after dosing was stopped.
Clinical Observations	At every weighing
Serial bleeding for fluorine and parent/metabolite analysis {Approx. 2 hrs after dosing}	Predosing bleed- Day (-4) During dosing- Day 1 & weekly Postdosing-Day 3, 14, 31, 45, 60, & 75 Blood for parent/metabolite analysis pooled per dose group and separated into plasma and RBC.
Necropsy (Anatomical Pathology)	
Select tissue/sample collection from males at steady state blood fluorine levels	5 males in the 0, 5, 25, and 125 mg/kg/day dose groups sacrificed on test day 81 (liver, kidney, and fat tissue collected and weighed for future analysis of parent/metabolites).
Select tissue/sample collection from males and females following elimination of blood fluorine levels	All rats in the 0, 5, 25, and 125 mg/kg/day dose groups sacrificed on post dose test day 75 (liver, kidney, and fat tissue collected and weighed for future analysis of fluorine levels).

Results

Total Blood Fluorine Analysis in Male Rats Dosed with Telomer 8-2 Alcohol



Total Blood Fluorine Analysis in Female Rats Dosed with Telomer 8-2 Alcohol



Mean Final Body and Organ Weights for Male Rats Sacrificed on Test Day 81

	8-2 Telomer B Alcohol (mg/kg/day)			
	0	**5**	**25**	**125**
Liver (g)	23.4 ± 3.4	21.1 ± 2.5	22.7 ± 2.1	27.9 ± 3.1
Kidneys (g)	4.5 ± 0.47	4.3 ± 0.34	4.1 ± 0.31	4.4 ± 0.68
Final body weight (g)	594.1 ± 44.35	564.1 ± 59.08	552.6 ± 33.89	508.9 ± 54.71#
Liver (% BW)	3.9 ± 0.28	3.7 ± 0.20	4.1 ± 0.19	5.5 ± 0.39#
Kidneys (% BW)	0.76 ± 0.03	0.76 ± 0.06	0.75 ± 0.04	0.87 ± 0.11

Mean ± standard deviation. The n = 5.
Statistically significant difference from control (p = 0.05).

Mean Final Body and Organ Weights for Male Rats Sacrificed on Post Dose Day 75

	8-2 Telomer B Alcohol (mg/kg/day)			
	0	**5**	**25**	**125**
Liver (g)	16.9 ± 3.2	18.7 ± 2.6	16.9 ± 2.2	17.2 ± 2.2
Kidneys (g)	3.6 ± 0.45	3.9 ± 0.32	3.9 ± 0.47	4.0 ± 0.39
Testes (g)	3.6 ± 0.28	3.5 ± 0.55	3.6 ± 0.34	3.4 ± 0.74
Final body weight (g)	553.5 ± 99.82	594.7 ± 57.07	573.5 ± 83.19	548.9 ± 36.78
Liver (% BW)	3.0 ± 0.13	3.1 ± 0.14	2.9 ± 0.13	3.1 ± 0.32
Kidneys (% BW)	0.65 ± 0.06	0.67 ± 0.03	0.69 ± 0.05	0.74 ± 0.07#
Testes (% BW)	0.7 ± 0.10	0.6 ± 0.08	0.6 ± 0.09	0.6 ± 0.11

Mean ± standard deviation. The n = 5.

Statistically significant difference from control (p = 0.05).

Mean Final Body and Organ Weights for Female Rats Sacrificed on Post Dose Day 75

	8-2 Telomer B Alcohol (mg/kg/day)			
	0	**5**	**25[a]**	**125**
Liver (g)	10.3 ± 1.7	11.1 ± 1.3	11.3 ± 2.5	12.7 ± 1.2
Kidneys (g)	2.2 ± 0.21	2.4 ± 0.04	2.4 ± 0.37	2.7 ± 0.17#
Final body weight (g)	322.2 ± 41.03	348.1 ± 29.21	332.1 ± 50.42	345.5 ± 31.34
Liver (% BW)	3.2 ± 0.14	3.2 ± 0.33	3.4 ± 0.37	3.6 ± 0.05#
Kidneys (% BW)	0.70 ± 0.03	0.71 ± 0.07	0.73 ± 0.06	0.78 ± 0.02#

Mean ± standard deviation. The n = 4 unless otherwise noted; [a] The n = 5.
Statistically significant difference from control (p = 0.05).

Mean Body Weight Gain for Male Rats

	8-2 Telomer B Alcohol (mg/kg/day)			
	0	**5**	**25[a]**	**125**
Days 0-81[a]	297.0 ± 70.4	287.6 ± 47.8	275.0 ± 41.2	238.4 ± 38.9#
Days 81-159 (recovery)[b]	-0.4 ± 7.7	9.1 ± 13.0	1.9 ± 12.0	2.2 ± 16.8

Mean ± standard deviation. [a] The n = 10. [b] The n = 5.
Statistically significant difference from control (p = 0.05).

Mean Body Weight Gain for Female Rats

	8-2 Telomer B Alcohol (mg/kg/day)			
	0	**5**	**25[a]**	**125**
Days 0-81[a]	80.5 ± 38.6	119.0 ± 22.0[a]	115.0 ± 24.8[a]	110.2 ± 12.1[a]
Days 81-159 (recovery)[b]	20.8 ± 27.2	4.3 ± 4.7	-3.2 ± 7.0#[a]	7.1 ± 7.4

Mean ± standard deviation.
The n = 4 unless otherwise noted; [a] The n = 5.
Statistically significant difference from control (p = 0.05).

