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Study Title

H-24159: Biopersistence Screening
Gavage Study in Rats

Laboratory Project ID: DuPont-6732

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CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

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STUDY INFORMATION

TEST SUBSTANCE:

Substance Tested: [redacted]

Synonyms/Codes: [redacted] H-24159 [redacted]

Haskell Number: 24159

Composition: [redacted]

Known Impurities: [redacted]

Sponsor: E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: June 11, 2001 / (see report cover page)

In-Life Initiated/Completed: June 15, 2001 / October 29, 2001

STUDY PERSONNEL

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SUMMARY

The objective of this study was to evaluate the potential for H-24159, when administered by gavage, to be absorbed and to accumulate in a mammalian system following the attainment of steady-state blood fluorine levels. Two groups of 10 male Crl:CD®(SD)IGS BR rats each were exposed to 35 mg/kg/day of H-24159 or deionzied water for 52 consecutive days. Blood was collected from the orbital sinus of 10 rats approximately 2 hours after dosing (except on test day 53) on test days 0, 4, 9, 14, 21, 28, 35, 42, 49, and 53. Beginning on test day 50, animals were placed in metabolism cages and urine and feces were collected for total fluorine analysis for 3 days at 24 hour intervals from the first 5 males in the control and 35 mg/kg/day group. The urine and feces obtained from a particular dose group was pooled. After the collection of urine and feces, the first 5 males in each dose group were sacrificed on test day 53 and liver, kidney and fat were collected, weighed and stored frozen until fluorine analysis. Blood was also collected for total fluorine analysis at 3, 7, 14, 21, 35, 56, and 84 days after the final dosing of each group. On postdosing day 84, the remaining rats were sacrificed and the liver, kidneys, and fat were collected, weighed and stored frozen until fluorine analysis. Body weights and clinical signs were evaluated twice per week.

No deaths occurred during the study. No test-substance-related clinical signs were observed. No statistically significant differences in mean body weights compared to control were observed for the group treated with the test substance. No statistically significant differences in mean body weight gain compared to control were observed for the treated group with the exception of a significant increase in mean body weight gain during the 95-98 and 102-105 test day intervals. Mean liver and kidney weights relative to body weight were similar to control in the test substance treated group.

The levels of total fluorine in rat blood rose rapidly and reached steady-state in approximately 45 days with a maximum equivalent concentration (C_{max}) of 62.1 ± 7.8 μM equivalents and a terminal elimination half-life of 38.0 days. Fluorine was present in each of the examined rat tissues with the relative ranking of liver > kidney > fat > blood at both the steady state and recovery time points. The ratio of tissue to blood fluorine was nearly equivalent at both time points. Under steady-state conditions, fluorine was eliminated in urine and feces at a rate of 5 mmoles/kg body wt/day.

INTRODUCTION

The test substance, H-24159 is [redacted] A 10-dose oral gavage biopersistence screening study was conducted with 35 mg/kg/day H-24159. Under the conditions of this initial study, the administration of H-24159 to male rats for 10 consecutive days resulted in moderate absorption and retention of fluorine in the blood. However, a steady-state for fluorine levels in whole blood was not achieved during the 10 consecutive days of dosing with 35 mg/kg H-24159. Therefore, the objective of this second biopersistence screening study was to dose males with 35 mg/kg/day H-24159 until a steady-state for fluorine levels in whole blood was achieved. The potential of H-24159 to be absorbed and to bioaccumulate in a mammalian system, as indicated by analytical determination of total fluorine in blood, liver, kidney, and fat was then evaluated.

MATERIALS AND METHODS

A. Test Substance

The test substance, H-24159, was supplied by the sponsor as [redacted] The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

B. Test Species

Male Crl:CD®(SD)IGS BR rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The Crl:CD®(SD)IGS BR rat was selected on the basis of extensive experience with this strain and its suitability with respect to longevity and low incidence of spontaneous diseases.

C. Animal Husbandry

1. Housing Environment

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

2. Feed and Water

Tap water was provided *ad libitum*. All rats were fed PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 chow *ad libitum*.

3. Identification

Prior to assignment to groups, each rat was assigned a unique identification number which was recorded on a card affixed to the cage. After assignment to groups, the last 3 digits of the individual identification number were tattooed on the tail of each rat and recorded on the cage card.

4. Animal Health Monitoring Program

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures were performed periodically to ensure that contaminant levels were 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. Evaluation of these data did not indicate any conditions that affected the validity of the study.

D. Quarantine and Pretest

Upon arrival at Haskell Laboratory, the rats were removed from shipping cartons and quarantined for 6 days. The rats were weighed 3 times during the pretest period and examined daily for any clinically apparent signs of disease or injury. The rats were observed daily for mortality and signs of illness, injury, or abnormal behavior.

On the bases of acceptable body weight gains and freedom from clinically apparent signs of disease or injury, the rats were released from quarantine by the laboratory animal veterinarian or designee.

E. Study Design

Substance	Vehicle	Dosage (mg/kg)	Number of Animals
Negative Control Deionized water	Not applicable	0	10
Test Substance H-24159	Deionized water	35	10

F. Assignment to Groups and Study Start

After the quarantine period, the rats were selected on the bases of adequate body weight gain and freedom from any clinical signs of disease or injury. The selected rats were distributed by computerized, stratified randomization into study groups as designated in the Study Design, so that there were no statistically significant differences between group body weight means.

After assignment to groups, each rat was housed individually. The rats were 7.5 weeks of age at the time of dosing. Dosing began on test day 0.

Rats that were not assigned to the study were released for other laboratory purposes, or were sacrificed by carbon dioxide asphyxiation and discarded without pathology evaluation.

G. Dosing Material Preparation and Administration

1. Test Substance

H-24159 was prepared daily as a solution in deionized water. The rats were dosed by intragastric intubation at a volume of 5 mL/kg body weight until steady state was attained and urine and feces collected. The amount of test substance each rat received was based on the most recent body weight collected and the dose solution concentration. The mixture was stirred on a magnetic stir plate throughout the dosing procedure to maintain homogeneity.

2. Negative Control

Deionized water was chosen as the negative control. The control rats were dosed at a volume of 5 mL/kg of body weight.

H. Body Weights

All rats were weighed twice per week at 3- to 4-day intervals.

I. Mortality and Clinical Observations

Cage-site examinations to detect moribund or dead rats and abnormal behavior and appearance among rats were conducted at least twice daily throughout the study. At every weighing, each rat was individually handled and examined for abnormal behavior and appearance.

J. Collection of Blood, Urine, and Feces for Total Fluorine Level Evaluation

On test day –4 (pre-bleed) and test days 0, 4, 9, 14, and weekly thereafter until attainment of steady-state blood fluorine levels (i.e. day 49), blood (approximately 0.6 mL) was collected from the orbital sinus of animals while they were under light carbon dioxide anesthesia. On the day of blood collection (except test day –4 and 53), blood was collected from the animals 2 hours (± 30 minutes) after dosing. The blood was collected in plastic tubes containing EDTA while on ice. For each bleeding, blood was collected at approximately the same time of day. Beginning on test day 50, the first 5 rats in the control and test groups were placed in metabolism cages and urine and feces were collected daily for 3 days at 24-hour intervals. The urine and feces obtained from a particular dose group was pooled. The exact time period of collection of urine and feces was documented along with the total volume of pooled urine and weight of pooled feces obtained. Urine and feces were stored frozen until analyzed for total fluorine. Blood was also collected for total fluorine analysis at 3, 7, 14, 21, 35, 56, and 84 days after the final dosing of each group and stored frozen.

K. Anatomical Pathology

1. Dosing Phase

Following the attainment of steady blood fluorine levels and subsequent collection of urine and feces, the first 5 rats in the control and treated groups were euthanatized by carbon dioxide anesthesia and exsanguination and the following tissues were collected and weighed: liver, kidneys, and fat. The tissues were stored frozen until analysis of total fluorine levels.

2. Postdosing Phase

On postdosing day 84, all remaining rats in the control and treated groups were euthanatized by carbon dioxide anesthesia and exsanguination and the following tissues were collected, weighed, and stored frozen until analysis of total fluorine levels: liver, kidneys, and fat.

L. Fluorine Analysis

The blood, tissue, and urine and feces samples were shipped frozen to Jackson Laboratory, Deepwater, New Jersey where they were analyzed for total fluorine. The total fluorine content of the samples was determined by using a Wickbold torch combustion method, followed by analysis with a fluoride ion selective electrode. The samples were decomposed or volatilized in the presence of wet oxygen and swept through an oxy-hydrogen flame in a closed quartz apparatus.