Male Clinical Observations

	8-2 Telomer B Alcohol (mg/kg/day)			
	0	**5**	**25[a]**	**125**
Striated teeth (striped)	0 (0%)	0 (0%)	0 (0%	7 (70%)#
Teeth clipped	0 (0%)	0 (0%)	0 (0%)	4 (40%)#

The n = 10.
Statistically significant difference from control (p = 0.05).

Female Clinical Observations

	8-2 Telomer B Alcohol (mg/kg/day)			
	0	**5**	**25[a]**	**125**
Striated teeth (striped)	0 (0%)	0 (0%)	0 (0%)	4 (80%)#

The n = 5.
Statistically significant difference from control (p = 0.05).

Analysis of 8-2 Telomer B Alcohol, PFOA, and PFNA in Serum and Selected Tissues

Pooled plasma samples obtained from males and females at steady state (test day 84) were analyzed for PFOA, parent compound, and total fluorine levels. The level of parent detected did not exceed 0.074 ppm in either males or females. The following plasma levels of PFOA were detected in males and females dosed with 0, 5, 25, and 125 mg/kg/day 8-2 Telomer B Alcohol.

Dose Group (mg/kg/day)	Male PFOA (ppm)	Male PFOA converted to ppm F	Male total plasma F (ppm)[a]	Female PFOA (ppm)	Female PFOA converted to ppm F	Female total plasma F (ppm)[a]
0	0.07	0.05	NM	0.0	0.0	NM
5	2.19	1.51	NM	0.08	0.05	NM
25	8.47*	5.83	8.11	0.54*	0.37	5.88
125	65.60*	45.16	53.24	1.85*	1.27	23.20

[a]Determined using Wickbold Torch; *mean of two separate runs; NM- not measured

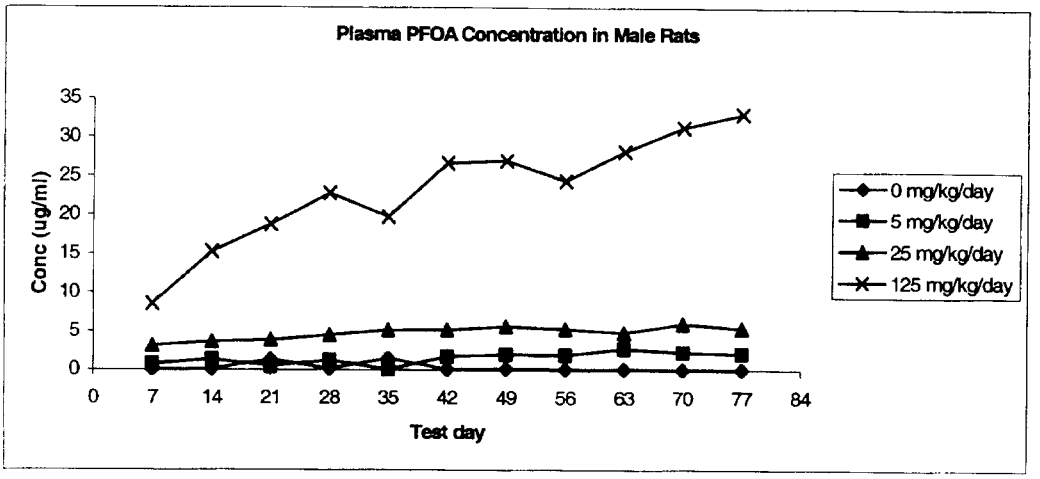
Based on the data presented above, the PFOA in males accounts for a large portion of the fluorine present in the plasma, however, this is not the case for females. While what is in the blood in males is predominately PFOA, it is not possible to determine what percentage of the 8-2 Telomer B Alcohol dose is converted to PFOA (e.g., information is lacking on mass balance and biodistribution). Further, it is less certain as to what the metabolites may be in females as both parent and PFOA were present in low levels and did not account for the total fluorine levels in the blood.

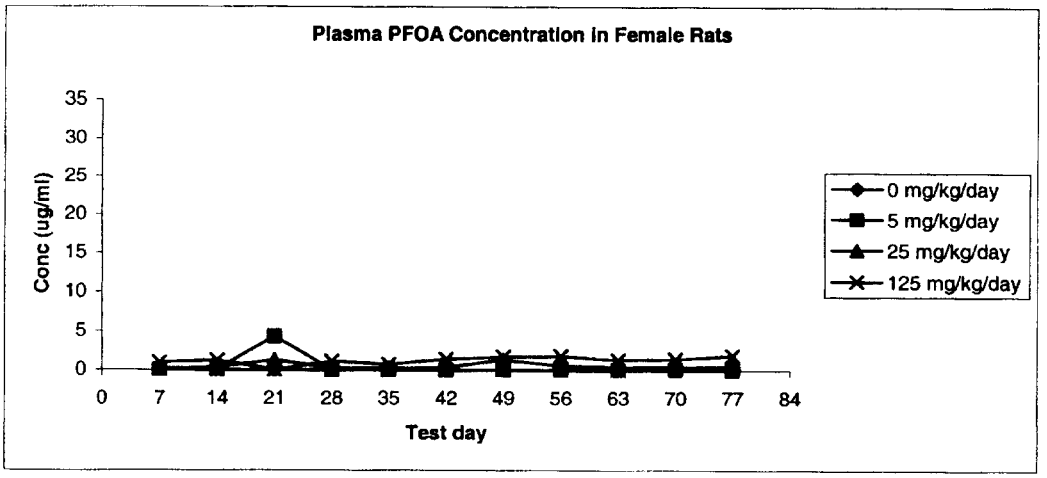
Tissue samples (fat, liver, and kidney) obtained at necropsy from males and females at steady state (test day 81) were analyzed for the concentration of parent compound, PFOA, and PFNA. The data are summarized below.