The combustion products were collected in an aqueous absorbing solution and analyzed with a fluoride ion selective electrode. Kinetic analysis of the ppm F in each sample was performed by Haskell Laboratory personnel for evaluation of fluorine biopersistence.

The percent of fluorine and molecular weight of the test substance was used as provided by the sponsor. The measured total fluorine values were used as received (ppm F) from Jackson Laboratory. For the Wickbold torch method, the background fluorine level is 0.2 ppm. This is the limit of detection (LOD) of this method and was subtracted from each sample. The limit of quantification (LOQ) for this method is 0.5 ppm, and any values listed as less than 0.5 ppm were excluded from further treatment.

Noncompartmental analysis was conducted on fluorine data derived from rats dosed with H-24159 using WinNonlin Version 3.1 software (Pharsight Corp, Mountain View, CA). WinNonlin software provided a means of computing derived pharmacokinetic parameters from experimental data.

The maximum observed concentration in blood was C_{max} (μM equivalent). Biopersistence was assessed by quantifying terminal elimination blood half-life ($T_{½}$, days). The points included in determination of the $T_{½}$ were selected manually and included only points after apparent log-linear elimination was achieved. Internal exposure was determined by calculating the blood area-under-the-curve (AUC). AUC, which is simply the integral of blood concentration over time, is the most common means for expressing internal dose. With the calculated half-life, the AUC can be extrapolated to infinity (AUCINF) to reflect the elimination of the compound.

M. Statistical Methods

Descriptive statistics (e.g. mean, standard deviation) were used for organ weights.

Significance was judged at $p < 0.05$.

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain	Test for lack of trend[1]	Sequential application[2] of the Jonckheere-Terpstra trend test[3]	Preliminary tests for pairwise comparison
	OR		
	Levene's test for homogeneity[4] and Shapiro-Wilk test[5] for normality[a]	One-way analysis of variance[6] followed with Dunnett's test[7]	Kruskall-Wallis test[8] followed with Dunn's test[9]
Incidence of Clinical Observations	None	Cochran-Armitage test for trend[6]	

a If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used.

RESULTS AND DISCUSSION

A. In-Life Toxicology
(Tables 1-2, Figure 1, Appendices A-B)

No deaths occurred during the study. No test-substance-related clinical signs were observed. No statistically significant differences in mean body weights compared to control were observed for the test-substance treated group. No statistically significant differences in mean body weight gain compared to control were observed for the test-substance treated group with the exception of a significant increase in mean body weight gain during the 95-98 and 102-105 test day intervals.

B. Liver Weights
(Table 3)

1. Test Substance

The absolute liver weight of rats dosed with the test substance, H-24159, was 9% lower on test day 53 compared to control, while the mean relative liver weights (liver/body weight) of the control and test substance treated animals were the same. At the end of the recovery period (test day 136), the absolute liver weights of the H-24159 treated animals were 15% higher than control, while the mean relative liver weights were similar to control.

C. Kidney Weights
(Table 4)

The absolute and relative kidney weights of rats dosed with H-24159 were similar to control on test day 53. At the end of the recovery period, absolute kidney weights of the H-24159 treated animals were 24% higher than control, while the mean relative kidney weights were similar to control.

D. Fluorine Data
(Tables 5-12, Figures 2-4, Appendices C-H)

Total blood fluorine concentrations were converted to micromolar (μM, micromoles per liter) equivalents of active fluorine-containing component for use in kinetic analysis (Appendix D). The level of fluorine in rat blood rose rapidly and reached steady-state in approximately 45 days (Figure 2). The maximum fluorine concentration (C_{max}) was 62.1 ± 7.8 μM equivalents (Mean ± SD). Under steady-state conditions, fluorine equivalents were eliminated in urine and feces at a rate of 5 mmoles/kg body wt/day. The fluorine in urine and feces on test days 51-53 are shown in Appendix H. The terminal elimination half-life was 38.0 days. The total internal exposure to

fluorine as presented in terms of the area under the blood curve extrapolated to infinity (AUCINF, μM x days) was 4.24×10^3. The calculations and equations are shown in Appendix C.

The concentration of fluorine in rat blood, liver, fat and kidney at study days 53 and 136 are shown in Appendices E - G. The relative ranking of fluorine in rat tissues was liver > kidney > fat > blood at both study days 53 and 136 (Figure 4). The ratio of tissue to blood fluorine was nearly equivalent at both the dosing and recovery time points. Fluorine concentrations in the control group blood and tissues were generally below the limit of quantification.

The data used in the kinetic analysis were derived from a limited screen, and therefore several caveats and considerations are important. These considerations include the limitations of single dose studies when extrapolating to other doses, small sample size, limited data points for estimating elimination kinetics, and the limiting of kinetic analysis to only the blood compartment. A more complete list of considerations is shown in Appendix C.

CONCLUSIONS

Rats dosed for 52 days with 35 mg/kg/day H-24159 exhibited no mortality, test substance-related clinical signs, or body weight effects. Mean liver and kidney weights relative to body weight were similar to control in the test substance treated animals.

The fluorine μM equivalents in rat blood rose rapidly and reached steady-state in approximately 45 days with a maximum fluorine concentration (C_{max}) of 62.1 $\pm$ 7.8 μM equivalents and a terminal elimination half-life of 38.0 days. Fluorine was present in each of the examined rat tissues with the relative ranking of liver > kidney > fat > blood at both the steady state and recovery time points. The ratio of tissue to blood fluorine equivalents was nearly equivalent at both time points. Under steady-state conditions, fluorine was eliminated in urine and feces at a rate of 5 mmoles/kg body wt/day.

RECORDS AND SAMPLE STORAGE

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 raw data such as personnel files and equipment records will be retained at the facility where the work was done.

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TABLES

TABLE 1

MEAN BODY WEIGHTS

Test Days	Negative Control Deionized Water	Test Substance H-24159
	Dosing	
0	260.8	258.7
4	282.9	280.1
7	301.6	298.4
11	318.4	316.3
14	337.5	322.2
18	350.1	347.3
21	363.9	360.0
25	374.9	368.6
28	387.6	380.2
32	396.0	386.8
35	403.8	394.5
39	411.6	400.1
42	421.3	409.1
46	431.1	418.2
49	442.3	428.3
53	441.3	431.1
	Recovery	
56	436.0	459.6
60	441.7	471.4
63	452.0	480.3
67	456.8	485.6
70	463.7	499.0
74	467.5	504.8
77	477.5	513.1
81	479.4	519.0
84	485.8	524.1
88	488.0	529.4
91	490.1	537.0
95	493.1	539.2
98	497.0	550.5
102	504.1	558.8
105	506.3	568.2
109	506.8	569.6
112	511.1	576.2
116	519.1	585.4

TABLE 1 (Continued)

MEAN BODY WEIGHTS

Test Days	Negative Control Deionized Water	Test Substance H-24159
119	523.9	587.8
123	532.1	592.6
126	536.6	597.7
130	542.5	604.5
133	545.7	607.8
136	546.2	604.5

There were no statistically significant differences from vehicle control at $p < 0.05$.

TABLE 2

MEAN BODY WEIGHT GAINS (g)

Dosing and Recovery

Test Days	Negative Control Deionized Water	Test Substance H-24159
Day 0 - Day 4	22.1 7.2(10)	21.4 6.5(10)
Day 4 – Day 7	18.7 6.1(10)	18.4 5.4(10)
Day 7 – Day 11	16.8 5.7(10)	17.8 4.3(10)
Day 11 - Day 14	19.1 6.4(10)	16.0 4.9(10)
Day 14 - Day 18	12.7 5.8(10)	15.1 4.2(10)
Day 18 - Day 21	13.8 4.6(10)	12.7 3.0(10)
Day 21 - Day 25	11 8.1(10)	8.6 7.2(10)
Day 25 - Day 28	12.8 4.1(10)	11.5 3.9(10)
Day 28 - Day 32	8.4 10.7(10)	6.6 4.4(10)
Day 32 - Day 35	7.7 4.0(10)	7.7 4.3(10)

TABLE 2 (Continued)

MEAN BODY WEIGHT GAINS (g)

Test Days	Negative Control Deionized Water	Test Substance H-24159
Day 35 - Day 39	7.8 11.1(10)	5.6 10.7(10)
Day 39 - Day 42	9.8 3.4(10)	9 3.4(10)
Day 42 - Day 46	9.7 11.2(10)	9.1 6.3(10)
Day 46 - Day 49	11.2 3.5(10)	10.2 4.1(10)
Day 49 - Day 53	-1 17.7(10)	2.8 18.3(10)
Day 53 - Day 56	0.2 4.7(5)	0.2 5.3(5)
Day 56 - Day 60	5.7 6.3(5)	11.8 6.0(5)
Day 60 - Day 63	10.3 4.4(5)	8.9 4.0(5)
Day 63 - Day 67	4.8 7.4(5)	5.3 4.8(5)
Day 67 - Day 70	6.9 6.8(5)	13.4 7.5(5)
Day 70 - Day 74	3.9 3.4(5)	5.9 4.2(5)

TABLE 2 (Continued)

MEAN BODY WEIGHT GAINS (g)

Test Days	Negative Control Deionized Water	Test Substance H-24159
Day 74 - Day 77	10 3.1(5)	8.2 3.8(5)
Day 77 - Day 81	1.8 9.2(5)	5.9 2.1(5)
Day 84 - Day 88	2.2 7.0(5)	5.3 4.0(5)
Day 81 - Day 84	6.4 3.4(5)	5.1 1.6(5)
Day 88 - Day 91	2.1 5.5(5)	7.6 2.2(5)
Day 91 - Day 95	3 4.9(5)	2.1 6.4(5)
Day 95 - Day 98	3.9 8.9(5)	11.4@ 3.7(5)
Day 98 - Day 102	7.1 3.2(5)	8.2 3.8(5)
Day 102 - Day 105	2.2 4.2(5)	9.5* 2.3(5)
Day 105 - Day 109	0.5 6.5(5)	1.4 4.7(5)
Day 109 - Day 112	4.3 6.1(5)	6.6 4.0(5)

TABLE 2 (Continued)

MEAN BODY WEIGHT GAINS (g)

Test Days	Negative Control Deionized Water	Test Substance H-24159
Day 112 - Day 116	8 7.1(5)	9.2 5.4(5)
Day 116 - Day 119	4.8 2.4(5)	2.4 3.6(5)
Day 119 - Day 123	8.2 5.6(5)	4.8 4.0(5)
Day 123 - Day 126	4.6 6.1(5)	5.2 2.2(5)
Day 126 - Day 130	5.9 3.2(5)	6.7 4.1(5)
Day 130 - Day 133	3.2 2.9(5)	3.3 4.4(5)
Day 133 - Day 136	0.5 4.5(5)	-3.3 10.1(5)
Day 0 – Day 53	180.5 64.1(10)	172.5 39.7(10)
Day 53 – Day 136	110.4 19.5(5)	145.1 46.2(5)

Data summarized as: Mean
Standard Deviation (n)

@ Nonparametric comparison to control (Dunn's) significant
* Parametric comparison to control (Dunnett/Tamhane-Dunnett) significant

TABLE 3

MEAN BODY AND LIVER WEIGHTS (g)

DEIONIZED WATER (NEGATIVE CONTROL)

Test Days	n	Absolute Body Weight	Absolute Liver Weight	Mean Relative Liver Weight (Liver/Body Weight)
53	5	446.8	15.192	0.034
136	5	546.2	18.379	0.034

H-24159 (TEST SUBSTANCE)

Test Days	n	Absolute Body Weight	Absolute Liver Weight	Mean Relative Liver Weight (Liver/Body Weight)
53	5	402.8	13.794	0.034
136	5	604.5	21.219	0.035

TABLE 4

MEAN BODY AND KIDNEY WEIGHTS (g)

DEIONIZED WATER (NEGATIVE CONTROL)

Test Days	n	Absolute Body Weight	Absolute Kidney Weight	Mean Relative Kidney Weight (Kidney/Body Weight)
53	5	446.8	3.153	0.007
136	5	546.2	6.382	0.012

H-24159 (TEST SUBSTANCE)

Test Days	n	Absolute Body Weight	Absolute Kidney Weight	Mean Relative Kidney Weight (Kidney/Body Weight)
53	5	402.8	3.178	0.008
136	5	604.5	7.937	0.013

TABLE 5

MEAN BLOOD FLUORINE LEVELS

Test Days	Negative Control: Deionized Water (ppm F)	Test Substance: H-24159 (ppm F)
-4	[a]	[a]
0	-	4.39 (0.8)[b]
4	[a]	9.30 (3.1)
9	0.84[c] (0.2)	10.37 (2.7)
14	0.57[d] (0.1)	11.53 (1.7)
21	-	11.08 (2.0)
28	-	14.46 (1.8)
35	-	14.09 (1.6)
42	-	15.58 (1.9)
49	[a]	14.04 (1.3)
53	[a]	12.76 (1.9)
55	[a]	7.98 (1.3)
59	[a]	8.42 (0.9)
66	-	5.74 (0.6)
73	0.80[e] (0.5)	4.74 (1.0)
87	-	3.96 (0.3)
108	0.70[f] (0.1)	2.54 (0.3)
136	[a]	1.74 (0.4)

a All values were below the limit of quantification (LOQ, < 0.5)
b Standard deviation is in parentheses.
c Mean of 7 of the 10 values. Three of the values were below the LOQ.
d Mean of 3 of the 10 values. Seven of the values were below the LOQ.
e Mean of 3 of the 5 values. Two of the values were below the LOQ.
f Mean of 2 of the 5 values. Three of the values were below the LOQ.
- Indicates no fluorine analysis performed on sample for this day.

Note: Only 5 values tested for days 53 to 136 (recovery).

TABLE 6

MEAN BLOOD FLUORINE CONCENTRATION EXPRESSED IN μM EQUIVALENTS

	Test Substance
Test Days	H-24159 μM Equivalents F
-4	[a]
0	16.93 (3.35)[b]
4	36.77 (12.51)
9	41.09 (11.03)
14	45.78 (6.99)
21	43.96 (7.93)
28	57.62 (7.25)
35	56.12 (6.59)
42	62.14 (7.80)
49	55.92 (5.37)
53	50.75 (7.51)
55	31.43 (5.14)
59	33.21 (3.81)
66	22.38 (2.30)
73	18.34 (4.04)
87	15.19 (1.09)
108	9.45 (1.09)
136	6.22 (1.75)

a All values were below the limit of quantification (LOQ, < 0.5)
b Standard deviation is in parentheses.

Note: Only 5 values tested for days 53 to 136 (recovery).

TABLE 7

MEAN LIVER FLUORINE LEVELS

	Negative Control	Test Substance
Test Days	Deionized Water (ppm F)	H-24159 (ppm F)
53	0.60[a]	176.04 (17.6)[b]
136	0.57 (0.1)	20.60 (5.7)

a Mean of 1 of the 5 values. Four of the values were below the limit of quantification (LOQ).
b Standard deviation is in parentheses.

TABLE 8

MEAN LIVER FLUORINE CONCENTRATION EXPRESSED IN μM EQUIVALENTS

	Test Substance
Test Days	H-24159 μM Equivalents
53	710.46 (70.97)[a]
136	82.42 (23.07)

a Standard deviation is in parentheses.

Note: Only 5 values tested for days 54 and 137 (recovery).

TABLE 9

MEAN FAT FLUORINE LEVELS

	Negative Control	Test Substance
Test Days	Deionized Water (ppm F)	H-24159 (ppm F)
53	0.75[a] (0.4)[b]	18.86 (1.6)
136	[c]	5.20 (0.9)

a Mean of 2 of the 5 values. Three of the values were below the limit of quantification (LOQ).
b Standard deviation is in parentheses.
c All values were below the LOQ.

TABLE 10

MEAN FAT FLUORINE CONCENTRATION EXPRESSED IN μM EQUIVALENTS

	Test Substance
Test Days	H-24159 μM Equivalents
53	75.39 (6.57)[a]
136	20.20 (3.65)

a Standard deviation is in parentheses.

Note: Only 5 values tested for days 54 and 137 (recovery).

TABLE 11

MEAN KIDNEY FLUORINE LEVELS

	Negative Control	Test Substance
Test Days	Deionized Water (ppm F)	H-24159 (ppm F)
53	0.86 (0.3)[a]	76.08 (14.1)
136	0.90[b]	9.66 (2.9)

a Standard deviation is in parentheses.
b Mean of 1 of the 5 values. Four of the values were below the limit of quantification (LOQ).

TABLE 12

MEAN KIDNEY FLUORINE CONCENTRATION EXPRESSED IN μM EQUIVALENTS

	Test Substance
Test Days	H-24159 μM Equivalents
53	306.59 (57.04)[a]
136	38.22 (11.82)

a Standard deviation is in parentheses.

Note: Only 5 values tested for days 54 and 137 (recovery).