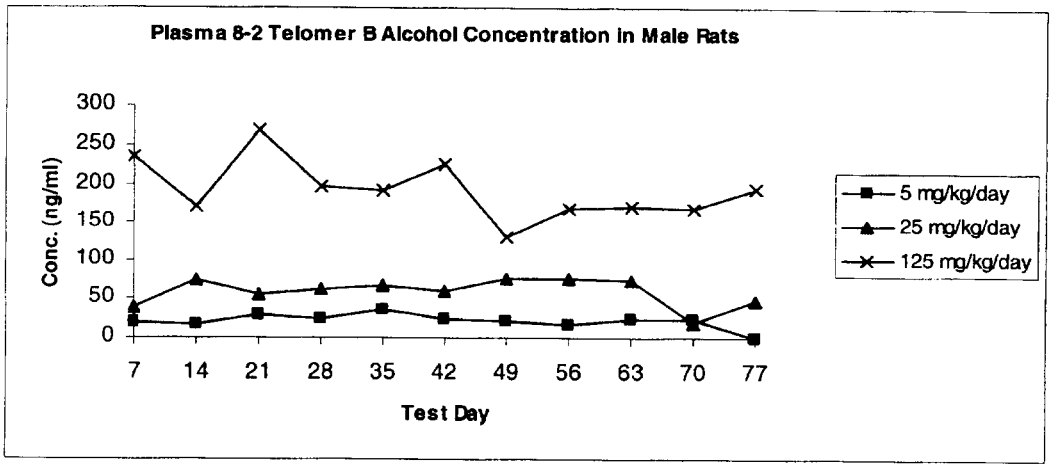
Tissue Analyses from Range-Finder Study (Test Day 81)

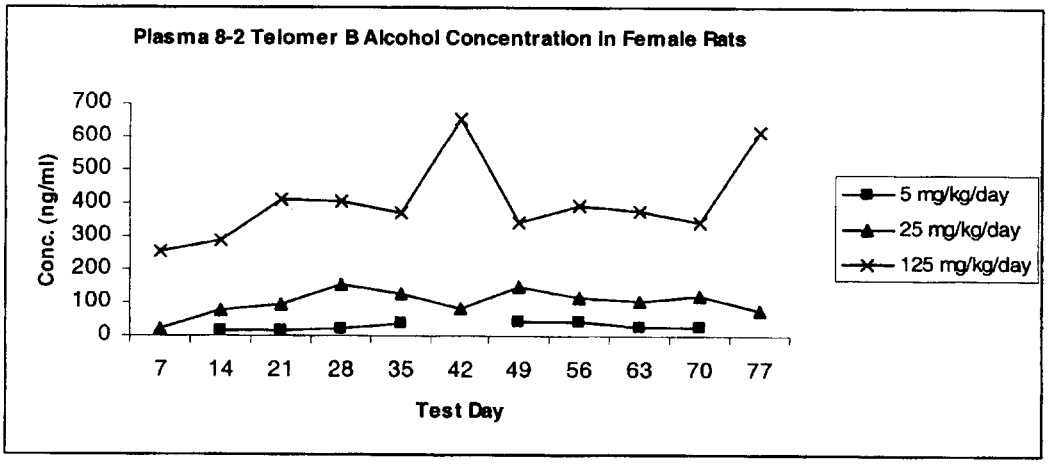
		8-2 Telomer B Alcohol (mg/kg/day)		
		5	25	125
C8-2A (ppm)	Fat	0.07 (0.01)	0.34 (0.13)	1.37 (0.55)
	Liver	0.02 (0.00)	0.13 (0.03)	0.77 (0.53)
	Kidney	ND	0.01 (0.00)	0.07 (0.07)
PFOA (ppm)	Plasma	2.19	8.47	65.60
	Fat	0.09 (0.04)	0.35 (0.12)	1.90 (1.66)
	Liver	6.96 (1.94)	18.68 (10.38)	30.69 (20.68)
	Kidney	1.64 (0.83)	8.43 (4.14)	29.11 (17.38)
PFNA (ppm)	Fat	0.00 (0.00)	0.00 (0.00)	0.01 (0.01)
	Liver	0.51 (0.09)	0.91 (0.23)	1.19 (1.25)
	Kidney	0.00 (0.00)	0.11 (0.09)	0.86 (0.62)