FIGURES

FIGURE 1

MEAN BODY WEIGHTS (g)

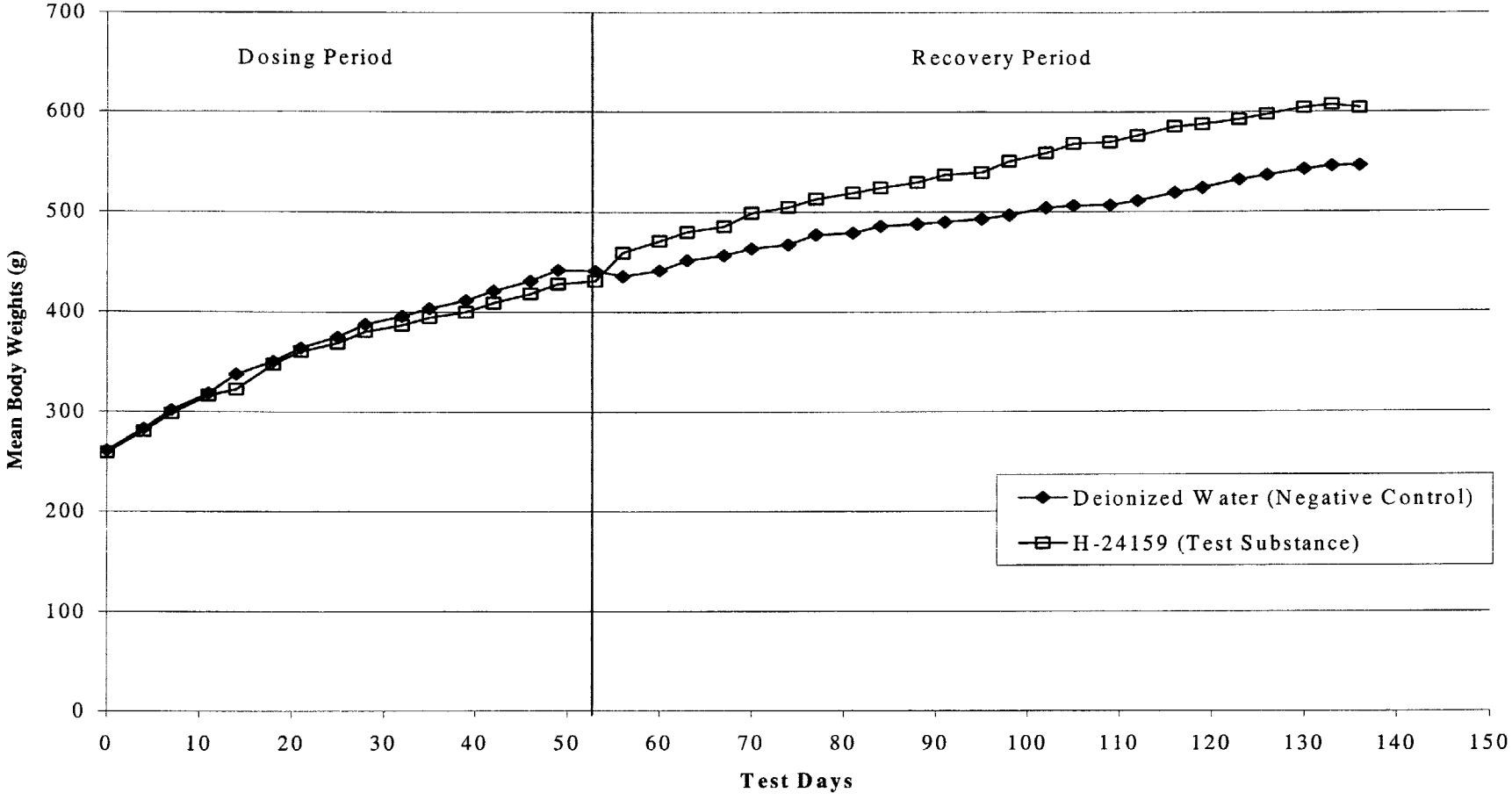
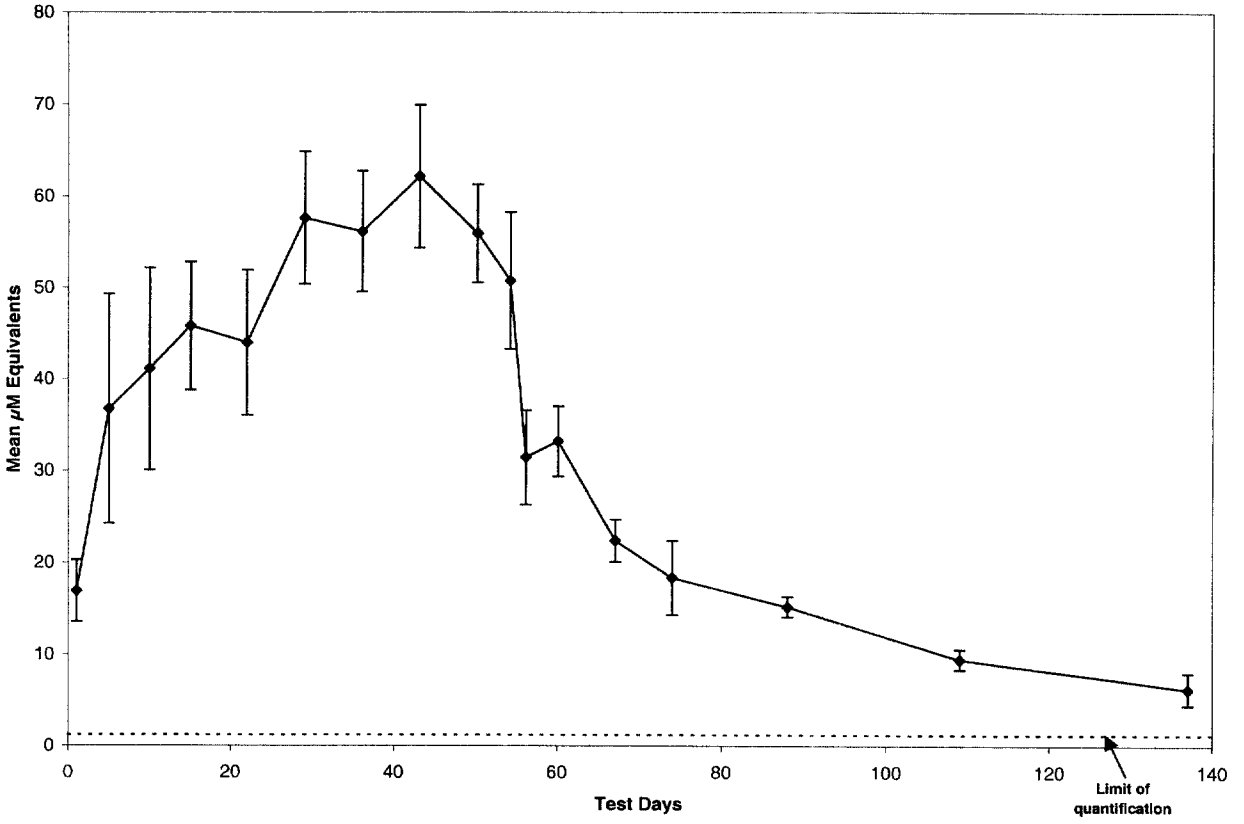


FIGURE 2

FLUORINE (μM EQUIVALENTS) IN RAT BLOOD



Micromolar (μM) equivalents fluorine in rat blood resulting from a 52 day oral gavage exposure. Values are means and error bars are standard deviation.

FIGURE 3

COMPARISON OF RELATIVE LIVER WEIGHT AND MEAN LIVER FLUORINE CONCENTRATION FOR H-24159 AND NEGATIVE CONTROL

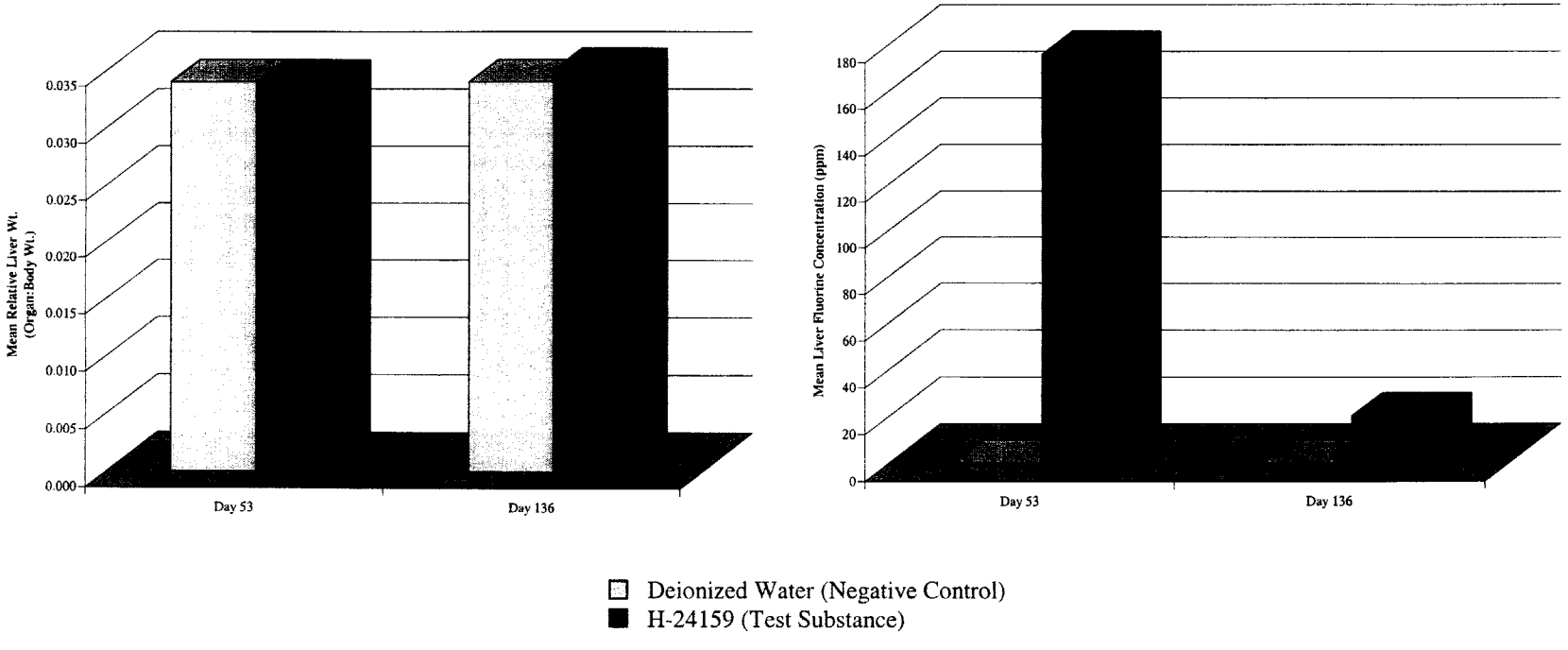
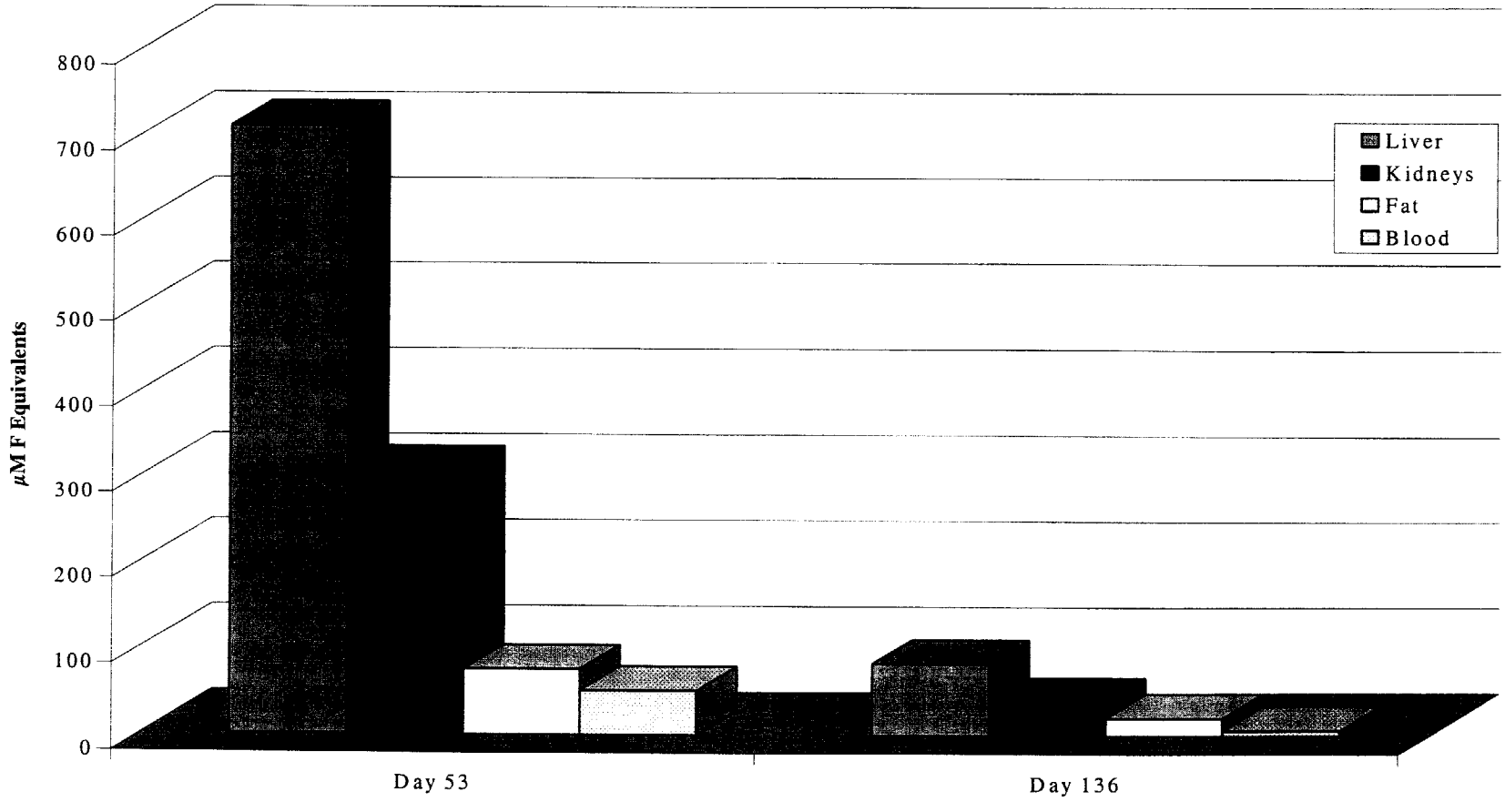


FIGURE 4

COMPARISON OF MEAN LIVER, KIDNEYS, FAT, AND BLOOD
FLUORINE CONCENTRATION NORMALIZED TO DOSE



APPENDICES

APPENDIX A

Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

SD - sacrificed by design

NOTE:

The first 5 animals were sacrificed by design on test day 53.

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP I

ANIMAL NUMBER	TEST DAY Day 0	Day 4	Day 7	Day 11	Day 14	Day 18	Day 21	Day 25	Day 28
649293	281.5	304.3	313.1	324.6	340.2	346.5	363.6	372.5	382.3
649294	265.3	287.6	306.8	328.1	355.2	368.6	385.9	404.6	423.7
649295	275.8	304.7	331.2	353.6	381.1	398.3	415.6	425.2	439.0
649296	242.7	263.6	278.2	295.5	310.8	313.3	326.7	323.3	335.1
649297	265.4	289.2	304.1	318.9	333.4	346.8	350.6	371.6	384.9
649298	226.8	241.8	258.1	264.7	272.0	283.9	293.5	303.6	309.6
649299	293.1	324.4	354.7	380.3	404.8	428.7	443.4	459.4	479.1
649300	268.9	298.9	316.5	336.8	354.7	370.2	382.4	403.0	415.4
649301	246.0	254.1	273.2	286.1	304.2	316.3	335.6	337.7	348.7
649312	242.8	260.4	280.3	295.2	318.1	328.4	341.7	347.7	358.5

ANIMAL NUMBER	TEST DAY Day 32	Day 35	Day 39	Day 42	Day 46	Day 49	Day 53	Day 56	Day 60
649293	397.0	399.6	416.3	428.4	435.3	444.4	437.2	SD test day 53	
649294	438.2	450.6	463.9	474.8	489.3	502.8	505.2	SD test day 53	
649295	450.0	464.8	476.2	485.5	506.2	515.9	525.8	SD test day 53	
649296	346.8	350.5	330.3	334.1	351.8	359.8	312.2	SD test day 53	
649297	398.2	407.2	418.0	430.1	446.0	452.0	453.4	SD test day 53	
649298	323.6	328.7	340.9	354.7	357.7	366.6	378.0	380.5	386.9
649299	493.7	501.5	520.6	531.5	545.5	560.9	571.9	569.8	581.5
649300	419.7	430.5	436.1	446.2	455.0	465.5	474.1	480.6	478.8
649301	354.8	359.0	366.5	377.3	359.0	374.7	381.7	375.6	375.9
649312	338.2	345.1	347.0	350.8	365.0	380.0	373.5	373.6	385.5

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP I Continued)