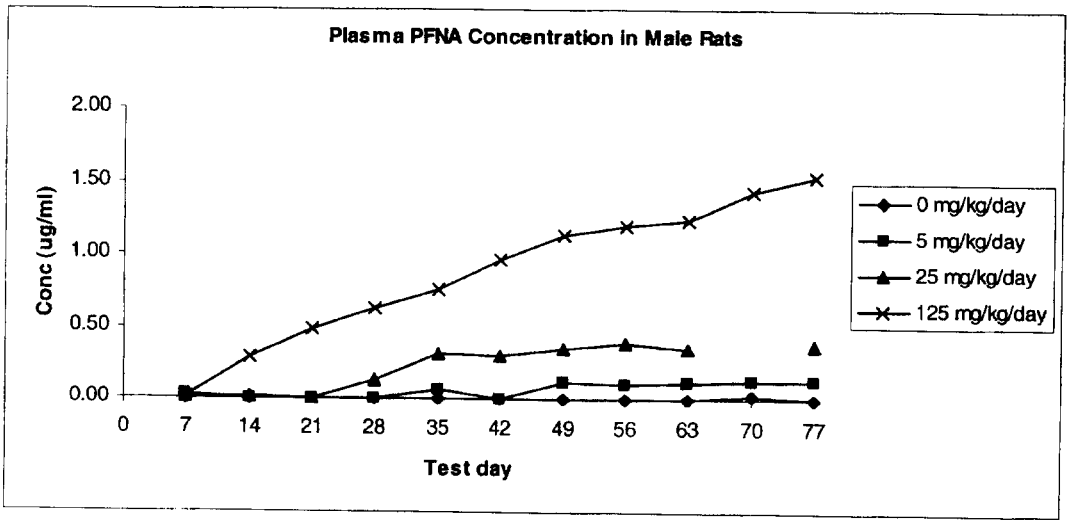
Serum samples were collected throughout the in-life phase of the study and pooled within each dose group (per sex) for analysis of parent compound, PFOA, PFNA, and the 8-2 acid ($C_{10}H_3O_2F_{17}$). Since there is no standard available for the 8-2 acid, we are unable to quantitate or positively identify this species in the plasma (fat, liver and kidney were not analyzed). However, there is an unknown at mass/charge ratio of 477, which is consistent with the 8-2 acid. This 477 species appears to have a dose response relationship, further supporting the premise that this species is related to the metabolism of the 8-2 alcohol. Since it is not possible to quantitate, we have presented the data as area counts rather than absolute concentrations. Although we cannot quantitate, if we assume that the response factor for this unknown metabolite is approximately equal to the response factor for PFNA, then the concentrations would be in the low ppm range (i.e. more similar to PFNA than PFOA). The data are presented below.

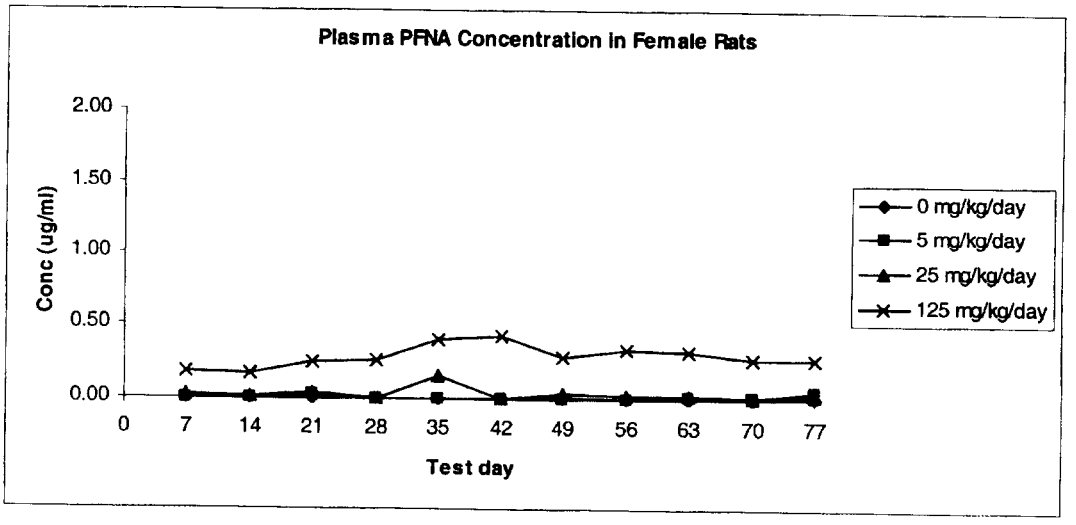

Plasma PFOA Concentration in Male Rats
Conc (ug/ml)
35
30
25
20
15
10
5
0
0 7 14 21 28 35 42 49 56 63 70 77 84
Test day
0 mg/kg/day
5 mg/kg/day
25 mg/kg/day
125 mg/kg/day

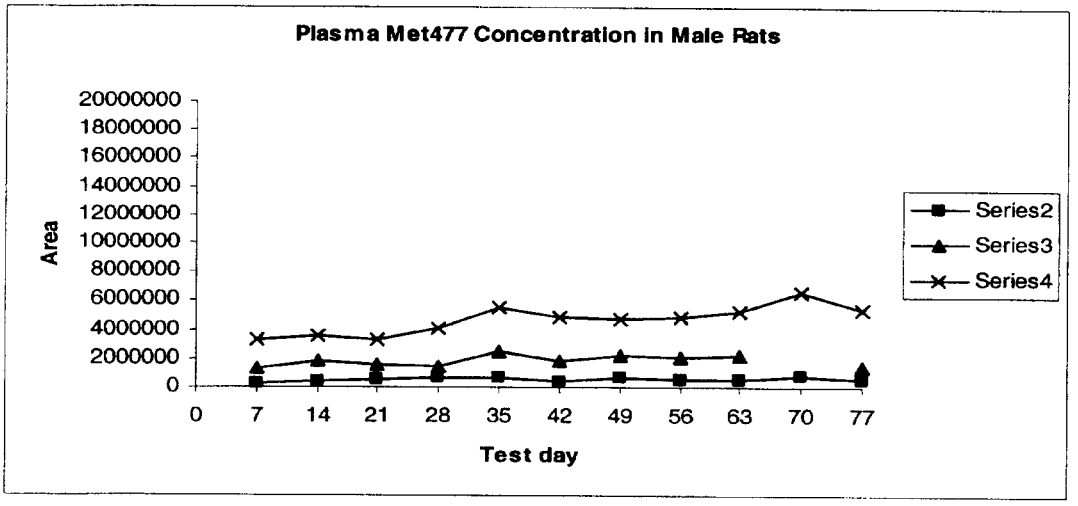

Plasma PFOA Concentration in Female Rats
Conc (ug/ml)
35
30
25
20
15
10
5
0
0 7 14 21 28 35 42 49 56 63 70 77 84
Test day
0 mg/kg/day
5 mg/kg/day
25 mg/kg/day
125 mg/kg/day

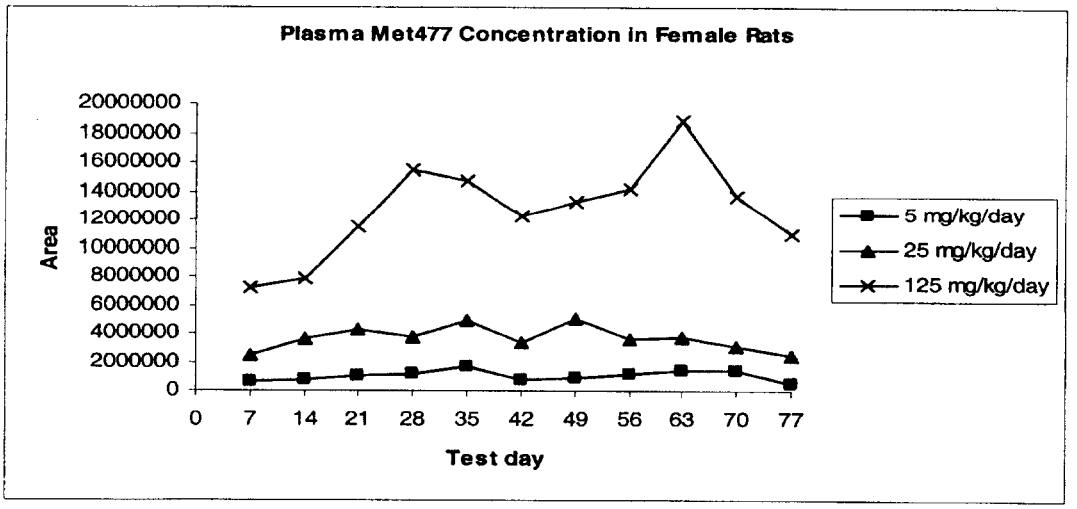

Plasma 8-2 Telomer B Alcohol Concentration in Male Rats
Conc. (ng/ml)
300
250
200
150
100
50
0
7
14
21
28
35
42
49
56
63
70
77
Test Day
5 mg/kg/day
25 mg/kg/day
125 mg/kg/day


Plasma 8-2 Telomer B Alcohol Concentration in Female Rats
Conc. (ng/ml)
700
600
500
400
300
200
100
0
7
14
21
28
35
42
49
56
63
70
77
Test Day
5 mg/kg/day
25 mg/kg/day
125 mg/kg/day


Plasma PFNA Concentration in Male Rats
Conc (ug/ml)
2.00
1.50
1.00
0.50
0.00
0
7
14
21
28
35
42
49
56
63
70
77
Test day
0 mg/kg/day
5 mg/kg/day
25 mg/kg/day
125 mg/kg/day


Plasma PFNA Concentration in Female Rats
Conc (ug/ml)
2.00
1.50
1.00
0.50
0.00
0
7
14
21
28
35
42
49
56
63
70
77
Test day
0 mg/kg/day
5 mg/kg/day
25 mg/kg/day
125 mg/kg/day


Plasma Met477 Concentration in Male Rats
Area
20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0 7 14 21 28 35 42 49 56 63 70 77
Test day
Series2
Series3
Series4


Plasma Met477 Concentration in Female Rats
Area
20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0 7 14 21 28 35 42 49 56 63 70 77
Test day
5 mg/kg/day
25 mg/kg/day
125 mg/kg/day

Appendix 1

8-2 Telomer B Alcohol Repeated-Dose Oral Toxicity Gavage Range-Finding Study in Rats

Work Request Number 13690

Service Code 1583

Protocol

DuPont Animal Welfare Committee Number: CBT-078-P

TABLE OF CONTENTS

INTRODUCTION

The test substance, 8-2 Telomer B Alcohol, is an organofluorine compound used as an intermediate in the production of numerous organofluorine products. The 5, 25, and 125 mg/kg/day dose levels for this study were selected based on the toxicity observed in subchronic studies with similar fluorotelomer-based alcohols and perfluorooctanoic acid (PFOA). Significantly lower mean body weights compared to control were observed in rats administered 100 mg/kg/day or greater of similar fluorotelomer-based alcohols. Liver and kidney weights were also significantly increased compared to control in rats administered dose levels of fluorotelomer-based alcohols as low as 25 mg/kg/day. Furthermore, data suggest that the PFOA metabolite may accumulate in animals dosed with the 8-2 Telomer B Alcohol. In repeat dose studies, PFOA at doses of 10 mg/kg/day or lower produced the following effects: increased liver weights, increased hepatic cell proliferation and beta oxidation, and altered serum hormone levels. The high-dose level of 125 mg/kg/day for this range-finding study was selected based on the ability of rats to tolerate a 100 mg/kg/day dose level in subchronic studies with similar fluorotelomer-based alcohols and is expected to produce toxicity without excessive mortality. The low dose of 5 mg/kg/day is expected to be the no-observed-adverse-effect level, while the 25 mg/kg/day dose is expected to induce minimal toxicity.