ANIMAL NUMBER	TEST DAY Day 63	Day 67	Day 70	Day 74	Day 77	Day 81	Day 84	Day 88	Day 91
649293									
649294									
649295									
649296									
649297									
649298	396.2	403.5	407.3	407.9	418.2	424.2	426.8	433.3	436.5
649299	591.0	583.7	598.4	604.9	619.4	625.0	632.1	630.5	636.8
649300	486.4	495.6	504.1	504.4	514.9	525.0	532.3	525.2	532.8
649301	393.8	397.1	407.5	411.5	420.2	406.5	417.7	420.3	414.2
649312	392.6	404.0	401.0	409.0	415.0	416.1	419.9	430.7	430.3

ANIMAL NUMBER	TEST DAY Day 95	Day 98	Day 102	Day 105	Day 109	Day 112	Day 116	Day 119	Day 123
649293									
649294									
649295									
649296									
649297									
649298	440.8	445.0	454.3	460.1	450.0	455.7	459.8	464.2	472.2
649299	640.2	648.8	658.1	661.6	661.4	655.8	669.9	677.1	683.4
649300	528.0	536.5	544.2	546.3	552.3	556.4	569.3	572.0	587.6
649301	417.5	427.2	435.0	430.0	431.3	442.2	439.5	446.9	447.4
649312	439.0	427.5	429.0	433.5	438.9	445.3	456.8	459.2	469.8

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP I

ANIMAL NUMBER	TEST DAY Day 126	Day 130	Day 133	Day 136	
649293					
649294					
649295					
649296					
649297					
649298	478.9	482.4	488.6	494.4	SD test day 136
649299	682.0	689.0	692.1	689.6	SD test day 136
649300	585.2	596.1	597.7	597.3	SD test day 136
649301	458.8	463.0	462.4	457.5	SD test day 136
649312	478.3	482.0	487.9	492.3	SD test day 136

H-24159 (TEST SUBSTANCE)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP III

ANIMAL NUMBER	TEST DAY Day 0	Day 4	Day 7	Day 11	Day 14	Day 18	Day 21	Day 25	Day 28
649302	215.0	228.1	240.4	256.9	270.2	277.0	285.9	290.5	300.6
649303	267.4	278.5	293.6	305.3	316.4	326.9	340.7	338.0	349.5
649304	254.7	281.9	303.4	324.3	343.0	360.6	374.6	389.8	404.0
649305	265.4	283.2	295.5	314.4	324.3	344.9	356.9	364.8	372.2
649307	259.0	285.0	309.5	330.1	350.2	363.6	378.2	386.0	396.1
649308	235.7	254.9	266.8	285.9	300.8	313.8	322.4	337.9	350.4
649309	280.4	310.7	334.5	343.4	353.9	372.6	383.8	394.0	414.0
649310	268.8	292.5	314.7	334.7	356.8	373.2	383.7	399.5	413.6
649311	235.8	254.1	269.5	290.0	305.7	320.6	336.8	351.7	360.2
649313	304.4	331.8	356.3	377.7	401.1	419.8	437.3	434.0	441.0

ANIMAL NUMBER	TEST DAY Day 32	Day 35	Day 39	Day 42	Day 46	Day 49	Day 53	Day 56	Day 60
649302	300.2	310.7	320.6	331.2	337.9	347.6	301.5	SD test day 53	
649303	363.5	368.8	376.8	387.6	397.5	406.3	409.2	SD test day 53	
649304	409.6	419.0	431.3	440.1	451.6	461.3	464.0	SD test day 53	
649305	374.4	371.9	378.1	387.1	390.2	400.4	400.4	SD test day 53	
649307	405.4	411.8	418.0	424.3	422.2	434.3	439.1	SD test day 53	
649308	356.6	366.5	373.9	381.2	396.3	405.4	410.2	417.1	421.2
649309	426.4	434.8	443.2	451.2	457.8	459.9	479.0	482.1	493.0
649310	421.3	426.7	437.1	442.0	452.2	462.1	475.5	473.4	483.9
649311	365.0	376.7	388.4	395.2	404.4	415.8	427.7	420.5	433.3
649313	445.7	457.8	433.5	450.7	471.4	490.2	504.6	505.1	525.8

H-24159 (TEST SUBSTANCE)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP III (Continued)

ANIMAL NUMBER	TEST DAY Day 63	Day 67	Day 70	Day 74	Day 77	Day 81	Day 84	Day 88	Day 91
649302									
649303									
649304									
649305									
649307									
649308	432.7	435.9	450.7	452.5	467.2	473.3	479.9	483.3	491.5
649309	498.0	509.5	513.7	519.0	526.6	530.2	533.8	534.7	543.6
649310	488.8	494.0	504.7	512.1	517.0	523.0	526.2	534.4	543.6
649311	442.2	440.9	453.3	455.9	463.2	467.9	473.2	476.7	480.4
649313	539.9	547.8	572.5	584.7	591.4	600.6	607.3	618.0	626.1

ANIMAL NUMBER	TEST DAY Day 95	Day 98	Day 102	Day 105	Day 109	Day 112	Day 116	Day 119	Day 123
649302									
649303									
649304									
649305									
649307									
649308	496.3	506.4	516.1	525.4	520.5	528.8	541.2	549.2	550.2
649309	545.9	557.5	561.5	574.9	577.1	587.6	594.0	594.8	601.0
649310	540.9	546.6	554.5	562.1	560.8	563.9	568.5	566.9	571.0
649311	475.5	490.3	496.1	503.9	507.5	509.0	514.6	517.5	519.2
649313	637.3	651.9	665.7	674.9	682.3	691.7	708.8	710.6	721.5

H-24159 (TEST SUBSTANCE)

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP III (Continued)

ANIMAL NUMBER	TEST DAY Day 126	Day 130	Day 133	Day 136	
649302					
649303					
649304					
649305					
649307					
649308	556.9	569.1	570.3	567.3	SD test day 136
649309	603.1	605.8	611.8	613.7	SD test day 136
649310	575.5	580.1	578.3	585.9	SD test day 136
649311	523.8	533.8	535.5	532.1	SD test day 136
649313	729.4	733.6	743.0	723.4	SD test day 136

APPENDIX B

Individual Clinical Observations

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

GROUP I

Sex	Group	Animal	Observation	Days
M	I	649293	General observation, No Abnormality Detected	0-7
			Eye Observations, Enophthalmus, Right	21-53
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,28,42,49
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,35
			Discharge, Eye bilateral, Black	21
			Discharge, Eye right, Black	11-14,25
			Hair Loss, Face, Right	18,28-35
			Sacrificed by design	53
M	I	649294	General observation, No Abnormality Detected	0
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	35
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,49
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28
			Hair Loss, Forelimb, Bilateral	4-7,11-53
			Hair Loss, Forepaw, Bilateral	18-53
			Sacrificed by design	53
M	I	649295	General observation, No Abnormality Detected	0-7,11-14,35-53
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,35
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,42,49
			Discharge, Eye left, Black	21-32
			Discharge, Eye right, Black	18
			Sacrificed by design	53
M	I	649296	General observation, No Abnormality Detected	0-4
			Eye Observations, Exophthalmus, Left	7,11
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	35
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,49
			Discharge, Eye bilateral, Black	25
			Discharge, Nose, Black	18-46
			Discharge, Eye left, Black	18-21,28-46
			Misshapen Observations, Ear, Right	14-53
			Sacrificed by design	53

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

GROUP I (Continued)

Sex	Group	Animal	Observation	Days
M	I	649297	General observation, No Abnormality Detected	0-7,11,18,32-35,49-53
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49
			Discharge, Nose, Black	14
			Discharge, Eye right, Black	39-46
			Hair Loss, Face, Right	28
			Wound, Superficial, Face	21-25
			Sacrificed by design	53
M	I	649298	General observation, No Abnormality Detected	0-7,11-32,39-53,56,60-63,67-70, 74,126-133
			Eye Observations, Enophthalmus, Right	77-84,88-105,109-123
			Eye Observations, Opaque, Right	136
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	42
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,66
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49,55,59,73,87,108
			Eye Observations, Dark, Right	77-84,88-105,109-123
			Discharge, Nose, Black	35
			Discharge, Eye right, Black	109
			Sacrificed by design	136
M	I	649299	General observation, No Abnormality Detected	0-7,11-53,56,60-63,67-70,74-84, 88-105,109-136
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	108
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,66,73
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49,55,59,87
			Sacrificed by design	136

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

GROUP I (Continued)