OBJECTIVE

The objective of this oral gavage range-finding study is to determine the dose levels for use in a 90-day oral subchronic study. The oral route of administration was selected because it is a potential route of human exposure.

SPONSOR AND TEST FACILITY

This study is sponsored by the Telomer Research Program. The sponsor's approval was effective the date the sponsor submitted the work on the Work Authorization Form. The study will be conducted at Haskell Laboratory for Toxicology and Industrial Medicine,
E. I. du Pont de Nemours and Company, Newark, Delaware.

STUDY DESIGN

The study design is as follows:

Group[a]		No./Group			Metabolism			
Male	Female	Male	Female	Serial bleed	Tissue collection (at steady state blood fluorine levels)	Tissue collection (following fluorine elimination from blood)	Dosage[b] mg/kg/day	Treatment
I	II	10[c,d]	5	First 5 males and all females	Second group of 5 males	All rats	0	Control
III	IV	10	5	First 5 males and all females	Second group of 5 males	All rats	5	8-2 Telomer B Alcohol
V	VI	10	5	First 5 males and all females	Second group of 5 males	All rats	25	8-2 Telomer B Alcohol
VII	VIII	10	5	First 5 males and all females	Second group of 5 males	All rats	125	8-2 Telomer B Alcohol

a Each rat will be assigned its own Haskell animal number. Rats will have the last 3 digits of the unique Haskell animal number marked on their tails.

b Weight/weight concentration of test substance.

c The first 5 rats in Groups I, III, V, and VII are designated for blood collection

d The second group of 5 rats in Groups I, III, V, and VII are designated for select tissue collection following attainment of steady state blood fluorine levels at all dose levels. When steady state is attained at all dose levels, these animals will be sacrificed and livers, kidneys, and fat will be collected and weighed.

Study Parameters	Frequency
Dose Administration	Test Day 0 – to attainment of steady state. All animals will continue to be dosed until last dose group attains steady state for total fluorine in blood.
Body Weight	Twice per week at 3-4 day intervals during dosing; once per week after dosing is stopped
Clinical Observations	At every weighing
Mortality/Moribundity Checks	At least twice daily
Serial bleeding for fluorine and parent/metabolite analysis [approximately 2 hrs after dosing (± 30 minutes)]	Predosing bleed – Day –4 During Dosing – Day 0 and weekly thereafter Postdosing – Day 3, 7, 14, 30, 45, 60, and 75 days
Necropsy (Anatomical Pathology)	
• Select Tissue/Sample Collection following attainment of steady state blood fluorine levels at all dose levels	The second group of 5 males from the 0, 5, 25, and 125 mg/kg/day dose groups will be sacrificed, and liver, kidneys, and fat tissue will be collected and weighed.
• Select Tissue/Sample Collection following elimination of blood fluorine levels	All rats in the 0, 5, 25, and 125 mg/kg/day groups. The date of sacrifice will depend on the rate of elimination of fluorine from the blood after dosing is stopped. Tissues from the control and high dose groups will be analyzed for fluorine levels. The low and middle dose groups may be analyzed as needed to evaluate the rate of elimination of fluorine.

MATERIALS AND METHODS

A. Test Substance

The test substances will be supplied by the sponsor. The test substance(s) were assigned Haskell Laboratory Number 24691.

B. Test Species

Male and female Crl:CD®(SD)IGS BR rats will be obtained from Charles River Laboratories, Inc. The address of the supplier (city/state) will be documented in the study records and final report. The Crl:CD®(SD)IGS BR rat has been selected on the bases of extensive experience with this strain at Haskell Laboratory and its suitability with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous diseases.

C. Animal Husbandry

All rats will be housed in stainless steel, wire-mesh cages suspended above cage boards. Animal rooms will be maintained at a temperature of 22 ± 3°C and a relative humidity of 50 ± 20%. Animal rooms will be artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle.

All rats will be provided tap water *ad libitum*. All rats will be fed PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 *ad libitum*.

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to assure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Feed samples are analyzed for total bacterial, spore and fungal counts.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Data are maintained separately from study records and may be included in the final report at the discretion of the study director.

D. Pretest Period

Upon arrival at Haskell Laboratory, all rats will be housed 1 per cage, sexes separate, in quarantine. The rats will be:

- quarantined for a minimum of 5 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine.
- observed with respect to weight gain and any gross signs of disease or injury.

The rats will be released from quarantine by the laboratory animal veterinarian or designee on the bases of body weights and clinical signs of all rats.

Rats that are accidentally killed or removed from study during the pretest period will be discarded without necropsy. Rats that are found dead or sacrificed *in extremis* during the pretest period will be sent to Pathology and given a gross examination to check for the presence of disease. Dependent upon these findings, further diagnostic procedures may be employed at the discretion of the study director, a pathologist, or the laboratory animal veterinarian. The results will not be reported in the final report unless considered significant to the evaluation of the study.

E. Assignment to Groups

Rats of each sex, selected on the bases of adequate body weight gain and freedom from any clinical signs of disease or injury will be distributed by computerized, stratified randomization into study groups as designated in the Study Design, so that there are no statistically significant differences among group body weight means within a sex. The weight variation of selected rats will not exceed ± 20% of the mean weight for each sex.

After assignment to groups, each rat will be housed individually. Information on the cage labels will include the unique Haskell animal number and the unique individual identification number assigned to each rat. The last 3 or 4 digits of the unique Haskell animal number will be tattooed on the tail of each rat.

At study start (test day 0) the rats will be 7 weeks old. On test day 0, when possible, rats with body weights that are not within ± 20% of the mean within a sex, will be removed from study and replaced with rats having body weights within that range (subject to the same selection criteria as the original rats).

Rats that have not been assigned to a test group or which have been removed from study on test day 0, for out-of-range body weight, will be released for other laboratory purposes, or be sacrificed by carbon dioxide asphyxiation and discarded without pathological evaluation, at the discretion of the study director.

F. Dosing Solution Preparation and Sampling

The test substance will be suspended in methylcellulose. Dosing solutions of the test substance will be prepared daily.

G. Administration of Dosing Suspensions

The test substance will be suspended in methylcellulose. The rats will be dosed by intragastric intubation until steady state is obtained at a volume of 5 mL/kg of body weight. Animals will be dosed 7 days a week. The amount of test substance each rat receives will be based on the body weights collected twice a week and the suspension concentration. Control rats will be dosed with methylcellulose at a volume of 5 mL/kg of body weight.

H. Body Weights

The rats will be weighed twice per week at 3 to 4 day intervals during the study.

I. Clinical Observations and Mortality

During the test period, cage-site examinations to detect moribund or dead rats and abnormal behavior and/or appearance among rats will be conducted at least twice daily throughout the study. Moribund rats will be sacrificed and discarded without necropsy. At every weighing, each rat will be individually handled and examined for abnormal behavior and appearance. Clinical signs noted will be recorded. Animals that do not exhibit clinical signs of toxicity will be recorded as "no abnormality detected" (NAD) in the study records.

Prior to sacrifice of moribund rats, if possible and at the discretion of the clinical pathologist and/or study director, blood samples for hematology and clinical chemistry measurements may be collected for clinical pathology evaluations.

J. Blood Collection for Total Fluorine and Parent/Metabolite Evaluation

Prior to dosing (test day –4), day 0, and 7 days after study start, and weekly thereafter until steady state for total fluorine in blood is attained, blood (approximately 1 mL) will be collected from the orbital sinus of both males and females while under light carbon dioxide anesthesia for total fluorine and parent/metabolite analysis approximately 2 hours (± 30 minutes) after dosing. For males, the first 5 animals in Groups I, III, V, and VII are designated for blood collection. The blood will be collected in plastic tubes containing EDTA while on ice. Approximately 0.5 ml of blood from each animal will be removed and stored frozen until analyzed for total fluorine levels. The remaining blood (approximately 0.5 ml) from each animal will then be pooled by dose group and separated into plasma and RBC. The separated plasma/RBC will be stored frozen until a qualitative analysis for parent and metabolites is performed. For each bleeding, blood should be collected at approximately the same time of day. All animals will continue to be dosed until the last dose group attains steady state for total fluorine in blood.

Blood will also be collected for total fluorine analysis at 3, 7, 14, 30, 45, 60, and 75 days postdosing for each dose group. The blood samples collected postdosing may be analyzed as needed to answer questions regarding distribution and metabolism of the test substances.

K. Anatomical Pathology

1. Pretest

See Materials and Methods, Section D. Pretest Period.

2. Dosing Phase

Moribund rats and rats scheduled for sacrifice will be sent to Pathology for sacrifice. Rats will be euthanatized by carbon dioxide anesthesia and exsanguination and will be discarded without necropsy. Rats found dead will be discarded without necropsy.

a) Select Tissue/Sample Collection following attainment of steady state blood fluorine levels of all dose groups.

The second group of five male rats in the control, low, intermediate, and high dose groups will be sent to Pathology for sacrifice. Rats will be euthanatized by carbon dioxide anesthesia and exsanguination. The following tissues will be collected and weighed:

Digestive System	Miscellaneous
liver	fat

Excretory System
kidneys

These tissues will be placed in plastic freezer bags and stored in the freezer until a qualitative analysis of parent and metabolites can be performed.

3. Postdosing Phase

a) Select Tissue/Sample Collection following elimination of blood fluorine levels

All remaining rats in the control, low, middle, and high dose groups will be sent to Pathology for sacrifice and a gross evaluation and collection of tissues. Rats will be euthanatized by carbon dioxide anesthesia and exsanguination.

The following tissues will be collected and weighed from rats sacrificed by design:

Digestive System
liver

Reproductive System
Male
testes

Miscellaneous
fat

The tissues will be placed in plastic freezer bags and stored in the freezer until analysis of total fluorine levels.

SAFETY AND HOUSEKEEPING

Good housekeeping procedures will be practiced to avoid contamination of dosing solution preparation facilities and potential health hazards. To avoid skin contact, gloves will be worn when handling the test substance or dosing solutions. In addition, the test substance will be handled in a chemical hood. Dosing solutions will be prepared in properly ventilated areas. Animal carcasses and feces will be incinerated.

RECORDS AND SAMPLE RETENTION

All original records will be retained at Haskell Laboratory, E. I. du Pont de Nemours and Company, Newark, Delaware or at Iron Mountain Records Management, 200 Todds Lane, Wilmington, Delaware. Laboratory-specific or site-specific records such as personnel files and equipment records will be retained at the facility where the work was done.