Sex	Group	Animal	Observation	Days
M	I	649300	General observation, No Abnormality Detected	0
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	87
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,66
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49,55,59,73,108
			Eye Observations, Dark, Right	60,88-91
			Discharge, Eye right, Black	74
			Hair Loss, Forelimb, Bilateral	4-7,11-53,56,60-63,67-70,74-84, 88-105,109-136
			Hair Loss, Forepaw, Bilateral	18-53,56,60-63,67-70,74-84, 88-105,109-136
			Hair Loss, Shoulder, Left	112-136
			Sacrificed by design	136
M	I	649301	General observation, No Abnormality Detected	0,21-53,56,60-63,67-70,74-84, 88-105,109-136
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	0-4,49
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,59,66,73,87,108
			Eye Observations, Bled via Orbital for Clin Path, Right	21,28,35,55
			Discharge, Nose, Black	14-18
			Discharge, Eye right, Black	4-7
			Hair Loss, Face, Right	7,11-18
			Sacrificed by design	136
M	I	649312	General observation, No Abnormality Detected	0-7,11-14,28,49-53,56,60-63, 67-70,74-84,88-105,109-136
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	35
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,59,66,73
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,49,55,87,108
			Discharge, Eye bilateral, Black	32-46
			Discharge, Nose, Black	18,32
			Discharge, Eye left, Black	18-25
			Sacrificed by design	136

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

GROUP III

Sex	Group	Animal	Observation	Days
M	III	649302	General observation, No Abnormality Detected	0-7,11-53
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49
			Sacrificed by design	53
M	III	649303	General observation, No Abnormality Detected	0-7,11-53
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	9,14
			Eye Observations, Bled via Orbital for Clin Path, Left	42
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49
			Sacrificed by design	53
M	III	649304	General observation, No Abnormality Detected	0-7,11-53
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49
			Sacrificed by design	53
M	III	649305	General observation, No Abnormality Detected	0,11-28,42-53
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	28
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,35,49
			Discharge, Eye right, Black	4-7
			Discharge, Eye right, Brown	32-39
			Sacrificed by design	53
M	III	649307	General observation, No Abnormality Detected	0-4
			Eye Observations, Exophthalmus, Left	49-53
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	42
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49
			Eye Observations, Dark, Left	49-53
			Hair Loss, Abdomen, Ventral	7,11-49
			Hair Loss, Nose	35-49
			Wound, Superficial, Nose	42-46
			Sacrificed by design	53

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

GROUP III (Continued)

Sex	Group	Animal	Observation	Days
M	III	649308	General observation, No Abnormality Detected	0-7,11-18
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,59,66,87
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49,55,73,108
			Discharge, Eye right, Black	109
			Discharge, Eye right, Red	56,60-63,67-70
			Misshapen Observations, Tail	21-53,56,60-63,67-70,74-84, 88-105,109-136
			Sacrificed by design	136
M	III	649309	General observation, No Abnormality Detected	0-7,39-42,49
			Eye Observations, Enophthalmus, Right	11-35
			Eye Observations, Opaque, Right	11-35,70,74-84,88-105,109-136
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	9,21,28
			Eye Observations, Bled via Orbital for Clin Path, Left	14,42,59,66,73,87,108
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,35,49,55
			Discharge, Nose, Black	14-21,28-35,46
			Discharge, Eye right, Red	18-25
			Hair Loss, Forepaw, Bilateral	53,56,60-63,67-70,74-77
			Hair Loss, Face, Right	28
			Sacrificed by design	136
M	III	649310	General observation, No Abnormality Detected	0-7,11-53,56,60-63,67-70,74-84, 88-105,109-136
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,59,66,87
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49,55,73,108
			Sacrificed by design	136
M	III	649311	General observation, No Abnormality Detected	0-7,11-53,56,60-63,67-70,77-84, 88-105,109-136
			Eye Observations, Bled via Orbital for Clin Path, Left	9,14,42,59,66,73,87
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,21,28,35,49,55,108
			Discharge, Eye left, Black	74
			Sacrificed by design	136

DEIONIZED WATER (NEGATIVE CONTROL)

INDIVIDUAL CLINICAL OBSERVATIONS IN MALE RATS

GROUP III (Continued)

Sex	Group	Animal	Observation	Days
M	III	649313	General observation, No Abnormality Detected	0-7,11-25,32-53,56,60-63,84,88-105
			Eye Observations, Enophthalmus, Right	109-136
			Eye Observations, Exophthalmus, Left	67-70,74-81
			Eye Observations, Bled via Orbital for Clin Path, Bilateral	9,14,21,28,49,55,73
			Eye Observations, Bled via Orbital for Clin Path, Left	42,59,66,87
			Eye Observations, Bled via Orbital for Clin Path, Right	0-4,35,108
			Eye Observations, Dark, Left	67-70,74
			Discharge, Nose, Black	28
			Sacrificed by design	136

APPENDIX C

Terms and Calculations
Factors Influencing Interpretation of Kinetic Analysis

TERMS AND CALCULATIONS

Terms:

Active	Fluorine containing compound(s)
% Active	The % of formulation that is made up of fluorine containing components
Mol Wt Active	The molecular weight of the fluorine containing components (g/mole)
% F in Active	The % fluorine in the fluorine containing components of the formulation (weight basis)

Individual Animal Measurement:

ppm F	The ppm fluoride measured

Individual Animal Calculations:

μmolar equivalents of active	The μmolar [μmol/L] concentration of fluorine containing compound based on the ppm fluorine measured minus the background fluoride level (0.2 ppm). This assumes that all fluorine is derived from the fluorine-containing component in the formulation. Note: 1 ppm = 1 mg/L = (ppm [mg/L] fluorine / % F in active [mg active/mg F] / Mol Wt Active [mg active/mmol active] x 1000 μmol/mmol

FACTORS INFLUENCING INTERPRETATION OF KINETIC ANALYSIS

Considerations:

- The data used for kinetic analysis was from a limited screen and extrapolation should be done cautiously.
- Sample size is low
- Analytical data used without validation
- Terminal phase elimination may not be reached
- H-24159 is a [redacted]

Assumptions: (May or may not be justified in all cases)

- Fluorine concentrations are linear with respect to dose
- Analytical method is appropriate for all types of compounds
- Elimination kinetics can be determined based on total fluorine rather than on concentrations of individual components
- Background Fluorine is 0.2 ppm
- % F data is the % Fluorine of the active (Fluorine containing component(s) in the formulation)
- Molecular weight is the molecular weight of the active component in the formulation

APPENDIX D

Individual Fluorine Levels in Blood

Data for Negative Control

Rat Number	Test Day Sample	ppm F in Blood
649293	-4	<0.5
649294	-4	<0.5
649295	-4	<0.5
649296	-4	<0.5
649297	-4	<0.5
649298	-4	<0.5
649299	-4	<0.5
649300	-4	<0.5
649301	-4	<0.5
649312	-4	<0.5
649293	4	<0.5
649294	4	<0.5
649295	4	<0.5
649296	4	<0.5
649297	4	<0.5
649298	4	<0.5
649299	4	<0.5
649300	4	<0.5
649301	4	<0.5
649312	4	<0.5
649293	9	0.7
649294	9	<0.5
649295	9	0.5
649296	9	<0.5
649297	9	<0.5
649298	9	0.9
649299	9	0.8
649300	9	0.9
649301	9	1.1
649312	9	1.0
649293	14	<0.5
649294	14	<0.5
649295	14	<0.5
649296	14	0.5
649297	14	<0.5
649298	14	<0.5
649299	14	<0.5
649300	14	<0.5

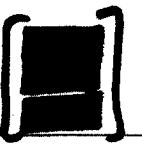
Rat Number	Test Day Sample	ppm F in Blood
649301	14	0.7
649312	14	0.5
649293	49	<0.5
649294	49	<0.5
649295	49	<0.5
649296	49	<0.5
649297	49	<0.5
649298	49	<0.5
649299	49	<0.5
649300	49	<0.5
649301	49	<0.5
649312	49	<0.5
649293	53	<0.5
649294	53	<0.5
649295	53	<0.5
649296	53	<0.5
649297	53	<0.5
649298	55	<0.5
649299	55	<0.5
649300	55	<0.5
649301	55	<0.5
649312	55	<0.5
649298	59	<0.5
649299	59	<0.5
649300	59	<0.5
649301	59	<0.5
649312	59	<0.5
649298	73	1.4
649299	73	<0.5
649300	73	0.6
649301	73	0.4
649312	73	<0.5
649298	108	0.8
649299	108	<0.5
649300	108	<0.5
649301	108	<0.5
649312	108	0.6
649298	136	<0.5
649299	136	<0.5
649300	136	<0.5

Rat Number	Test Day Sample	ppm F in Blood
649301	136	<0.5
649312	136	<0.5

Data for H-24159

Given:

Mol Wt. Active (g/mole):
% Active (F Containing) in Formulation:
% F in Active:



Rat Number	Test Day Sample	ppm F in Blood	μmolar Equivalents of Active in Blood
649302	-4	<0.5	*
649303	-4	<0.5	*
649304	-4	<0.5	*
649305	-4	<0.5	*
649307	-4	<0.5	*
649308	-4	<0.5	*
649309	-4	<0.5	*
649310	-4	<0.5	*
649311	-4	<0.5	*
649313	-4	<0.5	*
649302	0	4.5	17.37
649303	0	2.6	9.70
649304	0	5.0	19.39
649305	0	4.2	16.16
649307	0	5.0	19.39
649308	0	3.6	13.74
649309	0	4.4	16.97
649310	0	4.1	15.76
649311	0	5.3	20.61
649313	0	5.2	20.20
649302	4	4.8	18.59
649303	4	6.3	24.65
649304	4	10.2	40.40
649305	4	5.4	21.01
649307	4	8.5	33.54
649308	4	11.7	46.46
649309	4	11.0	43.64
649310	4	13.1	52.12
649311	4	8.6	33.94
649313	4	13.4	53.33
649302	9	5.9	23.03
649303	9	14.0	55.76

Rat Number	Test Day Sample	ppm F in Blood	μmolar Equivalents of Active in Blood
649304	9	14.3	56.97
649305	9	7.5	29.49
649307	9	11.5	45.66
649308	9	10.7	42.42
649309	9	8.3	32.73
649310	9	10.3	40.81
649311	9	9.1	35.96
649313	9	12.1	48.08
649302	14	8.6	33.94
649303	14	10.5	41.62
649304	14	13.5	53.74
649305	14	8.7	34.34
649307	14	12.7	50.51
649308	14	12.6	50.10
649309	14	11.4	45.25
649310	14	12.8	50.91
649311	14	11.9	47.27
649313	14	12.6	50.10
649302	21	9.0	35.56
649303	21	10.7	42.42
649304	21	8.8	34.75
649305	21	9.8	38.79
649307	21	11.8	46.87
649308	21	9.9	39.19
649309	21	14.5	57.78
649310	21	11.1	44.04
649311	21	11.0	43.64
649313	21	14.2	56.57
649302	28	10.9	43.23
649303	28	14.5	57.78
649304	28	16.2	64.65
649305	28	15.5	61.82
649307	28	12.8	50.91
649308	28	15.0	59.80
649309	28	16.8	67.07
649310	28	13.2	52.53
649311	28	13.9	55.35
649313	28	15.8	63.03
649302	35	12.1	48.08
649303	35	12.8	50.91

Rat Number	Test Day Sample	ppm F in Blood	μmolar Equivalents of Active in Blood
649304	35	16.1	64.24
649305	35	16.3	65.05
649307	35	12.6	50.10
649308	35	15.8	63.03
649309	35	15.5	61.82
649310	35	13.2	52.53
649311	35	13.1	52.12
649313	35	13.4	53.33
649302	42	11.7	46.46
649303	42	15.6	62.22
649304	42	18.9	75.56
649305	42	13.9	55.35
649307	42	16.5	65.86
649308	42	15.6	62.22
649309	42	15.2	60.61
649310	42	15.9	63.43
649311	42	15.1	60.20
649313	42	17.4	69.49
649302	49	11.2	44.44
649303	49	13.0	51.72
649304	49	14.9	59.39
649305	49	13.4	53.33
649307	49	13.4	53.33
649308	49	14.8	58.99
649309	49	15.4	61.41
649310	49	14.6	58.18
649311	49	14.1	56.16
649313	49	15.6	62.22
649302	53	14.5	57.78
649303	53	11.4	45.25
649304	53	13.8	54.95
649305	53	10.2	40.40
649307	53	13.9	55.35
649308	55	5.9	23.03
649309	55	8.3	32.73
649310	55	8.7	34.34
649311	55	7.8	30.71
649313	55	9.2	36.36
649308	59	8.4	33.13
649309	59	7.1	27.88

Rat Number	Test Day Sample	ppm F in Blood	μmolar Equivalents of Active in Blood
649310	59	8.0	31.52
649311	59	9.1	35.96
649313	59	9.5	37.58
649308	66	5.1	19.80
649309	66	5.7	22.22
649310	66	6.6	25.86
649311	66	5.9	23.03
649313	66	5.4	21.01
649308	73	3.8	14.55
649309	73	4.2	16.16
649310	73	6.4	25.05
649311	73	4.8	18.59
649313	73	4.5	17.37
649308	87	3.8	14.55
649309	87	3.6	13.74
649310	87	4.1	15.76
649311	87	4.3	16.57
649313	87	4.0	15.35
649308	108	2.1	7.68
649309	108	2.6	9.70
649310	108	2.8	10.51
649311	108	2.7	10.10
649313	108	2.5	9.29
649308	136	1.1	3.64
649309	136	1.7	6.06
649310	136	1.9	6.87
649311	136	1.7	6.06
649313	136	2.3	8.48

* Below LOQ (Limit of Quantification)

APPENDIX E

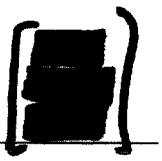
Individual Fluorine Levels in Liver

Data for Negative Control

Rat Number	Test Day Sample	ppm F in Liver
649293	53	<0.5
649294	53	<0.5
649295	53	<0.5
649296	53	<0.5
649297	53	0.6
649298	136	0.5
649299	136	0.5
649300	136	0.7
649301	136	<0.5
649312	136	<0.5

Data for H-24159

Given:
Mol Wt. Active (g/mole):
% Active (F Containing) in Formulation:
% F in Active:



Rat Number	Test Day Sample	ppm F in Liver	μmolar Equivalents of Active in Liver
649302	53	189.8	766.06
649303	53	169.3	683.23
649304	53	198.8	802.42
649305	53	156.8	632.73
649307	53	165.5	667.88
649308	136	13.7	54.55
649309	136	19.3	77.17
649310	136	26.0	104.24
649311	136	17.1	68.28
649313	136	26.9	107.88

APPENDIX F

Individual Fluorine Levels in Fat

Data for Negative Control

Rat Number	Test Day Sample	ppm F in Fat
649293	53	0.5
649294	53	1.0
649295	53	<0.5
649296	53	<0.5
649297	53	<0.5
649298	136	<0.5
649299	136	<0.5
649300	136	<0.5
649301	136	<0.5
649312	136	<0.5

Data for H-24159

Given:

Mol Wt. Active (g/mole):
% Active (F Containing) in Formulation: 
% F in Active:

Rat Number	Test Day Sample	ppm F in Fat	μmolar Equivalents of Active in Fat
649302	53	20.6	82.42
649303	53	19.1	76.36
649304	53	17.5	69.90
649305	53	20.2	80.81
649307	53	16.9	67.47
649308	136	5.6	21.82
649309	136	5.9	23.03
649310	136	5.9	23.03
649311	136	4.8	18.59
649313	136	3.8	14.55

APPENDIX G

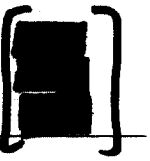
Individual Fluorine Levels in Kidneys

Data for Negative Control

Rat Number	Test Day Sample	ppm F in Kidneys
649293	53	0.8
649294	53	1.2
649295	53	0.9
649296	53	0.5
649297	53	0.9
649298	136	<0.5
649299	136	<0.5
649300	136	<0.5
649301	136	<0.5
649312	136	0.9

Data for H-24159

Given:
Mol Wt. Active (g/mole):
% Active (F Containing) in Formulation:
% F in Active:



Rat Number	Test Day Sample	ppm F in Kidneys	μmolar Equivalents of Active in Kidneys
649302	53	93.4	376.57
649303	53	60.1	242.02
649304	53	63.0	253.74
649305	53	82.9	334.14
649307	53	81.0	326.46
649308	136	6.0	23.43
649309	136	10.3	40.81
649310	136	8.4	33.13
649311	136	14.0	55.76
649313	136	9.6	37.98

APPENDIX H

Urine and Feces Data

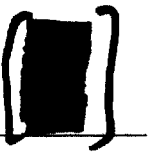
Data for Negative Control

	Test Day	ppm F
Urine	51	8.9
Urine	52	10.2
Urine	53	6.8
Feces	51	5.1
Feces	52	1.1
Feces	53	1.1

Data for H-24159

Given:

Mol Wt. Active (g/mole):
% Active (F Containing) in Formulation:
% F in Active:



	Test Day	ppm F	g or mL/day	μg F/day	μmole Equivalents of Active/Day
Urine	51	12.7	70.0	889.00	3591.11
Urine	52	9.9	46.0	455.40	1839.19
Urine	53	8.7	54.0	469.80	1897.37
Feces	51	39.6	3.5	139.39	562.39
Feces	52	38.9	24.3	945.27	3818.46
Feces	53	41.5	15.3	634.12	2561.29