PROTOCOL APPENDIX

Study Function	Study Personnel
Study Director:	Gregory S. Ladics, Ph.D. Senior Research Scientist
Anatomical Pathologist	G. Tracy Makovec, D.V.M. Senior Research Scientist.
Study Dates	
Prebleed	April 16, 2001
Initiation of Test Substance Administration	April 20, 2001
Scheduled Sacrifice	Dependent upon the elimination rate of fluorine from the blood of the various dose groups

SIGNATURES

Approved by:

Janine Britton, B.S.
Primary Technician
13 April 01
Date

G. Tracy Makovec, D.V.M.
Diplomate A.C.V.P.
Senior Research Scientist
13-APR-2001
Date

Judith C. Stadler, Ph.D., D.A.B.T.
Director
General Toxicology
13-APR-2001
Date

Gregory S. Ladics, Ph.D., D.A.B.T.
Senior Research Scientist
Study Director
12-April-2001
Date

cc: Merralyn Vaillancourt
John O'Connor
Sue Craven
Charlene Smith
Janet Maslanka
Gary Jepson
Paul Hinderliter
Vladamir Capka
Shawn Gannon
Nita Baker

Work Request Number 13690

Service Code 1583

Protocol Amendment No. 1

The protocol is amended as follows

1. Page 8, Materials and Methods, F. Dosing Solution Preparation and Sampling, add as second paragraph:

   "Homogeneity and concentration verification and stability for 8-2 Telomer B Alcohol will be conducted during the dose range-finding study."

Rationale: To establish mixing uniformity and stability (5-hour room temperature and 7-day refrigerated along with 5-hour room temperature) for the 90-day repeat dose study.

Approved by: Gregory S. Ladics 24-April-2001

Gregory S. Ladics, Ph.D., D.A.B.T.
Senior Research Scientist
Study Director

cc: Merralyn Vaillancourt
John O'Connor
Judy Stadler
Tracy Makovec
Janine Britton
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Work Request Number 13690

Service Code 1583

Protocol Amendment No. 2

The protocol is amended as follows (changes are bolded):

1. Page 4, Study Design, Study Design table, footnote d, change to read

   "The second group of 5 rats in Groups I, III, V, and VII are designated for select tissue collection following attainment of steady state blood fluorine levels at all dose levels. When steady state is attained at all dose levels, these animals will be sacrificed and livers, kidneys, and fat will be collected and weighed, **then placed in plastic freezer bags and stored in the freezer until a qualitative analysis of parent and metabolites can be performed.**"

2. Page 5, Study Design, Study Parameter table, Select Tissue/Sample Collection following attainment of steady state blood fluorine levels at all dose levels, change to read

   "The second group of 5 males from the 0, 5, 25, and 125 mg/kg/day dose groups will be sacrificed, and liver, kidneys, and fat tissue will be collected and weighed, **then placed in plastic freezer bags and stored in the freezer until a qualitative analysis of parent and metabolites can be performed.**"

Rationale: To complete the collection and storage methods for the specified tissues.

Approved by: _____ 11-July-2001
Gregory S. Ladics, Ph.D., D.A.B.T. Date
Study Director

cc:

Merralyn Vaillancourt	John O'Connor
Judy Stadler	Tracy Makovec
Janine Britton	Sue Craven
Charlene Smith	Janet Maslanka
Gary Jepson	Paul Hinderliter
Vladamir Capka	Shawn Gannon
Nita Baker	

Work Request Number 13690

Service Code 1583

Protocol Amendment No. 3

The protocol is amended as follows (changes are bolded):

Page 8, H. Body Weights, change to read

The rats will be weighed twice per week at 3 to 4 day intervals during dosing, **then once per week after dosing is stopped.**

Rationale: To accurately describe the frequency of body weight collection.

Approved by: _____ 19-July-2001
Gregory S. Ladics, Ph.D., D.A.B.T. Date
Study Director

cc:

Merralyn Vaillancourt	John O'Connor
Judy Stadler	Tracy Makovec
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Work Request Number 13690

Service Code 1583

Protocol Amendment No. 4

The protocol is amended as follows (changes are bolded):

1. Pages 5, Study Design, parameter for serial bleeding, change post dosing blood collection day from 30 to **31**.

2. Page 9, J. Blood Collection for Total Fluorine and Parent/Metabolite Evaluation, second paragraph, first sentence, change blood collection day 30 to **31**.

Rationale for items 1 and 2: The blood collection schedule was changed.

3. Page 9, K. Anatomical Pathology, 2. Dosing Phase, first paragraph is changed to read:

   Moribund rats will be euthanatized by carbon dioxide anesthesia and exsanguination and will be discarded without necropsy. Rats that are found dead will be discarded without necropsy.

Rationale: To accurately describe the disposition of moribund rats.

Approved: _____ 9-Aug-2001
Gregory S. Ladics, Ph.D., D.A.B.T.
Study Director

cc:
Merralyn Vaillancourt
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Work Request Number 13690

Service Code 1583

Protocol Amendment No. 5

The protocol is amended as follows:

Page 10, 3. Postdosing Phase, a. Select Tissue/Sample Collection following elimination of blood fluorine levels, add the following to the list of tissues.

Excretory System
kidneys

Rationale: The kidneys were inadvertently omitted from this list of tissues in the protocol.

Approved by: _____ 6-Sept-2001
Gregory S. Ladics, Ph.D., D.A.B.T.
Study Director

cc:

Merralyn Vaillancourt	John O'Connor
Judy Stadler	Tracy Makovec
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